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CONTENTS

	Page
CPJ 2018013/24201 The Potential Source Rocks for Petroleum Generation in the Dahomey Basin, Southwestern Nigeria <i>Olabisi A. Adekeye, Samuel O. Akande and Olukayode J. Samuel</i>	1-20
CPJ 2018014/24202 Effect of Sublethal concentration of sniper 1000EC on weight and length growth of <i>Oreochromis niloticus</i> obtained from Asa dam under laboratory conditions <i>Abubakar Musa Idi-Ogede</i>	21-37
CPJ 2018015/24203 Physicochemical Changes of <i>Citrullus Lanatus</i> (Watermelon) Seeds during <i>Bacillus</i> Species mediated Fermentation and Sensory Evaluation of Condiment Produced <i>Balogun, M. A., Akintayo, O. A., Kolawole, F. L., Karim, O. R., Rowland, E and Olalere, M. O.</i>	39-54
CPJ 2018016/24204 Evaluation of <i>Oligochaeta</i> Fauna of Ozomu Lake, Benin City, Nigeria. <i>J.O. Odigie and J.O. Olomukoro</i>	55-66
CPJ 2018017/24205 Physicochemical Properties of Starch obtained from Tubers of <i>Anchomanes difformis</i> and <i>Tacca involucrata</i> <i>Ameen, O. M., Olatunji, G. A., Abdulrahman A. A., Adenusi, B. F., Folorunsho, O. F., Okeola O. F. and Samoh, F. T.</i>	67-79
CPJ 2018018/24206 Antimalarial activity and heamatological parameters of <i>Plasmodium berhgei</i> - infected mice treated with combination of aqueous extract of <i>Nigella sativa</i> and Honey <i>Fatimah, Aluko, Abubakar, Abdulai, Suliat, Iyabode, Mohammed, Sheriff, Mohammed, Aisha, Abisola, Akinbami, Azeemat, Titilayo, Abdulazeez</i>	81-94

CPJ 2018019/24207	
Practice of safety measures and work related injuries among sawmill workers in Kwara South Senatorial District of Nigeria <i>Salaudeen A. G., Musa O. I., Ahmed A., Yusuf A. S., Gobir A. A., Awoyemi A. O.</i>	95-107
CPJ 2018020/24208	
Site Suitability Delineation for Sanitary Landfill Development Using Vertical Electrical Sounding Geophysical Method in Gosa Area of Abuja, Nigeria <i>O.O. Ige, T.A. Alafiatayo and B.A. Ojulari</i>	109-119
CPJ 2018021/24209	
Antibacterial activities and effects of dosage of Methanol and “Omidun” Extract of <i>Vitellaria paradoxa</i> (C.F. Gaertn) root on some diarrheagenic bacteria <i>M. B. Falana and Q. O. Nurudeen</i>	121-138
CPJ 2018022/24210	
Extraction, Physicochemical, Phytochemical, Biochemical, GC-MS Constituents and Environmental Effects of <i>Petiveria alliacea</i> Leaves <i>A.M.O. Abdul Raheem; F.A. Sulaiman; O.L. Malomo; M.M. Oyewo; A. Hassan; O. Ahmed; G.O. Alimi; D. Afolayan; O.T. Odeniran; H.A. Abdulrahim; O.K. Yusuf; A.A. Mukadam</i>	139-173
CPJ 2018023/24211	
Unsteady Non-Newtonian Magnetohydrodynamic Radiative Thin Film Flow in a Porous Medium Past an Oscillating Vertical Belt <i>M.S. Dada*, L. Yakub, A.S. Adeyemo</i>	175-195
CPJ 2018024/24212	
Magnetohydrodynamic free convection flow of viscoelastic fluid past a vertical porous plate with thermal conductivity and Chemical reaction <i>Idowu, A.S. and Jimoh, A.</i>	197-223
CPJ 2018024/24213	
Toxicological studies of <i>Terminalia glaucescens</i> using an animal model <i>Okutue, J.U. and Omojasola, P.F</i>	224-236

The Potential Source Rocks for Petroleum Generation in the Dahomey Basin, Southwestern Nigeria

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Abstract

The Upper Cretaceous (Maastrichtian) Araromi Shale formation in the Nigeria sector of the Dahomey basin has been investigated for its petroleum generation potential. From three exploratory wells, Araromi, Bode Ashe and Gbekebo in the eastern end (west of Niger Delta), source rock potential has been evaluated for over one hundred (100) drill core and ditch cutting samples. The investigated shallow marine shale facies have Total Organic Carbon (TOC) value range of 0.50-4.78wt% and Hydrogen Index (HI) value range of 1-327mgHC/gTOC with the maceral composition of liptinite (av. 26.0%) and abundance of vitrinite (av. 38.1%) plus inertinite (av. 35.9%) in all the samples investigated. Vitrinite reflectance values vary from 0.51-0.68%R_o. The T_{max} values vary from 398°C-437°C and the kerogen Types include type II, II/III, III, and IV in all the samples. The Source Potential (SP) values range from 0.01-14.56kgHC/ton of rock. Biomarker analysis of the shale samples generally reveal a bimodal n-alkane envelope with maxima between (nC₁₆ and nC₁₈) and (nC₂₇ and nC₂₉) suggesting that the source organic matter were derived from a variable mixture of algae and higher plant materials with q relative higher input from marine algae as reveal by the presence of the C₃₀ 24-n-propyl cholestane (%C₃₀ sterane range from 0.45 to as high as 5.23%). The presence of organic matter of marine algae (%C₂₇ sterane av. 52% and positive detection of C₃₀ sedimentary n-propyl cholestane av. 3.7%) suggest that the control on the HI as an indication of source rock quality in

varying level of organic matter preservation. The source rocks have predominantly gas prone with lesser oil prone organic matter ranging from immature to marginally mature at shallow levels but reaching proven mature levels in the subsurface. Hydrocarbons in the basin would be predominantly gas dominated, generated from deeper Cretaceous source rock (marine shale) in the subsurface levels with greater chances of heavy oils and tars at shallow levels.

Keywords: Total Organic Carbon, Biomarker analysis, Hydrocarbons

Introduction

The search for hydrocarbons in the Cretaceous source rocks in the Nigerian in land sedimentary basins has been a major task for many decades now. The efforts were to bust the oil and gas exploration and production activities and also adequately tap the natural resources in the country. Geoscience research in the aspects of hydrocarbon search in the Nigeria sector of the Dahomey basin has been so few for many years. In the last decade, there have been considerable interest and geosciences researches in the aspects of hydrocarbon search in the basin. Recent exploration and prospect re-evaluation efforts in the Nigeria segment of the basin have been encouraged following oil discovery and production in the neighboring Benin republic sector west of the basin and the social unrest in the prolific Niger Delta, east of the basin.

The Dahomey basin is an extensive sedimentary basin in the Gulf of Guinea. It extends from southeastern Ghana through Togo and Benin Republic on the west side to the Okitipupa ridge/Benin Hinge line on the west of Niger Delta. Shale samples (drill cores and ditch cuttings) for this study came from the Araromi and Gbekebo wells drilled in the eastern part of the Dahomey basin, southwestern Nigeria (Fig. 1). The drill core samples were collected from Geological Survey of Nigeria Agency (GSNA), Kaduna main office. This work is to contribute to the geosciences research and data generation activity by assessing and documenting the organic geochemical parameters and hydrocarbon generation potential of the Araromi shales in the Nigeria sector of the Dahomey basin. This paper will also throw more light on the possibility of Cretaceous sourced oil in the overlying prolific Tertiary Niger Delta which is at the eastern side of the basin.

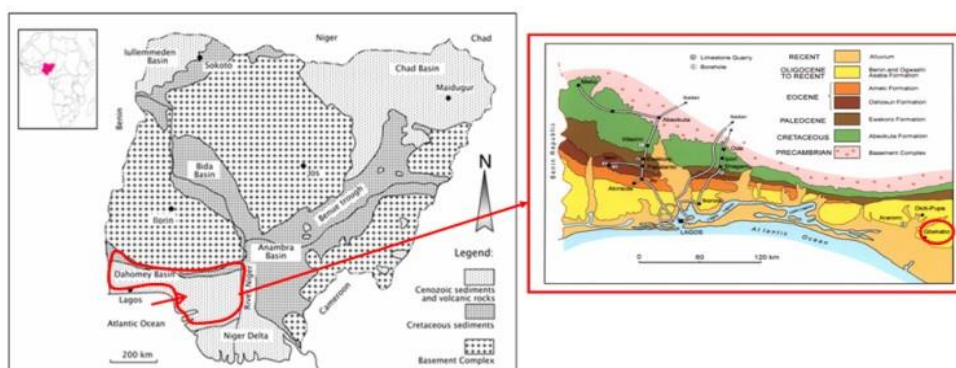


Figure 1

Fig. 1: Map showing the sedimentary basins of Nigeria. The arrow points to the location of the Dahomey Basin on the western margin of the Niger Delta. The map to the right is an enlarged outline geological map of Dahomey basin showing the location of the Araromi and Gbekebo wells (red ring) on the eastern margin of Dahomey (west of the Niger Delta). Map modified after Adekeye et al. (2006).

Geological setting

The break-up of the Gondwanaland as a result of “continental drift” led to the subsequent opening of the South Atlantic Ocean during the Mesozoic Era (Stoney, 1995; Mpanda, 1997). This event is also connected to the evolution of the Dahomey Basin. Several hypotheses have been developed as to the origin and evolution of the basin. The rift hypothesis is widely supported, on the basis of several salient features characterising the basin.

During the rifting stage in the Lower Jurassic – Early Cretaceous, there was basement fracturing and initial separation between the African and South American plates. At this time, several marginal basins developed consequent to block faulting, fragmentation and subsidence on the central Paleozoic basement rock (Asmus and Ponte, 1973; Ojeda, 1982; Omatsola and Adegoke, 1981). The early movements were in the Early Cretaceous when there was transcurrent movements on the oceanic fracture systems especially the Romanche, Chain and Charcot fractures during the drifting stages of separation of South America from Africa in the Campanian to Tertiary times. Consequent to the opening of the South Atlantic Ocean, there was landward extensional and transtensional

movement (drift stage) during the Upper Cretaceous to Tertiary. Horizontal movements along the oceanic fracture zones were translated to vertical movements leading to block faulting and subsequent development of horsts and grabens (Omatsola and Adegoke, 1981).

Adediran and Adegoke, 1987 proposed a four stage evolutionary model for the Gulf of Guinea basin (Dahomey Basin inclusive); as follows:

Stage 1 – The deposition of thick clastic sediments mostly immature sandstones and fresh water shales in the intracratonic basin.

Stage 2 – Reworked sands and silts intercalated with shales of fluvial – lacustrine origin deposited within the grabens during a period of tectonic activity, erosion and sedimentation.

Stage 3 – Paralic sequence (in the northern basins) and evaporitic deposits (in the southern basins) marking the beginning of marine incursion into the basin after the separation of South America from Africa.

Stage 4 – Marine sediments rich in fauna and flora marking the final stage of the development of the Gulf of Guinea basins.

The stratigraphic setting of the Dahomey Basin has been described in detail in the works of Adegoke, 1969; Ogbe, 1970; Kogbe, 1974; Billman, 1976; Omatsola and Adegoke, 1981; Ako et al., 1980; Okosun, 1990; Adekeye, 2005 and Adekeye et al., 2006. These authors reported five lithostratigraphic formations covering the Cretaceous to Tertiary ages. The formations from the oldest to the youngest include: Abeokuta Group comprises of Ise, Afowo and Araromi formations (Cretaceous), Ewekoro Formation (Paleocene), Akinbo Formation (Late Paleocene-Early Eocene), Oshosun Formation (Eocene) and Ilaro Formation (Eocene) Fig.2. Substantial amount of sediments were deposited in fault-controlled depressions in the Dahomey Basin during the Late Cretaceous. Post Santonian marine transgression accompanied the subsidence and drowning of continental margins, which brought about the deposition of very thick sequence of continental grits and pebbly sands over the entire basin (Lehner and Ruiters, 1977). In some places, mudstones and shales with thin limestone beds were formed. This lithostratigraphic formation is referred to as Abeokuta Group comprising Ise, Afowo and Araromi formations (Omatsola and Adegoke, 1981). A continuation of the marine

transgression during the Paleocene led to the deposition of shallow marine limestones of the Ewekoro Formation and the shales of the Akinbo Formation (Ogbe, 1970; Okosun, 1990). On top of the Paleocene sequence is the Eocene shales of the Oshosun Formation and the sandstones of the Ilaro Formation (Okosun, 1990; Idowu et al., 1993).

AGE		FORMATION		LITHOLOGY
		Ako et al., 1980	Omatsola and Adegoke, 1981	
TERTIARY	EOCENE	ILARO FM	ILARO FM	SANDSTONE
		OSHOSUN FM AKINBO FM	OSHOSUN FM AKINBO FM	SHALE
	PALEOCENE	EWEKORO FM	EWEKORO FM	LIMESTONE
CRETACEOUS	MAASTRICHTIAN	ABEOKUTA FM	ABEOKUTA GROUP	SHALE
	TURONIAN			SANDSTONE AND SHALE
	BARREMIAN			SANDSTONE

Fig. 2: Generalised stratigraphic setting of the Dahomey Basin in the southwestern Nigeria (modified and redrawn from Idowu et al., 1993)

Materials and methods

Hydrocarbon Source Rock Potential

Representative core samples of the Late Cretaceous Araromi Formation in the Dahomey Basin, SW Nigeria were collected from the Araromi and Gbekebo wells (Fig. 1) drilled on the eastern margin of the basin where it shares a border with the western Niger Delta. The samples were analysed for their total organic carbon content (TOC) and hydrocarbon source quality by Rock-Eval Pyrolysis using standard experimental techniques. Bitumen extracts from selected organic rich source rock samples were analysed for their biomarker and stable carbon isotope composition of *n*-alkanes using the GC, GC-MS and GC-MS-MS techniques described below.

Selected shale samples were crushed to less than 2mm and impregnated in epoxy for qualitative reflected light microscopy. Where organic constituents are sparse, kerogen concentrates were prepared, mounted and

polished. Organic petrology studies were carried out on a Reichert Jung Polyvar Photomicroscope equipped with halogen and HBO lamp, a photomultiplier and computer unit at the Zentraleinrichtung für Elektronenmikroskopie (ZELMI), Technische Universität Berlin, Germany. Mean random reflectance of vitrinite in oil ($R_o\%$, cf. Bustin et al., 1983) was calculated from the reflectance of at least 30 grains of vitrinite measured in random orientation using monochromatic (546nm) non-polarised light in conjunction with a x40 oil immersion objective. Calibration of the microscope photometer was achieved using standards of known reflectance (1.23 and 3.16%). Measured R_{om} values of reflectance standards confirmed the photomultiplier to be consistently linear within the range of the measurements. Data collection and evaluation were done using the coal programme by Reichert Jung and macerals were identified through the use of white light and blue light excitation at 546 and 460nm respectively. The mean reflectance as compared to the median or modal reflectance appear to be an adequate measure of thermal maturity in this study (Tissot and Welte, 1984; Pollastro and Barker, 1986).

The rock Eval pyrolysis was carried out at the Organic Geochemistry Laboratory of the Department of Mineralogy, Geochemistry and Petrology, University of Szeged, Hungary following the standard procedure of Rock Eval pyrolysis experimentation on Rock Eval II machine. The Rock-Eval II pyroanalyses has the ability of measuring the total organic carbon (TOC) content on the pulverized samples at elevated temperatures of ca. 600°C (Espitalie et al., 1977). Pyrolysis of 10-40mg of samples at 300°C for 4mins was followed by programmed pyrolysis at 25°C/min to 550°C in an atmosphere of helium.

Biomarker

Gas chromatography was performed on the saturated hydrocarbon fractions in order to obtain *n*-alkane and isoprenoid data as well as to determine sample concentration and complexity before GC-MS and gas chromatography-isotope ratio mass spectrometry (GC-IRMS) analyses. An HP 5890 series II gas chromatograph equipped with an HP-5 coated capillary column (60m x 0.25mm, 0.25µm film thickness) was used. The GC oven was initially set at 50°C for 2 min. and then the temperature was ramped from 50°C at 4°C/min to 300°C and held at final temperature for

20 minutes with hydrogen as the carrier gas (flow rate approx 2ml/min and initial pressure of 100kPa).

Gas chromatography-mass spectrometry (GC-MS) and gas chromatography-mass spectrometry-mass spectrometry (GC-MS-MS) analyses of saturated hydrocarbon fractions were performed on a Varian CP3800 GC split/splitless injector (280°C) linked to a Varian 1200 triple quadrupole mass spectrometer (electron voltage 70eV, filament current 50uA, source temperature 230°C, quad temperature 40°C, multiplier voltage 1400V, interface temperature 300°C). After addition of 5 β -cholane internal standard, the samples were analysed in scan mode (50-550 amu/sec) or selected ion monitoring mode (SIM) for 30 ions (dwell 35ms per ion) or in MS-MS mode where up to 8 parent/daughter transitions could be monitored, using argon as the collision gas at a pressure of 2mTorr and with a collision energy of -10ev. The sample (1 μ l) in dichloromethane was injected by a Varian CP8400 autosampler and the split opened after 1 minute. Separation was performed on a fused silica capillary column (60m x 0.32mm i.d) coated with 0.25 μ m methylsilicone (HP-1). The GC was temperature programmed over 3 ramps from 4°C-300°C (40°C-75°C at 10°C/min, held for 1min at 175°C, followed by 175°C-225°C at 6°C/min and held for 1 min at 225°C, and 225-300 at 4°C/min) and was held at final temperature for 20 minutes with helium as the carrier gas (flow approx 1ml/min, initial pressure of 30kPa, split at 30 mls/min).

Results, Interpretations and Discussions

Organic Matter Richness

The quantity of organic matter (OM) in a rock is a direct measure of the Total Organic Carbon content (TOC). The analysed Araromi shale samples have a value range of 0.5-4.78wt% (Tables 1 and 2), which indicates variable source rock organic carbon quality from poor to excellent. There is an overall trend of increasing TOC values down the well (Fig. 3) suggesting better source rock quality in deeply buried intervals. Most of the samples have TOC >0.5wt% which is the minimum threshold value for hydrocarbon generation in siliciclastic source rock (Tissot and Welte, 1978, Hedberg et al., 1979). The TOC values suggest

that Araromi shales have adequate organic matter constituents for hydrocarbon generation.

Hydrogen Index (HI)

The hydrogen index (HI) values vary from 1-327mgHC/gTOC (Tables 1 and 2) thus, suggesting source rock quality that spans from non-source potential through to gas/oil and oil generating potentials. In general, the OM is considered to be a mixture of Type II and Type III and a downhole profile of increasing HI concomitant with the TOC trend is apparent in the well logs. Deeper sections of the formation seem to be more organic rich and thus presumably have greater potential to generate liquid hydrocarbon. Additionally as shown in Fig. 3, two organic rich intervals (A and B) are obvious from the geochemical log and some core samples within the interval B contain HI values as high as 327mgHC/gTOC suggesting that more organic rich sections in the deep offshore equivalent of the strata may have greater potential to generate liquid petroleum.

Samples with HI value range of 1-45mgHC/gTOC having kerogen Type IV indicate a highly reworked terrestrially derived OM perhaps in a highly oxic depositional environment. They form inert source which have no potential for hydrocarbon generation. The samples with HI values of 45-132mgHC/gTOC with kerogen Type III suggest woody or herbaceous organic matter origin having gas prone characteristics. Those samples with in the range of 151-327mgHC/gTOC consisting of kerogen Type II and Type II/III usually at the basal part of the wells contain oil and mixture of oil and gas prone kerogens.

Table 1: Rock Eval pyrolysis data of the Araromi (Maastrichtian) and Imo (Paleocene) formations in the Araromi borehole.

S ample Numbe r	Dept h (m)	Locati on	Form a tion	S1(m gHC/ g rock)	S2 (mg HC/g rock)	SP (kg/ t)	T _{max}	PI	%R _o	TO C wt%	HI(m gHC/ g rock)	Kero gen Type
Ar-46	256. 03	Araro mi BH	Imo Shale	0.19	0.44	0.63	417	0.3 1	-	0.75	58	III
Ar-53	277. 37	Araro mi BH	Imo Shale	0.16	0.38	0.54	399	0.3 0	0.45	0.63	60	III
Ar-60	298. 70	Araro mi BH	Imo Shale	0.09	0.09	0.18	427	0.5 0	0.64	0.39	23	n.d.
Ar-68	329. 18	Araro mi BH	Imo Shale	0.07	0.09	0.16	391	0.4 4	-	0.34	26	n.d.
Ar-74	350. 52	Araro mi BH	Imo Shale	0.09	0.13	0.22	399	0.4 1	0.55	0.50	26	IV
Ar-81	371. 86	Araro mi BH	Imo Shale	0.09	0.35	0.44	420	0.2 0	0.55	0.58	60	III
Ar-88	393. 19	Araro mi BH	Imo Shale	0.08	0.31	0.39	419	0.2 1	-	0.74	41	IV
Ar-91	405. 38	Araro mi BH	Araro mi FM	0.02	0.01	0.03	403	1.0 0	0.51	0.65	1	IV
Ar-97	435. 86	Araro mi BH	Araro mi FM	0.05	0.10	0.15	419	0.3 6	-	0.61	16	IV
Ar-100	454. 46	Araro mi BH	Araro mi FM	0.05	0.10	0.15	412	0.3 6	-	0.75	13	IV
Ar-105	463. 29	Araro mi	Araro mi	0.04	0.05	0.09	410	0.5 0	-	0.66	7	IV

		BH	FM									
Ar-110	475. 49	Araro mi BH	Araro mi FM	0.17	0.95	1.12	424	0.1 5	-	1.69	56	III
Ar-114	487. 68	Araro mi BH	Araro mi FM	0.11	0.57	0.68	428	0.1 6	-	1.05	54	III
Ar-119	495. 30	Araro mi BH	Araro mi FM	0.15	0.75	0.87	422	0.1 7	-	1.77	40	IV
Ar-124	505. 36	Araro mi BH	Araro mi FM	0.18	0.54	0.72	420	0.2 5	-	1.19	45	III
Ar-132	518. 16	Araro mi BH	Araro mi FM	0.08	0.26	0.34	409	0.2 4	-	1.02	25	IV
Ar-139	539. 49	Araro mi BH	Araro mi FM	0.08	0.18	0.26	411	0.3 1	0.51	1.01	17	IV
Ar-145	557. 78	Araro mi BH	Araro mi FM	0.21	3.10	3.31	423	0.0 6	0.66	2.05	151	II-III
Ar-148	566. 93	Araro mi BH	Araro mi FM	0.28	5.18	5.46	424	0.0 5	-	2.75	188	II-III
Ar-151	572. 72	Araro mi BH	Araro mi FM	0.52	3.70	4.22	426	0.1 2	-	1.83	202	II-III

Table 2. Bulk geochemical and selected biomarker parameters for the analysed Araromi shale from Gbekebo well in Dahomey Basin

Sample Number	Depth (ft)	TOC (wt%)	S1	S2	T _{max} (°C)	HI	PI	Pr/Ph	%C27	%C28	%C29	%C30	OL	C29S/S+R
GB 304	2735.7	0.50	0.00	0.01	430.00	2.00	0.00							
GB 305	2739	0.63	0.12	0.36	425.00	57.14	0.25							
GB 306	2758.8	1.02	0.14	0.51	425.00	50.00	0.22							
GB 307	2772	1.01	0.13	0.48	432.00	47.52	0.21	1.06	36.94	25.55	37.06	0.45	0.00	0.23
GB 309	2788.5	0.69	0.05	0.24	425.00	34.78	0.17							
GB 310	2798.4	0.91	0.04	0.34	430.00	37.36	0.11	1.28	45.28	26.62	28.09	0.00	0.00	0.18
GB 311	2801.7	0.99	0.06	0.55	430.00	55.56	0.10							
GB 312	2818.2	0.95	0.08	0.41	425.00	43.16	0.16							
GB 313	2824.8	1.00	0.28	0.42	432.00	42.00	0.40							
GB 314	2828.1	0.68	0.12	0.44	435.00	64.71	0.21	0.78	24.10	41.81	32.77	1.32	0.00	0.25
GB 315	2831.4	0.91	0.07	0.36	435.00	39.56	0.16	0.93	23.62	35.50	38.88	2.00	0.06	0.31
GB 316	2834.7	0.76	0.06	0.21	437.00	27.63	0.22							
GB 318	2838	0.82	0.06	0.30	431.00	36.59	0.17	1.18	22.16	39.74	36.59	1.51	0.00	0.26
GB 320	2867.7	1.24	0.06	0.15	422.00	12.10	0.29							
GB 323	2897.4	1.67	0.12	1.29	435.00	77.25	0.09							
GB 324	2904	1.70	0.06	0.83	432.00	48.82	0.07	1.25	31.95	41.15	24.29	2.61	0.00	0.31
GB 325	2910.6	1.87	0.27	3.01	427.00	160.96	0.08							
GB 326	2917.2	2.13	0.18	5.25	430.00	246.48	0.03		38.20	38.47	20.83	2.50	0.00	0.26
GB 327	2920.5	2.12	0.17	3.01	427.00	141.98	0.05							
GB 402	2963.4	1.97	0.21	1.39	432.00	70.56	0.13		31.39	31.00	33.51	4.11		0.23
GB 421	3144.9	1.66	0.28	0.42	415.00	25.30	0.40							
GB 450	3319.8	2.24	0.31	5.01	433.00	223.66	0.06	1.13	33.68	38.20	25.09	3.02	0.20	0.29
GB 451	3323.1	2.52	0.32	6.44	432.00	255.56	0.05							
GB 454	3333	4.78	0.81	13.75	434.00	287.66	0.06							
GB 455	3336.3	3.33	0.73	10.30	431.00	309.31	0.07	2.02	30.15	30.74	33.89	5.23	0.21	0.16
GB 456	3342.9	3.94	1.29	12.88	426.00	327.07	0.09	1.39	37.91	33.23	26.73	2.13	0.12	0.47
GB 460	3372.6	1.54	0.31	1.09	434.00	70.78	0.22	0.96	34.20	33.56	29.00	3.24	0.09	0.19

HI = Hydrogen index (100*S2/TOC). PI = production Index S1/(S1+S2). Pr/Ph = pristane/phytane. % C27-C30 = % individual C27-C30 $\alpha\alpha\alpha$ sterane to sum of C27-29 $\alpha\alpha\alpha$ regular steranes. OL = 18 α (H) + 18 β (H) oleanane/C₃₀ 17 α (H),21 β (H)- Hopane. C29 S/S+R = ratio of 5 α (H),14 α (H),21 α (H) - steranes (20S/S+R) thermal maturity parameter.

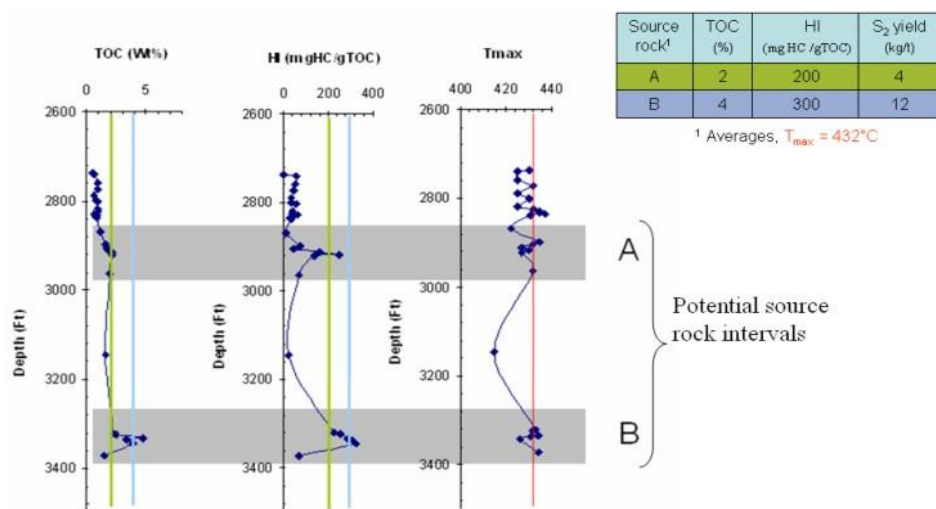


Figure 3: Geochemical log of the Gbekebo well. Two organic rich intervals are apparent (A and B). Only one sample within the interval A contains oleanane whereas most samples in the interval B contain oleanane, with oleanane index values as high as 0.21, despite the high marine algal biomarker composition of interval B.

Thermal Maturation

The thermal maturation (T_{max}) measured from the Rock Eval pyrolysis varies from 398°C to 437°C while the vitrinite reflectance values vary from 0.50 to 0.68% R_o . These values suggest that the source rocks are thermally immature to marginally mature for hydrocarbon generation. The plots of Rock Eval T_{max} against hydrogen index also show thermally immature to early mature source rocks with respect to hydrocarbon generation and dominance of a mixture of oil prone Type II and gas prone Type III kerogens (Fig. 4).

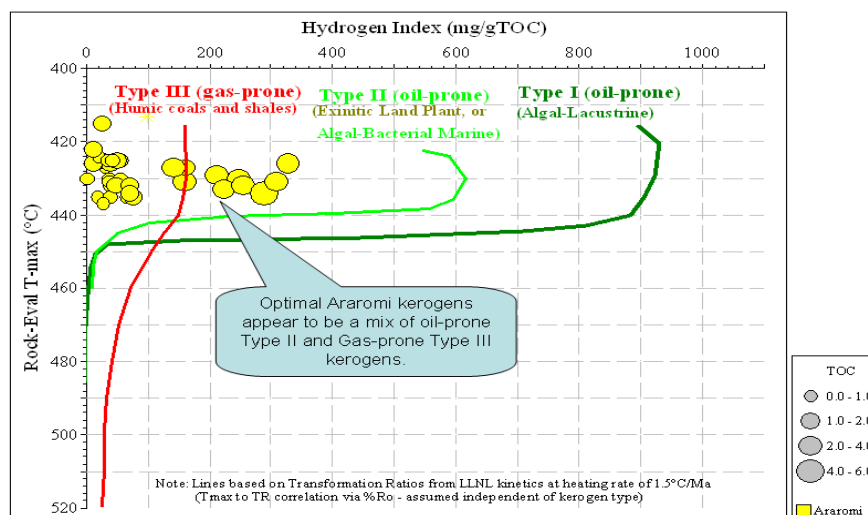


Figure 4: Plot of Rock-Eval HI against Tmax indicating the kerogen types and their respective hydrocarbon potentials in the analysed Araromi Formation sediments. Overlay field after Integrated Geochemical Interpretations Ltd P: IGI 2.7 geochemical interpretation software.

Source Potential

The source potential (SP) values vary from 0.01-14.56kgHC/ton of rock (Tables 1 and 2). The SP values are generally lower than the 2kgHC/ton of rock expected for good source rock (Dymann et al., 1996), suggesting little or no oil source rock potential but some potential for gas. Samples with higher SP values ranging from 3.01kgHC/ton of rock to 12.88kgHC/ton of rock suggest a moderate source rock with fair oil potential.

Maceral Analysis

The organic petrological study of the disseminated organic matter in the shale samples was carried out using the techniques in studying optical characteristics of finely dispersed organic matter particles in coals, coaly shales and carbonaceous shales. Terminologies of Stach et al., 1982 modified in Teichmuller, 1987 were used here. Selected shale samples were polished and observed under organic petrological microscope. The different macerals of vitrinite, liptinite and inertinite were observed. Photomicrographs of representative macerals are shown in Fig. 5. In

general, the maceral composition include liptinite (av. 26.0%), vitrinite (av. 38.1%) and inertinite (av. 35.9%) in all the samples investigated. The dominance of vitrinite and inertinite macerals suggests terrestrially derived, reworked and oxidized organic matter in the shale sediments. There is no particular maceral variation pattern or zonation down deep in all the wells.

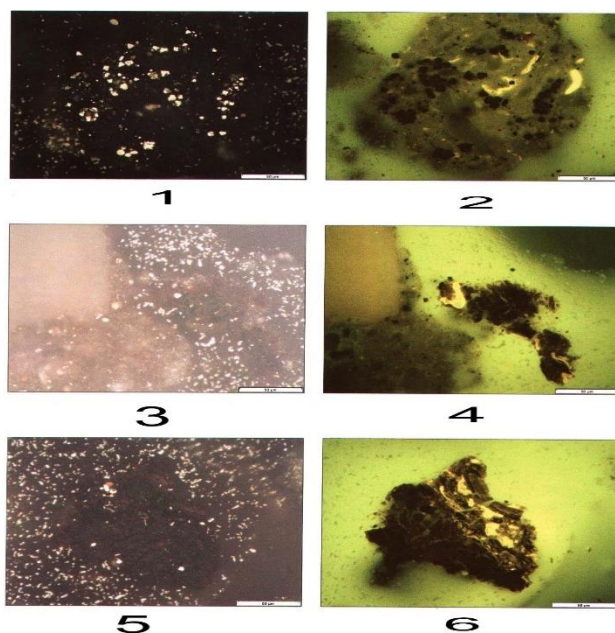


Figure 5: Photomicrographs of the macerals observed in the shale samples from the Araromi Formation in the Araromi borehole.

Biomarker compositions of the shales

Core samples analysed by Gas Chromatography (GC) display a bimodal n-alkane GC fingerprint envelope with maxima between $nC_{16} - nC_{18}$ and $nC_{27} - nC_{29}$ (Fig. 6), suggesting variable inputs from both terrigenous and non-terrigenous (probably marine algal) organic matter. However, some samples display a unimodal n-alkane GC fingerprint typical of dominantly normal marine algal contribution (maximizing between nC_{16} and nC_{18}). There is a relatively high input from alga-bacterial precursors indicated by the abundance of the low molecular weight relative to high molecular weight n-alkane derived from terrigenous higher plants (Bray and Evans, 1961). Additionally pr/ph ratios vary from 0.78-2.02 reflecting anoxic to

sub-oxic source rock depositional environment (Didyk et al., 1978, Volkman and Maxwell, 1986).

Steranes in the saturated hydrocarbon fractions of the bitumen extracts of the Araromi shale samples analysed by GC-MS and GC-MS-MS show the C27-C29 distributions to be nearing a ratio of 1.1:0.9:1.0, with higher C27 and C28 compounds on occasions relative to the C29 sterane homologues for the 5 α (H),14 α (H),17 α (H) 20R configuration isomers. Figs. 7 and 8 show the sterane distributions of the bitumen extracts from the Araromi shales. The above observation suggests a relatively higher input from the marine red algae and a low level of land plant contribution to the source organic matter (Goodwin, 1973). Additionally, the presence of the C₃₀ 24-*n*-propyl cholestane (detected by GC-MS-MS m/z 414-217 parent to daughter ion transition), confirms the relatively high marine algal contribution to the organic matter in the Araromi shales, as values of %C₃₀ sterane range from 0.45 to as high as 5.23%, even in oleanane rich intervals in “Zone B” (Figure 3).

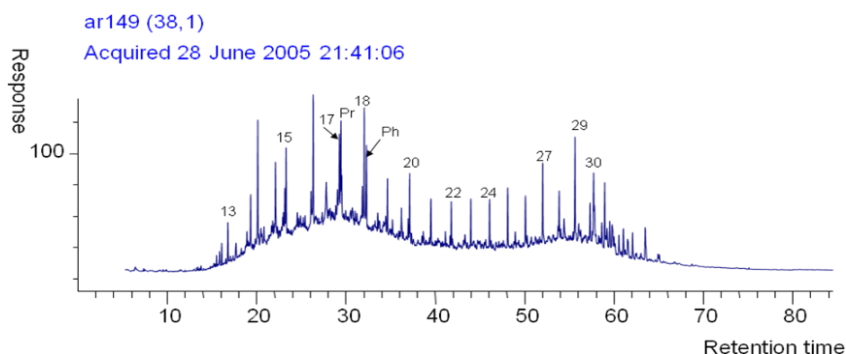


Figure 6: A bimodal peaks *n*-alkane envelope of sample Ar-149 maximizing at nC16 and nC29 typical of source rock whose organic matters have been derived from a variable mixture of algal and terrigenous higher plant materials. Note 13,15, e.t.c represent the *n*-alkane carbon numbers and Pr; Pristane , and Ph; Phytane.

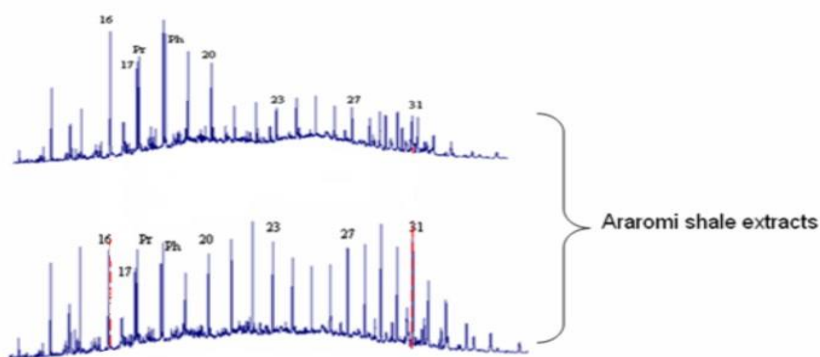


Figure 7: Figure 9. Gas chromatograms of representative saturated hydrocarbon fractions of the Araromi shale (samples GB314 and GB 307) extracts showing how *n*-alkane and isoprenoid distributions in the Araromi shale.

m/z 217 mass chromatograms

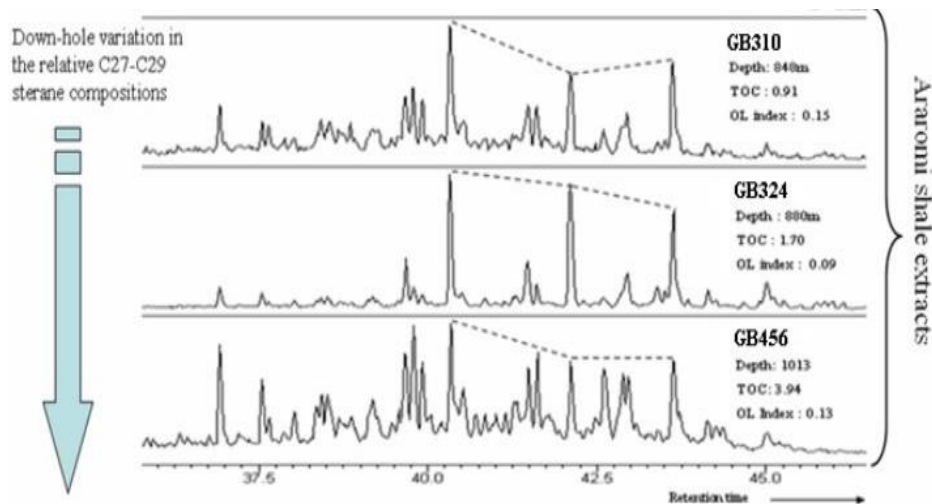


Figure 8: m/z 217 mass chromatograms showing the distribution of C₂₇-C₂₉ steranes in representative intervals within the Araromi. Note the downhole variation in the sterane distributions within the Araromi shale extracts.

Conclusion

Rock Eval pyrolysis data from this study suggest that the organic rich core samples of Late Cretaceous Araromi Formation in the Dahomey Basin, southwestern Nigeria contain predominantly gas prone with lesser oil prone organic constituents ranging from immature to marginally mature at shallow levels but reaching proven maturity in the subsurface. Hydrocarbons in the basin will be predominantly gas dominated generated from deeper Cretaceous source rock in the subsurface levels with greater chances of heavy oils and tars at shallow levels.

Gas chromatographic analyses of the bitumen extract show bimodal and unimodal *n*-alkane distributions, suggesting elevated contributions of marine relative to terrigenous higher plant matter to the source organic matter. The source rock organic matter accumulations probably occurred under anoxic to sub-oxic conditions (pr/ph ratios range from 0.78-2.02). Sterane distributions in the shale extracts are dominated by C₂₇ relative to C₂₈ and C₂₉ carbon homologues and the presence of significant C₃₀ 24-*n*-propyl cholestane concentrations are both diagnostic of marine algal inputs. These biomarker characteristics are comparable with the deepwater oil. Araromi bitumen extracts have nearly flat *n*-alkane CSIA stable carbon isotopic profiles that suggest derivation from organic carbon from a uniform $\delta^{13}\text{C}$ composition marine algal source.

References

- Adediran, S. A. and Adegoke, O. S. 1987. Evolution of the sedimentary basins of the Gulf of Guinea. In: Current Research in Africa Earth Sciences, Matheis and Schandeimeier (eds). Balkema, Rotterdam., p. 283-286.
- Adegoke, O. S. 1969. Eocene stratigraphy of Southern Nigeria. Mem. Bur. Rech. Geol. mins., p. 23-46.
- Adekeye, O.A. 2004. Aspects of sedimentology, geochemistry and hydrocarbon potentials of Cretaceous-Tertiary sediments in the Dahomey Basin, south-western Nigeria. Unpublished Ph.D thesis, University of Ilorin, p 202.
- Adekeye, O.A., Akande, S.O., Erdtman, B.D., Samuel, O.J. and Hetenyi, M. 2006. Hydrocarbon Potential Assessment of the Upper Cretaceous-

Lower Tertiary Sequence in the Dahomey Basin Southwestern Nigeria. NAPE Bulletin. v. 19 No 1, p.50-60.

Ako, B. D., Adegoke, O. S. and Petters, S. W. 1980. Stratigraphy of the Oshosun Formation in South-Western Nigeria. Jour. Min. Geol. v. 17, p. 97-106.

Asmus, H. E. And Ponte, F. C. 1973. The Brazillian marginal basins: In: Naim, A. E. M and Stehli, F. G. (Eds). Ocean Basins and margins: The South Atlantic, The Plenum Press, v. 1, 250p.

Billman, H. G. 1976. Offshore stratigraphy and paleontology of the Dahomey embayment. Proc. 7th Afri. Micropal. Coll. Ile-Ife, p. 27-42.

Bray, E.E. & Evans, E.D. 1961. Distribution of *n*-paraffins as a clue to recognition of source beds. *Geochimica et Cosmochimica Acta*, 22, p.2-5.

Bustin, R. M., Cameron, A. R., Grieve, D. A. and Kalkreuth, W. D. 1983. Coal petrology: its principles, methods and applications: Geology Association of Canada Short Course Notes, v. 3, 230p.

Didyk, B. M., Simoneit, B. R. T., Brassell, S. C. & Eglinton, G. 1978. Organic geochemical indicators of paleoenvironmental conditions of sedimentation. *Nature*, 272, p.216-222.

Dymann, T. S., Palacos, J. G., Tysdal, R. G., Perry, W. J. and Pawlewicz, M. J. 1996. Source Rock Potential of Middle Cretaceous Rocks in Southwestern Montana: AAPG Bulletin, v. 80, p. 1177-1184.

Espitalie, J., Madec, M., Tissot, B. P., Menning, J. J. and Leplat, P. 1977. Source rocks characterization method for exploration. Offshore Technology Conf. Paper 2935, 11th Annual OTC, Houston, v. 3, p. 439-444.

Goodwin, T.W., 1973. Comparative Biochemistry of Sterols in Eukaryotic Micro-organisms. In: Erwin , J. A. (ed.), *Lipids and Biomembranes of Eukaryotic Micro-organisms*, Academic Press, New York, p. 1-40.

Hedberg, H. D., Moody, J. O. and Hedberg, R. M. 1979. Petroleum prospects of deep offshore. AAPG Bull. v. 63, p. 286-300.

- Idowu, J. O., Ajiboye, S. A., Ilesanmi, M. A. and Tanimola, A. 1993. Origin and significance of organic matter of Oshosun Formation south-western Dahomey Basin Nigeria. *Jour. Min. Geol.* v. 29, p. 9-17.
- Kogbe, C. A. 1974. Paleo-ecologic significance of vertebrate fossil in the Dukamaje and Dange Formations (Maastrichtian and Paleocene) of northwestern Nigeria. *Jour. Min. Geol. (Nigeria).* v. 8, p. 49-55.
- Lehner, P. and de Ruiter, P. A. C. 1977. Structural history of the Atlantic margin of Africa. *AAPG Bull.* v. 61, p. 961-981.
- Mpanda, S. 1997. Geological development of East African coastal basin of Tanzania: *Acta Universities Stockholmiensis*, v. 45, 121p.
- Ogbe, F. A. G. 1970. Stratigraphy of strata exposed in the Ewekoro Quarry Western Nigeria. In: (Dessauvague, T. F. J. and Whiteman, A. J. (eds). *African Geology* University of Ibadan Press, Nigeria. p.305-324.
- Ojeda, H. A. 1982. Structural framework, stratigraphy, and evolution of Brazilian basins. *AAPG Bull.* v. 66, p.732-749.
- Okosun, E. A. 1990. A review of the Cretaceous stratigraphy of the Dahomey Embayment, West Africa. - *Cretaceous Research*, v. 11, p. 17-27.
- Omatsola, M. E. and Adegoke, O. S. 1981. Tectonic evolution and Cretaceous Stratigraphy of the Dahomey Basin. *Jour. Min. Geol.* v. 8, p. 30-137.
- Pollastro, R. M. and Barker, C. E. 1986 . Application of clay mineral, vitrinite reflectance and fluid inclusion studies to the thermal and burial history of the Pinedale anticline, Green River basin, Wyoming. *SEPM Special Publication.* v.28, p. 73-83.
- Stach, E. M., Mackowsky, T. H., Teichmuller, M., Taylor, G., Chandra, D. and Teichmuller, R. 1982. Stach's text book of coal petrology. Gerbruder Borntraege, Berlin. 535p.
- Storey, B. C. 1995. The role of mantle plumes in continental break up: case history from Gondwanaland – *Nature*, v. 377, p. 301-308.

Teichmuller, M. 1987, Organic material and very low grade metamorphism. In: M. Frey (ed). Low temperature metamorphism. Blackie and Sons Pub. London, p.114-161.

Tissot, B. P. and Welte, D. H. 1978. Petroleum formation and occurrence., Springer-Verlag, New York, 538p.

Tissot, B. P. and Welte, D. H. 1984, Petroleum Formation and Occurrence, 2nd ed. Springer Verlag, Berlin 699p.

Volkman J.K. & Maxwell, J.R 1986. Acyclic isoprenoids as biological markers. In: Johns, R.B. (ed.) Biological markers in sedimentary record, Elsevier, New York, pp. 1-42.

Effect of Sublethal concentration of sniper 1000EC on weight and length growth of *Oreochromis niloticus* obtained from Asa dam under laboratory conditions

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Abstract

The study investigated the effect of sublethal concentration of sniper 1000EC on the weight and length growth of *Oreochromis niloticus* obtained from Asa dam under laboratory conditions. Indiscriminate use of Sniper 1000EC by fishermen has become a serious problem in Northern region of Nigeria. Juveniles of *Oreochromis niloticus* (mean body weight 7.05 ± 1.02 ; mean standard length 9.60 ± 0.38 cm) were subjected to 5 sublethal treatment levels: 0.00mg/L is the control, 0.12, 0.15, 0.19 and 0.25mg/L. There was no significant difference between water quality parameters of the exposed and control groups. A 56 day exposure to sublethal concentrations of the toxicant led to a significant decrease ($p < 0.05$) in specific and relative growth rate in the exposed fish species in comparison with their controls. Change in weight and length decreases with increasing sublethal concentrations of sniper 1000EC. It is concluded that depressive growth rate in the exposed fish species were consequences of exposure to the toxicant (sniper 1000EC). It is recommended that the use of Sniper 1000EC by local fishermen be banned to save the aquatic environment from destruction.

Keywords: Sniper 1000EC, *Oreochromis niloticus*, chronic toxicity and growth rates.

Introduction

Weight and length data provide a very useful tool for estimating growth rates, length, and age structures and for the improvement of the knowledge regarding fish population dynamics (Froese, 2006). Weight-length relationship is very useful for fisheries research because they allow the conversion of growth-in-length equation to growth-in-weight for use in stock assessment models (Moutopoulos and Stergiou, 2000). Length-weight relationship allow for estimation of biomass from length observations as well as comparisons of life histories of certain species (Goncalves *et al.*, 1996; Froese and Pauly, 1998). They are important component of FishBase (Froese and Pauly, 1998). Pauly (1993) stated that length-weight relationship provides valuable information on the habitat where the fish lives while Kulbicki *et al.*, (2005) highlighted the significance of length-weight relationship in modeling of aquatic system. Length-weight is useful for the conversion of growth-in-length to growth-in-weight for use in stock assessment models and to estimate stock biomass from limited sample sizes (Ecoutin *et al.*, 2005). Length and weight measurements can give information on the stock composition, life span, mortality, growth and production (Bolger and Connolly, 1989; Erkoyuncu, 1995; King, 1996; Moutopoulos and Stergiou, 2000).

Tilapia fishes are important source of animal protein to humans. They are obtained through capture fisheries activities and also farmed in ponds. They dominate major inland water bodies including rivers, lakes and reservoirs in major fish landings (Trewavas, 1982). Tilapia is one of the fisheries resources that suffers from environmental effects and needs to be protected because world production of tilapia exceeds two million tons per year far exceeding the harvest of Atlantic salmon and secondary only to carp as a culture food fish (FAO, 2005). Tilapia has become the shining star of aquaculture across the globe (Waleed, 2012). Fagbenro (2002) stated that tilapia species are of major economic importance in tropical and sub-tropical countries throughout the world, particularly in Africa where farms stock mixed-sex tilapia in production ponds. Arrington (1998) describes *Oreochromis niloticus* as the best species for culture among the tilapia family-with squat shape. Trewavas (1982) recognized *Oreochromis niloticus* as macrophages and herbivorous used in irrigation channels and dams to control weed. Fagbenro (2002) stated that tilapia species are of major economic importance in tropical and sub-tropical

countries throughout the world, particularly in Africa where farms stock mixed-sex tilapia in production ponds. There are different species of tilapia fish in Asa dam but *Oreochromis niloticus* is more abundant. *Oreochromis niloticus* can survive in bad environmental conditions because their resistance to disease is physically powerful, and their respiratory demands are slight so that they can accept low oxygen and high ammonia levels (Zhou et al., 1998). They are disease resistant, highly prolific; feed on wild variety of foods, tolerant of poor water quality with low dissolved oxygen level (Fagbenro, 2002).

Widespread application of various pesticides has aggravated the problem of pollution to aquatic environment. Due to these synthetic chemicals, environment has failed to keep its healthy characteristics. The insecticides of proven economic potentialities could not do well in the ecosystem when viewed on extra fronts since these revenue poisons, in a residual form or as a whole, get into the aquatic ecosystem. They cause a series of problems to aquatic organisms (Mastan and Ramayya, 2010).

Sniper 1000 EC is the common pesticide among the local farmers cultivating crops around Asa dam, Ilorin. In line with the saying, “When Sniper and Mussel struggles inside water, it is the local fishermen that smile home” (Abubakar, 2013). Indiscriminate use of Sniper 1000EC by fishermen around Asa dam has become a serious problem to be studied. Sniper 1000EC (2, 3-dichlorovinyl dimethyl phosphate), a brand of dichlorvos, is contact acting and fumigant insecticide (Abubakar, 2013). Like all organophosphates, it kills insects and other target organisms because of its toxicity to the nervous system. This is achieved by inhibition of enzyme acetylcholinesterase (AChE) that breaks down acetylcholine at the receptor site for partial uptake into the nerve terminal. Without functioning AChE, accumulation of acetylcholine results in depolarizing block of muscle membrane, producing rapid twitching of involuntary muscles, convulsions, paralysis and early death. Indiscriminate use of Sniper 1000EC is common among local fishermen from Northern parts of Niger state.

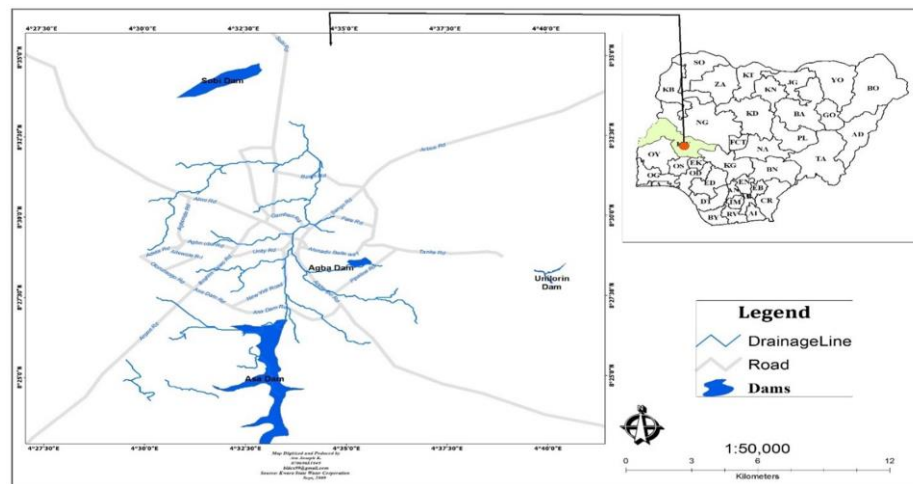
There is paucity of information on toxicity of sublethal concentrations of sniper 1000EC on weight and length growth of tilapia fish despite its indiscriminate use by the fish farmers.

Accordingly, this investigation is aimed at evaluating the effect of sublethal concentrations of sniper 1000EC on weight and length growth rates of *Oreochromis niloticus* under laboratory conditions.

Materials and methods

The Study Area

The study was conducted in Asa dam which is located in Ilorin, the capital of Kwara State, Nigeria.



Source: Clement *et al.*, 2015

Figure 1: Map of Ilorin showing the location of Asa dam. Inset: map of Nigeria showing the states

Asa dam is located on River Asa (8_ 4400 N; 4_ 5600 E) at approximately 5 km from the city centre. The dam has an overall length of 597 m and storage capacity of 43 million m³ with lake extension of 18 km. The length and breadth of the spillway are about 65 m and 14 m respectively with discharge capacity of 79,000 m³ (Araoye, 2009; Ayanshola, 2013). Asa River is the major river in Ilorin city. Ilorin is mainly drained by Asa River which flows in a South-North direction (Ajadi *et al.*, 2016). The river has a total surface area of about 303 hectares (Olayemi, 1994; Eleta *et al.*, 2005). Other rivers in Ilorin that drains into Asa river are river

Agba, river Alalubosa, river Okun, river Osere, river Aluko, river Yalu, river Odota and river Loma.

Sample collection, transportation and test chemical

Juveniles of *Oreochromis niloticus* (mean body weight 7.05 ± 1.02 kg; mean standard length 9.60 ± 0.38 cm) were obtained from Asa dam using drag nets with the help of the local fishermen. The samples were transported to the laboratory in plastic container of 100L capacity filled with water to two-third volume between 0700 hours and 0900 hours. They were held in large water baths of 160L capacity and acclimated for 14 days to laboratory conditions. The top of water bath was covered with netted material to prevent jumping out of the fish. A slit was made at middle of the net to allow for feeding fish and cleaning of the bath. Feeding commenced two days after the arrival and stopped twenty-four hours before the commencement of the experiment. During acclimation, fish were fed twice daily (0800 and 1600 hours) with formulated feed (35% crude protein) at 3% body weight. The fishes were accepted as well as adapted to laboratory conditions when less than 5% death was recorded for the 14 days. The water in the bath was changed daily and uneaten food and faecal matters were siphoned out. Dead fish were also removed to minimize contamination of water.

Test chemical (2, 3-dichlorovinyl dimethyl phosphate), a brand of Dichlorvos with the trade name Sniper 1000EC was obtained from Ilorin central market and was used for the study. The test concentrations were prepared after range finding tests had been conducted.

Experimental Design

The experimental design was a complete randomized design. A total of one hundred and fifty (150) juvenile of *Oreochromis niloticus* were randomly distributed into the tanks at a stocking rate of 10 fish per tank. The fifteen (15) tanks were assigned to 5 treatments (control inclusive). In order to determine the LC_{50} , the *O. niloticus* were exposed to four different concentrations of sniper 1000EC for 96hr. LC_{50} value obtained using EPA Probit Analysis programme version 1.5 was 3.81mg/L. Using the method of Abubakar and Abdulsalami (2013), One fifteen (1/15), one twenty (1/20), one twenty fifth (1/25) and one thirty (1/30) of 3.81mg/L were used to produced the sublethal concentrations used in the study.

$$1/15 \times 0.00\text{mg/L} = 0.00\text{mg/L}$$

$$1/15 \times 3.81\text{mg/L} = 0.25\text{mg/L}$$

$$1/20 \times 3.18\text{mg/L} = 0.19\text{mg/L}$$

$$1/25 \times 3.18\text{mg/L} = 0.15\text{mg/L}$$

$$1/30 \times 3.18\text{mg/L} = 0.12\text{mg/L}$$

The Aqua feed used for feeding the fish composed of 35% of ground nut cake, 30% of fish meal 20% of rice bran, 5% of bone meal, 5% of vegetable oil and 5% of vitamin and mineral premix respectively (Table 1).

Table 1:Ingredient of the Aqua feed used:

INGREDIENTS	% COMPOSITION
Groundnut cake	35
Fish meal	30
Rice bran	20
Bone meal	5
Vegetable oil	5
Vitamin and mineral pre-mix	5
Aqua feeds total	100

The fish were fed 3 times daily .The test solutions were renewed forthrightly. The average weight and length of the fish were measured bi-weekly using a 3- digit sensitive weighing balance Meter pm 2500 Delta range ®. For clarity purpose, a transparent ruler was used for the measurement of length. The experiment lasted for 56 days (8weeks).Growth was based on relative and specific growth rates which were calculated using standard formulae:

$$GR = \frac{W_t - W_0}{t_2 - t_1} \times 100 \quad \text{----- Haghighi, 2009:}$$

W_0

$$RG_R = \frac{W_t - W_0}{W_0} \quad \text{----- Goda *et al*, 2007:}$$

$t_2 - t_1$

$$SGR = \ln \frac{W_t}{W_0} \quad \text{----- Akintola *et al.*, 2010:}$$

W_0

Where:

W_t = Final body weight at time t (weeks);

W_0 = Initial body weight at time 0(weeks);

GR = Growth rate;

RG_R = Relative growth rate;

SG_R = Specific growth rate;

t_1 = Initial time;

t_2 = Final time;

Water quality parameters

Dechlorinated municipal tap water was used. It was allowed to stand for 72 hours during which it was aerated. The water quality parameters determined were temperature, dissolved oxygen, pH, water hardness and total alkalinity which could adversely affect survival and growth of fish in tanks. Temperature and dissolved oxygen were monitored on daily basis (09:00 and 14:00 hours) using a thermometer and oxygen meter (Cole Parma model 5946; Sigma Chemical, Berlin, Germany). The values of pH were measured daily using an Orion digital pH meter (Model 210; Sigma Chemical, Lisbon, Portugal). Water hardness was monitored daily using a German hardness scale while total alkalinity were monitored on weekly basis using standard method of APHA (1987). These were done to ensure proper recordings and calculations of the parameters.

Statistical Analysis

All the data generated were managed with Microsoft office Excel 2003; they were analyzed with one-way analysis of variance (ANOVA) using Statistical Product for Service Solution (SPSS), version 16.0 for window. Statistical significance of difference among means was compared for significant differences using Duncan's New Multiple Range tests at 95% probability (Snedecor and Cochran, 1980).

Results

Effects of sublethal concentrations on water quality parameters

The water quality variables at various sub lethal treatments for the exposed and control groups did not differ significantly ($p < 0.05$). The mean values recorded for various sublethal concentrations compared with those of the controls are presented in Table 2.

Table 2: Physio -chemical parameters of chronic test solutions for *Oreochromis niloticus*

(mean±SD)					
Concentration	pH	Temperature (OC)	Dissolved oxygen (mg/L)	Hardness (mg/L)	Alkalinity (mg/L)
0.00	6.64±1.70	28.6±1.52	7.50±0.30	39.1±2.81	33.7±2.13
0.12	6.67±0.51	28.8±2.09	7.63±0.28	40.2±3.26	33.3±1.54
0.15	6.56±0.42	28.6±1.51	7.65±0.29	40.4±2.22	33.0±2.37
0.19	6.59±0.49	28.5±2.04	7.68±0.27	40.2±3.42	34.1±2.14
0.25	6.62±0.45	28.6±1.61	7.67±0.22	39.4±3.86	34.3±1.56

values of parameters along the same column are not significantly different at ($p < 0.05$)

Effect on growth rates

Differences in weight and length among the exposed fish species at various

sublethal concentrations were significant ($p < 0.05$). Weight ranged from 16.4g in the control tank to 13.6g in the highest concentration tank

(0.25mg/L). Change in length ranged from 9.5cm in the control tank to 7.6cm in the highest concentration tank (0.25mg/L). Differences were also observed in the growth rates, specific growth rates and relative growth rates of the two fish species exposed to the various concentrations of sniper 1000EC. The toxicant exhibited depressive effect on the exposed groups with increase in concentration levels in the exposed fish species. These are shown in Tables 3 to 6. The change in weight of *Oreochromis niloticus* at different concentration levels of sniper 1000EC are presented on Table 3. The results show that the bi-weekly change in weight declined with increase in concentrations of sniper 1000EC.

Table 3: Weekly change in weight (g) at different concentration level of sniper 1000EC in *Oreochromis niloticus*

Concentration	Mean change in weight			
	WK2	WK4	WK6	WK8
0.00mg/L	16.4 ^a	16.6 ^a	16.6 ^a	16.7 ^a
0.12mg/L	16.2 ^a	15.9 ^a	15.8 ^a	15.6 ^a
0.15mg/L	15.8 ^a	15.3 ^a	15.2 ^a	14.9 ^a
0.19mg/L	14.7 ^a	14.5 ^a	14.3 ^a	14.2 ^a
0.25mg/L	14.0 ^a	13.8 ^a	13.7 ^a	13.6 ^a

Mean with the same superscript along column are not significantly different at ($p < 0.05$)

NB

WK denotes week

WK2 denotes Second week

WK4 denotes Fourth week

WK6 denotes Sixth week

WK8 denotes Eight week

Data for change in length of *Oreochromis niloticus* at different concentration levels of sniper 1000EC are shown in Table 4. The Table revealed that the mean change in length decreased insignificantly ($p > 0.05$) with increase in concentrations of sniper 1000EC.

Table 4. Bi-weekly change in length (cm) at different concentration levels of sniper 1000EC in *Oreochromis niloticus*.

Concentration	Mean change in length (cm)			
	WK2	WK4	WK6	WK8
0.00mg/L	9.5 ^a	9.5 ^a	9.6 ^a	9.7 ^a
0.12mg/L	9.2 ^a	9.1 ^a	9.0 ^a	9.0 ^a
0.15mg/L	9.0 ^a	8.9 ^a	8.7 ^a	8.6 ^a
0.19mg/L	8.5 ^a	8.4 ^a	8.2 ^a	8.1 ^a
0.25mg/L	8.0 ^a	7.8 ^b	7.7 ^a	7.6 ^a

Mean with the same superscript along column are not significantly different at ($p < 0.05$)

NB

WK denotes Week

WK2 denotes Second week

WK4 denotes Fourth week

WK6 denotes Sixth week

WK8 denotes Eight week

Table 5 shows the growth rate, specific growth rate and relative growth rate in weight at various concentration levels of sniper 1000EC in *Oreochromis niloticus*. The Table shows that growth rate, specific growth rate and relative growth rate declined with increase in concentration of sniper 1000EC.

Table 5. Mean Initial weight, Mean final weight, Specific growth rate and Relative growth rate in *Oreochromis niloticus*.

Concentration	Mean Initial weight (g)	Mean Final weight (g)	Growth rate (g)	Specific growth rate(g)	Relative growth rate (g)
0.00mg/L	13.0 ^a	16.7 ^a	28.5 ^a	1.2 ^a	0.29 ^a
0.12mg/L	13.4 ^a	16.2 ^a	21.0 ^b	0.9 ^a	0.21 ^a
0.15mg/L	12.9 ^a	15.3 ^b	18.6 ^c	0.8 ^a	0.19 ^a
0.19mg/L	13.2 ^a	15.0 ^b	13.6 ^d	0.6 ^a	0.14 ^a
0.25mg/L	13.5 ^a	15.3 ^b	13.3 ^d	0.6 ^a	0.13 ^a

Mean with the same superscript along column are not significantly different at ($p > 0.05$)

The growth rate, specific growth rate and relative growth rate in length of *O. niloticus* at different concentration levels of sniper 1000EC are shown in Table 6. The Table shows a similar trend as in weight. Growth rate, specific growth rate and relative growth rates also decreased with increase in concentrations of sniper 1000EC.

Table 6. Mean initial length, Mean final length, Specific growth rate and Relative growth rate in *Oreochromis niloticus*:

Concentration	Mean Initial length (cm)	Mean Final length (cm)	Growth rate (cm)	Specific growth rate(cm)	Relative growth rate (cm)
0.00mg/L	7.8 ^a	9.7 ^a	24.4 ^a	0.6 ^a	0.24 ^a
0.12mg/L	7.4 ^a	8.9 ^b	20.3 ^b	0.5 ^a	0.20 ^a
0.15mg/L	7.2 ^a	8.4 ^b	16.7 ^c	0.4 ^a	0.17 ^a
0.19mg/L	7.1 ^a	8.2 ^b	15.5 ^d	0.4 ^a	0.16 ^a
0.25mg/L	7.7 ^a	8.6 ^b	11.7 ^e	0.3 ^a	0.12 ^a

Mean with the same superscript along column are not significantly different at (p>0.05)

Discussion

The effect of sublethal concentrations of sniper 1000EC has depressive effect on the growth rate of *Oreochromis niloticus*. The fish exhibited a progressive decline in growth at various concentration levels. This is in agreement with the work of Abubakar and Abdulsalami (2013) who worked on toxicological effects of sublethal concentrations of sniper 1000EC on growth of *Clarias gariepinus* under laboratory conditions. The results of water quality parameters of the media were within the optimal range recorded by Omoniyi *et al.*, (2002) as requirements for fish culture which implied that the parameters did not seem to influence the toxicity of the pesticide to the test fish. Exposure to toxicant without causing death to organisms can still cause harm (Stephan, 1982) and survival of estuarine and marine organisms in relatively low concentrations of toxicant on the first day, does not necessarily indicate that they are resistant to the toxicant pollution (Mironov, (1972). Decreased growth was reported on *Mesidotea etemon* by Percy (1978). A similar reduction in growth was also observed by Abubakar and Abdulsalami (2013) when they worked on toxicological effects of sublethal concentrations of sniper 1000EC on growth of *Clarias gariepinus*. Reduction in growth was also observed by Toussain, *et al.*, (2001) and Onusiriuka (2002) when they exposed

Japanese Medaka fish and *Clarias gariepinus* to sub-lethal concentrations of chloroform and formalin respectively, better growths were reported in control groups of certain fish than those exposed to toxicants as observed in this study. This might be due to the fact that they were able to utilize the feeds or that the feeds were palatable. This observation was in agreement with the reports of Omoregie and Okpanach (1995) in *Tilapia zilli*; Omoregie *et al.*, (1998) in *Oreochromis niloticus*; Omoregie and Onuogu (2000) in *Aphyosemion gardneri*. Literature reports often attributed the decline in growth rates to the impairment of feeding by fish in the toxicant polluted area as observed in this study. Several workers have reported similar findings (Shanmugavel, *et al.*, 1988; Auta 2001 and Toussain, *et al.*, 2001). This might also be due to the presence of a dominant aggressive fish that caused an increased activity for others and consequently a reduction in their growth rates as well as an increase in their sensitivity of the pollutant. The dose- dependent effects of sniper 1000EC on the growth rates of the fish species suggested that high concentration of the toxicant inhibited the feeding rate of the fish species or make the feed unpalatable for them. Pal and Konar (1987) similarly reported that growth rate of *Oreochromis mossambicus* was considerably reduced by sublethal concentrations of organophosphorus insecticides. Other pollutants have also been reported to decrease fish growth and survival as recorded in Petroleum effluent by Omoregie *et al.*, (1997), Paraquat by Babatunde (1997) and Tannery by Adakole (2005). Decrease in weight and standard length of the treated fish species may be attributed to the stress they experience while adjusting to attain a tolerance level with the toxicant (Abubakar and Abdulsalami, 2013).

Conclusion

In conclusion, abnormalities in growth rates of the exposed fish species were associated with the effects of sublethal concentrations of sniper 1000EC. By this context, the toxicant has to be taken into more consideration as an environmental contaminant. The use of sniper 1000EC by fishermen should be banned to save the aquatic ecosystem and more studies recommended for further evaluation of this toxicant.

References

- Abubakar, M.I. (2012). Comparative growth and survival rate of the African Catfish (*Clarias gariepinus*) larvae reared in water sourced from Stream and Tap water. *Journal of Biological Sciences and Bioconservation*. 4: 32-37.
- Abubakar, MI (2013). Toxicity of 2, 3-dichlorovinyl dimethyl phosphate(Sniper 1000EC) on *Clarias gariepinus* (Burchell, 1822) and *Oreochromis niloticus*(Trewavas, 1983) under laboratory conditions. Ph.D Thesis, Department of Aquaculture and Fisheries Management, Funaab.184pp.
- Adakole, JA (2005). Acute toxicity of metal-finishing company waste water to *Clarias gariepinus* fingerlings. *Journal of Aquatic sciences*. 20(2):69-73.
- Ajadi, BS., Adaramola, M.A., Adeniyi, A. and Abubakar, M.I. (2016): Effect of Effluents Discharge on public health in Ilorin Metropolis, Nigeria. *Ethopian Journal of Environmental Studies and Management*. 9(4):389-404
- Akintola, S.L, Anetekhai, MA, Fakoya, KA. and Adewolu, MA (2010). Seasonal distribution and aspect of biology of Genus Macrobranchium from Badagry Creek, Nigeria.*Nigeria journal of Fisheries*. Vol 7 (1&2) Oct, 2010. 16p.
- APHA, (1987). Amer. Publ. Hlth. Assoc., Washington, D.C.
- Araoye, PA (2009). The seasonal variation of pH and dissolved oxygen (DO₂) concentration in Asa lake Ilorin, Nigeria. *Int. J. Phys. Sci*. 4 (5): 271–274.
- Arrignon, JCV (1998). Tilapia; The Tropical Agriculturalist, C.T.A macmillan Education Ltd. Pp.78.
- Auta, J (2001). Toxicity of Dimethoate to Juveniles of *Oreochromis nilotius* (Trewavas) and *Clarias gariepinus*(Tengels). Ph.D. Thesis. Biological Sciences Department, Ahmadu Bello University, Zaria, Nigeria

Ayanshola, AM (2013). Evaluation of supply reliability and sustainability of household water use in Ilorin, Kwara State, Nigeria. Unpublished PhD Thesis submitted to the Department of Civil Engineering, University of Ilorin, Ilorin

Babatunde, MM (1997). Toxicity of paraquat (Gramazone) to *Oreochromis niloticus*: M. Sc thesis; Department of Biological Science, AhmaduBelloUniversity, Zaria Nigeria 212 P.

Bolger, T and Connoly, PL (1989). The selection of suitable indices for the measurement and analysis of fish condition. *J. Fish Biol.*, 34: 171–182.

Concalves, JMS, L. Bentes, PG.Lino, J.Ribeiro, AVM Canario and K.Erzini. (1996). Weight-Length relationships for selected fish species of small-scale demersal fisheries of the south and south-west coast of Portugal. *Fish. Res.*30:253-256.

Ecoutin, JM, Albert, JJ and Trape, S (2005). Length-weight relationships for fish populations of a relatively undisturbed tropical estuary: The Gambia, *Fisheries Resources*, 72: 351-374.

Elleta, OAA and Adekola, FA. (2005). Studies of the physical and chemical properties of Asa river water, Kwara State, Nigeria. *Science Focus* 10(1) pp 72-76.

EPA, (2002). Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. Fifth edition. U.S. Environmental Protection Agency Report 821-R-02-012.

Erkoyuncu, İ. (1995). Balıkçılık Biyolojisi ve Populasyon Dinamiği. Ondokuz Mayıs Üni.Yayınları, Samsun, 265 pp.

Fagbenro, OA (2002). Tilapia; Fish for thought. Inaugural Lecture Series 32. Delivered at Federal University of Technology, Akure.Pp.77.

FAO, (2005). The state of world Fisheries and Aquaculture, 2004: FAO Rome.

Fitzsimmons K, Martinez-Garcia R and Gonzalez-Alanis P (2011): Why tilapia is becoming the most important food fish on the planet. In: Liu LP

and Fitzsimmons K. (eds.), *Proceedings of the 9th International Symposium on Tilapia in Aquaculture*. Shanghai, 409 pp

Froese, R. and D. Pauly (1998). Fish Base concepts, design and data sources. Manila, *ICLARM*, 293p.

Froese, R. (2006). Cube law, condition factor and weight-length relationships: history, meta-analysis and commendations. *Journal of Applied Ichthyology* 22: 241-253. Le Cren, C.D. 1951.

Goda, AM, El-Haroun, ER, and Chowdhury, MAK (2007). Effect of totally or partially replacing fish meal by alternative protein sources on growth of African catfish *Clarias gariepinus* (Burchell, 1822) reared in concrete tanks. *Aquaculture Research*, 38:279-287.

Haghighi, DCR, Saad, SH, hosseinzadeh and D. Mansouri. (2009). The effect of dietary lipid level on the growth of kutum fry (*Rutilus frisii kutum*). *Iran. J. Fish. Sci.*, 8: 13-24.

King, RP (1996): Length-weight relationship of Nigerian Coastal water fishes *Fishbyte*, 19(4): 53-58.

Kulbicki, M, Guillemot, N and Amand, M (2005). A general approach to length-weight relationships for New Caledonian Lagoon species. *Cybium*, 29:235-252

Mastan, S and Ramayya, PJ (2010). Biochemical profile of *Channa gachua* (Ham) exposed to sublethal doses of Dichlorvos (DDVP). *The internet journal of Toxicology* Volume 8, Number 1.

Mironov, OG (1972). Effect of oil pollution on the Flora and Fauna of the black sea. In marine pollution and sea life (Ed). M. Ruwo. *FAO Fishing News Book*, London 222-224 pp.

Moutopoulos, DK and Stergiou, KI (2000). Weight-length and length-length Relationships for 40 fish species of the Aegean Sea (Hellas). *Journal of Applied Ichthyology* 18: 200-203.

Olatunde, AA (1989). Approach to the study of fisheries Biology in Nigerian inland waters. *Proc. Nat. Con. of two decades of Research on Lake Kainji*: Ayeni and Olatunde (Eds) pp. 538-541

Olayemi, A B (1994). Bacteriological water assessment of an urban river in Nigeria. *International J. Environ. Health Res.* 4:156-164

Omoniyi, IT, Agbon, AO and Sodunke, SA (2002). Effect of lethal and sub lethal concentrations of tobacco (*Nicotiana tobaccum*) leaf dust extract on weight and haematological changes in *Clarias gariepinus* (Burchell). *J. Appl. Sci. Environ. Mgt*; 6(2): 37-41.

Omoriege, E and Okpanachi, AF (1995). Growth of Tilapia zilli exposed to sublethal concentrations of crude extracts. *Azadiraccta indica*. *Acta Hydrobiologia* 34(30): 281-286.

Omoriege, E, Ofojekwu, PC and Amali, EI (1997). Effects of sublethal concentrations of formalin on weight gain in the Nile Tilapia, *Oreochromis niloticus*(Trewavas) *Asian Fisheries Science*, 10; 323-327.

Omoriege, E, Ofojekwu, PC and Amali, EI (1998): Effects of sublethal concentrations of formalin on weight gain in the Nile Tilapia, *Oreochromis niloticus*(Trewavas) *Asian Fisheries Science*, 10; 323-327.

Omoriege, E., and Onwogu, VI (2000). Growth and survival of the Cyprind, *Aphyosemion gardneri* exposed to water extracts of *Balanite aegyphaca* and *Azadiracta indica*. *Hamdard Medicine* 43(2):5-10.

Onusiriuka, BC (2002). Effects of sublethal concentrations of formalin on weight gain in the African catfish, *Clarias gariepinus*(Tengel). *Journal of Aquatic sciences*.17:66-68

Pauly, D (1993). Fishbyte section editorial. *Naga. ICLARM Quarterly*, 16:26

Percy, JA (1978). Effects of chronic exposure to petroleum upon the growth and Moulting Juveniles of the Artic marine Isopod [Crustacea] *Mesidatea isomom*. *J. fish Res. BdCanada* 35:650-656.

Petrakis, G and Stergiou, KI (1995). Weight-length relationships for 33 fish species in Greek waters. *Fisheries Research* 21: 465-469.

Shanmugavel, S, Sampath, K, Swakumar, V, Geotha, B and Gladys, DJ (1988). Sublethal effect of phosphamidon and methyl parathion on food

intake, growth and conversion efficiency of the fish, *Oreochromis mossambicus* *Environmental and Ecology* 6 (2): 257-261.

Snedector, GW and Cochram, WG (1980). Stastistical methods (7th Ed). The Iowa State University Press, Aves, Iowa, USA.

Stephan, EC (1982). Increasing the usefulness of acute toxicity test. In Aquatic Toxicity and Hazard Assessment. Proceedings of the fifth Annual symposium on Aquatic Toxicology: Ed. J.G. Pearson, R.B. Foster and W.E., Bishop. *ASTM Spec. Tech. Publ. Vol. 766*, pp. 69-81.

Toussain, MW, Rosencrance, AB, Brenna, LM, Beaman, JR, Wolfe, MJ, Hoffmann, FJ and Gardner, HS (2001). Chronic toxicity of chloroform to Japanese Medaka fish. *Environmental Health Perspective. 109(1)* : 35-40

Trewavas, E (1982). Tilapias: Taxonomy and Speciation, P.3-13. In the Biology and culture of tilapias (R.S.V Pullin and R.H Lowe-McConnell, eds). International Centre for Living Aquatic Resources Management, Manila Philippines. *Conference Proceedings* 7:432pp.

Waleed, NH (2012). Biochemical and Non-specific immune- parameter of healthy Nile Tilapia (*Oreochromis niloticus*), Blue Tilapia (*Oreochromis aureus*) and their interspecific hybrid (Male *O.aureus* x Female *O. niloticus*) maintained in semi-intensive culture system. *Online Journal of Animal and Feed Research* Vol.2 (1):84-88.

Zhou, HY, Cheung, RYH, Chan, KM and Wong, MH (1998). Metal concentrations in sediments and tilapia collected from inland waters of Hong Kong. *Water Res* 32(11):3331-3340

**Physicochemical Changes of *Citrullus Lanatus* (Watermelon) Seeds
during *Bacillus* Species mediated Fermentation and Sensory
Evaluation of Condiment Produced**

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Abstract

Watermelon (*Citrullus lanatus*), a drought tolerant and ubiquitous fruit, contains a lot of seeds that are generally discarded after consumption of the juicy portion. This study was designed to assess changes in proximate, amino acid, mineral and phytochemical compositions of watermelon seeds during a 72 hr fermentation period with mixed inocular of *Bacillus subtilis* and *Bacillus licheniformis* in the production of a local condiment similar to *ogiri* (a condiment produced from *Citrullus vulgaris* popularly consumed in Nigeria). The sensory quality was also evaluated for the resulting watermelon condiment. Proximate analysis revealed significant ($p < 0.05$) with an increase in crude protein (14.00-21.10 %), and ash (1.90-2.55%) at the end of fermentation. Some of the essential amino acids obtained include: threonine, valine, isoleucine, tryptophan and leucine, all of which increased with days of fermentation. Similar trend was observed in the mineral components: magnesium (41.02-45.06 mg/100 g), zinc (5.05-7.15 mg/100 g), phosphorus (14.52-17.00 mg/100 g), potassium (22.03-33.02 mg/100 g), calcium (13.45-16.03 mg/100 g), sodium (5.40-7.14 mg/100 g), and manganese (1.10-2.53 mg/100 g). Results showed that pH of the condiment ranged from 5.6 to 8.6 over 72 hrs of fermentation. Length of fermentation caused a steady decrease in hydrocyanic acid (1.57-0.03 mg/100 g), tannin (22.05-16.00 mg/100 g) and phytate (7.05-3.52 mg/100 g) but an increase in flavonoids (1.58-2.30 mg/100 g) and phenol (0.11-1.41 mg/100 g). Sensory evaluation showed

that *ogiri* produced from *C. vulgaris* was more generally accepted but that produced from *C. lanatus* was more preferred in taste with a score of 6.20. Increased days of fermentation with *B. subtilis* and *B. licheniformis* in consortium had varying effects on the nutritional and phytochemical compositions of watermelon condiment.

Keywords: Watermelon seed, *Bacillus* species, condiment, fermentation, physicochemical.

Introduction

Watermelon (*Citrullus lanatus*) is the fruit of an herbaceous creeping plant belonging to the family *Curcubitaceae* (Aguilera *et al.*, 2011). It has been described as one of the most widely cultivated crops with more than 1,200 varieties grown all over the world (Alfa-nla, 2014). Nigeria is one of the leading producers of watermelon in Africa, producing over 139, 000 tonnes in 2011 (Alfa-nla, 2014). Watermelon is highly nutritious with good contents of vitamin C as well as carotenoids, which are good antioxidant agents. In addition to the nutritional quality, as a fresh fruit, watermelon has an excellent thirst quenching property. The flesh of the fruit provides a sweet refreshing juice. As a result, only this part of the fruit is consumed. Obizoba *et al.* (2005) reported that only about 45-50 % of the total weight of watermelon is consumed; the rinds and seeds are usually discarded. This practice, aside constituting an economic loss, leads to increase in solid waste pollution and wastage of valuable biomass and nutrients (Acar *et al.*, 2012). Some recent researches have again reiterated the potential of some underutilised leguminous seeds, such as tamarind seeds (Olagunju *et al.*, 2018) and watermelon seeds (Adebayo, 2018; Adebayo *et al.*, 2018) for the production of protein-dense condiment.

Watermelon seeds are a good source of protein (16.33 – 17.75 %) (El-Adway and Jaha, 2001; Tabiri *et al.*, 2016), consisting of both essential and non-essential amino acids while watermelon rinds can be pickled and used as a condiment (Foster, 2015). Adebayo (2018) also showed that watermelon seeds are good potential for condiment production, and that *Bacillus subtilis* is one of the active microorganisms involved in the wild fermentation process.

Condiment is a supplemental food ingredient, such as a sauce, added to foods in some cultures to impart or enhance flavour or to complement the

dish (Agboola and Oguntuase, 2006). Some locally produced condiments in Nigeria have been identified as low-cost meat substitutes, contributing to protein and calorie intake (Oboh, 2006) and helping to reduce the incidence of protein energy malnutrition among low-income families who cannot afford animal protein. Also, the controversy surrounding the safe consumption of monosodium glutamate-based seasonings has made some homes shift preference to the use of locally produced condiments (Oboh, 2006). Some locally produced condiments include *iru* or *dawadawa* (fermented locust bean), *okpehe* (fermented *Prosopis africana*) and *ogiri* (fermented melon seed). However, the production of these fermented condiments is still largely being done spontaneously (village art method) which leads to longer fermentation time, inconsistent condiment quality and unguaranteed microbiological safety. This has made it necessary for the use of starter cultures in the production of condiment.

Many genera of bacteria including *Bacillus*, *Micrococcus*, *Leuconostoc*, *Staphylococcus* and *Enterobacteria*, have been shown to be involved in fermentation during indigenous condiment production (Enujiugha, 2009; Adebayo *et al.*, 2018). Achi (2005) noted that the use of mixed cultures, through their physiological and metabolic properties, might be the best approach to achieving product of most desired nutritional and sensory qualities. Mono and mixed cultures of *Staphylococcus* and *Streptococcus* have been successfully used in the fermentation of soy *iru* (Agboola and Oguntuase, 2006). Balogun *et al.*, (2017) have also reported the production of *okpehe* using a mixed culture of *Bacillus subtilis* and *Bacillus licheniformis*. However, scanty information is available on the fermentation of watermelon seeds using starter cultures for condiment production, despite its established high protein content.

It was therefore necessary to determine the physicochemical changes and sensory properties of a fermented condiment produced from watermelon seeds using starter cultures of *Bacillus subtilis* and *Bacillus licheniformis*. The use of watermelon seed for the production of a locally fermented condiment will improve the economic importance of the seed, while also serving as a cheap source of plant protein for low-income populations in developing countries.

Materials and Methods

Source of materials

Dried watermelon seeds were obtained from Obasanjo Farms, Igbo-ora, and authenticated at the Herbarium of the Department of Plant Biology, University of Ilorin with the Voucher No: UILH/003/1056 Nigeria. Pure cultures of *Bacillus subtilis* and *Bacillus licheniformis* were obtained from the Microbiology Laboratory of Federal Institute of Industrial Research, Oshodi, Nigeria.

Production of fermented water melon condiment

The method of Balogun and Oyeyiola, (2011) was used with some modifications. The seeds were first softened by heating for 1 hr on a hot plate set at 60°C. The water used in heating the seeds was thereafter decanted, and the seeds were washed with water at ambient temperature (30±2 °C). The testae of the washed seeds were then removed using gloved hands. The cotyledons obtained were thoroughly washed with clean water, after which they were submerged in a clean pot filled with water, and then cooked at 60 °C for 30 mins. The cotyledons were drained over a sterile, dirt-free muslin cloth, and cooled to about 25-30 °C before being inoculated with the selected starter organisms.

The method described by Balogun *et al.*, (2014) was used for the inoculation of the starter organisms. Unfermented cotyledons in two separate portions (100 g each) were inoculated with mono cultures: one with *B. subtilis* and the other with *B. licheniformis*. Another 100 g was inoculated with 1 ml of a mixed starter culture prepared from 0.5 ml each from the mono cultures. Prior to inoculation, a suspension of each starter organism was made in nutrient broth and incubated at 37 °C for 24 hrs. The resulting inoculated mash was placed in banana leaves, which had been disinfected using a 75 % ethyl alcohol and rinsed with distilled water prior to their use. The mash was then wrapped in a 2-layered foil. The wrapped mash was kept at 30±2 °C for different time regimes (0, 24, 48, and 72 hrs).

Determination of physicochemical parameters of fermented watermelon seeds condiment

The protein content of each watermelon condiment was determined and the sample with the highest crude protein content after 72 hrs of fermentation was selected for subsequent analyses. The pH, proximate and mineral compositions of the fermented watermelon condiments were determined using the method described by AOAC (2005). The carbohydrate content was determined by difference (i.e., 100 – moisture + crude protein + crude fibre + crude fat + ash). Amino acid profile was analysed using post-column Ninhydrin method described by Frank *et al.* (2000) while flavonoids and phenol contents were determined using AOAC (1990) method.

Some phytochemicals were also determined using standard methods. These include tannin (Jaffe, 2003), hydrocyanic acid (Drochioiu *et al.*, 2000) and phytate (Penuel *et al.*, 2013).

Sensory evaluation of fermented watermelon condiment

A local vegetable soup was prepared with the fermented watermelon seed condiment. This was then compared with vegetable soup prepared using *ogiri* (a fermented condiment produced from *Citrillus vulgaris*). Thirty (30) panelists, who were familiar with the peculiar sensory attributes of *ogiri*, were selected from the Department of Home Economics and Food Science, University of Ilorin. A duo-trio test was utilized. The panelists were given three samples, in which two were the same and one was different. The panelists were to identify the odd sample and compare it with the other two based on some stated sensory parameters (colour, taste, aroma, texture and general acceptability). A 9-point hedonic scale in which 9 represents ‘like extremely’ and 1 represents ‘dislike extremely’ was used.

Statistical analysis

Data generated from the various analyses above were subjected to Analyses of Variance using SPSS (Version 20.0; IBM SPSS) and the mean scores were separated using Multiple Range of Comparison Test.

Results and Discussions

Proximate compositions of *Bacillus subtilis* and *Bacillus licheniformis* fermented watermelon seeds

The protein content of watermelon seeds fermented with a mixed starter culture of *B. subtilis* and *B. licheniformis* was significantly ($p < 0.05$) higher (21.10 %) than those of other samples (those fermented singly and wildly) at the end of each fermentation period (Table 1). This shows a benefit of inoculation with either organism and an additional benefit when both organisms are present in the inoculum. Generally, the protein contents of all the condiments (both mono and mixed starter culture fermented watermelon seeds) increased with increased days of fermentation (Table 1). The increased protein content obtained in this study is similar to the findings of Gberikon *et al.*, (2010) who noted that the protein contents of *Parkia biglobosa*, *Glycin max*, and *Prosopis africana* seeds inoculated with a consortium of *B. subtilis* and *B. pumilus* were higher than those of the naturally fermented seeds. Balogun *et al.*, (2017) also reported an increase in the protein content of *Prosopis africana* seeds fermented with starter cultures.

There was a significant ($p < 0.05$) increase in the crude protein contents of the mixed-culture fermented watermelon seeds with increased days of fermentation (Table 2). This increase in protein could be as a result of significantly reduced carbohydrate contents with increased days of fermentation. It is plausible that the combined effects of *B. subtilis* and *B. Licheniformis* enhanced the fermentation process. Starter organisms are also known to secrete extracellular enzyme, which are protein in nature, during their metabolic activities (Oboh and Akindahunsi, 2003).

Table 1: Crude protein content (%) of watermelon seeds fermented with culture of either or both organisms (*B. subtilis* and *B. licheniformis*)

Bacterial isolate	0 hr	24 hrs	48 hrs	72 hrs
BS1M	14.00 ^a ± 0.20	15.06 ^b ± 0.02	16.50 ^b ± 0.00	18.43 ^c ± 0.01
BL1M	14.04 ^a ± 0.01	15.02 ^b ± 0.01	16.25 ^c ± 0.05	19.05 ^b ± 0.01
*B_sB_L	14.05 ^a ± 0.01	16.28 ^a ± 0.01	17.18 ^a ± 0.01	21.10 ^a ± 0.05
Control	14.05 ^a ± 0.00	15.00 ^b ± 0.02	15.05 ^d ± 0.05	16.05 ^d ± 0.01

Data presented as means ± S.D

Mean values with different superscripts within the same column are significantly (p<0.05) different

Keys: BS1M: *Bacillus subtilis*; BL1M: *Bacillus licheniformis*; B_sB_L: *Bacillus subtilis* and *Bacillus licheniformis*

* Sample selected for further analyses

Table 2: Proximate composition (%) of fermented watermelon seeds using mixed cultures of *Bacillus subtilis* and *Bacillus licheniformis*

Time (hr)	Moisture	Carbohydrate	Protein	Ash	Fiber	Fat
0	6.05 ^c ±0.01	52.9 ^a ±0.02	14.05 ^d ±0.01	1.90 ^b ±0.01	7.05 ^a ±0.03	18.05 ^d ±0.13
24	6.07 ^c ±0.00	47.58 ^b ±0.10	16.28 ^c ±0.01	2.00 ^b ±0.00	6.05 ^b ±0.00	22.02 ^c ±0.20
48	7.02 ^b ±0.0	45.25 ^c ±0.00	17.18 ^b ±0.03	2.04 ^b ±0.02	5.01 ^c ±0.01	23.50 ^b ±0.00
72	7.75 ^a ±0.05	38.35 ^d ±0.02	21.10 ^a ±0.15	2.55 ^a ±0.01	5.05 ^c ±0.01	25.20 ^a ±0.02

Data presented as means ± S.D

Mean values with different superscripts within the same column are significantly (p<0.05) different

The crude fat content of watermelon condiment produced from a mixed starter culture of *B. subtilis* and *B. licheniformis* significantly ($p < 0.05$) increased from 18.05 % at 0 hr to 25.20 % after 72 hrs (Table 2). This is likely due to the significant reduction of carbohydrate and crude fibre contents. The high amount of crude fat obtained is in line with the finding of Murkovic *et al.*, (2002) that watermelons are a good source of fat. It has also been reported that the fat content of locust beans increased when fermented (Achi, 2005). There was a significant reduction in the carbohydrate content of the watermelon seeds fermented with a mixed culture as increased days of fermentation. This could be associated with the utilization of carbohydrate by fermenting organisms. Crude fibre ranged from 5.05 to 7.05 % while ash content was between 1.90 and 2.55 %. Oboh (2006) earlier reported 5.9-11.2 % and 1.9-4.7 % as the crude fibre and ash contents, respectively, for some fermented underutilized leguminous seeds. Crude fiber is of great importance in human metabolism; it helps to form complexes with triglycerides, and tends to reduce low density lipoprotein (LDL) thereby reducing the risks of heart attack (Bello *et al.*, 2008). Ash content on the other hand is a measure of the amount of minerals present in food.

Amino acid profile of fermented watermelon seeds using mixed cultures of *Bacillus subtilis* and *Bacillus licheniformis*

Seventeen amino acids were found present in varying proportions in the seeds (Table 3). These include: all the essential amino acids (threonine, valine, isoleucine, tryptophan, lysine, histidine, methionine, leucine and phenylalanine), most of which steadily increased with increased days of fermentation. A similar trend was reported by Balogun and Oyeyiola (2012). The rapid increase in total amino acids is consistent with the increase in protein content. Low molecular weight peptides and amino acids produced through enzymatic breakdown of protein exhibit a desirable flavouring property (Achi, 2005). It was actually found that serine decreased steadily with increase in fermentation days, suggesting its depletion by microbial activity during fermentation.

Table 3: Amino acids profile (g/100 g) of fermented watermelon seeds using mixed cultures of *Bacillus subtilis* and *Bacillus licheniformis*

Amino acids	0 hr	24 hr	48 hr	72 hr
Lysine	0.52	1.05	1.55	1.79
Histidine	0.39	0.56	0.64	0.79
Asparagine	0.73	0.88	1.46	1.58
Aspartic acid	0.85	0.92	1.04	1.07
Threonine	1.04	1.45	3.88	2.64
Serine	4.03	3.07	3.03	2.07
Glutamine	0.03	0.07	0.53	0.87
Proline	2.85	2.82	2.90	2.98
Glycine	2.30	2.42	1.65	2.45
Alanine	1.51	1.64	2.01	2.75
Valine	2.54	2.73	2.02	3.53
Cysteine	0.06	0.10	0.19	1.10
Methionine	1.25	1.29	2.20	2.43
Isoleucine	2.00	2.50	2.43	2.44
Leucine	1.65	2.75	1.05	2.02
Tryptophan	2.11	1.32	2.21	2.45
Phenylalanine	0.25	1.44	1.77	1.97

Results are expressed as means of two determinations (n=2)

Mineral composition of fermented watermelon seeds using mixed cultures of *Bacillus subtilis* and *Bacillus licheniformis*

Major minerals found in the watermelon condiment are magnesium (41.02-45.06 mg/100 g), potassium (22.03-33.02 mg/100 g), phosphorus (14.52-17.00 mg/100 g), and calcium (13.45-16.03 mg/100 g) (Table 4). This is similar to the finding of Tabiri *et al.* (2016) who reported that watermelon seeds had appreciable contents of the aforementioned minerals. Oboh (2006) also reported that condiments produced from underutilised legumes in Nigeria such as soybeans (*Glycine max*), locust beans (*Parkia filicoidea* L.), pigeon pea (*Cajanus cajan*) and watermelon seed (*Citrullus lunatus*) were generally high in potassium, calcium, magnesium and sodium. The mineral contents analysed increased with increased days of fermentation. This might be linked with reduction of some antinutrients such as phytate (Table 5) which are known to bind with some minerals thereby making them biounavailable. Minerals are important for vital physiological functions in the body. Calcium is essential for bone formation and strength (Samir-El *et al.*, 2012) while potassium is required for proper sodium-potassium balance in the body.

Table 4: Mineral composition (mg/100 g) of fermented watermelon seed using mixed cultures of *Bacillus subtilis* and *Bacillus licheniformis*

Time (hr)	Magnesium m	Zinc	Phosphorus	Potassium	Calcium	Sodium	Manganese
0	41.02 ^d ±0.02	5.05 ^d ±0.05	14.52 ^c ±0.02	22.03 ^c ±0.01	13.45 ^c ±0.05	5.40 ^c ±0.02	1.10 ^d ±0.01
24	42.05 ^c ±0.05	5.52 ^c ±0.02	15.64 ^b ±0.02	25.04 ^b ±0.02	15.00 ^b ±0.00	6.01 ^b ±0.01	1.57 ^c ±0.03
48	44.00 ^c ±0.09	6.20 ^b ±0.01	16.75 ^b ±0.05	25.01 ^b ±0.01	16.01 ^a ±0.01	7.00 ^a ±0.00	2.01 ^c ±0.01
72	45.06 ^a ±0.02	7.15 ^a ±0.00	17.00 ^a ±0.02	33.02 ^a ±0.02	16.03 ^a ±0.01	7.14 ^a ±0.12	2.53 ^a ±0.03

Data presented as means ± S.D

Mean values with different superscripts within the same column are significantly ($p < 0.05$) different

pH of fermented watermelon seeds using a mixed starter culture of *Bacillus subtilis* and *Bacillus licheniformis*

Significant increase was noticed in the pH (5.8 – 8.6) of the watermelon seeds fermented with a mixed starter culture of *B. subtilis* and *B. licheniformis* with increase in duration of fermentation from 0 to 72 hrs (Fig. 1). The initially acidic pH became basic after 72 hrs. This may be as a result of ammonia, a by-product of protein, produced during fermentation (Balogun and Oyeyiola, 2012). Ammonia production is accompanied by a characteristic off-flavour. Fermented condiments from vegetable seeds have a fairly alkaline pH (Ogunshe *et al.*, 2007).

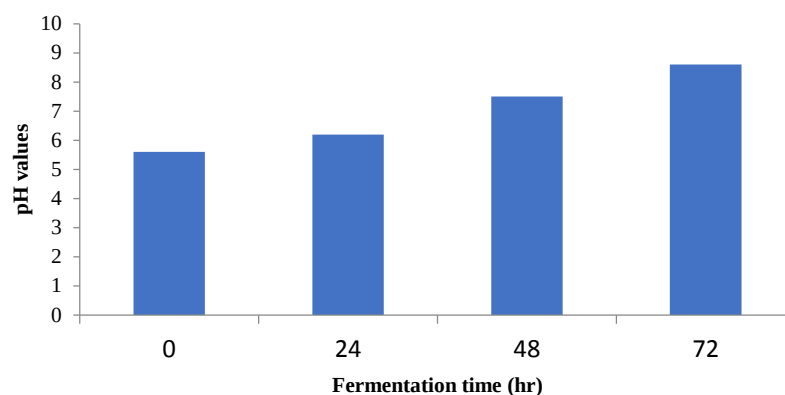


Figure 1: Changes in pH during fermentation of watermelon seeds using mixed cultures of *Bacillus subtilis* and *Bacillus licheniformis*

Phytochemicals present in watermelon seeds fermented using mixed cultures of *Bacillus subtilis* and *Bacillus licheniformis*

Fermentation brought about a significant reduction in the tannin, hydrocyanic acid and phytate contents of the watermelon seeds (Table 5). This is desirable as these food components, when present in high quantity, can interfere with the bioavailability of essential nutrients. For example, phytate forms insoluble complexes with zinc, iron, copper, and calcium (Lee and Kader, 2000) thereby limiting the absorption of these minerals. Tannin binds with protein forming a protein-limiting complex. Cyanohydric acids, also known as prussic acid, is extremely toxic due to its ability to link with metals such as Fe^{2+} , Mn^{2+} , and Cu^{2+} , which are functional groups of many enzymes (Alfred, 2010). This consequently

inhibits physiological processes like reduction of oxygen in the cytochrome respiratory chain as well as the activity of some important metabolic enzymes in the body (Doymaz, 2007). There was however an increase in the flavonoid (1.58-2.30 mg/100 g) and phenol (0.11-1.41mg/100g) contents. Egbebi *et al.* (2016) reported that these phytochemicals are in low amounts in fermented locust bean (*Parkia biglobosa*) and fermented melon (*Citrullus vulgaris*).

Table 5: Phytochemical composition (mg/100 g) of watermelon seeds fermented using mixed cultures of *Bacillus subtilis* and *Bacillus licheniformis*

Time	Hydrocyanic acid	Tannin	Flavonoids	Phytate	Phenol
0 hr	1.57	22.05	1.58	7.05	0.11
24 hr	1.02	20.94	1.80	5.78	0.58
48 hr	0.82	17.01	2.08	4.45	1.11
72 h	0.03	16.00	2.30	3.52	1.41

Sensory evaluation of fermented watermelon seeds

Though the soup prepared with *ogiri* was more generally accepted, the one prepared with the fermented watermelon seeds had higher mean score in taste and also compared closely in other sensory attributes (Table 6). In terms of taste, the mean score (6.20) obtained for fermented watermelon seed is similar to that reported for *ogiri-iru* blend (Ibeabuchi *et al.*, 2013). These results showed that, with regards to taste, the condiment from fermented watermelon seeds is a promising substitute for *ogiri*.

Table 6: Sensory scores of watermelon seed condiment

Samples	Colour	Taste	Aroma	Texture	General acceptability
A	4.05 ^b ±0.03	6.20 ^a ±0.01	5.00 ^b ±0.50	5.03 ^b ±0.51	5.32 ^b ±0.20
A1	5.05 ^a ±0.03	5.03 ^b ±0.00	6.55 ^a ±0.30	6.04 ^a ±0.41	7.30 ^a ±0.20

Data presented as means ± S.D

Means with different subscript letters in the same column are significantly different ($p < 0.05$)

Keys:

A: Watermelon seed condiment

A1: *Ogiri*

Conclusion

This study has revealed the suitability of starter cultures of *Bacillus subtilis* and *Bacillus licheniformis* as inocula for fermentation of watermelon seeds in the production of condiment. The combined use of *Bacillus subtilis* and *Bacillus licheniformis*, as well as increased days of fermentation resulted in an increase in protein and ash contents, and thus nutritional value. The condiment was rated quite close to *ogiri* by the panelists, and was, in fact, more preferred in taste. These findings indicate that through fermentation, the proportionate contents of protein and other nutrients can be increased at the expense of carbohydrate.

References

- Acar, R., Özcan, M.M., Kanbur, G. and Dursun, N. (2012). Some physicochemical properties of edible and forage watermelon seeds. *Iran. Journal of Chemistry and Chemical Engineering*, 31(4): 41-47.
- Achi, O.K. (2005). Traditional fermented protein condiments in Nigeria. *African Journal Biotechnology*, 4: 1612-1621.
- Adebayo, F.O. (2018). Microbiological Profile of 'Ogiri' Condiment Made from Seeds of Watermelon (*Citrullus lanatus*). *Asian Food Science Journal*, 1(1): 1-9.
- Adebayo, F.O., Mukhtar O.A. and Abdussalaam, R.B., (2018). Microbiological, Proximate and Organoleptic Analyses of a Fermented Condiment Made from Seeds of *Citrullus lanatus* (Watermelon). *South Asian Research Journal of Natural Products*, 1 (1): 1-7
- Agboola, Y.A. and Oguntuase O.S. (2006). Effect of *Bacillus sphaericus* on proximate composition soybean *iru* (*Glycine max*) for the production of soy-*iru*. *Pakistan Journal of Nutrition* 5(6): 606-607

Aguilera, Y., Benítez, V., Mollá, E., Esteban, R.M. and Martín-Cabrejas, M.A. (2011). Influence of dehydration process in *castellano* chickpea: changes in bioactive carbohydrates and functional properties. *Plant Food, Human Nutrition* 22(5): 43-49

Alfa nla, M.B.A. (2014). Economic analysis of watermelon production in selected local government areas of Kano State. Masters Thesis, Department of Agricultural Economics and Rural Sociology, Uploaded at Open Access Institutional Repository, Ahmadu Bello University, Nigeria, pp 12 and 22 <http://hdl.handle.net/123456789/6660>

Alfred O.U. (2010). Cyanogenic Glycosides and the fate of cyanide in soil. *Australian Journal of Crop Science*, 4(4):223-237

AOAC (1990). Official Method of analysis. Association of official Analytical Chemists, Washington DC, pp. 205-228A

AOAC (2005). Official Methods of Analysis (18th Edition): Association of Official Analytical Chemist, Washington D.C.

Balogun, M.A. and Oyeyiola, G.P. (2011). Microbiological and Chemical Changes During the Production of *Okpehe* from *Prosopis africana* Seeds. *Journal of Asian Scientific Research*, 1 (8): 390 – 398.

Balogun, M.A. and Oyeyiola, G.P. (2012). Changes in the Nutrient Composition of *Okpehe* during fermentation. *Pakistan Journal of Nutrition*, 11(3): 270-275

Balogun, M. A., Oyeyiola, G. P. and Kolawole, F. L. (2014). Physicochemical and sensory characteristics of fermented seeds of *Prosopis africana*. *Ethopian Journal of Environmental Studies and Management*, 7 (4): 411-422

Balogun, M.A., Oyeyiola, G.P. and Kolawole, F.L. (2017). Comparative study of physicochemical analysis of *Prosopis africana* seeds fermented with different starter cultures. *Croatian Journal of Food Science and Technology*, 9(1): 25-30

Bello, M.O., Falade, O.S., Adewusi, S.R.A., and Olawore, N.O. (2008). Studies on the chemical compositions and anti-nutrients of some lesser known Nigeria fruits. *African Journal of Biotechnology*, 7 (21): 3972-3979

- Doymaz, I., (2007). The Kinetics of forced convective air drying of pumpkin slices, *Journal of Food Engineering* 76 (1): 562-567
- Drochioiu, G., Mangalagiu, I. and Tataru, V. (2000). Specific spectrophotometric determination of hydrocyanic acid in the environment. *Analyst*, 125: 939-941
- Egbebi, A.O., Seidu, K.T. and Muhammad, A.A. (2016). Nutritional and Microbiological Analyses of Fermented Locust Beans (*Parkia biglobosa*) and Fermented Melon (*Citrullus vulgaris*). *Savant Journal of Agricultural Research*, 2 (1): 1-6
- El-Adway, T. A. and Jaha, K. M. (2001). Characteristics and composition of water melon, pumpkin and paprika seed oils and flours. *Journal Food Chemistry*, 49(3): 1253-1259
- Enujiugha V.N. (2009). Major fermentative organisms in Some Nigerian Soup Condiments. *Pakistan Journal of Nutrition*, 8: 279-283.
- Foster, K. (2015). 5 Reasons to Reconsider Trashing Your Watermelon Rind, Tips from the Kitchn. Kitchn, <https://www.thekitchn.com/5-reasons-to-reconsider-tossing-your-watermelon-rind-tips-from-the-kitchn-219949>
- Frank, D.M., Felicity, J.S, Rodney, G.K. and Reed J.H. (2000). Amino Acid Analysis, Using Postcolumn Ninhydrin Detection, in a Biotechnology Laboratory. *Methods in Molecular Biology*, 159: 9-30
- Gberikon, G. M., Ameh, J. B., Ado, S. A. and Umoh, V. J. (2010). Comparative studies of the nutritional qualities of three fermented African legumes seeds using *Bacillus subtilis* and *Bacillus pumilus* as starters. *Croatian Journal of Food Science and Technology* 4: 60-64.
- Ibeabuchi, J.C., Ojukwu, M. and Olawunmi, I. (2013). Comparative study of proximate and sensory qualities of *iru* powder, ogiri powder, and *iru*-ogiri powder blend. *Indian Journal* 9(7): 288-293
- Jaffe, C.S. (2003). *Analytical Chemistry of Food*. Blackie Academic and Professional, New York, pp.1: 200.
- Lee, S.K. and Kader, A.A. (2000). Pre-harvest and Post-harvest factors influencing vitamin C content of horticultural crops. *Post-harvest Biology and Technology*, 256-271.

Murkovic, M., Mulleder, U. and Neunteufl, H. (2002). Carotenoid content on different varieties of pumpkins: *Journal of Food Composition and Analysis*, 15(6): 633-38.

Obizoba, A.S., Vellon, L., Colomer, R. and Lupu, R. (2005). Oleic acid: the main monounsaturated fatty acid of olive oil suppresses. *Annals of Oncology*, 16(3): 359-371

Oboh, G. and Akindahunsi, A.A. (2003). Biochemical changes in cassava products (flour and gari) subjected to *Saccharomyces cerevisiae* solid media fermentation. *Food Chemistry*. 82, 4: 599-602.

Oboh, G. (2006). Nutrient and antinutrient composition of condiments produced from some fermented underutilized legumes, *Journal of Food Biochemistry*, 30 (5): 579-588

Ogunshe, A.A.O., Omotoso, M.O., Ayansina, A.D.V. (2007). Microbial studies and biochemical characteristics of controlled fermented ajiya- a Nigerian fermented food condiment from *Prosopis africana* (Guill and Perr.). *Pakistan Journal of Nutrition*, 6 (6): 620-627.

Olagunju, O.F., Ezekiel, O.O., Ogunshe, A.O., Oyeyinka, S.A. Ijabadeniy, O.A. (2018). Effects of fermentation on proximate composition, mineral profile and antinutrients of tamarind (*Tamarindus indica* L.) seed in the production of daddawa-type condiment, *LWT*, 90: 455-459

Penuel, B.L., Khan, E.M. and Maitera, M.O. (2013). Properties of proximate composition and elemental analysis of *Citrullus vulgaris* seed. *Life Science*, 2(2): 39-46.

Samir-El, S.F., Rabab, H.S. and Abd El-Ghany, M.E., (2012). Chemical and nutritional evaluation of different seed flours as novel sources of protein. *World Journal of Dairy and Food Science* 7(1): 59-65

Tabiri, B., Agbenorhevi, J.K., Wireko-Manu, F.D. and Ompouma, E.I. (2016). Watermelon seeds as Food: nutrient composition, phytochemicals and anti-oxidant activity. *International Journal of Nutrition and Food Science*, 5 (2): 139-144.

Evaluation of *Oligochaeta* Fauna of Ozomu Lake, Benin City, Nigeria

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Abstract

The evaluation of *Oligochaeta* fauna of Ozomu Lake was investigated for a period of twelve months (July 2014-June 2016). The findings revealed a total of 23 *Oligochaeta* taxa from a total of 271 individuals comprising of 14 species of *Naididae* family, 2 species of *Aeolosomatidae* family, and 7 species of *Tubificidae* family. The percentage abundance of *Oligochaeta* in the study stations showed that *Naididae* had the highest abundance of 70% followed by *Tubificidae* and *Aeolosomatidae* recording 27% and 3% respectively. *Naididae* was predominant and widespread in the study stations. The abundance and community structure of *Oligochaeta* in this study showed significant variations ($P < 0.05$). The diversity indices indicated that taxa richness (d), Shannon –Wiener (H) and Evenness (E) varying differences at the 2 study stations respectively. Jaccard's coefficient of similarity of species revealed a significant similarity when comparing the presence and absence of species found between the stations ($P > 0.05$). The impact of seasonal variations, human activities and environmental factors influencing the distribution and abundance of *Oligochaeta* are observed. There is a need for freshwater ecologists to investigate the developments in *Oligochaeta* ecology in Lakes and other freshwater ecosystems. Furthermore, *Oligochaeta* plays a vital role biomonitoring, as their presence or absence in a water body serves as a pointer to the true quality of water, be it in a fresh, brackish or, marine water ecosystem.

Keywords: *Oligochaeta*, *Naididae*, *Aeolosomatidae* and *Tubificidae*, Ozomu Lake

Introduction

Oligochaeta is a subclass of animals in the Phylum Annelida. They are made up of many types of aquatic and terrestrial worms, and including all of the various earthworms (Plisko, 2013; Odigie and Olumokoro, 2016). Specifically, the Oligochaetes contain the terrestrial megadrile earthworms (some of which are semi-aquatic or entirely aquatic), and freshwater or semi-terrestrial microdrile forms, including the tubificids, pot worms and ice worms (*Enchytraeidae*), black worms (*Lumbriculidae*) and several interstitial marine worms (Plisko, 2013; Odigie and Olumokoro 2016). With around 10,000 known species, the *Oligochaeta* makes up about half of the Phylum Annelida. These worms usually have few setae (chaetae) or "bristles" on their outer body surfaces, and lack parapodia, unlike Polychaeta members of the Class *Oligochaeta* include common earthworms (Odigie and Olumokoro 2016). The aquatic sludge worms (Family-*Tubificidae*) are best known for their value as environmental indicators (Plisko, 2013). The name *Oligochaeta* means "few bristles," which refers to the small bundles of hair-like bristles that occur on each body segment. Tubificid worms have long been recognized as pollution-tolerant because of their ability to thrive under poor water quality conditions (Odigie and Olumokoro, 2016). Their blood contains haemoglobin, which enables them to survive in waters where oxygen is lacking. Haemoglobin (the same oxygen-transporting protein in our blood) absorbs and retains oxygen from the water (Odigie and Olumokoro, 2016). Their high tolerance for low oxygen also means that they can survive in the anaerobic conditions caused by severe nutrient pollution, such as sewage discharge, that kills off other invertebrates (Odigie and Olumokoro, 2016). An aquatic invertebrate community dominated by large red worms in an area that isn't naturally oxygen-deprived is often an indicator of organic pollution (Voshell *et al.*, 2002).

Generally, studies of *Oligochaeta* fauna in Lakes have received little or no attention especially in benthic ecology research as the *Oligochaeta* fauna on Lakes in Benin City is yet to be harnessed hence the need for this present study. Ozomu Lake serves as a major source of water for domestic use and cassava processing in the community. Furthermore, there remains paucity of information and dearth of literature on the *Oligochaeta* fauna of Ozomu Lake situated in Benin City, Nigeria. The present will investigate the diversity, composition, and abundance of Oligochaetes in Ozomu Lake

which may serve as a reference point for further research studies in the lentic water body.

Materials and Methods

Study Area

This study was carried out in Ozomu Lake with two sampling stations chosen. Ozomu Lake is situated in the interior of Ozomu village 60km away from Benin- City, Edo State, Nigeria at Ovia North East local government area of Edo State. It lies between latitude $5^{\circ}26'25.940\text{E}$ and longitude $6^{\circ}16'16.154\text{N}$. The Lake takes its source from underground water. The Lakes reveal various man made segments that are specially designed to retain water during the dry season of the year. Tropical rainforest vegetation surrounds Ozomu Lake, as a result, the Lake is always cool. Some of the trees shed their leaves into the water thereby resulting in a high amount of decomposing organic matter content. The inhabitants are mostly farmers; they till the land for production of crops like cassava, maize, and yam. Small scale plantation farming and processing of farm produce into semi-finished products for local consumption is also common. For example, cassava is processed into garri and oil palm is processed into palm oil using crude implements. Some people also engage in hunting for bush meat, tree felling for timber and fish farming.

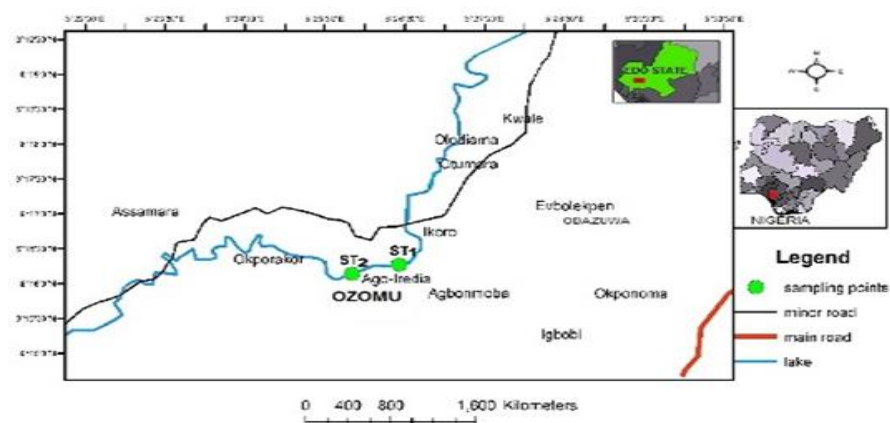


Figure 1. Map of Ozomu Lake showing sample stations.

Station 1

Station 1 is located 200m away from the village and lies between latitude 5°26'25.946E and longitude 6°16'16.155N. It had enormous vegetation composed of large shrubs *Alchomelaxiflora* sp and *Nephrolepsis biserrata*, thick vegetation of *Bambusa* sp and aquatic weeds. Organic matter from dead and decaying leaves from trees was present in the station. Surface dwelling frogs, snails, and other organisms frequented the station. The water level is about 3.20m overlaid.

Station 2

Station 2 lies between latitude 5°25'50.509E and longitude 6°16'8.47N. In this station, there is thick vegetation of *Bambusa* sp, few numbers of aquatic plants *Pistia* sp, *Azolla* sp, and *Salvinia* sp. The bottom is muddy, and organic matter from trees was also present in the station. Surface dwelling frogs, snails, and other organisms frequented the station. The water level is about 2.24m overlaid.

Sampling collection, duration, and analysis

Oligochaeta fauna was sampled in the months of July 2014 to June 2015. Sampling was conducted fortnightly at each sampling stations between the early hours of 8:00 am and 9:00 am. Water samples and bottom sediments were collected for analysis in the laboratory. Bottom sediments were collected using a modified Ekman's grab and sodium chloride added for three to five minutes to facilitate floatation of the organisms. The liquid medium and floating fauna were sieved through a mesh sieve of size 0.5 mm. The retained macroinvertebrates were stored in sampling plastic containers, preserved with 10% formalin and appropriately labeled. Benthic organisms were sorted out in the laboratory using the American Optical dissection microscope (Model 570) with X25-40 magnification. The sorted samples were preserved in 4% formalin for identification and counting. Specimen identification was achieved using the American Optical dissection microscope (model 570), with a binocular light microscope® (Olympus, England). Identification keys adopted in this study were according to Brinkhurst (1971); Ogbeibu (1991); Olomukoro (1996); Milligan and Hulbert (1997) and Pinder (2010).

Data Analysis

PAST Graph-MS word was used to analyze the Biological indices (Green, 1971). The fauna of the stations was compared using Jaccard index (Jaccard, 1912). All statistical analysis and graphical presentations were done with SPSS version 16.0, ANOVA and MS-Excel pack.

Results and Discussion

Twenty three taxa from a total of 271 individuals are recorded in this study (Table 1). The taxa are represented according to study stations. The family *Aeolosomatidae* represented by 2 taxa *Aeolosoma* sp, *Aeolosoma hemprichi*, accounted for (3%) of the total number of individuals recorded in all stations, *Aeolosoma* sp. had the highest abundance in station 1 with *Aeolosoma hemprichi* occurring once in station 1 (Fig 2). The family *Tubificidae* accounted for (27%) of an overall number of individuals encountered and was represented by 7 taxa (Fig. 2). *Tubifex* sp had the highest abundance in Station 1 followed by *Psammoryctides albicola* in station 1 and *Branchiura sowerbyi* in station 2 (Table 1). Furthermore *Monopylephorus irroratus* and *Potamothrix hammoneniensis* had a slightly high abundance in station 2 with *Limnodrilus udekemianus* recording the least abundance in the two study stations (Table 1). The family *Naididae* had the highest abundance (70%) representing the number of individuals collected from the study stations (Fig 2). Fourteen (14) taxa of *Naididae* were recorded in the study station (Table 1). *Nais barbata* was the most abundant occurring mostly in station 2, followed by *Dero* sp occurring at a high number at both stations with a peak value was recorded in station 2. *Nais* sp. had a high abundance mostly in station 1, also *Chaetogaster* sp. recorded a peak value in station 1 with no occurrence recorded in station 2 during the duration of the study. *Pristina longiseta* had a slightly high abundance in station 2 with *Branchiodrilus hortensis* recording the least value in station 1 and also occurred once (Table 1). The monthly abundance of *Oligochaeta* sp in the study showed that the highest abundance was recorded in the months of April and May 2015 with the least abundance occurring in the month of March 2015 which is usually the end of the dry season (Fig 3).

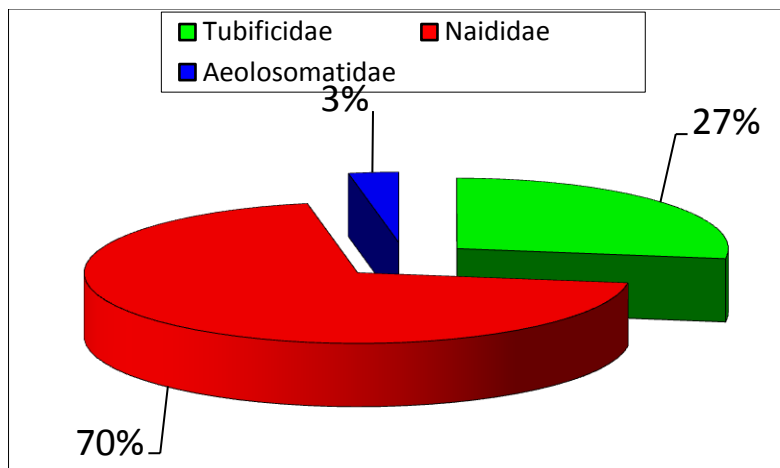


Fig 2: Percentage relative abundance of *Oligochaeta* in the study stations.

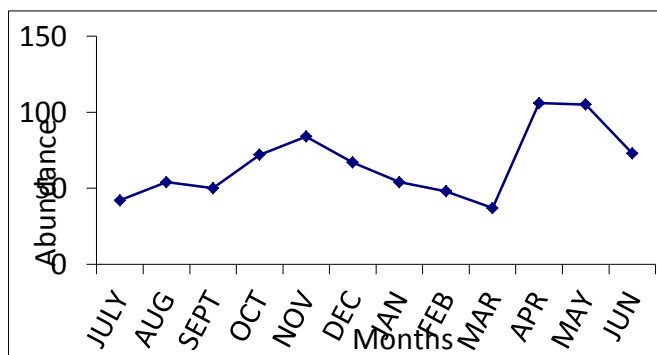


Figure 3: Monthly abundance of *Oligochaeta* for the duration of study

TABLE 1: The Distribution of *Oligochaeta* in Ozomu Lake

TAXA	Station 1	Station 2	TOTAL
Phylum Annelida			
Class Oligochaeta			
Order Haplotaxida			
Family Tubificidae			
<i>Potamothrix hammonensis</i>	-	5	5
<i>Pranonyctides albicola</i>	15	-	15
<i>Mononyctephorus irroratus</i>	-	7	7
<i>Limnodrilus udekemianus</i>	1	1	2
<i>Branchiura caverhyi</i>	2	15	17
<i>Tubifex</i> sp.	21	5	26
<i>Tubifex tubifex</i>	-	2	2
Family Naididae			
<i>Pristinella osborni</i>	4	-	4
<i>Pristina like</i> sp.	-	5	5
<i>Pristina longiceta</i>	2	13	15
<i>Pristina aequiceta</i>	-	3	3
<i>Ophidonais serpentina</i>	-	5	5
<i>Branchiodrilus hortensis</i>	1	-	1
<i>Chaetogaster</i> sp.	15	-	15
<i>Nais</i> sp.	21	2	23
<i>Nais barbata</i>	7	29	36
<i>Sylaxia lacustris</i>	8	9	17
<i>Aulonophorus vagus</i>	5	7	12
<i>Dero</i> sp.	19	23	42
<i>Dero digitate</i>	-	3	3
<i>Dero furcatus</i>	8	-	8
Order Plesiopora			
Family Aeolosomatidae			
<i>Aeolosoma</i> sp.	5	2	7
<i>Aeolosoma hemprichi</i>	1	-	1
Total	135	136	271

Community structure

Table 2 shows the indices of diversity, taxa richness and evenness of species distribution in the study stations. *Oligochaeta* species had a high dominance in Station 1 (0.1131) and least dominance in Station 2 (0.1074). The maximum value of species abundance recorded is 19% in station 2 while Station 1 had a slightly high abundance of 17%. The taxa richness of *Oligochaeta* species was high at station 1. However, Station 2 had a higher diversity value of 2.447 with Station 1 having (2.329) respectively (Table 2) Ozomu Lake had an evenness index value of 0.7336 and 0.7219 in Station 1 and Station 2 respectively. Therefore, the

evenness index in this study showed that *Oligochaeta* was evenly distributed in Ozomu Lake. The Jaccard Similarity Index showed that the stations in the present study were similar when comparing the presence and absence of species found at the study stations (Table 3).

Table 2: Species Diversity of *Oligochaeta* in Ozomu Lakes

Indices	Station 1	Station 2
Taxa_(S)	14	16
Individuals	131	149
Dominance_(D)	0.1131	0.1074
Relative abundance (%)	17	19
Shannon_(H)	2.329	2.447
Evenness_(e ^{H/S})	0.7336	0.7219
Margalef (d)	2.667	2.998
Equitability_(J)	0.8826	0.8824

Table 3: Jaccard similarities of *Oligochaeta* species of Ozomu Lake

Jaccard similarity	STATION 1	STATION 2
STATION 1	1	
STATION 2	0.57895	1

The family *Naidiade* had a high occurrence and dominance in the study stations which is sometimes constant in fresh water bodies. Odigie and Olumukoro (2016), suggested that the abundance of *Oligochaeta* is usually associated with the type of substratum in the study station. This is in agreement with Odigie and Olumokoro (2016), who reported that the abundance of *Oligochaeta* has been associated with muddy substratum rich in organic matter, even at a short interval of 10 to 50m of the bottom substratum. The fauna characteristics in Ozomu Lakes showed that *Oligochaeta* is predominant in station 2. This recorded increase is in

agreement with the study of Odigie and Olumokoro (2016) reported for Obazuwa Lake, that the organic matter contents of sediments and the dissolved oxygen concentration near the bottom are the most important factors determining the abundance of *Oligochaeta* in Lakes. The abundance of *Tubificidae* in the two stations of Ozomu Lake was characterised by increased abundance of *Tubificidae* in station 2 with over 27%. However, the Family *Tubificidae* was the most dominant with *Tubifex* sp and the least dominant was *Limnodrilus udekemianus*. These patterns of observations may be due to human pressures in the form of anthropogenic stress and domestic waste which could lead to escalation of taxa that are tolerant to pollution. This is in agreement with the report of Odigie and Olumokoro (2016) in the study of the species diversity and abundance of *Oligochaeta* fauna in Obazuwa Lake. In this study, it was revealed that anthropogenic impacts change the benthic community composition of a lentic waterbody. Furthermore, in the Family *Naididae*, the most dominant species were the *Nais barbata* and the least dominant were *Branchiodrilus hortensis*. Also, the abundance of individual species of the Family *Naididae* according to the stations showed the species was most dominant in stations 2 and with a slightly high dominance in station 1. The abundance of *Nais* sp was due to the abundance of aquatic plants at Ozomu Lake, these results are in agreement with Timm and Veldhuijzen van Zanten (2003) who stated that *Nais* sp is well known to inhabit an aquatic plant zone and live among macrophytes when in abundance in fresh waters. The abundance of individual species in the Family *Aeolosomatidae* was observed to be abundant at station 1 and least abundant at station 2. The abundance of *Aeolosomatidae* at Ozomu Lake, was due to the location of fish ponds in some areas demarcated in both Lakes thereby increasing the abundance of *Aeolosomatidae*, which are known to inhabit fish ponds. Chatarpaul et al. (1979) showed that *Aeolosomatidae* accelerated nitrogen loss from coarse streams sediments, indicating their importance in rivers as well as Lakes. The monthly occurrence of *Oligochaeta* in the duration of the study (July 2014- June 2015) showed that *Oligochaeta* had the peak abundance in the months of April and May with the least abundance in the month of March. This result is in agreement with the report of Sharma et al. (2011) who observed a peak abundance of *Oligochaeta* in the months of April and May when studying the water quality of the tributary of Behlol Nullah in India.

The diversity of *Oligochaeta* in the study area estimated by the Margalef, Shannon-Wiener, Evenness and Jaccard Similarity indexes in the stations were generally low and compared favorably with Odigie and Olumokoro (2016) who reported low values for Margalef's species richness and Shannon – Wiener diversity Index in Obazuwa Lake. The presence of good organic detritus content further contributed to the maximum quantity of *Oligochaeta* as has also been reported by Chakraborty and Das (2006); Gasim *et al.* (2006); Manoharan *et al.* (2006) and Odigie and Olumokoro, 2016. *Oligochaeta* normally favors the organically rich environment and remains dominant in severally polluted conditions (Odigie and Olumokoro, 2016). Some aquatic *Oligochaeta* species are abundant in organically polluted water because of a lack of competition for food and space, which is also linked to tolerance to reduced oxygen conditions (Brinkhurst *et al.*, 1983). However, in this study, *Oligochaeta* fauna was observed to be available all year round while differences seen in some species which may be due mainly to the recruitment and reproductive cycle of *Oligochaeta* occurring all year round (Odigie and Olumokoro, 2016).

Conclusion

The low diversity of *Oligochaeta* fauna in this study is not unusual. It is, therefore, important that freshwater biologists pay attention to the developments in *Oligochaeta* ecology in Lakes and other freshwater ecosystem, as *Oligochaeta* play a vital role biomonitoring, as their presence or absence in a water body serves as a pointer to the true quality of water, be it in a fresh, brackish or, marine water ecosystem.

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References

- Brinkhurst, R.O. and Jamieson, B.G.M. (1971). Aquatic *Oligochaeta* of the World, Toronto University, Toronto, 860pp.
- Brinkhurst, R.O., Chapman, P.M. and Farrell, M.J., (1983). A comparative study of respiration rates of some aquatic Oligochaetes in relation to sublethal stress. *Internationale Revue der Gesamten Hydrobiologie*, **2**:1-89.
- Chakraborty, D. and Das, S. K. (2006). Alteration of macroinvertebrate community in tropical aquatic system in relation to sediment redox potential and overlying water quality. *Journal of Environmental Science and Technology*, **2**(4): 327-334.
- Chatarpaul, L., Robinson, J. B., and Kaushik, N. K. (1979). The role of tubificid worms on nitrogen transformations in stream sediment. *Journal of Fisheries Research Board of Canada*, **36**: 673-678.
- Gasim, M. B., Toriman, M. E., Rahim, S. A., Islam, M. S., Chek, T. C. and Juahir, H. (2006). Hydrology and water quality and land use assessment of Task Chini's Feeder Rivers, Pahang Malaysia. *Geographia*, **3**(3): 1-16.
- Green, R.H. (1971). Sampling design and statistical method for environmental biologist. John Wiley and Sons, Toronto, 257pp.
- Jaccard, P. (1912). The distribution of the flora in the alpine zone. *New Phytologist*, **11**(2):37-50.
- Manoharan, S., Murugesan, V. K. and Palaniswamy, R. (2006). Numerical abundance of benthic macroinvertebrates in selected reservoirs of Tamil Nadu. *J. Inland Fish. Soc. India*. **38**(1): 54-59.
- Milligan, M.R. and Hulbert, J.L. (1997). Identification Manual for Aquatic *Oligochaeta* of Florida (Freshwater Oligochaetes). Final report for the Department of Environmental Protection, Tallahassee, Florida. **1**:3- 173.
- Odigie, J. O. and Olomukoro J.O. (2016). Species Diversity and Abundance of *Oligochaeta* fauna in a Tropical Rain Forest Lake in South-Southern, Nigeria. *Journal of Aquatic Sciences*, **31** (2b):391-400.

Ogbeibu, A.E. (1991). Hydrobiological characteristics of water bodies in the Okomu forest reserve (sanctuary), Benin, Nigeria. Ph.D. Thesis. University of Benin, Nigeria.

Olomukoro, J.O. (1996). Macrobenthic fauna of Warri River. Ph.D. Thesis, University of Benin. 205pp.

Pinder, A. (2010). Tools for identifying selected Australian Aquatic Oligochaetes (Clitellata: Annelida). *Museum Victoria Science Reports*, **13**: 1–26.

Plisko, J.D. (2013). A new family *Tritogeniidae* for the genera *Tritogenia* and *Michalakus* earlier accredited to the composite *Microchaetidae* (Annelida: *Oligochaeta*). *African Invertebrates*, **54** (1): 69–92.

Sharma, K. K., Langer, S. and Sharma, R. (2011). Water Quality and Macrobenthic Invertebrate Fauna of Behlol Nullah, Jammu. *The Echscan*, **5**(3&4): 111-115.

Timm, T. and Veldhuijzen van Zanten, H.H. (2003). Freshwater *Oligochaeta* of North-West Europe. ETI Information Services Ltd, The Netherlands.

Voshell, J.R., Reese, J. and Wright, A. B. (2002). A Guide to Common Freshwater Invertebrates of North America. Blacksburg (VA): The McDonald & Woodward Publishing Company. 442pp.

Physicochemical Properties of Starch obtained from Tubers of *Anchomanes difformis* and *Tacca involucrata*

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Abstract

Anchomanes difformis and *Tacca involucrata* are wild tuberous plants rich in starch. Starch granules were isolated from the tubers of these plants using wet milling method of extraction and further subjected to physicochemical characterization by different experimental procedures. *A. difformis* afforded starch with a percentage yield of 28.15 % while *T. involucrata* yielded 12.25 % starch. The physicochemical analysis of isolated starch compounds revealed a swelling power of 8.5 and 12.0, gelatinization temperature of 73 and 75 °C, pasting temperature of 79.96 and 77.89 °C, with a pH of 5.8 and 6.5 and bulk density 0.77 and 0.76 g/cm³ for *A. difformis* and *T. involucrata* respectively. The microscopic and X-ray diffraction analysis indicated that the starch granules are generally small in size, with clustered arrangement. This study has revealed that both *A. difformis* and *T. involucrata* are not only rich in starch but that the investigated starch compounds have high potential for industrial applications and could serve as alternative sources to avoid competition with the staple crop, cassava.

Keywords: Starch, *Anchomanes difformis*, *Tacca involucrata*, physicochemical properties

Introduction

Starch is a naturally occurring, biodegradable, and widely available polysaccharide molecule. It is composed of the chains of glucose units $(C_6H_{12}O_6)_n$, with n ranging from 300 to 1000, bound with a glycosidic linkage. Starch occurs widely as reserve for carbohydrate in stems, grains, and fruits of all forms of green plants. Cereal grains such as corn, wheat, sorghum and tubers and some roots such as potato, tapioca, arrowroot, etc. are some of the commercial sources of starch for industrial exploitation. Different starches differ depending on their botanical source, biochemistry and morphological properties (Neelam *et al.*, 2012). Starch is also one of the most widely used biomaterial in the food, textile, cosmetics, plastics, adhesives, paper and pharmaceutical industries. The diverse industrial usage of starch is based on its availability at low cost, high calorific value and inherent excellent physicochemical properties (Omojola *et al.*, 2010).

Tacca involucrata (Sch. and Thonn.) is a rhizome, usually found in the savannah grassland. It belongs to the family *Taccaceae*. It is a perennial herb with a round swollen tuber underneath the ground from which it shoots out a leaf stalk up to 1 m long. The plant had a long history of being a major source of carbohydrate in the savannah belt of Nigeria until the advent of cassava when its importance waned, it is now harvested wild (Nwokocha *et al.*, 2011). The tuber is first boiled to remove the toxic element and then converted to flour and employed in a variety of food preparations. *T. involucrata* is an under-utilized crop with a high potential as a source of industrial starch (Nwokocha *et al.*, 2011).

Anchomanes difformis, a member of Araceae family (Burkill, 1985), is an herbaceous plant with prickly stem having huge divided leaf and spathe that arise from a horizontal rhizome occurring in the forest of West Africa. It is sometimes called forest Anchomanes in English (Oyetayo, 2007), while among the Yoruba speaking populace of the Southwestern Nigeria, it is known as Abirisoko, Ogirisako or Igo (Soladoye *et al.*, 2005).

The quest for cheaper and higher quality starch from biological materials has necessitated this research into lesser known plants which have better physicochemical properties over the present commercial starch sources. Therefore, the purpose of this study is to compare the physicochemical properties of starch granules from tubers of *Tacca involucrata* and *Anchomanes difformis* obtained from South Western Nigeria.

Materials and Methods

Freshly harvested rhizomes of *Anchomanes difformis* and *Tacca involucrata*, grown naturally were collected from Ekiti East local government area of Ekiti State, and authenticated at the Herbarium unit of the Department of Plant Biology, University of Ilorin, Ilorin, Kwara state. Voucher specimens were deposited, the following voucher numbers were thus issued; UILH/001/1850 and UILH/001/1930 for *T. involucrata* and *A. difformis* respectively.

Isolation of Starch

The tubers were weighed, washed, peeled and cut into 2 cm long chips. The chips were homogenized in a Phillips HR1720 blender with 50 ml of water. The slurry obtained was mixed with five times its volume of water and sieved with a muslin cloth. The starch milk was allowed to settle overnight and the supernatant decanted. This was washed several times with fresh water. The resultant white starch was sun dried, pulverized, weighed and stored in a plastic container (Nwokocha *et al.*, 2009).

Determination of physicochemical properties

Swelling power:

The method described by Daramola and Osanyinlusi (2006) was used to determine the swelling power with slight variations. The starch sample (0.1 g) was weighed into a test tube and 10 ml of distilled water was added. The mixture was heated in a water bath at a temperature of 50 °C for 30 min with continuous shaking. In the end, the test tube was centrifuged at 1500 rpm for 20 min in order to facilitate the removal of the supernatant which was carefully decanted and weight of the starch paste taken. This was carried out over a temperature range of 50 – 100 °C.

The swelling power was calculated using the expression 1.

$$\text{Swelling power: } \frac{\text{Weight of starch paste}}{\text{Weight of dry starch sample}} \cdot 1$$

Solubility Index:

Solubility index was determined over a temperature range of 50 – 100 °C as follows: Starch sample (0.5 g) was added to 10 ml distilled water in a

test tube. This was subjected to heating in a water bath with a starting temperature of 50 °C for 30 min. It was then centrifuged at 1500 rpm for 30 min. 5 ml of the supernatant was decanted and dried to constant weight. The solubility was expressed as the percentage (%) by weight of dissolved starch from heated solution (Mandala and Bayas, 2004).

Gelatinization Temperature:

This was evaluated using the method of Attama *et al.* (2003). The starch sample (1 g) was placed in a 20 ml beaker and 10 ml of distilled water was added. The dispersion was heated on a hot plate at between 60 – 80 °C. The gelatinization temperature was then read with a thermometer suspended in the starch slurry.

pH:

A 20 % w/v dispersion of the sample was shaken in water for 5 minutes and the pH was determined using a pH meter (ATPH-6 water proof pocket pH tester meter).

Pasting Properties:

Pasting characteristics such as pasting temperature and viscosity of the starch obtained from the tubers were determined with a Rapid Visco – Analyser (RVA), (Model RVA 3D+, Network Scientific, Australia). The starch (2.5 g) sample was weighed into a dried empty canister, 25 ml of distilled water was dispensed into the canister containing the sample. The solution was thoroughly mixed and the canister was well filled into the RVA as recommended. The slurry was heated from 50 – 95 °C with a holding time of 2 minutes followed by cooling to 50 °C with 2 minutes holding time. The rate of heating and cooling were at constant rate of 11.25 c/min viscosity and pasting temperatures were read from the pasting profile with the aid of thermocline (Adewumi *et al.*, 2015).

Microscopy:

A scanning electron microscope (SEM) (model EVO/MA 10) was used to determine the particle size at 50 X and 100 X magnifications. The sample was imaged with the secondary electron detector at an accelerating voltage of 5 kV, probe current of 200 PA and variable pressure of 50 Pa (Fanon *et al.*, 1992).

X-ray Diffraction:

The starch samples were pulverized to powder particle size of less than 63 μm mesh sieve and equilibrated in a desiccant of a desiccator overnight. The samples were placed in the cavity of a disc sample holder of the diffractometer. Diffraction diagrams were recorded using Inel X-ray equipment (Inel CSP120, 45410 Artenay, France) operating at 40 kV and a generator current of 30 mA. Cu K-1 radiation ($\lambda = 0.15405 \text{ nm}$) was selected using a quartz monochromator and scanned at $\theta - 2\theta$ degrees. A curved position detector was used to monitor the intensities using 2 h exposure periods. PeakFit software (Systat software Inc., Chicago, USA) was used to quantitatively estimate the degree of crystallinity using the ErfcPk type in peak fitting and analyze the amorphous area ($r^2 > 0.99$), the percentage crystalline area was obtained by difference.

Results and Discussion

Chemical Composition:

The starch compounds obtained were slightly off white in colour with no smell and yields of about 28.15 % and 10.25 %. Both yields were considered to be appreciable especially when compared with starch compounds from other sources such as cassava, 30 % (Fakir *et al.*, 2012) corn, 66.31 % (Khan *et al.*, 2014) and *Mesua ferrea*, 5.85 % (Sayeed *et al.*, 2004). The pH measurement showed that starch *A. difformis* is very close to that of corn starch properties (Omojola *et al.*, 2010), comparable with the previous pH values reported for starch from tubers and within the pH range of 3 – 9 obtained for most used in the pharmaceutical, cosmetics and food industries (Coursey and Rasper, 1967).

Amylose and amylopectin composition varied in both starch compounds with values from 20.68 to 10.30 % and 75.60 to 90.70 %, respectively. While, *Tacca involucrata* shows the highest amylose content this is also higher than 20.68 % compared to that obtained from Cassava starch. High amylose starch compound would be suitable for the coating of fried products, pasta, sweet and bread production (Vignaux *et al.*, 2005). The very low amylose content obtained in *Anchomanes difformis* makes it a good choice food for diabetics and other health conscious individuals (Agbo and Odo, 2010). Table 1 presents physicochemical properties of the starch.

Gelatinization temperature:

The temperature at which starch granules undergo transition from the crystalline state to a gel is referred to as gelatinization temperature. This is an important parameter in starch characterization. The gelatinization temperature of *Tacca* starch (75 °C) obtained is comparable to that of cassava starch (Omojola, 2013) while that of *Anchomanes* starch is 73 °C which falls within the range of gelatinization temperatures commonly observed for starch compounds (Afolayan *et al.*, 2012). It has however been established that high amylose starch sometime have high gelatinization temperature and since *Tacca* starch has higher amylose content which well related to the reduced gelatinization temperature (73 °C) for *Anchomanes* starch.

Bulky Density:

Bulk density is a function of particle size; particle size is inversely proportional to bulk density. Table 1 showed that there is little significant difference in values of the bulk densities for both *Tacca* and *Anchomanes*. Their relatively high value of bulk density supported their suitability as drugs binder and disintegrant in pharmaceuticals (Zaku *et al.*, 2009].

Table 1: Physicochemical properties of starch from *Tacca involucrata* and *Anchomanes difformis*

S/N	Physicochemical Analyses	<i>Tacca involucrata</i>	<i>Anchomanes difformis</i>
1	Starch yield (%)	28.15	12.25
2	pH	6.50	5.80
3	Gelatinizaton Temperature (°C)	75.00	73.00
4	Bulk density (g/cm ³)	0.76	0.77
5	Pasting Temperature (°C)	77.89	79.96
6	Amylose (%)	24.40	10.30
7	Amylopectin (%)	75.60	90.70

Morphology:

The variability in the granular shape of the starch samples are shown in Plate 1. The shapes of *Tacca involucrata* and *Anchomanes difformis* starch granules were heterogeneous, having different shapes of oval and polyhedral for *Tacca* and hexagonal for *Anchomanes*. From particle size distribution analysis of the starch compounds, the results showed that the mean granule size of 6.49 μm for *Tacca involucrata* and a granule size range of 1-5 μm for *Anchomanes difformis*. This clearly showed that both starch compounds are smaller than cassava starch which has a mean granule size of 11.82 μm and average range of 1.05 – 36.08 μm . Granule size and particle size distribution of starch influences the functional properties of starch this includes; swelling power, solubility and digestibility (Moorthy et al., 2002). Generally, small and medium sized starch granules have been reported to have varied utilization in the food and pharmaceutical industries properties (Omojola et al., 2010) which gives credence to the industrial potentials of the starch.

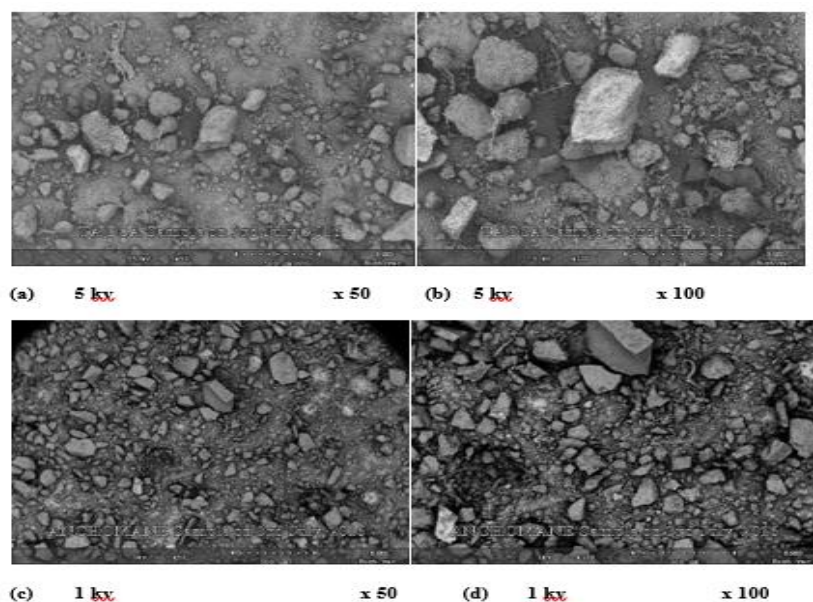


Plate 1: Photomicrograph of (a) *Tacca* Starch and (b) *Anchomanes* Starch sample at 5 KV, and 1 KV respectively

X-ray diffraction:

The x-ray diffractogram of starch compounds presented in Fig. 1 and Fig. 2 showed that *Tacca* gave characteristic peaks at 10.8° , 18.2° , 20.5° , 24.0° , 24.8° 2θ which are diffraction peaks corresponding to A-type diffraction pattern while *Anchomanes* gave characteristic peaks at 18.12° , 18.27° , 23.7° , 24.0° 2θ which are diffraction peaks corresponding to A-type. Cassava starch was also reported by Rocha *et al.*, (Rocha *et al.*, 2010) to exhibit A-type diffraction pattern. It has been shown in previous studies that an A-type X-ray diffraction starch, that has a high proportion of short branch chain amylopectin, has inferior crystallinity and thus is more susceptible to digestion by α -amylase compared to its opposite, B-type starch (Jane *et al.*, 1997).

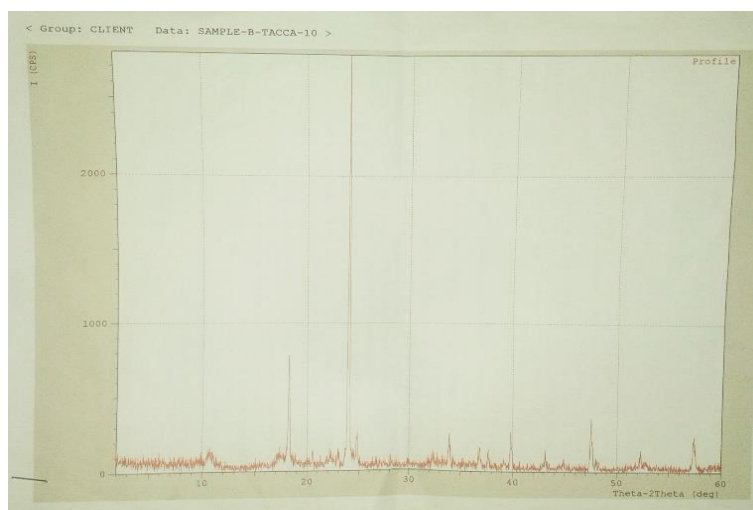


Fig.1: X-ray Diffractogram of *Tacca involucreta* Starch

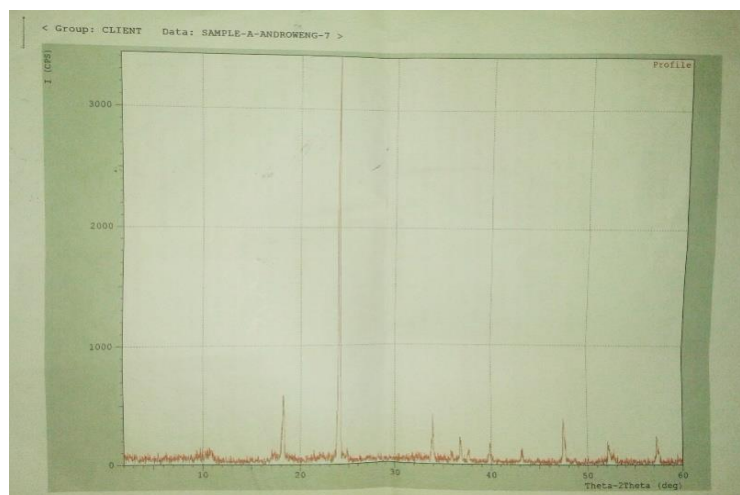


Fig. 2: X-ray diffractogram of *Anchomanes difformis* Starch

Swelling and Solubility properties

Figure 3 and 4 showed the swelling power and solubility properties starch compounds from *Tacca involucrata* and *Anchomanes difformis* as a function of temperature.

Solubility and swelling power provides the evidence of interactions between the water molecules and the starch chains in the crystalline and amorphous regions (Ratnayake *et al.*, 2002). Swelling power increased with increased temperature (60 to 100 °C) in the starch samples with *Tacca* possessing a higher swelling power than *Anchomanes*. The solubility profile also shows an increase in solubility with temperature rise which relaxes at 90 °C before rising again for *Anchomanes*, this was an indication that the starch granules has a greater intra granular organization in which most of the bonding forces required energy supplied in the temperature range 80 to 90 °C to cause a relaxation (Nwokocha *et al.*, 2009). Factors that influence the solubility of starches include: origin, granule size and other component s of the starch. Solubility has been shown to positively correlate with swelling, indicated that solubilization occurred along with granular swelling (Srichuwong *et al.*, 2005). Increased swelling power is an indicative of the suitability of such starch for use as a disintegrant in the pharmaceutical industry.

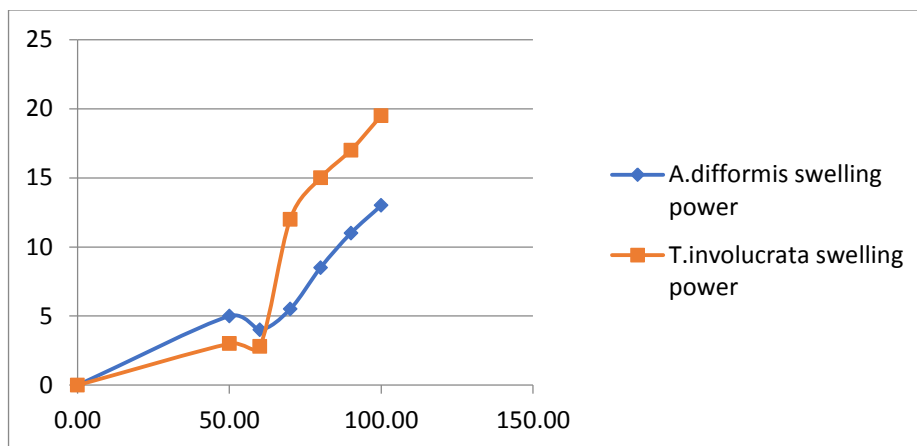


Fig. 3: Swelling Power Profile of *Anchomanes difformis* and *Tacca involucreta* starch

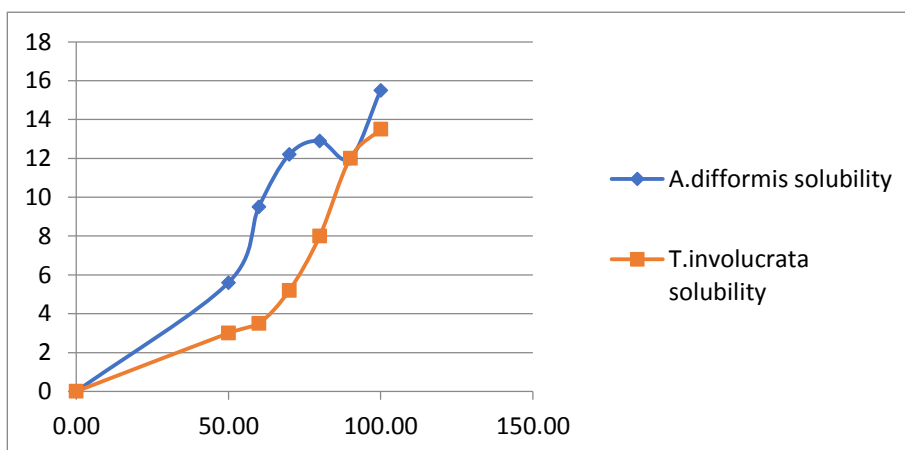


Fig. 4: Solubility Index Profile of *Anchomanes difformis* and *Tacca involucreta* Starch

Conclusion

The results of the physicochemical and functional properties of starch compounds from *Tacca* and *Anchomanes* revealed that the starch granules can compare favourably with starch from cassava plant. This confirms the potential of starch compounds from *Tacca* and *Anchomanes* in both food

and non-food industries. These results may allow variety of choice when sourcing new ingredients or ingredients with properties to enhance production and development of starch. This will help to lessen stress on starch from other well-known sources such as cassava.

References

- Adewumi D.F., Adewole E., Ogunmodede O.T., Ojo A. (2015). Effect of Chemical Modifications on Pasting Properties of Cocoyam Starch (*Xanthosoma Sagittifolium*). *Journal of Natural Sciences Research*. 5(15): 2225 – 0921.
- Afolayan M.O., Omojola M.O., Oriajogun J.O., Thomas S.A. (2012). Further physicochemical characterization of *Anchomanes difformis* starch. *Agriculture and Biology Journal of North America*. 3(1), 31 – 38.
- Agbo I.U., Odo G.E.. (2010). The effect of hypochlorite oxidation and acetylation on some of the physicochemical properties of *Ipomoea trichantha* starch; *Bio – Research*. 8(1): 593 – 597.
- Attama A.A., Nnamani P.O., Mbonu I.K., Adiku M.U. (2003). Effect of hypochlorite oxidation on the physicochemical properties of gladiolus starch; *Journal of Pharm and allied Science*. 1, 28 – 35.
- Burkill, H. M. (1985). *The Useful Plants of West Tropical Africa*,. Vol 2, 2nd Ed., pp196.
- Coursey, D.G., Rasper V. (1967). Properties of starches of some West African yams; *J. Sci. Food Agric*. 18: 240 – 248.
- Daramola B., Osanyinlusi S.A. (2006). Investigation on modification of cassava starch using active components of ginger roots (*Zingiber officinale* Roscoe); *African Journal of Biotechnology*, 5: 917 – 920.
- Fakir M.S.A., Jannat M., Mostafa M.G., Seal H. (2012). Starch and flour extraction and nutrient composition of tuber in seven cassava accessions. *J. Bangladesh Agril. Univ*. 10(2), 217–222.
- Fanon J.E., Hauber R.J., BeMiller J.N. (1992). Surface pores of starch granules. *Cereal Chemistry*, 69: 284 – 288.

Jane J., Wong K.S., McPherson A.E. (1997). Branch-structure difference in starches of A- and B- type X-ray patterns revealed by their Naegali dextrans. *Carbohydrate Research*. 300: 219 – 227.

Khan A.H., Minhas N.M., Asad M.J., Iqbal A., Ilyas M., Mahmood R.T. (2014). Estimation of Protein, Carbohydrate, Starch and Oil Contents of Indigenous Maize (*Zea mays* L.) Germplasm. *European Academic Research*. 2(4): 5230 – 5240.

Mandala I.G., Bayas E. (2004). Xanthan effect on swelling, solubility and viscosity of wheat starch dispersions, *Food Hydrocolloids*, 18: 191–201.

Moorthy S.N., Vimala B., Mukherjee A. (2002). Physicochemical and functional properties of *Canna edulis* starch. *Trop. Sci.* 42: 75 – 77.

Neelam, K., Vijay S., Lalit S. (2012). Various techniques for modification of starch and the applications of its derivatives. *International Research Journal of Pharmacy*. 3(5): 2230 – 8407.

Nwokocha L.M., Aviara N.A., Senan C., Williams P.A. (2009). A comparative study of some properties of cassava (*Manihot esculenta*, Crantz) and cocoyam (*Colocasia esculenta*, Linn) starches. *Carbohydr. Polym.* 76(3): 362 – 367.

Nwokocha, L.M., Senan C., Williams P.A. (2011). Structural, physicochemical and rheological characterization of *Tacca involucreta* starch. *Carbohydrate Polymers*, 86: 789 – 796.

Omojola M.O. (2013). *Tacca* Starch: A Review of its production, Physicochemical Properties, Modification and Industrial Uses. *African Journal of food, Agriculture, Nutrition and Development*; 13(2): 1684 – 5374.

Omojola, M.O., Akinkunmi Y.O., Olufunsho K.O., Egharevba H.O., Martins E.O. (2010). Isolation and physicochemical characterization of cola starch. *African Journal of Food Agric Nutrition & Dev.* 10(7): 2884 – 2900.

Oyetayo, V.O. (2007). Comparative studies of the phytochemical and antimicrobial properties of the leaf, stem and tuber of *Anchomanes difformis*. *Journal of Pharmacology and Toxicology*. 2(4): 407- 410.

Ratnayake W.S., Hoover R., Warkentin T. (2002). Pea starch: composition, structure and properties - A review. *Starch/Staerke*, 54(6): 217 – 234.

Rocha T.S., Carnero A.P.A., Franco C.M.L. (2010). Effect of enzymatic hydrolysis on some properties of root and tuber granular starches. *Ciencia e tecnologia de Alimentos*. 30: 544 – 551.

Sayeed M.A., Ali M.A., Soheli F.I., Astaq Mohal Khan G.R.M., Yeasmin Mst.S. (2004). Physico-chemical characteristics of *Mesua ferrea* seed oil and Nutritional composition of its seed and leaves. *Bull. Chem. Soc. Ethiop.* 18(2): 157 – 166.

Soladoye M.O., Sonibare M.A., Nadi A.O., Alabi D.A. (2005). Indigenous angiosperm biodiversity of Olabisi Onabanjo University permanent site. *African Journal of Biotechnology*. 4(5): 554 – 562.

Srichuwong S., Sunarti T.C., Mishima T., Isono N., Hisamatsu M. (2005). Starches from different botanical sources II: contribution of amylopectin fine structure to thermal properties and enzyme digestibility. *Carbohydrate Polymers*, 62: 25 – 34.

Vignaux N., Doehlert D.C., Elias E.M., McMullen M.S., Grant L.A., Kianian S.F. (2005). Quality of spaghetti made from full and partial waxy durum wheat. *Cereal Chem.* 82: 93 – 100.

Zaku S.G., Aguzue O.C., Thomas S.A., Barminas J.T. (2009). Studies on the functional properties and the nutritive values of amura plant starch (*Tacca involucrata*) a wild tropical plant. *African Journal of Food Science*. 3(10): 320 – 322.

Antimalarial activity and heamatological parameters of *Plasmodium berthgei*- infected mice treated with combination of aqueous extract of *Nigella sativa* and Honey

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Abstract

Malaria is one of the most deadly parasitic diseases in endemic areas including Nigeria. The aim of the study was to investigate antimalarial potential of the aqueous extract of *Nigella sativa* in combination with honey in ratio 1: 1 (w/v) and its effect on heamatological parameters of *P. berthgei* infected mice. Dose levels of the combined extract and honey at 50, 100, and 200 mg/kg body weight were evaluated for the curative effects against *Plasmodium berthgei* (NK 65 chloroquine sensitive) infected mice. Chloroquine (20 mg/kg body weight) was used as standard drug. The results showed significant ($p < 0.05$) decrease in the parasitaemia levels in treated mice when compared to those of the untreated group. Chemosuppressive inhibition (91.37%) of the parasite in *P. berthgei* treated mice at 100 mg/kg body weight of the combination was relatively higher when compared to those treated with the standard drug (81.06%). Also, there was significant ($p < 0.05$) increase in white blood cell, red blood cell, heamoglobin, packed cell volume and platelets counts of the treated *P. berthgei* mice when compared with the untreated *P. berthgei* mice. The findings suggested that the combination of aqueous extract of *N. sativa* and honey has antimalarial property and restored the deranged hematological indices caused by the *P. berthgei* parasite.

Keywords: Antimalarial, *Plasmodium berthgei*, *Nigella sativa*, Honey, Hematological parameters

Introduction

Malaria is a major global health problem and a predisposition risk to 97% of Nigeria's population where it accounts for more clinical episodes (100 million/ year) and deaths (300,000/ year) than in any other country in the world (WHO, 2015). This devastating parasitic infectious disease competes with death rate (215,000/ year) from HIV/AIDS in the country. Children and pregnant women are the most susceptible groups to the disease resulting in an estimated 11% of maternal mortality rate (WHO, 2015). Recent report from World Health Organization reveals that nearly half of the world's population is at risk of malaria, with about 216 million malaria cases and about 445,000 cases of malaria-related deaths (WHO, 2016).

Most common complications in malaria infection are changes or alteration in the hematological parameters of the infected individuals. These changes involve the major cell lines such as red blood cells, leucocytes and thrombocytes. As such they play a major role in malaria pathology (Maina *et al.*, 2010). Some degree of anaemia is linked to most malaria infections (Lamikanra *et al.*, 2007). Severe injury and death may arise from malaria anaemia particularly in children and pregnant women (Menendez *et al.*, 2000).

Plant materials have been a major source of natural therapeutic remedies for treatment of various infectious diseases in many countries (Wendy *et al.*, 2017). Among various medicinal plants, *Nigella sativa* (*N. sativa*) (Family Ranunculaceae) is emerging as a miracle herb with a rich historical and religious background (Al-Buhari, 1976), since many researches revealed its wide spectrum of pharmacological potential (Khare, 2004). *N. sativa* is commonly known as black seed. Pharmacological properties include: antioxidant activity (Kruk *et al.*, 2000), analgesic effect and anti-inflammatory activity (Al-Ghamdi, 2001; Ali and Blunden, 2003; Hajhashemi *et al.*, 2004), hepato-protective activity (Sultan, *et al.*, 2012; Yesmin *et al.*, 2013; Rahmani and Aly, 2015). Antimalarial activity (Abdulelah *et al.*, 2007; Hadi *et al.*, 2016). They stimulate the body's energy and help recovery from fatigue (Tembhurne *et al.*, 2014; Krishnaveni and Saranya, 2016; Botnick *et al.*, 2012). Also they can be administered internally in intermittent fever (Tembhurne *et al.*, 2014).

Honey is a natural substance produced, when the nectar and sweet deposits from plants are gathered, modified and stored in the honeycombs by honeybees of the genera *Apis* and *Meliponini* (Namias, 2003; Al-jabri, 2005). Honey forms part of traditional medicine in many cultures (Viuda-Martos *et al.*, 2008), although it is most widely used as sweetener. There is currently resurgence in interest in the use of honey and honey products by the public. This alternative branch of medicine is called apitherapy (Manyi-Loh *et al.*, 2011; Mamo, 2018). It is composed of at least 181 components and is basically a solution supersaturated in sugars (Wei *et al.*, 2009). The actual composition of honey varies; depending on many factors such as the pollen source, climate, environmental conditions, and the processing it undergoes (Bertoncelj *et al.*, 2007).

Pharmacological properties include: anti-oxidant activity (Jaganathan *et al.*, 2010, Manyi-Loh *et al.*, 2011, Khan *et al.*, 2018), anti-inflammatory activity (Kassim *et al.*, 2010, Candiracci *et al.*, 2012, Vallianou *et al.*, 2014), anti-malarial activities (Jirattikarn *et al.*, 2012, Olayemi, 2014) and boosting of the immune system (Jones *et al.*, 2000, Molan, 2001, Tonks *et al.*, 2007). The objective of the present work was to assess the antimalarial potential of the combination of aqueous extract of *N. sativa* seed and honey in ratio 1:1 and its effects on the hematological parameter on the *P. berghei* infected mice.

Materials and methods

Plant materials and authentication

Nigella sativa seeds and honey were obtained from Alhamed shop in Taiwo Road Ilorin, Kwara State, Nigeria. The seeds were authenticated at herbarium unit of the Department of Plant Biology, University of Ilorin, Nigeria with voucher number UIH039.

Plant Preparation

100g of dry black seed was ground using a mortar and pestle, the powdered seed was dissolved in 2L of distilled water in a plastic container and shaken intermittently for 48 hours. The resulting mixture was filtered and the filtrate was freeze dried.

Laboratory Animals and Ethical protocol

Adult albino mice, with average weight of $21.5 \pm 1.4\text{g}$, obtained from Small Animal Holding unit, Department of Biochemistry, University of Ilorin, were used for the experiment. The mice were placed on standard pellet feed and allowed to drink water freely. They were acclimatized for 2 weeks before the commencement of the study. The guide line on animal handling and treatment were duly followed (CCAC, 1993).

Malaria parasites

NK65 strain of *P. berghei* was obtained from the Institute for Advance Malaria Research and Training (IMRAT), College of Medicine, University of Ibadan, Ibadan Nigeria, and sustained in the laboratory by continuous passage of parasitized blood into mice.

Antimalarial assay

Curative antimalarial test used was a modified method of Ryley and Peters (1970).

Preparation of smear

A drop of blood was collected from the tail end of each mouse and was placed on slides, smear, and stain to determine its parasitemia using a method described by Adebayo *et al.* (2013).

Blood preparation

The mice were sacrificed by anaesthetizing them in a jar containing cotton wool soaked with diethyl ether. Blood was collected in well labeled EDTA sample bottles for the hematological analysis.

Results

There was a significant ($p < 0.05$) decrease in percentage parasitemia level of treated parasitized mice when compared with untreated parasitized mice as shown in figure 1. All the treated group shown a significant ($p < 0.05$) reduction in parasitemia level from day 4 to day 8 with the treatment at 100 mg/kg bw of the combination demonstrating more reduction in parasite level when compared with the chloroquine treated (fig. 1).

There was a significant ($p<0.05$) increase in percentage chemo suppression of parasite inhibition in all the treated groups with 100 mg/kg bw of the combination (91.37%) demonstrated a significant parasite inhibition when compared with chloroquine treated (87.73%) as shown in figure 2.

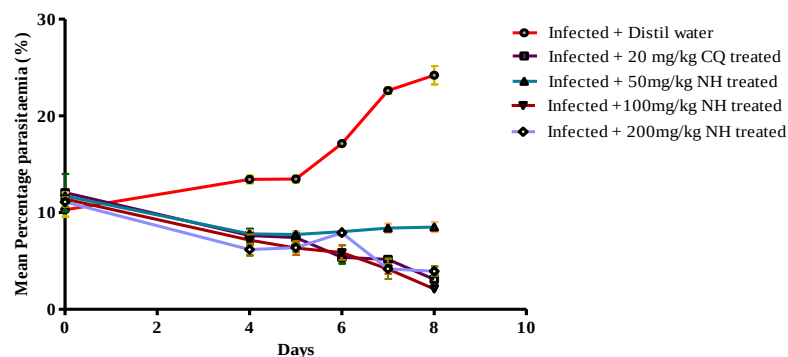


Figure 1: Mean percentage parasitaemia of *P. berghei* infected mice treated with combination of aqueous extracts of *N. sativa* seed and honey

lines are mean $\pm$ SEM; $n = 5$, the alphabets show the statistical different with $P<0.05$. NH: *Nigella sativa* aqueous extract and honey

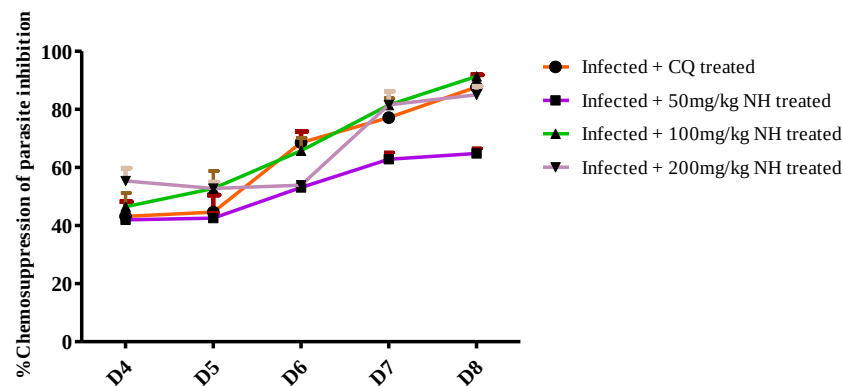


Figure 2: Percentage chemosuppression of parasite inhibition of *P. berghei* infected mice treated combination of aqueous extracts of *N. sativa* seed and honey

line are mean $\pm$ SEM; $n = 5$, the alphabets show the statistical different with $P < 0.05$. NH: *Nigella sativa* aqueous extract and honey

There was a significant ($p < 0.05$) decrease in the red blood cell (RBC) count, hemoglobin (Hb) and packed cell volume (PCV) of untreated infected *P. berghei* mice when compared with the treated parasitized mice as shown in figure 3,4 and 5. The rate of increase in RBC, Hb, and PCV of treated mice at 100 mg/kg bw of the combinations and chloroquine (standard drug) was compare favorably with the normal control (uninfected mice). Also, there was a significant ($p < 0.05$) decrease in the white blood cell (WBC) count and platelet count of the untreated parasitized mice when compared with treated parasitized mice as presented in figure 6 and 7. However, the treatment at 100 mg/kg bw of the combination and chloroquine treated shown appreciable increase in the level of WBC count and platelet count when compared with the normal control (figure 6 and 7).

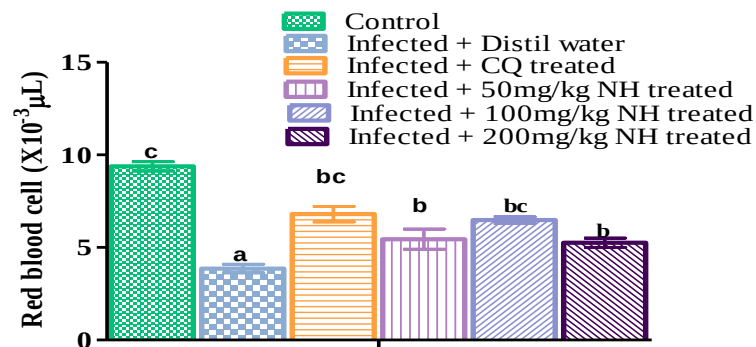


Figure 3: Red blood cell of *P. berghei* infected mice treated with combination of aqueous extracts of *N. sativa* seed and honey

Bars are mean $\pm$ SEM, $n = 5$, the alphabets show the statistical different with $P < 0.05$

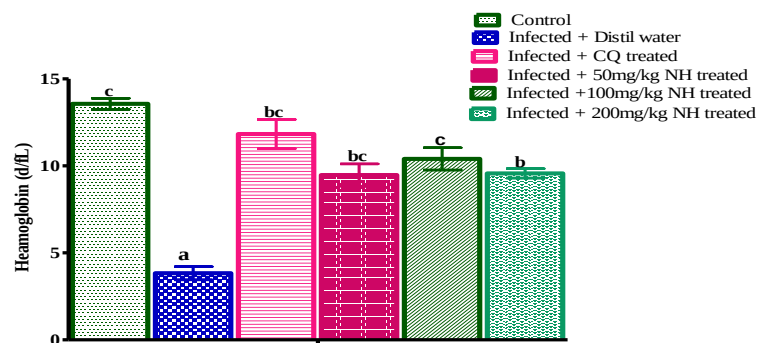


Figure 4: Heamoglobin of *P. berghei* infected mice treated with combination of aqueous extracts of *N. sativa* seed and honey

Bars are mean $\pm$ SEM, $n = 5$, the alphabets show the statistical different with $P < 0.05$. NH: *Nigella sativa* aqueous extract and honey

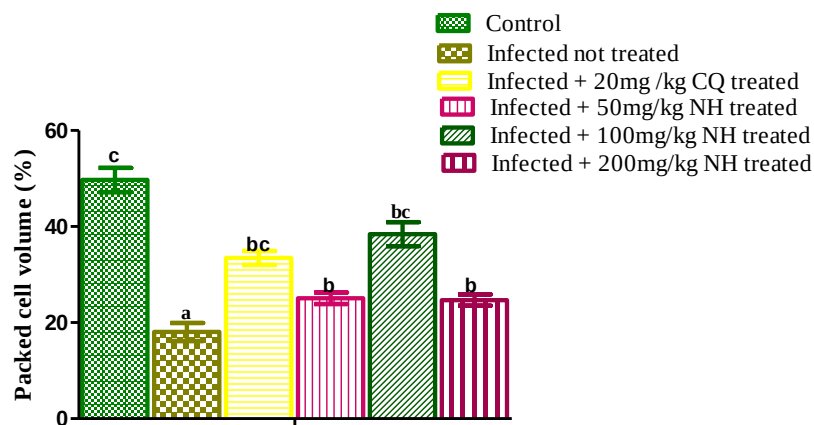


Figure 5: Pack cell volume of *P. berghei* infected mice treated with combination of aqueous extracts of *N. sativa* seed extract and honey

Bars are mean $\pm$ SEM, $n = 5$, the alphabets show the statistical different with $P < 0.05$ NH: *Nigella sativa* aqueous extract and honey

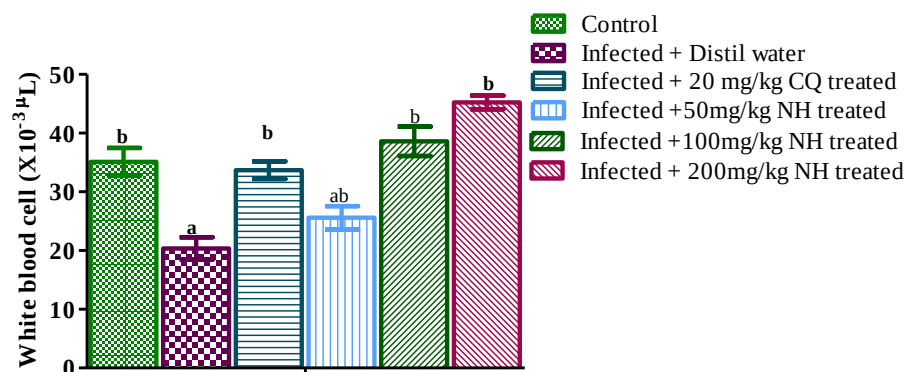


Figure 6: White blood cell of *P. berghei* infected mice treated with combination of aqueous extracts of *N. sativa* seed and honey

Bars are mean ± SEM, n = 5, the alphabets show the statistical different with P<0.05. NH: Nigella sativa aqueous extract and honey

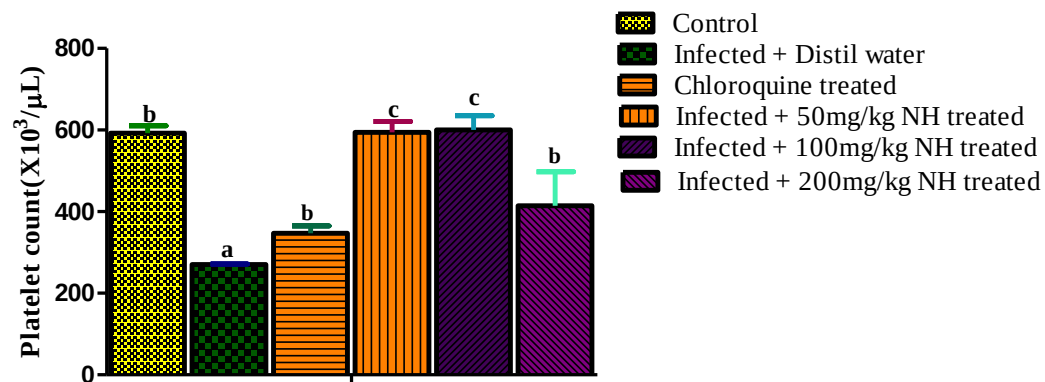


Figure 7: Platelet count of *P. berghei* infected mice treated with combination of aqueous extracts of *N. sativa* seed and honey

Bars are mean ± SEM, n = 5, the alphabets show the statistical different with P<0.05. NH: Nigella sativa aqueous extract and honey

Discussion

The results show that the combination of aqueous extract *N. sativa* seed and honey at 100 mg/kg bw was able to reduce the level of the parasite in the *P. berghei* infected mice. The treatment at various dosages of the combination and chloroquine also exhibit growth inhibition of parasite above 50%. This is in line with the work of Isha *et al.* (2003) who reported that an extract is considered active if it demonstrates 50% growth inhibition. In addition, the growth inhibition of the combination at 100 mg/kg bw (91.37%) was significantly higher than that of the chloroquine (81.06%) treated. Therefore, the treatment at 100 mg/kg bw of the combination was considered more effective for the treatment of malaria.

Hematological parameter plays a major role in malaria pathology (Maina *et al.*, 2010). The hematological assay revealed that RBC, Hb and PCV level significantly decrease ($p < 0.05$) after infection with *P. berghei* parasite. This is an indication of anemia due to hemolysis. Anemia is a consistent finding in blood protozoa parasite infection (Ajagbonna *et al.*, 2002). The increase in RBC, Hb and PCV counts after the treatment with the combination at 100 mg/kg bw that was brought to near normal was an indication that the combination at 100 mg/kg bw can revert the anaemic condition of the infected parasitized mice to near normal. The WBC and platelets counts of the treated *P.berghei* mice also increased significantly ($p < 0.05$) following the treatment at 100 mg/kg bw of the combination. This could also be an indication that the immune system of the *P. berghei* infected mice could have been boosted after the treatment.

Conclusion

The combination of *N. sativa* aqueous extract and honey at 100 mg/kg bw has antimalarial potential which was higher than that of standard drug (chloroquine) and demonstrated the ability to restore the deranged hematological indices caused by *P. berghei* infected mice.

Reference

- Abdulelah, H. A. A., & Zainal-Abidin, B. A. H. (2007). In vivo anti-malarial tests of *Nigella sativa* (Black Seed) different extracts. *Am J Pharmacol Toxicol*, 2(2), 46-50.
- Adebayo, J. O., Balogun, E. A., Malomo, S. O., Soladoye, A. O., Olatunji, L. A., Kolawole, O. M., & Andrade, I. M. (2013). Antimalarial activity of *Cocos nucifera* husk fibre: further studies. *Evidence-Based Complementary and Alternative Medicine*, 2013.
- Ajagbonna, O. P., Esaigun, P. E., Alayande, N. O., & Akinloye, A. O. (2002). Anti-malarial activity and haematological effect of stem bark water extract of *Nuclea latifolia*. *Bioscience Research Communication*, 14(5), 481-486.
- Al-Bukhari MI. In: Sahi Al-Bukhari, (1976), editor. The collection of authentic sayings of Prophet Muhammad (peace be upon him), division 71 on medicine. 2nd ed. Ankara: Hilal Yayinlari.
- Al-Ghamdi, M. S. (2001). The anti-inflammatory, analgesic and antipyretic activity of *Nigella sativa*. *Journal of ethnopharmacology*, 76(1), 45-48.
- Ali, B. H., & Blunden, G. (2003). Pharmacological and toxicological properties of *Nigella sativa*. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, 17(4), 299-305.
- Al-Jabri, A. A. (2005). Honey, milk and antibiotics. *African Journal of Biotechnology*, 4(13).
- Al-Waili, N., Salom, K., & Al-Ghamdi, A. A. (2011). Honey for wound healing, ulcers, and burns; data supporting its use in clinical practice. *The scientific world journal*, 11, 766-787.
- Bertoncelj, J., Doberšek, U., Jamnik, M., & Golob, T. (2007). Evaluation of the phenolic content, antioxidant activity and colour of Slovenian honey. *Food Chemistry*, 105(2), 822-828.

Botnick, I., Xue, W., Bar, E., Ibdah, M., Schwartz, A., Joel, D. M., ... & Lewinsohn, E. (2012). Distribution of primary and specialized metabolites in *Nigella sativa* seeds, a spice with vast traditional and historical uses. *Molecules*, 17(9), 10159-10177.

Candiracci, M., Piatti, E., Dominguez-Barragán, M., García-Antrás, D., Morgado, B., Ruano, D., & Castaño, A. (2012). Anti-inflammatory activity of a honey flavonoid extract on lipopolysaccharide-activated N13 microglial cells. *Journal of Agricultural and Food Chemistry*, 60(50), 12304-12311.

CANADIAN COUNCIL ON ANIMAL CARE (1993). Guide to the Care and Use of Experimental Animals, Vol. 1, 2nd Edn., 212 pp. Ottawa, ON: CCAC

Hadi, M. Y., Mohammed, G. J., & Hameed, I. H. (2016). Analysis of bioactive chemical compounds of *Nigella sativa* using gas chromatography-mass spectrometry. *Journal of Pharmacognosy and Phytotherapy*, 8(2), 8-24.

Hajhashemi, V., Ghannadi, A., & Jafarabadi, H. (2004). Black cumin seed essential oil, as a potent analgesic and antiinflammatory drug. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, 18(3), 195-199.

Isah, A.B., Ibrahim, Y.K., Iwalewa, E.O., (2003). Evaluation of the antimalarial properties and standardization of tablets of *Azadirachta indica* (*Meliaceae*) in mice. *Phytotherapy Research* 17, 807–810.

Jaganathan, S. K., Mandal, S. M., Jana, S. K., Das, S., & Mandal, M. (2010). Studies on the phenolic profiling, anti-oxidant and cytotoxic activity of Indian honey: in vitro evaluation. *Natural product research*, 24(14), 1295-1306.

Kassim, M., Achoui, M., Mustafa, M. R., Mohd, M. A., & Yusoff, K. M. (2010). Ellagic acid, phenolic acids, and flavonoids in Malaysian honey extracts demonstrate in vitro anti-inflammatory activity. *Nutrition research*, 30(9), 650-659.

Khan, M. A., & Afzal, M. (2016). Chemical composition of *Nigella sativa* Linn: part 2 recent advances. *Inflammopharmacology*, 24(2-3), 67-79.

Khan, S. U., Anjum, S. I., Rahman, K., Ansari, M. J., Khan, W. U., Kamal, S., ... & Khan, H. U. (2018). Honey: Single food stuff comprises many drugs. *Saudi Journal of Biological Sciences*, 25(2), 320-325.

Khare, C. P. (2004). *Indian herbal remedies: rational Western therapy, ayurvedic, and other traditional usage, Botany*. Springer science & business media.

Krishnaveni, M., & Saranya, S. (2016). Phytoconstituent analysis of *Nigella Sativa* seeds using analytical techniques. *Bull. Environ. Pharmacol. Life Sci*, 5(3), 25-38.

Kruk, I., Michalska, T., Lichszeld, K., Kładna, A., & Aboul-Enein, H. Y. (2000). The effect of thymol and its derivatives on reactions generating reactive oxygen species. *Chemosphere*, 41(7), 1059-1064.

Lamikanra, A. A., Brown, D., Potocnik, A., Casals-Pascual, C., Langhorne, J., & Roberts, D. J. (2007). Malarial anemia: of mice and men. *Blood*, 110(1), 18-28.

Maina, R. N., Walsh, D., Gaddy, C., Hongo, G., Waitumbi, J., Otieno, L., & Ogutu, B. R. (2010). Impact of *Plasmodium falciparum* infection on haematological parameters in children living in Western Kenya. *Malaria Journal*, 9(3), S4.

Mamo, T. (2018). Review on Medicinal and Nutritional Importance of Honey. *Advances in Research*, 1-9.

Manyi-Loh, C. E., Clarke, A. M., & Ndip, N. (2011). An overview of honey: therapeutic properties and contribution in nutrition and human health. *African Journal of Microbiology Research*, 5(8), 844-852.

Menendez, C., Ordi, J., Ismail, M. R., Ventura, P. J., Aponte, J. J., Kahigwa, E., ... & Alonso, P. L. (2000). The impact of placental malaria on gestational age and birth weight. *The Journal of infectious diseases*, 181(5), 1740-1745.

Namias, N. (2003). Honey in the management of infections. *Surgical infections*, 4(2), 219-226.

Rahmani, A. H., & Aly, S. M. (2015). Nigella sativa and its active constituents thymoquinone shows pivotal role in the diseases prevention and treatment. *Asian J Pharm Clin Res*, 8(1), 48-53.

Rayley, J. F and Peters, W. (1970). The antimalaria activity of some quinolone esters. *Ameri. J. Trop. Med .parasitol.* 84: 213-225

Sultan, M. T., Butt, M. S., Ahmad, R. S., Pasha, I., Ahmad, A. N., & Qayyum, M. M. N. (2012). Supplementation of Nigella sativa fixed and essential oil mediates potassium bromate induced oxidative stress and multiple organ toxicity. *Pakistan journal of pharmaceutical sciences*, 25(1).

Tembhurne, S. V., Feroz, S., More, B. H., & Sakarkar, D. M. (2014). A review on therapeutic potential of Nigella sativa (kalonji) seeds. *Journal of Medicinal Plants Research*, 8(3), 167-177.

Vallianou, N. G., Gounari, P., Skourtis, A., Panagos, J., & Kazazis, C. (2014). Honey and its anti-inflammatory, anti-bacterial and anti-oxidant properties. *Gen Med (Los Angel)*, 2(132), 1-5.

Viuda-Martos, M., Ruiz-Navajas, Y., Fernández-López, J., & Pérez-Álvarez, J. A. (2008). Functional properties of honey, propolis, and royal jelly. *Journal of food science*, 73(9), R117-R124.

Wei, Z., Wang, J., & Liao, W. (2009). Technique potential for classification of honey by electronic tongue. *Journal of Food Engineering*, 94(3-4), 260-266.

World Health Organization (2015). World Malaria Report 2015. Geneva Switzerland: World Health Organization pp32 – 42

World Health Organization (2016). World Malaria Report. World Health Organization.

WHO Document Production Services, Geneva, Switzerland

<<http://www.who.int/malaria/publications/world-malaria-report-2016/report/en/>>.

Yesmin, F., Rahman, Z., Dewan, J. F., Helali, A. M., Rahman, N. I. A., Alattraqchi, A. G., & Haque, M. (2013). Hepato-protective role of the aqueous and n-hexane extracts of *Nigella sativa* Linn. in experimental liver damage in rats. *Asian J Pharm Clin Res*, 6(3), 205-209.

Practice of safety measures and work related injuries among sawmill workers in Kwara South Senatorial District of Nigeria

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Abstract

Workplace accidents and illness are on the increase in the Nigerian Sawmill industries, this is because safety measures were not given desirable attention. The objective of the study was to assess practice of safety measures and work related injuries of saw mill workers in Kwara South Senatorial District of Nigeria. This is a descriptive cross sectional survey carried out among sawmills workers in four Local Government Areas of Kwara State. Multistage sampling technique was used in selecting respondents and interviewer administered validated questionnaire was used to obtain information. Data collected were processed and analyzed using Epi-Info version 2007 software. Chi square and simple percentages were used and p value < 0.05 was set as the significant level. The findings from this study showed that majority of the workers who were within age group 28-47 years and mainly involved in technical aspect of the job had fair knowledge of sawmill hazards. The use of personal protective equipment (PPE) was high among respondents who

had spent many years on the job. Those who did not use PPE considered it inconvenient, irrelevant and disturbing. The workers with secondary education and above were found to record use of protective devices compared with others. Sawmill machine and technical operators, Sawmill labourers (wood loaders and machine off loaders) and workers with less than 10 years working experience recorded the highest cases of hazards. Government and management of the industry should make adequate provision for regular health education, PPE at subsidized rate and mandate regular inspection and monitoring of sawmills by occupational health officers. Management should enforce environmental cleanliness and hazard free environment, educate and enforce formal training on work place hazards and need for periodic medical examination, provide first aid box and adequate workers compensation plans in case of occupational disability.

Keywords: Sawmill workers, safety measures, work-related injuries

Introduction

Healthy work force is vital for sustainable social and economic development of a country. This has not been possible due to poor compliance to health and safety standards in developing countries as a result of proliferation of small and medium size workplace. The lucrative nature of sawmilling industries in Nigeria have attracted a high considerable number of the work forces, and everyday these workers are exposed to various hazards of wood processing such as physical, chemical, and biological hazards (Fatusi and Erhabor. 1996). Sawmill occupational accident and illnesses are on the increase in developing countries because workers are often migrating from rural areas with few skills and very little training in safe work practices (Federal Ministry of Agriculture. 2003). It was estimated that at least two million people are routinely exposed occupationally to wood dust worldwide (Osagbemi. 2010).

Sawmills are mainly owned by individuals in Nigeria who are not keen on the standard safety practice for their employees. Studies have also revealed that in Nigeria, health and safety standards were neither practiced nor enforced leading to proliferation of hazards in this growing industry. Research had shown that sawmill workers are at high risk of ergonomic hazards resulting from lifting of heavy loads, repetitive work, and poor work posture (Osagbemi. 2010). Manual handling involved in sawmilling

operations has exposed workers to higher levels of risks associated with log handling and machine operation, work related body injuries including fractures, and in extreme cases, death (Aruofofor. 2000, Dosunmu. 1995, Hind and Davies 2010, Larama and Anoek.2005).

In Nigeria, it has been reported that availability and use of occupational safety devices were less than 20% (Osagbemi. 2010). Factors responsible for the poor practices of occupational safety measure among saw mill workers include, ignorance on the part of saw millers, unavailability and poor attitude to use of personal protective equipment, lack of training on the various hazards of their occupation and ways of controlling them (Hind and Davies. 2010). Study done in the past revealed no exposure control equipment such as exhaust ventilation fittings were available in any of the sawmills, which are majorly situated in populated areas (Mwaiselage and Bratweit. 2005).

Since work-related hazards are largely preventable, information obtained from this study will go a long way in planning appropriate intervention such as increasing the awareness and promotion of safety practices of sawmill workers ultimately reducing work place fatalities; accidents and illness in sawmill industries in future. This study is aimed at determining the level of awareness; safety measures practiced and work related injuries among sawmill workers in Kwara South Senatorial District of Nigeria.

Methodology

This is a descriptive cross-sectional survey of safety practices and occurrence of hazards among sawmill workers in Kwara South Senatorial District of Nigeria. The study population were the registered workers in the sawmill industries. Sawmill workers were grouped into; machine operators, saw technicians, dust packers, overseers, wood loaders, machine off loaders, and administrative staff. All registered sawmill workers who had spent at least six months in continuous employments in the industry were included to participate in the study.

In Kwara South Senatorial District of Nigeria, there are seven Local Government Areas (LGA); these are Oyun, Offa, Ekiti, Oke Ero, Isin, Ifelodun and Irepodun LGAs. Four LGAs were selected by simple random sampling using balloting method. These are Ifelodun, Offa, Ekiti and

Irepodun LGAs. These LGAs have similar characteristics with other LGAs in Kwara South senatorial district.

All sawmill workers available and willing to participate in all the four LGAs selected were engaged for the study. Six hundred and sixty respondents were involved in this study. The research instrument used in data collection was semi-structured interviewer administered questionnaire. The pre-testing of the questionnaire was done in sawmills in Moro LGA, Kwara North Senatorial District using 40 sawmill workers who had been in continuous employment for not less than 6 months and are exposed to similar occupational hazards as respondents. Corrections and modifications were done based on the findings from the pretest. Data was collected by four trained research assistants after informed consent has been taken and proper introduction done. All data generated from the study were collected and checked manually for errors in the filling of responses. Analysis was done using EPI INFO version 2007 computer software package. The test statistics used was Chi square and p-value of ≤ 0.05 was significant for the study.

Results

The age of respondents ranged from 18 to 58 years with a mean age of 34.3 ± 7.8 years. About half 300 (45.5%) had secondary education while 93 (14.1%) had no formal education. The machine operators constituted more than half 366 (55.5%) of the sawmills work force. Majority of respondents 264 (40.0%) had spent 6-10 years in the sawmill employment. Less than one-third 210 (31.8%) mentioned that regular wear and tear check was done at the work place and only 423 (64.1%) said environmental sanitation was done in their factory while none carried out wet drilling as maintenance/precautionary measures. Almost two-thirds 393 (59.5%) did not use protective devices and only 4.5% of respondents mentioned availability of first aid box in their work places (Table 1).

Less than two-thirds 369 (55.9%) of respondents always use boots during work activities in sawmill. About a third (30.0%) had never used face mask during working hours to prevent dust while almost half 48.2% never use safety goggle at places of work (Table 2). More respondents with the longest working years (21 years and above) recorded the highest (72.2%) use of protective devices than those with the least working years (< 10 years). There was a statistically significant difference ($p=0.0072$) in the

use of personal protective devices by those who had spent longer years on the job compared with those who had less years (Table 3). Of the respondents who did not use protective devices 354 (90.0%) said it was not convenient, 165 (42.0%) said they often forget to use them, 120 (30.5%) mentioned lack of knowledge while 21 (5.3%) said it was not necessary to use them.

More than three-quarters 609 (92.3%) of sawmills had canteens while none had health clinic, ventilators for impurities and incinerators. Less than three-quarters 465 (70.5%) of the sawmills had toilet facilities and running water available in 288 (43.6%) of the factories. Only 27 (4.1%) of the respondents reported occasional visit by the officers from Local Government Area in charge of monitoring and supervision of work places to observe the level of compliance with safety precautions. Majority 633 (96.0%) of the respondents had not gone for medical examination since their engagement in the sawmill work and only 27 (4.0%) had visited hospital for medical examinations. Of the respondents that had medical examinations done, only three persons had eye test done while none had done hearing and skin test although all of them were examined physically and did chest X-ray.

Although, there was no statistically significant difference ($p = 0.84721618$) between use of protective devices and literacy level of respondents, however, the proportion of respondents that used protective devices were more among those that had primary (42.3%) and secondary education (42.2%) compared with others. However, there was no statistically significant difference ($p = 0.84721618$) in the use of protective devices and literacy level of respondents. Majority of respondents with many working years (21years and above) had experienced more trauma from falls (83.3%) than those with the lesser working years of 11-20 years and 1-10 years.

More respondents 237 (61.7%) that had the least working experience (1-10 years) had highest occurrence of fracture compared with 21 years and above. More respondents in technical/ machine operations 282 (68.6%) experienced the highest cases of strains and sprains than workers in administrative department, as this was found to be significant ($p=0.0004$).

More respondents working as machine off loaders and wood loaders 90 (63.8%) had the highest cases of trauma from fall (63.8%) while the least occurrence was recorded from administrative workers 6 (11.8%).

Discussion

This study revealed that majority (80%) of the respondents falls within age group 28-47 years, this is similar to a study done among saw millers in South West Nigeria, where most of the sampled respondents age falls within 25-44 years (Iyun and James. 2005). This could justify the fact that milling activities are done by those in active age group that can hustle around for the demands of the job. Also, a larger proportion of the respondents are males 618 (93.6%) as this finding confirms the United Nations agency report of the male tendency towards sawmill work.

Many (45.5%) of the respondents had secondary education, 31.4% had primary education, 14.1% had no formal education and 9.1% had tertiary education. The pattern of educational level of the study population is closely related to a study done in South West Nigeria where respondents with secondary education were the highest (58%) while those with tertiary education constituted the least among the study respondents (Iyun and James. 2005). This might be due to the fact that no formal qualification requirement before enrolling in sawmill industry. The occupational structure shows that technical sawmill workers (machine operators and saw technicians) were the majority 62.3%, followed by sawmill manual labourers (machine off loaders, wood loaders and sawdust parkers) and administrative workers were the least among respondents (7.7%). This is similar to a study done by Uhumwangho in Benin where technical sawmill workers constituted 45.9%, and sawmill manual labourers were 30.4% while 23.1% were administrative staff (Uhumwangho and Gordon. 1993). This is due to sawmilling activities being complex and mechanized.

This study also revealed that hand glove is known by most 600 (90.9%) of the respondents as a protective device. This might be due to their work involving carrying of woods thus, hand glove would be the most known and used protective device. The high number (50.9%) of hospital attendance after sustaining injuries by the workers might be due to non-availability of first aid services in most of the sawmills. More respondents with post-secondary education recorded the highest (65%) in the use of protective devices, however, the educational attainment of the respondents

and their use of protective devices were observed not to be statistically significant. The insignificant finding in this study might be due to the number of respondents involved in the study. It has been observed that educational level of a person exert influence on his or her action more positively. According to Mwaiselage, workers behavior could be influenced by education and training (Mwaiselage and Bratweit. 2005) and those who are well informed are more likely to use safety measure than those who were not. This could also be due to better access to safety information they might have come across while in school on the importance of safety devices thus, informing their higher usage. This is in agreement with Asogwa which stressed that part of personal measure is education, understanding and motivation (Asogwa. 2000).

The department and unit of operation of the respondents had influence on the incidence of sprains and strains as it revealed that most cases of strains and sprains were among the machine/technical operators 282 (68.6%) and the least being among the administrative staff 3 (17.6%). This is similar to findings from study done in Southwestern Nigeria where the high percentage of body injuries recorded were sprain and stain due to manual hollowing of wood and logs by a number of workers rather than the conventional conveyors of lift cranes (Segun and Yahaya.). The workers in technical departments were more involved in physical activities, and most time these work processes are done manually unlike administrative workers who are involved with light paper work.

Working years of the respondents were also observed to exert a strong influence on the occurrence of trauma from fall as most 83.3% of the respondents that had being on the job for more than 21 years had more experience of falls compared with 46.9% respondents that had been on the job within 10 years. This is similar to a study by Demers that revealed poor working conditions and poor housekeeping in work environment of developing countries increase the risk of slip and falls, which workers with longer working years have cumulative exposure (Demers and Teschke. 1998). In developing countries the workplaces have lot of potential hazards which workers become more susceptible to as they become more exposed to their environment.

More than half of the workers 61.7% that had spent the least years on the sawmilling job had more occurrence of fracture than respondents with 21

years and above. This is related to a report from Canadian Center for Occupational Health and Safety where young untrained workers with few weeks in the job were seriously fractured when caught between a conveyor and a roller. This is supported by analysis of the causes of 37 sawmill fatalities in British Colombia, Canada, where 35% of the fatality occurred within the first year of employment and 5% of this occurred within the first week of the employment.

More respondents with the longest working years (21 years and above) recorded the highest practice 58.3% of using protective devices than those with the least working years. There was a statistically significant difference in the use of protective devices and duration on the job. The highest practice recorded by these workers can be due to their various experiences with all aspect of the factory activities including hazards and ways of protecting themselves from such hazards. This assertion is supported by a study in British Colombia where sawmill workers studied over 8 years had increased awareness of occupational hazards and use of protective device compared with fresh apprentice recorded among them.

More than half of machine off loaders/wood loaders 63.8% had highest occurrence of trauma from fall while the least recorded was from administrative unit of the sawmill 11.8%. The administrative workers are involved in secretariat job and are less exposed to wood logs and straying objects which are often scattered around due to poor housekeeping. Also their movement around the mill is limited when compared with other workers who become more prone to falls. This is similar to a study done by Uhumwangho in Benin where falls was reported in 1% of administrative workers and 12.9% in sawmill technical and labourers (Uhumwangho and Gordon. 1993).

The management of sawmill should enforce regular general cleaning and improve on workplace safety to prevent frequent fall injuries. There should be regular inspection and monitoring of the workplace by the occupational health officers to ensure compliance with safety practices and use of personal protective equipment.

References

- Aruofor R. Improvement of data related to wood products in Nigeria 2000 annual Review. EC.FAO partnership Programme (1998-2001) Tropical forestry Budget line.
- Asogwa S. Heat and the tropical worker. In a guide to occupational health practice in developing countries, fourth dimension publisher, 2nd Edition 2000: 222-235.
- Bean T. L. Wood dust exposure hazard. 2001 (revised) <http://healthy.org/idl.html>, Accessed on 1/11/10
- Demers P, Teschke, 1998 Lumber Industries Based on Biological Resources, In Stellman , J.M. (Ed.) Encyclopaedia of Occupational Health and Safety, 4th Edition, Vol. 3. Geneva, 71:1-71.
- Dosunmu O. Lagos The right to Health at work, WHO/OCH/95-1. Geneva, 1995.
- Fatusi A, Erhabor G. Occupational Health station of saw mill workers in Nigeria: JRSOC Health. 1996 Aug; 116 (4): 232-6.
- Federal Ministry of Agriculture. Wood industry of Nigeria situation report.(Forestry Statistics, 2003) pg 20-23.
- Gordon K, Osagbemi. Awareness of occupational hazard, health problems and safety measures among sawmill workers in North Central Nigeria. TAF preventive medicine bulletin, 2010:9(4); 325-328
- Hind S, Davies H. Hypertension in noise-exposed saw mill workers: a cohort study available on: www.sawmilimag.com. Accessed on 3/11/10
- Iyun B, James O. A study of Occupational hazards and prevention among saw Millers and plank dealers in Oshogbo LGA Osun state: Research project. October 2005; Pg 2, 4.
- Larama M, Anoek B. Lung function among low and high exposure workers in small scale wood industries in Tanzania .East African Journal of Public health, Vol. 2, No. 1, April 2005, pp. 17-20

Mwaiselage J, Bratweit M. Variability in dust exposure in a cement factory in Tanzania. *Annals of occupational hygiene* 2005; 49 (6): 511-519.

Segun B. yahaya M. Assessment of injures in small scale sawmill industry of SouthWestNigeria:Agric.Eng,Int:CIGRJJournal.Accessedat<http://www.cigrjournal.org>.Vol.12, No 1.

Uhumwangho O, Gordon J. The Epidemiology of eye diseases. Ocular trauma at place of work.1st Edition, Cambridge University Press, U.K, 1993 pp. 278.

WHO. The right to health at work. World Health Organization document, Geneva, 1995; 1-3.

Table 1: Precautions or Safety measures practiced by respondents. (N=660)

MAINTENANCE/PRECAUTIONARY MEASURES	Yes		No	
	Frequency (%)		Frequency (%)	
Regular wear and tear check	210	(31.8)	450	(68.2)
Servicing of machine	375	(56.8)	366	(55.5)
Environmental sanitation	423	(64.1)	237	(35.9)
Availability of First Aid box	30	(4.5)	630	(95.5)
Medical Examination	72	(10.9)	588	(89.1)
Wet drilling	0	(0)	660	(100.0)
Enclosure of dangerous	294	(44.5)	285	(43.2)
Use of protective device	267	(40.5)	393	(59.5)

Table 2: Types of protective devices used by respondents (N=660)

PROTECTIVE DEVICES	ALWAYS		OCCASSIONALY		NEVER	
	Frequency (%)		Frequency (%)		Frequency (%)	
Goggle	102	(15.5)	240	(36.4)	318	(48.2)
Protective wears	264	(40.0)	249	(37.7)	147	(22.3)
Boot	369	(55.9)	225	(34.1)	66	(10.0)
Hand glove	279	(42.3)	270	(40.9)	111	(16.8)
Face mask	252	(38.2)	210	(31.8)	198	(30.0)
Helmet	171	(25.9)	300	(45.5)	189	(28.6)

Table 3: Influence of working years on the use of protective devices

Working years	Use of protective devices		Total
	Yes	No	
< 10 years	129 (35.2)	237 (64.8)	366 (100)
11-20 years	99 (41.3)	141 (58.7)	240 (100)
≥ 21 years	39 (72.2)	15 (27.8)	54 (100)
Total	267 (40.5)	393 (59.5)	660 (100)
	$X^2 = 9.8633$ df =2, p= 0.0072		

Table 4: Influence of educational attainment on the use of protective device

Literacy level	Use of protective device		Total
	Yes	No	
None	36 (36.4)	63 (63.6)	99 (100)
Primary	90 (42.3)	123 (57.7)	213 (100)
Secondary	114 (42.2)	156 (57.8)	270 (100)
Post-secondary	27 (34.6)	51 (65.4)	78 (100)
Total	267 (40.5)	393 (59.5)	660 (100)
	$X^2 = 0.81$ df =3, p= 0.84721618		

Table 5: Relationship between occurrence of trauma and fracture from falls with number of working years of the respondents

Variable	Frequency (%)		Total	X ²	p-value
Working experience (years)/ History of falling trauma	Yes	No			
1-10 yrs	180(46.9)	204(53.1)	384(100.0)		
11-20 yrs	87(36.3)	153(63.8)	240(100.0)		
21 and above yrs	30(83.3)	6(16.7)	36(100.0)		
TOTAL	297(45.0)	363(55.0)	660(100.0)	9.7811	p=0.0075
History of bone fracture					
1-10years	237(61.7)	147(38.3)	384(100.0)		
11-20years	90(37.5)	150(62.5)	240(100.0)		
21years and above	21(58.3)	15(41.7)	36(100.0)		
Total	348(52.7)	312(47.3)	660(100)	11.7450	0.0028

Table 6: Relationship between occurrence of Strains/ Sprains and trauma from falls in the various departments.

Variable	Frequency (%)		Total	X ²	p-value
Sprains & strains/department	Yes	No			
Administrator	9(17.6)	42(82.4)	51(100.0)		
Machine /wood off-loaders	75(53.2)	66(46.8)	141(100)		
Machine operator/Technician	282(68.6)	129(31.4)	411(100)		
Saw-dust packers	39(61.4)	18(38.6)	57(100)		
TOTAL	405 (61.4)	255(38.6)	660 (100)	18.4635	0.0004
Trauma from fall/department					
Administrator	6(11.8)	45(88.5)	51(100.0)		
Machine /wood off-loaders	90(63.8)	51(36.2)	141(100.0)		
Machine operator/Technician	168(40.9)	243(59.1)	411(100.0)		
Saw-dust packers	33(57.9)	24(42.1)	57(100.0)		
TOTAL	297(45.0)	363(55.0)	660(100.0)	16.5390	0.0009

Site Suitability Delineation for Sanitary Landfill Development Using Vertical Electrical Sounding Geophysical Method in Gosa Area of Abuja, Nigeria

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Abstract

Geophysical investigation of Gosa dumpsite in Abuja, North-Central Nigeria was carried out to provide information on lithologic and structural characteristics of subsurface rock with a view of determining groundwater pollution potential should the landfill be eventually developed. 19 Vertical Electrical Soundings were carried with a view of determining presence and depth of linear structures, soil layers resistivity and resistivity values to infer degree of water saturation.

Results show that depth to bedrock VES-DC data indicated the presence of three to four geoelectric layers namely topsoil, dry lateritic soil/clay unit, and weathered basement overlying the fresh basement rock. Curve matching with the Orellana-Mooney master curves and 2-D forward modeling with *IPI2WIN* software revealed no evidence of fracture in the underlying rock that may promote large scale groundwater pollution.

Depth to basement and aquiferous zone varies from 4.08m and 27.2m. The presence of a clay layer with thickness ranging from 3.03m to 17.37m is significant in siting a sanitary landfill because of its impermeable nature that makes it qualify as a seal, hence protecting the underlying aquifer from being polluted. However, the southern part of the proposed sanitary landfill should be excluded as a result of a thin lateritic clay layer and shallow depth to the basement rock.

Introduction

Abuja became the Capital city of Nigeria in 1991 as a result of its central geographical position. It is approximately at latitude 9° 12' North of the equator and along longitude 7° 11' East of the Greenwich Meridian (CIA, 2012). The city has an estimated population of 1.4 million people of which 405,000 live and work within the municipality (NPC, 2012). It has a total land area of approximately 713 km² which is divided into six area councils: Abuja Municipal, Abaji, Bwari, Gwagwalada, Kuje and Kwali. Like other Africa capital cities, commercialization, urbanization and industrialization have generated much waste that will not only compete for the limited land space but causes air, soil groundwater pollution if not properly disposed. Limitations and implications of several methods of waste disposal such as burning, ocean and street dumping, incineration, open dumpsite have been reported (Ige, 2010).

Engineered landfill, which is an environment-friendly waste disposal facility with records of success in developed countries (Lumsden, 2002) has been proposed for development at the investigated site in order to alleviate pollution due to solid waste. The success of this waste containment technique in Nigeria is guaranteed because of the availability of part of construction materials (clayey soils) and equipment (earth moving machines) commonly used in other civil works.

Thus, this study sets out to evaluate the suitability of the GOSA dumpsite for possible conversion to an engineered Sanitary Landfill, using geophysical approach. This is with the view of determining the thickness of lithologic units and depth to basement rock, delineating fracture and identifying aquiferous zones, identifying potential materials for construction and the predicting of suitable areas for eventual development.

Location and Characteristics of Study Area

Gosa dumpsite is located about 10 km off the Nnamdi Azikwe International Airport road, Abuja, Nigeria (Fig.1) within coordinates GS 01 - GS 04 (GS 01- N09° 01.270' E007° 19.59', GS 02- N09° 01.463' E007° 20.120', GS 03- N09° 01.002' E007° 20.100' and GS 04- N09° 01 173' E007° 20.510'). Accessibility is through a rugged earth road that maybe difficult during raining season. The topography of the terrain shows an undulating features marked by rock exposures and stream within

the area of study. The site is already used as an open landfill as some parts of the land is occupied by indiscriminate-dumped waste. It has a concrete fence which runs through the perimeter of average 0.97 by 0.97km² land. Scavengers have inhabited the site for the purpose of sorting waste to be recycled.

The average annual rainfall of the area is between 1200 and 1400 mm (Akande *et al.*, 2010). The vegetation cover consists of shrubs and trees typically of savannah belt region of north central Nigeria. Abuja city is warm and humid during the dry season and cool during the raining season. Elevation above sea level varies from 430 m and 540 m with settlement concentrated at the low-lying areas. The dumpsite is drained by the tributary of river Wupa and a stream both trending in the North-East direction.

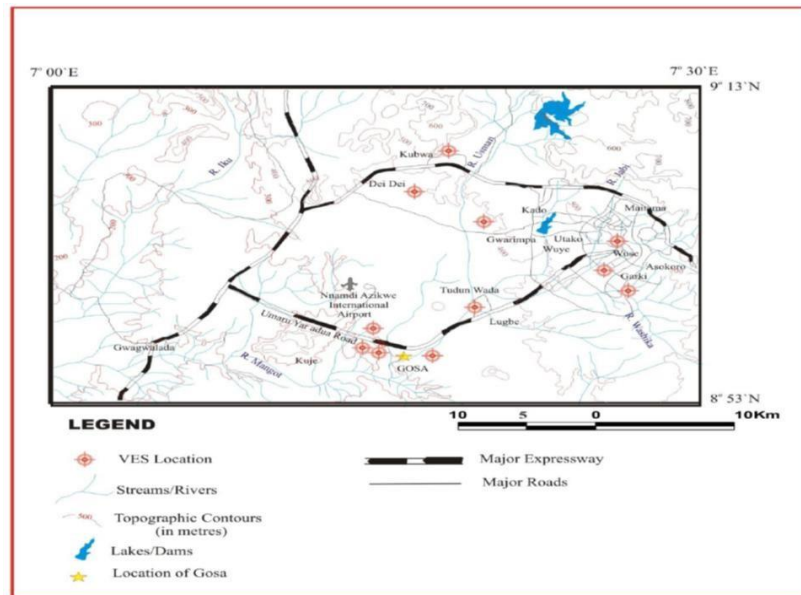


Fig. 1: Road network and drainage map of Abuja (Omeje *et al.*, 2013)

Geologically, Abuja and its environs are underlain by the basement complex rocks which are described as the Migmatite-gneiss complex (Rahaman, 1988; Dada, 2008). Exposures of these rocks define the general topography of the entire Capital city. According to Omeje *et al* (2013), Gosa site is underlain by porphyroblastic gneiss and Hornblendbiotite gneiss (Fig. 2). These rock types where not fractured, are competent

enough to provide required supports to the proposed sanitary landfill (Taha and Kabir, 2003)

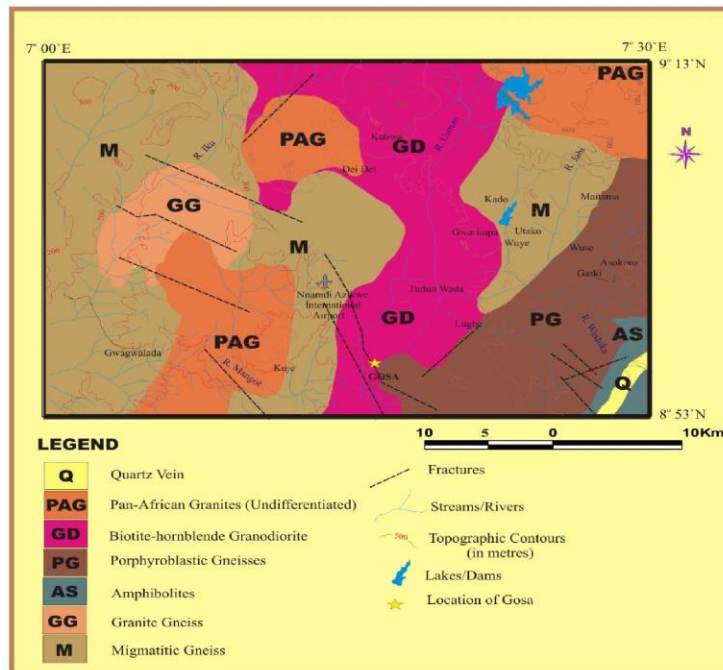
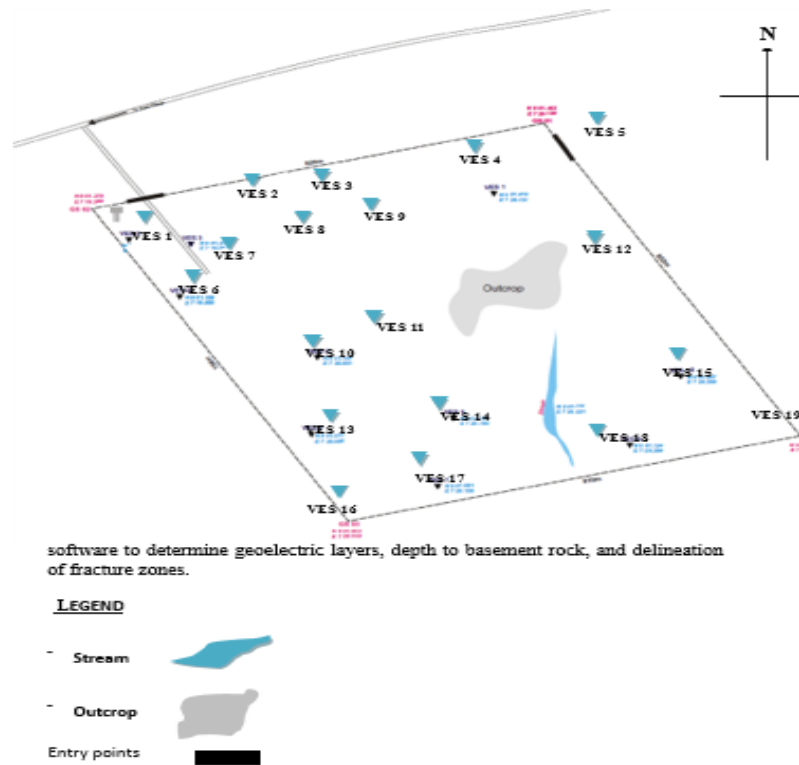


Fig 2: A Geological map of the study area (Omeje *et al.*, 2013)

Materials and Method

Geophysical investigation was carried out in the study area using the Vertical Electrical Sounding technique of Electrical Resistivity method. Six (6) traverses were selected in the east-west direction to enable detail investigation of the area. Vertical Electrical Sounding (VES) was carried out along the traverses using the Schlumberger Array. A total of 19 VES spread over the traverses were carried out at selected positions marked out in such a way that the whole area is investigated (Fig. 3). The electrodes were hammered into the ground until firm grip is achieved to enable readings to be taken from the Allied Omega terrameter. The readings were plotted on a log-log graph sheet to get the visual overview of the curves. The data were later subjected to computer iteration and interpreted using the IPI2WIN software to determine geoelectric layers, depth to basement rock, and delineation of fracture zones.



Results and Interpretation

Vertical Electrical Sounding

Partial curve matching and computer iteration of the results produced a system of three to five geo-electric layers (Fig. 4 a-c). The pseudo and geoelectric sections obtained after data inversion and computer iteration for Traverses 1 to 5 delineates four subsurface layers which are the top soil, Lateritic layer, clay soil (Sandy clay and clayey sand), weathered basement which overly the fresh basement (Fig. 5 a-d). The top soil apparent resistivity values vary $796 \Omega\text{m} - 1000 \Omega\text{m}$ (VES 1-VES 5), $490\Omega\text{m} - 885 \Omega\text{m}$ (VES 6 -VES 9), $5686 \Omega\text{m}- 686 \Omega\text{m}$ (VES 10 -VES 12) , $2945 \Omega\text{m} - 2659 \Omega\text{m}$ ((VES 13 -VES 15) and $752 \Omega\text{m}- 110 \Omega\text{m}$ (VES 16 -VES19) and depth vary from 1.45 m– 1.85 m (VES 1 -VES 5), 1.73 m– 1.13 m (VES 6 -VES 9), 2.1 m– 2.17 m (VES 10 VES 12) , 1.08 m– 1.02 m (VES 13 -VES 15), 2.17 m– 0.13 m (VES 16 -ES19)

respectively for Traverses 1 to 5. The variation in the top soil resistivity values is as a result of possible reworked surface or presence of compacted lateritic hard pan. This is characteristic of typical basement complex.

The second and third layer is the lateritic/clay soil layer whose apparent resistivity values vary from 1883 Ωm -5128 Ωm (VES 1-VES 5), 3537 Ωm – 1699 Ωm (VES 6-VES 9), 2087 Ωm – 416 Ωm (VES 10-VES 12) , 408 Ωm – 1358 Ωm (VES 13-VES 15) and 2353 Ωm - 736 Ωm (VES 16-VES 19) and depth vary from 9.83 m– 9.83 m (VES 1-VES 5), 7.99 m– 3.92 m (VES 6-VES 9), 18 m– 8.16 m (VES 10-VES 12) , 6 m– 5.1 m (VES 13VES 15), 7.67 m– 7.13 m (VES 16-VES19) respectively for Traverses 1 to 5. This layer plays significant role in siting appropriate location for landfill because of the impermeable nature of clay. The layer serves as a seal and hence protects the underlying aquifer from being polluted by any form of contaminant as such contaminant will be prevented from percolating into the aquifer by the porous but impermeable clay layer. The fourth layer is the weathered basement which represents the deep seated aquiferous layer in the area. The layer resistivity varies from vary 903 Ωm – 983 Ωm (VES 1-VES 5), 941 Ωm – 922 Ωm (VES 6-VES 9), 9054 Ωm – 1010 Ωm (VES 10-VES 12) , 1503 Ωm – 9124 Ωm (VES 13-VES 15) and 7072 Ωm - 5934 Ωm (VES 16-VES 19) and depth vary from 27.2 m– 22.0 m (VES 1-VES 5), 25.5 m– 19.2 m (VES 6-VES 9). VES 10 and VES 12 depth to basement is shallow showing only two layers above the basement at 15.9m and 8.16m, VES 13 third layer has a depth of 14.7 m. VES 15 depth to basement is shallow showing only two layers above the basement at 5.1 m. VES 16 and VES 19 depth to basement is shallow showing only two layers above the basement at 5.5 m and 7 .0 m, for Traverses 1 to 5.

It was observed that the basement is shallow towards the southern flank of the study area particularly for the third, fourth and fifth traverses and this accounts for general high resistivity observed across the waste dumpsite. This observation is confirmed with the presence of an outcrop at the centre

of the high resistivity soundings. The presence of the clay layer is of great importance to landfill siting as a result of both the attenuation and containment properties of clay (Jones et al., 1995, Comeau et al., 1998). The overburden thickness ranges from 4.08 m– 27.2 m indicating different degrees of weathering of the rocks over the study area. Only the Northern

part of the proposed sanitary landfill which represent about 35% of the total study area is suitable for sanitary landfill (Fig. 6) Hence, the southern part of the proposed sanitary landfill should be excluded as a result of a thin lateritic clay layer and shallow depth to the basement rock.

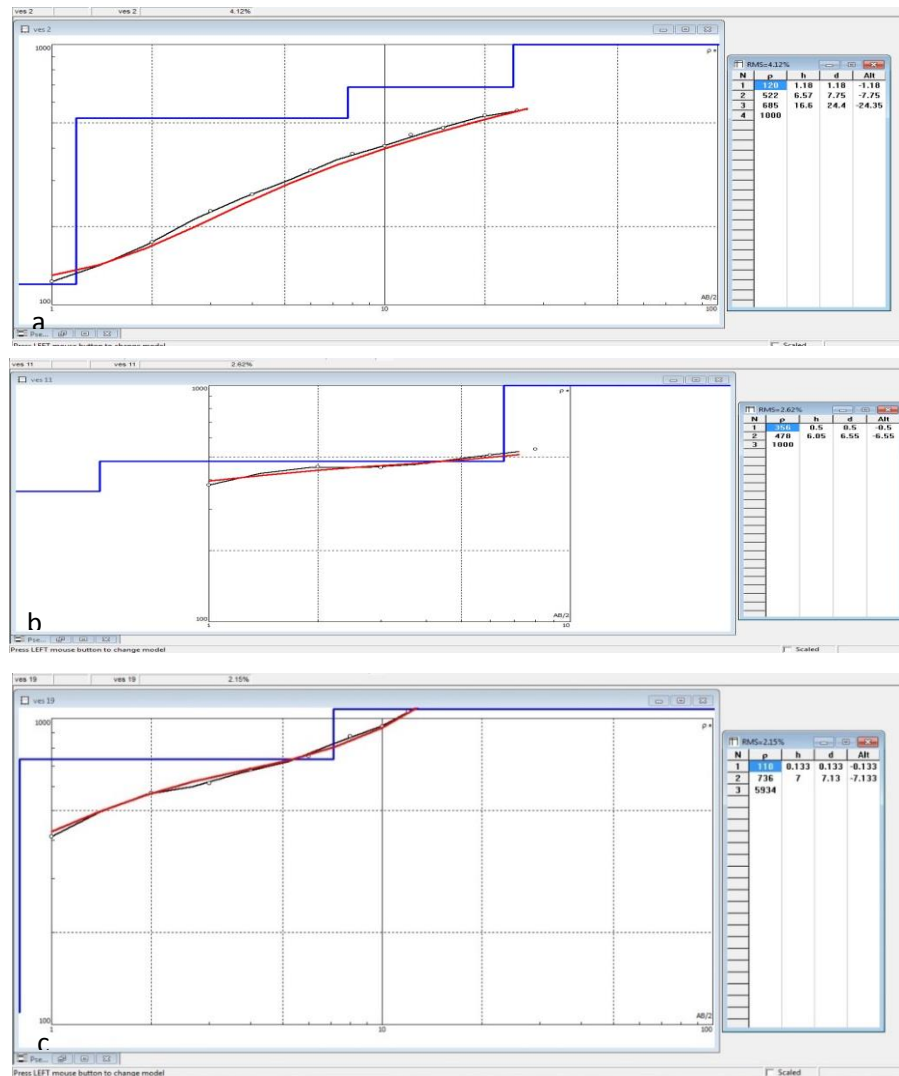
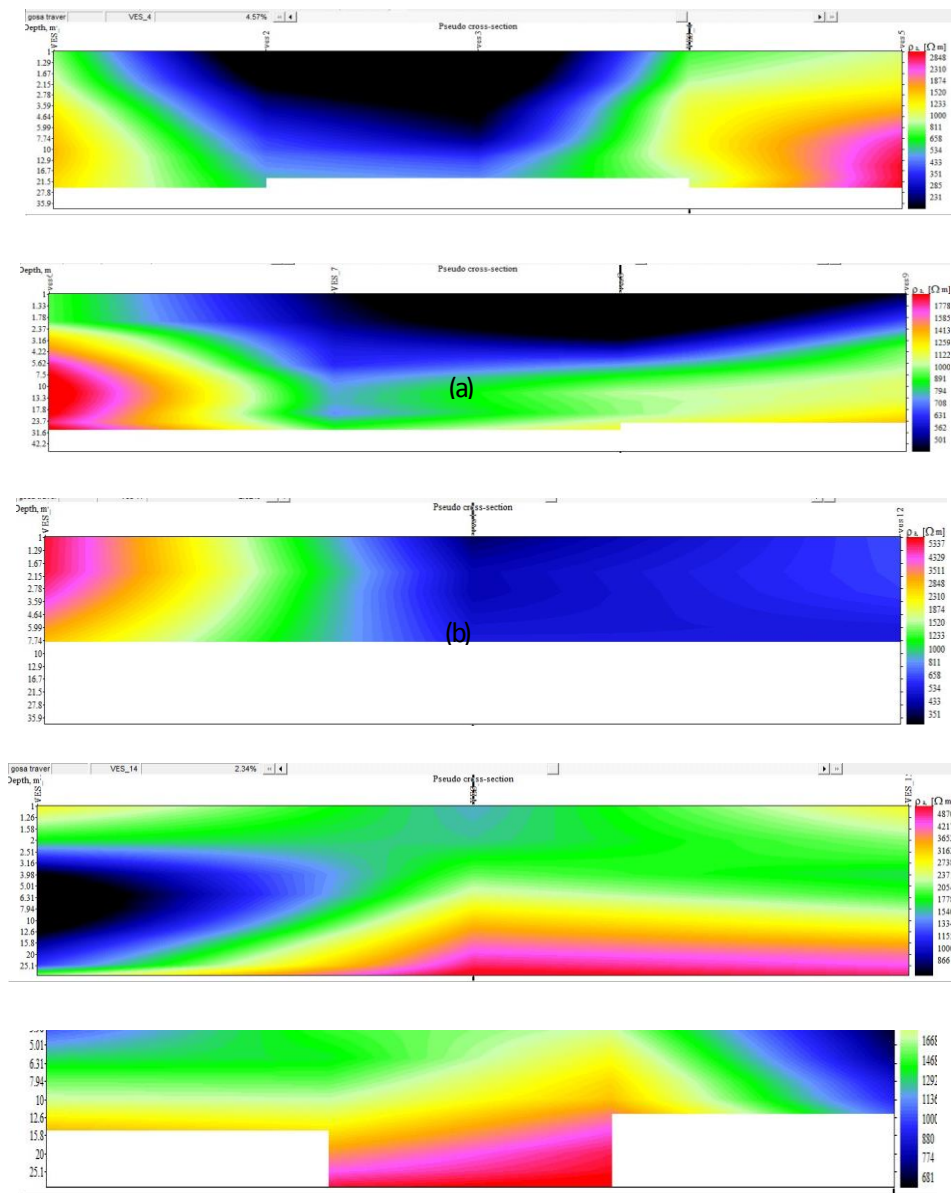
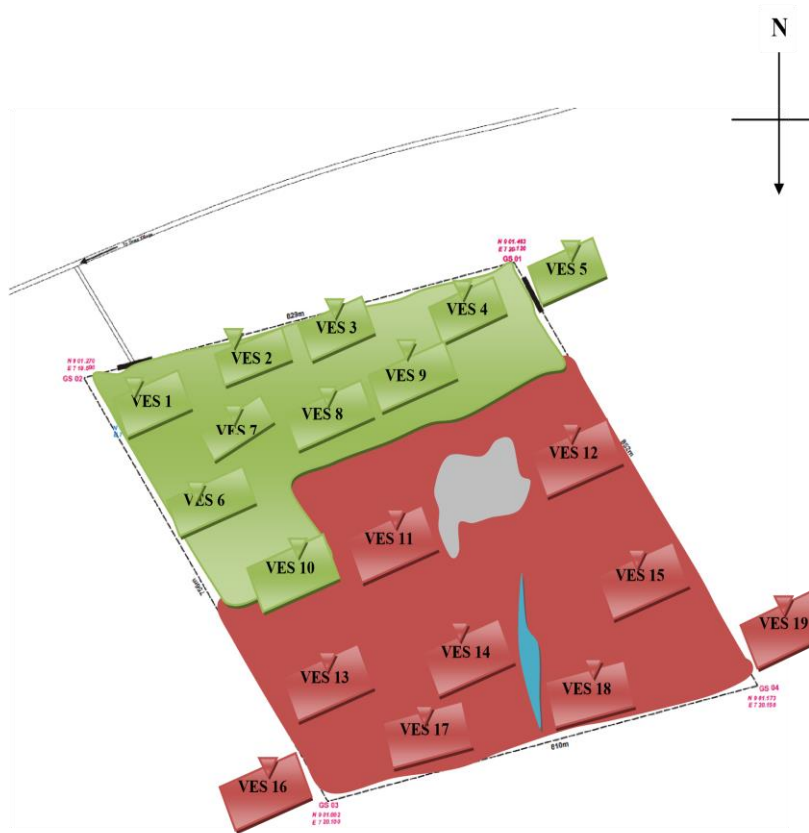


Figure 4: Typical VES Curves over the Site at: (a) VES 2 (b) VES 11 (c) VES 19

Fig 6: Schematic diagram showing arrangement of VES Positions an Level of suitable of the areas for the proposed sanitary landfill.





Areas with moderate clay overburden suitable for siting of the proposed of the impermeable nature of clay. The layer serves as a seal and hence protects the underlying aquifer from being polluted by any form of contaminant as such contaminant will be prevented from percolating into the aquifer by the porous but impermeable clay layer.

- Areas with shallow or thin overburden, characterized mostly of sandy soil, which are porous can promote pollution in the aquiferous zone through percolation.

Conclusions and Recommendations

Comprehensive investigation using VES geophysical approach in the study area has helped in arriving at the following conclusions;

Presence of a clayey layer of greater than 5.00 m thick, as recommended by previous researchers and agencies. This layer is recommended for geotechnical investigation as a potential leachate barrier or containment during development of the sanitary landfill.

Absence of linear features such as fractures or faults that may promote large scale groundwater pollution.

The average depth to basement rock head in the entire study area is about 4.08 m in the southern region and 27.2 m in the northern segment.

Only about 35% of the total area (which is restricted to the northeastern part) is suitable for development of sanitary landfill based on this study if the thick layer of clayey unit is suitable as barrier for sanitary landfill development. A simplified schematic diagram of the proposed landfill site has been used to classify the site into suitable and unsuitable zone, based on results obtained.

Groundwater in the vicinity of the landfill is expected to be less vulnerable to pollution as the bedrock is overlain by 5-15m of clayey and impermeable unit.

References

Akande, SO, Ojo OJ, Erdtmann BD, Hetenyi M: Paleoenvironments, Organic Petrology and RockEval studies on source rock facies of the Lower Maastrichtian Patti Formation, Southern Bida

Basin, Nigeria. Journal of African Earth Sciences Vol.41:pp.394–406. 2010

CIA: Central Intelligence Agency US. Available on: www.cia.gov. 2012

Comeau, S., Chihson, P., Massiera, M: Liner construction using tills composed of weakly cemented particles. Proceedings of Third Int. Congress on Environmental Geotechnics, Lisbon, Portugal. 77-82. 1998.

Dada, SS: Proterozoic Evolution of the Nigeria – Boborema Province. Geologic Society London Special Publications. Vol.294: pp121- 136. 2008.

Ige, OO: Landfill Site Selection For Municipal Solid Waste and Assessment of Soils as Mineral Seals Around Ilorin, Southwestern, Nigeria. Unpublished Ph.D. Thesis University of Ilorin. pp 256. 2010.

Jones RM, Murray EJ, Rix DW, Humphrey RD: Selection of clays for use as landfill liners. Waste disposal by Landfill GREEN'93, Sarsby (ed). Balkema, Rotterdam. Pp 345. 1995

Lumsden, TK: Pattern of waste management in developed countries. International Journal of water and sediment contamination. vol.49, 2, 63-68. 2002

NPC: National Population Commission Nigeria, Retrieved from: www.population.gov.ng. 2012

Omeje M, Husin W, Nooriddin I, Oha IA, Onwuka OS, Ugwuoke PE and Meludu O: Geoelectrical investigation of aquifer problems in Gosa area of Abuja, North Central, Nigeria International Journal of Physical Sciences Vol.18(13),pp.549-559. 2013.

Rahaman, MA: Review of the Basement Geology of Southwestern Nigeria In: Kogbe C.A. (ed): Geology of Nigeria, Elizabethan Pub. Co. Lagos, 41-58. 1988.

Taha, MR and Kabir, MH: Sedimentary Residual Soil as a Hydraulic Barrier in Waste Containment Systems, In: Proceedings of the International Conference on Recent Advances in Soft Soil Engineering and Technology, 2-4 July 2003. Putrajaya, Malaysia. 2003.

Antibacterial activities and effects of dosage of Methanol and “Omidun” Extract of *Vitellaria paradoxa* (C.F. Gaertn) root on some diarrheagenic bacteria

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Abstract

Root of *Vitellaria paradoxa* extracted using methanol and amidun (supernatant solution of fermented cereal mash) as solvent was tested for efficacy by *in vitro* assay (Minimum Inhibitory Concentration (MIC) and disc diffusion assay). Effects of dosage (50 mg/mL and 100 mg/mL) on microbial induced albino rats was also determined. The test organisms used include *Salmonella typhi*, *Shigella flexneri*, Enteropathogenic *Escherichia coli* (EPEC ATCC 43887), Enterohaemorrhagic *Escherichia coli* (EHEC ATCC 43889) and *Escherichia coli* ATCC 25922. Seven groups (I-VII) of five rats each were treated thus: Group I rats received organisms and Ciprofloxacin (positive control), group II rats received organisms only (negative control), group III rats received organisms and 50 mg/mL methanol extract of *V. paradoxa* roots, group IV rats received organisms and 100 mg/mL methanol extract of *V. paradoxa* roots, group V rats received organisms and 50 mg/mL amidun extract of *V. paradoxa* roots, group VI rats received organisms and 100 mg/mL amidun extract of *V. paradoxa* roots and group VII rats received nothing. The treatment procedures were replicated for other test organisms. Liver and kidney samples of the treated rats were collected after six hours of observation for diarrhea symptom. Methanol extract gave higher percentage yield (20.80 %) than amidun (19.2 %) extract. MIC of methanol extract (3.15 -50.0 mg/mL) was significantly different ($P<0.05$) from that of amidun extract (6.25 - 50.0 mg/mL) on all the tested microorganisms. Higher concentration tested and methanol extract (17.00 mm) gave wider

inhibition zones than lower concentration and omidun extract (12.67 mm). Amounts of active constituents were slightly higher in methanol extracts than in omidun extracts. Livers and kidneys of rats in groups I and IV showed no abnormality. Mild inflammation to focal lymphocytic aggregate was observed in the portal areas of liver and kidneys of rats treated with 50 mg/mL of omidun and methanol extracts. Intense infiltration, marked distortion of architecture and vacuolar degeneration was seen in group II rats and in rats treated with 100 mg/ml concentration omidun and methanol extract of *V. paradoxa* root. Hence, omidun is a good extraction solvent and the root extract of *V. paradoxa* can be toxic at high concentration.

Keywords: Omidun, Sapotaceae, *Vitellaria paradoxa*, Vacuolar, *in vivo*, *in vitro*, diarrheagenic bacteria

Introduction

Medicinal plant is defined as any substance with one or more of its organs containing substances that can be used for therapeutic purposes or which can be used as precursors for the synthesis of antimicrobial drugs (1,2). Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been generated from natural products (3).

In developing countries, it is estimated that about 80 % of the population rely on herbal medicine for their primary health care (4), because of better cultural acceptability, belief of better compatibility with the human body and fewer side effects (5). It has been used in many parts of the world as a rich tradition for the treatment of many infectious diseases, including diarrhea and abdominal pain (6). The major cause of diarrhea among children in developing countries is malnutrition. To nullify the problem of diarrhea which is a leading cause of mortality in developing countries, the World Health Organization constituted a diarrheal disease control programme which includes studies of traditional medicinal practices, together with the evaluation of health education and prevention approaches (6).

Search for potent herbal drugs require the bringing together of ethnobotanical, ethnopharmacological, chemical, biological, pharmacological and toxicological studies (7). Considering the importance

of plants as a source of medicine, in the present study, a plant *Vitellaria paradoxa*, often known as Shea butter tree is a tree of the Sapotaceae family, which is in use traditionally in the treatment of many diseases was selected.

Vitellaria paradoxa CF Gaertn, also known as locally as *Ori* (Yoruba), *òkwùma* (Igbo), *èkó* (Nupe), is a thin, tart, nutritious pulp that surrounds a moderately large, oil-rich seed from which shea butter is removed. It is a deciduous tree usually 7 – 15 m tall, but can reached 25 m and a trunk diameter of 2 m. Shea butter has many uses and may or may not be refined. In Nigeria, it is mostly used for cosmetics as cream. All through Africa, it is used extensively for food, being a major source of dietary fat, and for medicinal purposes (8). The antibacterial activity of molecules isolated from the root bark of *V. paradoxa* had been documented (9). Also, the leaves and stem bark of *V. paradoxa* have been used for the treatment of skin diseases, rheumatism, typhoid fever and microfilaria (in association with other medicinal plants) (10). The present study seek to investigate the antibacterial and effects of dosage of methanol and “omidun” extract of *Vitellaria paradoxa* root on some diarrheagenic bacteria and also add to the existing information on the toxicity of the plant extract as it relates to the histopathology of the liver and kidney of infected rats. Hence, this plant has been extracted traditionally using omidun along with conventional methanol as solvents of extraction and the different solvent extracts comparatively investigated for potential use in treating diarrhea.

Materials and Methods

Materials

Collection of Diarrheagenic Bacteria

Clinical diarrheagenic isolates (*Salmonella typhi* and *Shigella flexneri*) and typed cultures Enteropathogenic *Escherichia coli* (EPEC ATCC 43887), Enterohaemorrhagic *Escherichia coli* (EHEC ATCC 43889) *Escherichia coli* ATCC 25922 used in this study were obtained from Sacred Heart Hospital Lantoro, Abeokuta Nigeria and National Institute of Medical Research, Yaba Lagos Nigeria respectively. Ciprofloxacin antibiotics was a product of Bayer Healthcare Pharmaceuticals Inc., New Jersey, United States. Pure cultures of the isolates were promoted by

further subjecting them to cultural and morphological identification and finally biochemical characterization using protocols described by Cheesbrough (11). The pure cultured isolates were then maintained on appropriate media for further use.

Inocula Preparation

An 18 hr broth culture was aseptically prepared from each microbial cell suspension; it was adjusted to McFarland turbidity standard (equivalent to 0.5×10^8 colony-forming units per mL) and kept for further studies.

Collection and preparation of roots of *V. paradoxa*

Roots of *Vitellaria paradoxa* (Shea butter tree) were collected around Onipako Village, Ilorin, Kwara State of Nigeria, and authenticated in the Herbarium unit of the Department of Forestry and Wildlife Management, Federal University of Agriculture, Abeokuta where Voucher specimen (UAHA NO. 015/001) was deposited. The roots were washed thoroughly with sterile water to remove all traces of dust and sand, air-dried for 7 days in a well-ventilated room. The dried roots were reduced into small pieces with a surface-sterilized scalpel and further milled with electric blender (Master Chef Blender, Model MC- BC 1980, China). A quantity of 150 g each of the fine powder were weighed into two separate 1000 ml-capacity conical flasks and 500 ml methanol and omidun were added to each powder in the conical flasks respectively. Each was allowed to stand for 48 hours with constant shaking at regular intervals to facilitate extraction (12). The percolates were then filtered and the resulting volume on filtration was reduced to dryness with a Rotary evaporator at $45 \pm 10^\circ$ C. The extracts were then collected, weighed, packed in sterile air tight containers and labeled. They were kept in the refrigerator at 4° C until needed for use.

Quantitative phytochemical screening

The phytochemicals which are present in the methanol and omidun extracts of *V. paradoxa* roots were determined and quantified by standard procedures (13 - 15).

Preparation of Dilute Stock of Extracts

Stock solution of root extract was prepared in three test tubes labeled 1 to 3. A stock concentration of 100 mg/mL of the extract was prepared in the first test tube, subsequently; 5 ml of distilled water was then introduced into the remaining two test tubes. 5 ml of the stock was withdrawn from the first tube and added to the second test tube which was mixed thoroughly to obtain a concentration of 50 mg/mL. Another 5 ml was withdrawn from the second tube and then transferred to the third tube which was also thoroughly mixed to give a concentration of 25 mg/mL.

Experimental Animal

Albino rats made up of both sexes (weighing between 180 – 220 g) but from the same genetic lineage were procured from the Animal holding unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. They were kept in Laboratory cages and maintained according to the NIH guidelines of care and use of laboratory Animals reported by Saha *et al.* (16). They were acclimatized to standard laboratory conditions (temperature 24 ± 1 °C and a 12 hours photoperiod), fed twice daily with standard commercial feeds (Vital Feeds, Ibadan, Nigeria) and distilled water *ad libitum* for one week before the commencement of the experiment.

Ethical Considerations

Albino rats used for this research were appropriately selected. The rats were kept in Laboratory wire mesh built cages and maintained according to the NIH guidelines of care and use of laboratory Animals while standards of the animal care and administration met those required by applicable international laws and regulations.

Experimental Design

A total number of 125 (male: 50 and female: 75) Wistar rats (weighing between 180-220 g) were randomly assigned into 7 groups (I- VI) of 5 rats each. They were fasted overnight prior to the experiment but allowed free access to water *ad libitum*. The various groups were treated as follows:

Group I: Received organisms and Ciprofloxacin (positive control).

Group II: Received organisms only (negative control).

Group III: Received organisms and 50 mg/mL methanol extract of *V. paradoxa* roots.

Group IV: Received organisms and 100 mg/mL methanol extract of *V. paradoxa* roots.

Group V: Received organisms and 50 mg/mL omidun extract of *V. paradoxa* roots.

Group VI: Received organisms and 100 mg/mL omidun extract of *V. paradoxa* roots.

Group VII: Received nothing.

Rats in all groups were weighed prior to and after the experiment to determine the growth index, each rat in groups I-IV were orally given 0.5 mL of 18 hr broth culture of *S. typhi* to induce diarrhea. One hour later, all groups I-VI rats were treated by intraperitoneal route as follows: Group I rats received 2 mls of commercially obtained Ciprofloxacin, groups II rats received no treatment, group III and IV received 2 mls of 50 mg/kg and 100 mg/kg of methanol extracts of *V. paradoxa* roots respectively, group V and VI received 2mls of 50 mg/kg and 100 mg/kg of omidun extracts of *V. paradoxa* roots respectively received while group VII rats received nothing. The treatment procedures were replicated for other test organisms and all treated rats were then placed in separate cages over clean white paper and observed for the presence of diarrhea every hour for six hours for the onset of diarrhea and number of diarrhea episodes.

Histological examination

At the end of the *In vivo* assay, laparotomy was carried out by placing the treated rats in a jar containing cotton wool soaked with followed by jugular puncture with a sharp sterile blade. Thin sections of each of liver and kidney samples (3 μ m) were quickly excised and fixed in 10 % formalin for histopathological studies. The organ sections were histologically processed, stained with Haematoxylin and Eosin (H & E) and observed with light microscope for histopathological changes.

Data Analysis

Data were expressed as the mean $\pm$ SEM of five determinations and subjected to statistical analysis using Duncan Multiple Range Test with the aid of Statistical Package for Social Sciences, version 16.0 (SPSS Inc., Chicago, IL, USA). Differences were considered statistically significant at $P < 0.05$.

Results

Percentage yield of methanolic extract of *V. paradoxa* roots was 20.80 % while that of omidun extract was 19.20 %.

Table 1: Percentage Yield after Extraction of *V. paradoxa* roots with methanol and omidun

Extraction solvent	Dry Raw powder (g)	Yield from dry raw powder (g)	Percentage yield%
Methanol	150	31.2	20.80
Omidun	150	28.8	19.20

Table 2 shows the result obtained for Minimum inhibitory concentration (MIC) of methanol and omidun extracts of *V. paradoxa* roots. There was significant difference ($p < 0.05$) in the MIC of methanol extract (3.15 - 50.0 $\mu\text{g/mL}$) and omidun extract (6.25 - 50.0 $\mu\text{g/mL}$) of *V. paradoxa* root on all the tested microorganisms.

Table 2: Minimum Inhibitory Concentration (MIC) of Methanol and Omidun Extracts ($\mu\text{g/mL}$) of *V. paradoxa* roots on Diarrhea Inducing Bacteria tested

Extract	EHEC ATCC 43889	EPEC ATCC 43887	<i>S. typhi</i> <i>Sh. Flexneri</i>	<i>E. coli</i> ATCC 25922
Omidun root	12.50 ^a	50.00 ^a	6.25 ^a 12.50 ^a	6.25 ^b
Methanol root	6.25 ^b	50.00 ^a	6.25 ^a 6.25 ^b	3.15 ^a

Values with different superscript on the same column are significantly different ($p < 0.05$)

The antibacterial assay result of omidun and methanol extracts of *V. paradoxa* roots revealed a concentration dependent zone of inhibitions (Table 3). Methanol extract yielded zones of inhibitions ranging from 2.67- 17.00 mm while omidun extract yielded inhibition zone ranging from 2.33- 12.67mm.

Table 3: *In vitro* Inhibition zone (mm) of Different Concentrations of Omidun and Methanol extracts of *V. paradoxa* roots on tested Microorganisms

Concentration of Extract	Extraction Solvent	EHEC ATCC 43889	EPEC ATCC 43887	<i>S.typhi</i>	<i>Sh.flexneri</i>	<i>E.coli</i> ATCC 25922
100 mg/mL	Methanol	12.67±1.16	6.67±2.08	17.00±1.00	14.67±1.16	15.00±1.00
	Omidun	11.00±1.00	4.67±1.16	12.33±1.53	10.33±0.58	12.67±1.53
50 mg/mL	Methanol	8.00±1.00	4.67±1.16	13.33±4.93	13.33±4.93	9.33±0.58
	Omidun	7.00±1.00	4.67±0.58	9.33±0.58	9.33±0.58	11.00±1.00
25 mg/mL	Methanol	6.33±1.16	3.00±1.00	2.67±1.16	3.00±1.00	4.67±0.58
	Omidun	4.67±0.58	2.67±0.58	2.67±0.58	2.33±0.58	3.67±0.58

Result expressed as mean ± SEM of five replicates

Qualitative phytochemical analysis of the extracts revealed the presence of all the tested active ingredients (tannin, saponin, flavonoids, alkaloids, oxalate, steroid, terpene, Phlobatannin, cardiac glycoside and anthraquinone) in methanol and omidun extracts of *V. paradoxa* roots (Table 4).

Table 4: Preliminary Phytochemical Constituents of Methanol and Omidun extracts of *V. paradoxa* Roots

Phytochemical Constituents	Methanol	Omidun
Tannin	+ve	+ve
Saponin	+ve	+ve
Alkaloid	+ve	+ve
Oxalate	+ve	+ve
Flavonoid	+ve	+ve
Steroid	+ve	+ve
Terpene	+ve	+ve
Phlobatannin	+ve	+ve
Cardiac glycoside	+ve	+ve
Anthraquinone	+ve	+ve

+ve Present

The active ingredients found in methanol and amidun extracts of *V. paradoxa* roots were found present in varying proportion (Figure 1). Although, these constituents were slightly higher in methanol extracts (2.60- 8.60) than amidun extracts (1.80 - 7.80). Oxalate was found present in larger amount (7.80 and 8.60) than other active ingredients. Tannin, saponin, flavonoid and anthraquinone were present in moderate quantities while alkaloid, steroid, terpene, phlobatannin and cardiac glycoside were present in small quantities.

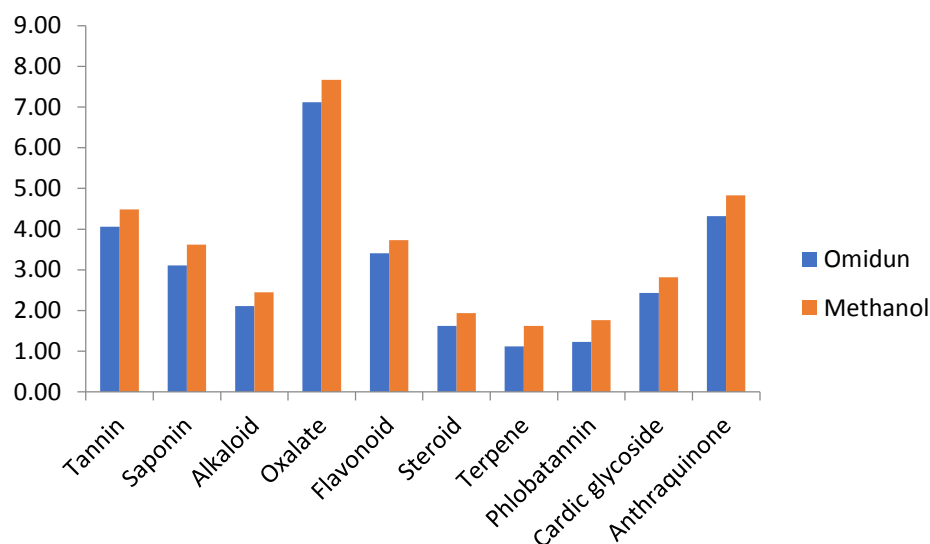


Fig 1: Comparative Phytochemical result of omidun and methanol extracts of *V. paradoxa* roots

Similar presentations with no histopathological changes (plate 1 (A) and B) were observed in Livers and kidneys of rats in the non-challenged (with the tested microorganisms) nor treated groups and those of rats in the group treated with commercial antibiotics. However, moderate inflammation was observed in liver of rats in the group treated with low concentration (50 mg/mL) of solvents extracts (methanol and omidun) of the root as in plate 1(A) while livers of rats in the group treated with high concentration (100 mg/mL) of root extract showed moderate vacuolar degeneration of centrilobular hepatocytes as shown in plate 1 (B). Kidneys of rats treated with 50 mg/mL concentration of solvent extracts of the root revealed mild to moderate mononuclear cellular infiltrate (Plate 2 (B)) and at high concentration (100 mg/mL), inflammation and moderate degeneration was observed in the kidneys of rats in this group as shown in plate 2 (C).

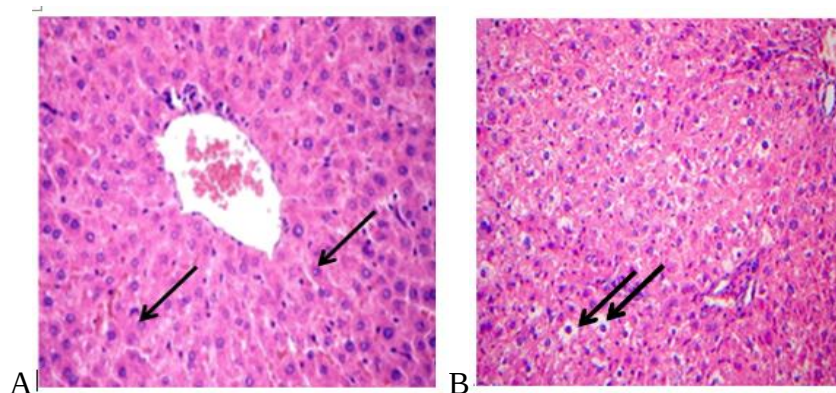


Plate 1: Microscopic Presentation of liver of Diarrhea Induced Albino Rats Treated with 50mg/mL and 100mg/mL root extracts of *V. paradoxa*

Normal hepatic presentation. (H & E. X400). B: moderate mononuclear cellular infiltrate in the portal area (H & E. X400).

Moderate mononuclear cellular infiltrate in the portal area (H & E. X400).

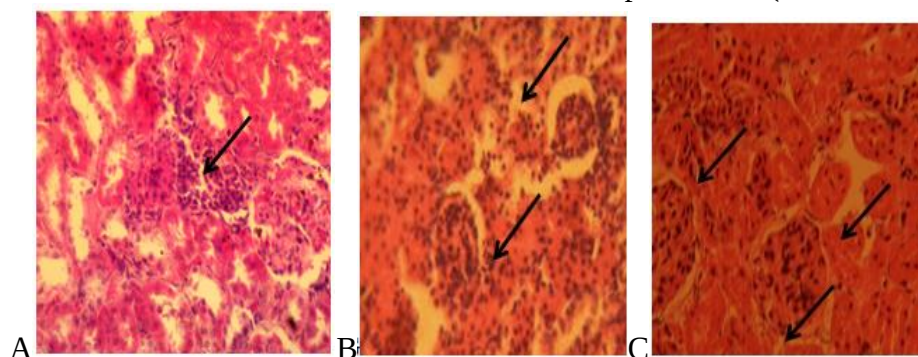


Plate 2: Microscopic Presentation of Kidney of Diarrhea Induced Albino Rats Treated with 50mg/mL and 100mg/mL of extract of *V. paradoxa* roots

A: Focal interstitial lymphocytic aggregate in the kidney. (H & E. X400).

B: Marked distortion of the architecture and vacuolar Degeneration. (H & E. X400).

C: Normal Kidney presentation with prominent structural. (H & E. X400).

Discussion

The present study was designed to evaluate the effects of conventional (using methanol) and traditional (using omidun) extraction methods on *V. paradoxa* roots on some diarrhea causing organisms using the *In vitro* and *In vivo* assay methods. Higher percentage yield of methanol extract *V. paradoxa* roots than omidun extract may be as a result of difference in polarity of the solvents. Reports by Ncube *et al.* (17) and De Boer *et al.* (18) also stated that that choice of solvent used in extraction of plants may have effect on the yield after extraction and active components of plants are more soluble in organic solvent. Similar report was given by Sun *et al.* (19) who stated that methanol was most effective in extracting active components from oat bran while higher yield (52 %) of water extract of *Senna obtusifolia* was reportedly obtained by Doughari *et al.* (20). Owolabi *et al.* (21) also reported a yield of 10.74 % for water extract and 3.78 % of ethanolic extracts of *Kigelia africana* stem bark.

Minimum inhibitory concentrations (MIC) of the extracts on the tested organisms were 50 mg/mL, 25 mg/mL, 12.5 mg/mL, 6.25 mg/mL and 3.25 mg/mL. Crude plant extracts are generally a mixture of active and non-active compounds and MICs of less than 100 mg/mL suggest good antimicrobial activity (22). Thus, the MICs observed in this work were lower than 100 mg/mL and this implies high antimicrobial activity of root of *V. paradoxa*. The low MIC is of great significance as this plant could be used as an alternative to orthodox medicine in the treatment of microbial infections, with rapid increase in microbial resistance to known antibiotics (23).

Methanol extract gave lower MIC (3.15 - 50.0 mg/mL) than omidun extract (6.25 -50.0 mg/mL). This could be an indication that there is a variation in the levels of compounds in the two solvent extracts (methanol and omidun) of *V. paradoxa* roots (24) and may also signify the presence of the more dissolved phytochemicals in methanol than omidun extract of the plant part. This is in agreement with the report of de Boer *et al.* (18) who reported that active components of plants are more soluble in organic solvent, also conforms with the work of Yahaya *et al.* (25) who reported that methanolic extract of stem bark of *Combretum glutinosum* showed high level of activity on *Salmonella typhi* and *E. coli* while the aqueous extract showed less response.

The antibacterial activities by disc diffusion method revealed that, at all concentrations tested, omidun and methanol extracts of root of *V. paradoxa* inhibited growth of the tested microorganisms (EPEC ATCC 43887, EHEC ATCC 43889, *S. typhi*, *Sh. flexneri* and *E. coli* ATCC 25922). Even though all the tested microorganisms were susceptible to all the tested concentrations of methanol and omidun extracts, the zones of inhibitions obtained were concentration dependent. Concentration dependent zones of inhibitions have also been reported in antimicrobial activities of roots of *R. sativus* (26). Akinpelu *et al.* (27) also reported the bioactivity of the methanolic crude leaf extract of *D. guineense* on some bacteria.

Better potency of omidun extracts may probably have resulted from the synergistic effects of microorganisms (such as lactic acid bacteria and *Aspergillus fumigatus*) found in it and the active compounds present in root of *V. paradoxa*. These microorganisms have been shown to control some diarrhea organisms in *in vitro* study by Falana *et al.* (28). Lactobacilli are important lactic acid bacteria in foods, which contribute not only to acidification of foods, but are also important in the preservation of food, prevention of pathogens and improvement in food palatability (29). Lactobacilli exert strong antagonistic activity against many microorganisms including food spoilage organisms and pathogens (30 - 32). Furtado *et al.* (33) has also reported the production of some antimicrobial metabolites from *A. fumigatus*.

Although, previous work on *V. paradoxa* by Ayankunle *et al.* (34) suggested that Gram- positive bacteria are more susceptible to the antimicrobial effect of ethanol extract of *V. paradoxa* than Gram-negative bacteria, this study however showed that the two tested solvent extracts of root of *V. paradoxa* were found inhibitory against the tested Gram-negative organisms. This agrees with the report of Akinsulire *et al.* (35) that omidun extracts of *Bryophyllum pinnatum* and *Kalanchoe crenata* were found inhibitory against some Gram-negative organisms. This result further support the report of Bibitha *et al.* (36) that variations exist in the antibacterial activities of different plant extracts.

The effect produced by the positive control agent (Ciprofloxacin) on the tested microorganisms was higher than the tested concentrations of methanol and omidun extracts of root of *V. paradoxa*, a clear indication

that orthodox medicine was more effective against the tested microorganisms than herbal medicine. This disparity may be due to the mixtures of bioactive compounds present in the extract compared to the pure compound contained in the standard antibiotics (37). The solvent extracts contain many active compounds that are in minor which are working either in synergy (38), some of which might have been degraded by the tested microorganisms and some might be having antagonistic effects with others, hence reducing their antimicrobial activities.

Based on this study, it is revealed that methanol and omidun extracts of *V. paradoxa* roots showed appreciable antimicrobial effects against all the tested diarrhea causing microorganisms. Also, methanol and omidun extracts conferred similar patterns of protection at both concentrations of treatments from diarrhea. The root extracts was safe on livers and kidneys at low concentration but toxic at high concentration and should therefore be carefully consumed and should be avoided at higher dosage.

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References

- Sofowora, A. (1980). The Present status of knowledge of the plants used in Traditional Medicine in Western Africa; A Medical approach and chemical evaluation. *Journal of Ethnopharmacology*, 2: 109 - 118.
- Tapsell, L. C., Hemphill, I., Cobiac, L., Sullivan, D. R., Fenech, M., Patch, C. S., Roodenrys, S., Keogh, J. B., Clifton, P. M., Williams, P.G., Fazio, V. A. and Inge, K. E. (2006). Health benefits of herbs and spices: the past, the present, the future. *Medical Journal of Australia*, 185(4), S1-S24.
- Lahlou, M. (2007). Screening of Natural Products for Drug Discovery. *Expert Opinion on Drug Discovery*, 2(5): 697-705.
- Matu, E.N. and Van Staden, J. (2003). Antibacterial and anti-inflammatory activities of some plants used for medicinal purposes in Kenya. *Journal of Ethnopharmacology*, 87(1): 35 - 41.

- Azadbakhta, M., Safapour, S., Ahmadi, A., Ghasemi, M. and Shokrzadeh, M. (2010). Anti-diabetic effects of aqueous fruits extract of *Diospyros lotus* L. on streptozotocin-induced diabetic rats and the possible morphologic changes in the liver, kidney and heart. *Journal of Pharmacognosy and Phytotherapy*, 2(2): 10 - 16.
- Brantner, A. and Grein, E. (1994). Antibacterial activity of plant extracts used externally in traditional medicine. *Journal of Ethnopharmacology*, 44(1): 35 - 40.
- Gurib-Fakim, A. (2006). Medicinal plants: traditions of yesterday and drugs of tomorrow. *Molecular Aspects of Medicine*, 27(1): 1 - 93.
- Moore, S. (2008). The role of *Vitellaria Paradoxa* in poverty reduction and food security in the Upper East region of Ghana. *Earth Environment*. 3: 209 – 245
- Garba, S. and Salihu, L. (2011). Antibacterial Activities of 2-O-butyl-1-O-(2'-ethylhexyl)benzene-1,8-dicarboxylate and 1-phenyl-1,4-pentanedione Isolated from *Vitellaria paradoxa* Root Bark. *Asian Journal of Scientific Research*. 4: 149 – 157.
- Siméon, P.C.F., Donatien, G., Benjamin, T.T., Richard, S.T., Sédrick, D.T., and Jules, R.K. (2015). Effect of *Salmonella typhimurium* infection on rat's cell oxidation and in vivo antioxidant activity of *Vitellaria paradoxa* and *Ludwigia abyssinica* aqueous extract. *Asian Pacific Journal of Tropical Diseases*. 5(1): 38 – 46.
- Cheesbrough, M. (2002). Medical laboratories manual for tropical countries. *Tropical Health Technology and Butterworth* London, 2: 479.
- Asuzu, I. U. and Onu, U. O. (1994). Anthelmintic activity of the ethanolic extract of *Piliostigma thonningii* bark in *Ascaridia galli* infected chickens. *Fitoterapia*, 65(4): 291 - 297.
- Obadoni, B. O. and Ochuko, P. O. (2002). Phytochemical studies and comparative efficacy of the crude extracts of some haemostatic plants in Edo and Delta States of Nigeria. *Global Journal of Pure and Applied Sciences*, 8(2): 203 - 208.

Kumaran, A. and Karunakaran, R. J. (2007). *In vitro* antioxidant activities of methanol extracts of five *Phyllanthus* species from India. *LWT-Food Science and Technology*, 40(2): 344-352.

AOAC. (2010). Official Methods of Analysis. Association of Official Analytical Chemists, 15th Edn. Washington D.C. USA. pp 184.

Saha, B. K., Sarkar, A., Basak, R. and Chatterjee, M. (2001). 1α , 25-dihydroxyvitamin D₃ suppresses the effect of streptozotocin-induced diabetes during chemical rat liver carcinogenesis. *Cell Biology International*, 25(3): 227-237.

Ncube, N. S., Afolayan, A. J. and Okoh, A. I. (2008). Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. *African Journal of Biotechnology*, 7(12): 1797 – 1806.

de Boer, H. J., Kool, A., Broberg, A., Mziray, W. R., Hedberg, I. and Levenfors, J. J. (2005). Anti-fungal and anti-bacterial activity of some herbal remedies from Tanzania. *Journal of Ethnopharmacology*, 96(3): 461 - 469.

Sun, T. and Ho, C. T. (2005). Antioxidant activities of buckwheat extracts. *Food chemistry*, 90(4), 743-749.

Doughari, J. H., El-Mahmood, A. M. and Tyoyina, I. (2008). Antimicrobial activity of leaf extracts of *Senna obtusifolia* (L). *African Journal of Pharmacy and Pharmacology*, 2(1), 007-013.

Owolabi, O. J., Omogbai, E. K. and Obasuyi, O. (2007). Antifungal and antibacterial activities of the ethanolic and aqueous extract of *Kigelia africana* (Bignoniaceae) stem bark. *African Journal of Biotechnology*, 6(14). 1677 – 1680.

Webster, D., Taschereau, P., Belland, R. J., Sand, C. and Rennie, R. P. (2008). Antifungal activity of medicinal plant extracts; preliminary screening studies. *Journal of Ethnopharmacology*, 115(1): 140-146.

Singleton, P. (1999). *Bacteria in Biology, Biotechnology and Medicine*, 4th edition. John Wiley and Sons Ltd, New York, pp 324-337.

- Anjorin, T. S., Ikokoh, P. and Okolo, S. (2010). Mineral composition of *Moringa oleifera* leaves, pods and seeds from two regions in Abuja, Nigeria. *International Journal of Agriculture and Biology*, 12(3): 431-434.
- Yahaya, O., Yabefa, J. A. and Usman, B. (2012). Phytochemical Screening and Antibacterial Activity of *Combretum glutinosum* Extract against Some Human Pathogens. *British Journal of Pharmacology and Toxicology*, 3(5): 233-236.
- El-Tohamy, M. M. and El-Nattat, W. S. (2010). Effect of on Lead-induced oxidative Damage and Reproductive Dysfunction in Male rabbits. *Journal of American Science*, 6(11): 613- 622.
- Akinpelu, A.D., Awotorebo, T.O., Agunbiade, O.M., Aiyegoro, A.O. and Okoh, I.A. (2011). Anti-Vibrio and preliminary phytochemical characteristics of crude methanolic extracts of the leaves of *Dialium guineense* (Wild). *Journal of Medicinal Plants Research*, 5(11): 2398 – 2404.
- Falana, M. B., Bankole, M. O., Omemu, A. M. and Oyewole, O. B. (2012). Antimicrobial potentials of some selected Microorganisms associated with supernatant solution of fermented maize mash omidun from two varieties of maize grains. *African Journal of Microbiology and Research*, 6(19): 4095-4101.
- Hounhouigan, D. J., Nout, M. J., Nago, C. M., Houben, J. H. and Rombouts, F. M. (1993). Characterization and frequency distribution of species of lactic acid bacteria involved in the processing of mawe, a fermented maize dough from Benin. *International Journal of Food Microbiology*, 18(4): 279-287.
- Obadina, A. O., Oyewole, O. B., Sanni, L. O. and Tomlins, K. I. (2006). Bio-preservative activities of *Lactobacillus plantarum* strains in fermenting Cassava ‘fufu’. *African Journal of Biotechnology*, 5(8): 620-623.
- Valenzuela, A. S., Ruiz, G. D., Omar, N. B., Abriouel, H., López, R. L., Cañamero, M. M., Ortega, E. and Galvez, A. (2008). Inhibition of food poisoning and pathogenic bacteria by *Lactobacillus plantarum* strain 2.9 isolated from ben saalga, both in a culture medium and in food. *Food Control*, 19(9): 842-848.

Ma, C. W., Cho, Y. S. and Oh, K. H. (2009). Removal of pathogenic bacteria and nitrogens by *Lactobacillus* spp. JK-8 and JK-11. *Aquaculture*, 287(3-4): 266-270.

Furtado, N. A. J. C., Fonseca, M. J. V. and Bastos, J. K. (2005). The potential of an *Aspergillus fumigatus* Brazilian strain to produce antimicrobial secondary metabolites. *Brazilian Journal of Microbiology*, 36(4), 357-362.

Ayankunle, A. A., Kolawole, O. T., Adesokan, A. A. and Akiibinu, M. O. (2012). Antibacterial activity and sub-chronic toxicity studies of *Vitellaria paradoxa* stem bark extract. *Journal of Pharmacology and Toxicology*, 7(16): 298- 304.

Akinsulire, O. R., Aibinu, I. E., Adenipekun, T., Adelowotan, T. and Odugbemi, T. (2007). *In vitro* antimicrobial activity of crude extracts from plants *Bryophyllum pinnatum* and *Kalanchoe crenata*. *African Journal of Traditional, Complementary and Alternative Medicines*, 4(3): 338-344.

Bibitha, B., Jisha, V. K., Salitha, C. V., Mohan, S. and Valsa, A. K. (2002). Antibacterial activity of different plant extracts. *Indian Journal of Microbiology*, 42(6): 361-363.

Gatsing, D., Nkeugoauapi, C. F. N., Nkah, B. F. N., Kuate, J. R. and Tchouanguep, F. M. (2010). Antibacterial activity, bioavailability and acute toxicity evaluation of the leaf extract of *Alchornea cordifolia* (Euphorbiaceae). *International Journal of Pharmacology*, 6(3): 173-182.

Davicino, R., Mattar, M. A., Casali, Y. A., Graciela, S., Margarita, E. and Micalizzi, B. (2007). Antifungal activity of plant extracts used in folk medicine in Argentina. *Revista Peruana de Biología*, 14: 247-251.

**Extraction, Physicochemical, Phytochemical, Biochemical, GC-MS
Constituents and Environmental Effects of *Petiveria alliacea* Leaves**

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Abstract

The study was carried out to determine the phytochemical compositions, physicochemical parameters, biochemical and Gas Chromatography-Mass Spectroscopy GC-MS analysis of *Petiveria alliacea* leave extracts collected at three different times of the day and the results compared. The extracts were prepared using successive extraction with both non-polar and polar solvents. The physicochemical analysis revealed no significant variation in the three samples. It showed that the extracts are green in colour and acidic with a total ash content of 18.95 %. The phytochemical analysis revealed that flavonoids, terpenoids, alkaloids, saponins, steroids, glycosides, tannins were present in the plant leaves but the composition varies in relation to the time of collection of the samples. GC-MS analysis revealed major constituents such as; 9,12,15-octadecatrienoic acid; 3,7,11,15-Tetramethyl-2-hexadecen-1-ol; Squalene and n-Hexadecanoic

acid in the n-Hexane extracts of the morning and afternoon samples and major constituents of Phytol, α -linolenic acid, Pinane, Palmitic acid and 5-nonadecen-1-ol in the evening sample. In the ethanol extract, n-Hexadecanoic acid, phytol, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol and 9,12-Octadecadienoyl chloride were found in the morning sample; campesterol, 9,12,15-octadecatrienoic acid and ethyl ester in the afternoon sample while Linolenic acid, Nonadecyl cyclohexane and 9,12,15-octadecatrienoate in the evening samples. It is thus concluded that *P.alliacea* leaves contain many bioactive components and that collection times and the extraction medium have significant effects on the chemical compositions of the leaves. These compounds possess many biological properties; however, these results confirmed the influence of the time of collection of the leaves on the composition which affect the biological activity and pharmacological applications of the plant. The variation in the constituents in the samples could be attributed to sunlight effect, since all other conditions are the same. Also leaf extracts of *P.alliacea* collected at three different times of the day showed varying and different toxicological effects when administered to Wistar rats.

Keywords: Phytochemicals, *Petiveria alliacea*, GC-MS, Physicochemical, Biochemical parameters

Introduction

Plant is man's friend in survival, giving him food, fuel and medicine from the days beyond dawn of civilization. Medicinal plants are those plant parts such as (stems, roots, leaves' extracts etc.) that are used in treating and preventing specific ailments and diseases that affect human beings (Akinmoladun *et al.*, 2007). Medicinal plants contain both organic and inorganic constituents, and are found to be rich in one or more individual elements, thereby, providing a possible link to the therapeutic properties of the medicines (Singh and Garg, 1997). Many active compounds include; alkaloids, steroids, tannins, glycosides, volatile oils, fixed oils, resins, phenols and flavonoids, which are deposited in their specific parts, such as leaves, flowers, barks, seeds, stems, fruits, and roots. The beneficial medicinal effects of these plant materials typically result from the combination of some of these secondary products (Tonthubthimthong *et al.*, 2001).

Petiveria alliacea (*P. alliacea*) is a perennial shrub in the pokeweed family (*Phytolaccaceae*), which grows wild in the South and Central American tropics, Africa and the Caribbean (Garcia-gonzalez, 2006). *P. alliacea* is known by a wide number of common names including: anamu in the Dominican Republic, Puerto Rico in Brazil (where it is also known as tipi), mucura in Peru, guine in many other parts of Latin America, mapurite (pronounced Ma-po-reete) in Trinidad, in Mexico as gully root and in Jamaica as guinea hen weed (Mendes, 1986). It is sometimes called "garlic weed" as the stems and roots have a strong garlic odour, which taints the milk and meat of animals that graze on it. *Petiveria alliacea* is described as a tall straight perennial herb, slightly branched and is 0.5 to 1 m tall, with alternate leaves in elliptic form, 6 to 19 cm long and up to 5 cm wide. Its flowers are small and white, bisexual, zygomorphic, slightly imbricate to rather remote; sepals are white or greenish to pinkish, linear-lanceolate to linear-oblong, 3.5-6 mm long with superior ovary; fruit is a cuneiform berry, narrowly oblong achenes, 6-8 mm long with four hooks turned downwards (Illnait, 2007; Alegre and Clavo, 2007); Seeds are solitary, erect and linear.

P. alliacea plants have been used for pain relief, as an anti-influenza, anti-inflammatory, anti-tumor, anti-bacterial, anti-fungal, anti-hyperlipidemia, anti-diabetic, anti-venereal, sedative, anti-helminthic, emmenagogue, anesthetic, and anti-cancer drug (Schmelzer and Gurib-Fakim, 2008; TPD, 2011; Hernandez *et al.*, 2014). It is used in folk medicine to enhance memory and in the treatment of common cold, flu, other viral or bacterial infections (Kim *et al.*, 2006), and it has depurative properties (De Lima *et al.*, 1991).

Due to the various reported biological effects of this plant, there arises a need to screen the plant for bioactive compounds. Also, secondary metabolism is known for showing response patterns related to environmental stimuli, and it is thought that environmental temperature might play a major role in the composition and metabolic activity of the plant (Blank *et al.*, 2005). This study aimed at carrying out phytochemical, physicochemical, and GC-MS analysis on the leaves' extracts of *P. alliacea* and assessing the effects of collection time and extraction medium on the chemical constituents. Similarly, despite the therapeutic uses of medicinal plants and medicinal plant products, the toxicological effects and effective dosage of most of these plants has not been

investigated to the best of our knowledge. Therefore, this research also determined the toxic and safety effects of n-hexane and ethanol extracts of *P. alliacea* leaves, collected at different times of the day (morning, afternoon and evening).

Materials and Methods

Chemicals and reagents

The assay kits for urea, creatinine, albumin (ALB), alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were products of RANDOX Laboratory Limited, Cumlin, UK. All other reagents used were of analytical grade and supplied by Sigma-Aldrich Inc., St. Louis, USA.

Distilled water, double-distilled ethanol and n-hexane, Ferric chloride, glacial acetic acid, chloroform, hydrochloric acid, concentrated sulphuric acid and Dragendorff reagent.

Plant Sample Collection and Preparation to Powder Form

The leaves of *P. alliacea* were collected at a flower garden in Ilorin, Kwara state, Nigeria at three different times (6:00 am, 1:00 pm and 6:00 pm) of the day. Identification was done at the Herbarium of the Department of Plant Biology, University of Ilorin, Ilorin, Kwara State and voucher specimen number UILH/001/1224 was deposited. The leaves were thoroughly washed with water, air dried until constant weights were obtained (163.28, 231.33 and 166.94 g) for morning, afternoon and evening samples respectively. The dried leaves were then pulverized using mortar and pestle and stored in an air tight, sealed plastic container at room temperature till the time of extraction.

Preparation of Plant Extracts

All solvents used in the extraction process were double-distilled using a distillation apparatus. The *P.alliacea* leaves' extracts were prepared using cold successive extraction at room temperature. The pulverized samples were soaked in extract jars with double-distilled n-hexane, left for three days (72 hours) with frequent agitation and then decanted. Double-distilled ethanol solvent was successively added to the samples in the jars and also left for another three days (72 hours) with frequent agitation

before being decanted. The extracts were then filtered using a Whatmann No.1 filter paper (Sringfield, Maidstone, Kent and England) and concentrated using a rotary evaporator (Buchi Rotavapor R110 Laboratories-Technik Ag. CH-9230 FLAWIL/SCHWE12, made in Switzerland) at 40 °C and kept at a temperature of 4°C until further analysis.

Experimental Animals

Twenty-one (21) healthy adult male Wistar rats weighing 165.5 g were obtained from the Animal House of Biochemistry Department, University of Ilorin, Ilorin, Nigeria. The animals were housed in well-ventilated plastic cages with sawdust as beddings and fed on standard rodent feed and water *ad libitum*. The animals were acclimatized for two weeks before treatment. Appropriate ethical clearance had been obtained.

Animal Grouping

All rats were maintained under standard laboratory conditions (12-h light/dark cycle, $25 \pm 2^{\circ}\text{C}$). The animals were then randomly distributed into seven groups, each with three rats. Group A is the positive control and Groups B to G are the test groups. Table 1 shows a list of the groups.

Table 1: Animal Grouping

Test Groups	Extracts administered daily
A	Control: distilled water
B	<i>Petiveria</i> ethanol morning
C	<i>Petiveria</i> n-hexane morning
D	<i>Petiveria</i> ethanol afternoon
E	<i>Petiveria</i> n-hexane afternoon
F	<i>Petiveria</i> ethanol evening
G	<i>Petiveria</i> n-hexane evening

Collection of Blood Samples and Preparation of Serum

After two weeks of administration of the extracts, the animals were sacrificed after inhalational anaesthesia with di-ethyl ether. This was followed by blood collection by cardiac puncture into sterile plain bottles for analysis. Blood samples were allowed to stand for 30 minutes, after which it was centrifuged at 1000 g for 10 minutes. The collected serum after centrifugation were appropriately labeled and stored in a freezer at -4 °C until required for analysis.

Isolation and Homogenization of Tissues

During the sacrifice, the rats were dissected and a known weight of selected tissue were isolated (liver, heart and kidney). The isolated tissues were cleaned with cotton wool to remove blood stains, weighed and immediately stored in ice cold 0.25 M sucrose solution. The isolated organs were subjected to homogenization in ice-cold 0.25 M sucrose solution (1:5 w/v). The homogenates were stored in the freezer (-4 °C) before used for analysis.

Preliminary Phytochemical Screening of Extracts

Qualitative tests for the presence of active compounds such as steroids, glycosides, saponins, alkaloids, flavonoids, tannins and phenols were carried out on the n-hexane and ethanol extracts, using standard procedures (Harborne, 1984, Trease and Evans, 1989, Sofowora, 1996).

Physicochemical Analysis

The pH, specific gravity, colour, moisture and ash content of *P. alliacea* leaves' extracts were determined. The pH of each extract solution was measured using a pH meter (Combo meter, New Zealand) and the colour was determined by measuring the absorbance of the extract solution on a Beckman Coulter DU 730 UV/VIS spectrophotometer. The moisture and ash contents were determined using standard methods (AOAC, 1980).

GC-MS Analysis

Three gram (3 g) each of the *P. alliacea* leave extracts were re-dissolved in 5 ml n-hexane and ethanol in two different sample tubes before they were subjected to GC-MS analysis for characterization.

Some Biological activities of reported Bioactives in *Petiveria*

The biological activities of some of the compounds identified as published by a researcher are as summarized in Table 2.

Table 2: Biological activities of reported Bioactives

S/No	Name of compounds	Compound's nature	Biological Activity
1	Hexadecanoic acid, methyl ester	Organic acid	Antimicrobial
2	n-Hexadecanoic acid	Palmitic acid	Antimicrobial, Lubricant, Antioxidant, Hemolytic, Hypocholesterolemic, Nematicide, Pesticide, Antiandrogenic, Flavour.
3	Squalene	Triterpene	Antibacterial, anticancer, Antioxidant, Pesticide, Antitumor, Immunostimulant, Chemo preventive, Lipoxxygenase-inhibitor, Adjuvant
4	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Terpene alcohol	Antimicrobial, Antituberculosis, insecticidal, anti-inflammatory, antioxidant
5	Phytol	Diterpene	Antimicrobial, Anticancer, Cancer preventive, Diuretic Anti-inflammatory.
6	Hexadecanoic acid, ethyl ester	Ester compound	Antioxidant, Flavor, Hypocholesterolemic, Nematicide, Pesticide, Lubricant, Antiandrogenic, Hemolytic, 5-Alpha reductase inhibitor
7	Hexadecanoic acid, methyl ester	Fatty acid ester	Antioxidant, Flavor, Hypocholesterolemic, Pesticide, 5-Alpha reductase inhibitor
8	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	Fatty acid ester compound	Antiinflammatory, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Nematicide, Insectifuge, Antihistaminic, Antiarthritic, Anticoronary, Antieczemic, Antiacne, 5-Alpha reductase inhibitor, Antiandrogenic,
9	Stigmasterol		Anti-inflammatory, inhibit tumor promotion, anti-HIV reverse transcriptase, anti-inflammatory
10	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R*-R*-(E)]-	Phytol	Antimicrobial, Anticancer, Anti-inflammatory, Diuretic

Source: Duke's Phytochemical and Ethnobotanical Databases (2016)

Roles of identified fatty acids in soap making

The fatty acids; stearic acid, palmitic acid, myristic acid, lauric acid and oleic acid contribute to lathering and washing properties of the soaps. These are characteristically different from soaps made from divalent metals such as calcium, magnesium, iron or aluminum which are not water soluble (Shoge, 2011).

Cosmetic effects

The presence of phytochemicals such as antioxidants, vitamins, proteins, tannins, terpenoids and other bioactive ingredients help rejuvenate, freshen and protect the hair and skin from various skin and hair conditions such as psoriasis, eczema, skin dryness, skin cancers, sun burn, skin dryness, boil, solar keratosis, dermatitis, impetigo, candidiasis, athlete's foot, chicken pox, carbuncles, staph infections, cyst, abscess, cracking, dandruff, flaking and others.

Increased attention has been given to the use of natural antioxidants for prevention of diseases caused by oxidative damage in human body and/or by lipid peroxidation in food (Teow *et al.*, 2007).

Biochemical Assays

Total Protein Determination

The protein content of the serum and homogenates was determined using the Biuret method as previously described by Sulaiman and Adeyemi, 2010.

Determination of other Biochemical Parameters

The concentrations of urea, creatinine and albumin (ALB), as well as the enzyme activities of alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were done according to the methods described in the RANDOX Laboratory assay kits.

Statistical Analysis

All data were expressed as the mean of 3 replicates $\pm$ standard error of mean (S.E.M). Statistical evaluation of data was performed by Graph pad prism version 5.02 using one way analysis of variance (ANOVA). Post-Hoc test analysis was done using the Tukey's Multiple Comparison Test. Values were considered statistically significant at $P < 0.05$.

Results and Discussion

Results of the Phytochemical Analysis

The results of the phytochemical analysis carried out on the extracts of *Petiveria alliacea* leaves collected in the morning (6:00 am.), afternoon (1:00 pm) and evening (6:00 pm) are as summarized in table 4:

Table 4: Phytochemical composition of *Petiveria alliacea* leaves collected at different times of the day

Phytochemicals	Morning (6:00 am)		Afternoon (1:00 pm)		Evening (6:00 pm)	
	n-Hexane	Ethanol	n-Hexane	Ethanol	n-Hexane	Ethanol
Alkaloids	+	+	-	-	+	-
Tannins	+	+	-	+	-	+
Flavonoids	+	+	+	-	-	+
Glycosides	+	+	+	+	-	+
Steroids	+	-	+	+	-	-
Saponins	+	+	-	+	+	+
Phenols	-	-	-	+	-	-
Terpenoids	+	+	+	-	+	-

Key: + present: - absent

The phytochemical screening of the n-hexane and ethanol extracts of the leaf of *Petiveria alliacea* revealed that tannins, glycosides, terpenoids, flavonoids and saponins are present in almost all the extracts, which shows that the plant is rich in these metabolites.

Flavonoids, tannins and phenols are phenolic compounds; they are a major group of compounds that act as primary antioxidants or free radical scavengers. Since these compounds were found to be present in the extracts, they may be responsible for the potent antioxidant capacity of *Petiveria* leaf. Previous studies have also reported that phenolic compounds possess other biological activities such as anti-inflammatory,

anti-ulcer, anti-spasmodic and anti-diarrheal (Galm and Bashen, 2007; Djeridane *et al.*, 2006). Alkaloids, according to literature, possess anti-inflammatory, anti-asthmatic, and anti-anaphylatic properties with consequences of altering immunological status *in vivo* study (Johnson *et al.*, 2012). Saponins are special class of glycosides that have been shown to possess anti-fungal activity, hypolipidemic and anti-cancer activity (Sarker and Nahar, 2007). Steroids have also been reported to possess antibacterial, anti-inflammatory, anti-ulcerative and anti-tumor actions (Kim *et al.*, 2006).

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Saponins are special class of glycosides that have been shown to possess anti-fungal activity, hypolipidemic and anti-cancer activity (Sarker and Nahar, 2007). Steroids have also been reported to possess antibacterial, anti-inflammatory, anti-ulcerative and anti-tumor actions (Kim *et al.*, 2006). However, there is variation in the phytochemicals obtained from the leaves in relation to the collection times. For instance, alkaloids are present in both n-hexane extracts of leaves collected in the morning and in the evening but absent in those collected in the afternoon (Table 3). Also, Phenol is absent in all the extracts except in the ethanol extract of leaves harvested in the afternoon while steroids are present in both morning and afternoon samples but not in the evening sample. These observations are indication that the time of collection depends on the ailment to be treated that is the metabolite of interest.

Physicochemical Analysis of Extracts

The results are as summarized in Tables 4a and 4b:

Table 4a: Results of physical parameters of *Petiveria alliacea* leaves collected at different times of the day

Physicochemical Parameters	Morning		Afternoon		Evening	
	n-Hexane	Ethanol	n-Hexane	Ethanol	n-Hexane	Ethanol
pH	4.58	5.836	6.955	6.342	6.129	5.777
Color	Green	Green	Green	Green	Green	Green
Specific gravity	0.8355	0.8148	0.633	0.801	0.7147	0.8299

Table 4b: Result of physicochemical parameters of *Petiveria alliacea* leaves collected at different times of the day

Physicochemical Parameter	Morning (6:00 a.m.)	Afternoon (1:00 p.m.)	Evening (6:00 p.m.)
Moisture content (%)	79.00	80.94	80.74
Total ash content (%)	18.59	18.59	18.59

As shown in table 4b, there was no significance variation in the physicochemical parameters in relation to the collection time. A total ash content of 18.59 % indicates that the amount of organic matters and other minerals in the leaves of *P. alliacea* is slightly high. It also showed that the extract is acidic (4.58) and green in colour (Table 4a).

Results of GC-MS Analysis

As revealed by GC-MS analysis, in n-hexane extracts 14 compounds were present in the morning, 17 compounds in the afternoon and 10 compounds in the evening (Table 5). However, in ethanol extracts, 13 compounds were present in the morning, 11 in the afternoon and 17 compounds in the evening extracts (Table 6).

Table 5: GC-MS results of n-hexane extracts of morning, afternoon and evening samples

S/No	Morning	%	Afternoon	%	Evening	%
1	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	16.80	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	17.22	Phytene 2	1.32
2	2(4H)-Benzofuranone,5,6,7,7a tetrahydro-4,4,7a trimethyl-, (R)-	1.49	2(4H)-Benzofuranone,5,6,7,7a-tetrahydro-4,4,7a-trimethyl	1.20	Pinane	11.21
3	2-Hexadecene,3,7,11,15-tetramethyl-[R-[R*,R*-(E)]]-	1.76	2-Hexadecen-3,7,11,15-Tetramethyl	1.51	5-nonadecen-1-ol	6.08
4	N-Hexadecanoic acid	11.07	N-Hexadecanoic acid	9.38	Palmitic acid	9.86
5	Hexadecanoic acid, ethyl ester	1.99	Hexadecanoic acid, ethyl ester	5.53	Squalene	2.10
6	Phytol	9.22	Phytol	8.02	Phytol	43.97
7	9,12,15-Octadecatrienoic acid,	32.51	9,12,15-octadecatrienoic acid	22.06	Ethyl palmitate	1.42
8	Octadecanoic acid	2.19	Ethyl-9,12,15-octadecatrienoate	9.23	Alpha linolenic acid	15.13
9	Ethyl iso-allocholate	4.04	Ethyl iso-allocholate	1.79	Linolenic acid, ethyl ester	5.73
10	Cholestan-3-one, cyclic 1, 2-ethanediyl acetal	1.02	4,8,12,16-	0.69	Palmitin	3.19

	(5 β)		Tetramethylheptadecan-4-olide			
11	7, 8- Epoxy lanostan-11-ol, 3-acetoxy-	2.31	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediylester	1.62	-	
12	Diisooctyl phthalate	2.11	Diisooctylphthalate	1.76	-	
13	Stigmasterol	1.42	1-heptatriacotanol	2.67	-	
14	Squalene	14.81	Squalene	12.57	-	
15	-		Octadecanoic acid, ethyl ester	1.30	-	
16	-		Docosanoic acid-1,2,3-propanetriylester	1.13	-	
17	-		Oleic acid, eicosyl ester	2.33	-	

NOTE:- sign – denote no compound detected.

From the GC-MS data, the variation in the compositions is morning < afternoon > evening for n-hexane sample extracts but significantly high in the afternoon while the order is morning > afternoon < evening for ethanol sample extracts but significantly high in the evening sample. Many compounds present in both the n-hexane extracts of the morning and afternoon samples; include 2(4H)-Benzofuranone, 5,6,7, 7a tetrahydro-4,4, 7a trimethyl-, (R)- ; 3,7,11,15-Tetramethyl-2-hexadecen-1-ol; 2-Hexadecene, 3,7,11,15-tetramethyl-[R-[R*,R*-(E)]]-; N-Hexadecanoic acid; Hexadecanoic acid, ethyl ester; Phytol; Ethyl iso-allocholate; Squalene and 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- in varying concentrations (Table 5).

In the ethanol extract, also present in the morning and afternoon samples are; Phenol, 2,4-bis(1,1-dimethylethyl); 3,7,11,15-Tetramethyl-2-hexadecen-1-ol; Hexadecanoic acid, methyl ester; Hexadecanoic acid, ethyl ester; 9,12,15-octadecatrienoic acid, ethyl ester; Stigmasterol; and Campesterol, n-Hexadecanoic acid, and Phytol are also present in the evening sample (Table 6).

Table 6: GC-MS result of Ethanol extracts of morning, afternoon and evening samples

S/No.	Morning	%	Afternoon	%	Evening	%
1	1-(2-Hydroxyethyl)-1,2,4-triazole	2.47	Cholestan-3-ol,2-methylene-	5.57	Nonadecyl cyclohexane	9.15
2	Phenol, 2,4-bis(1,1-dimethyl)	2.06	Phenol,2,4-bis(1,1-dimethylethyl	2.88	2(E)-hexenoicacid (4s)-amino 5-methyl	5.79
3	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	8.46	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	4.12	Ethylene diammine	1.51
4	n-Hexadecanoic acid	10.37	n-Hexadecanoic acid	7.48	n-Hexadecanoic acid	13.05
5	Hexadecanoic acid, methyl ester	2.35	Hexadecanoic acid, methyl ester	1.79	2,4-di-tert butyle phenol	1.90
6	Hexadecanoic acid, ethyl ester	1.49	Hexadecanoic acid,ethyl ester (3.19 %)	3.19	2,6,6-trimethyl-bicyclo(3,1,1) heptane	1.12
7	Phytol	27.15	Phytol (36.68 %)	36.68	Phytol	24.72
8	9,12-Octadecadienoyl chloride, (Z,Z)-	10.66	Cholest-5-en-3-ol,24-pyrolidene (3.12 %)	3.12	4-hydroxy-trimethyl pyroline	5.82
9	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	17.08	9,12,15-octadecatrienoic acid,ethyl ester (20.15 %)	20.15	9,12,15-octadecatrienoate	7.57
10	Stigmasterol	2.32	Stigmasterol	1.16	Methyl palmitate	2.11
11	Campesterol	3.94	Campesterol	13.87	Linolenic acid	14.70
12	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl	3.15	-		Methyl ricinoleate	3.31

	ester					
13	1-Heptatriacotanol	2.73	-		Cyclopropane (3-chloropropyl) methylene	1.45
14	-		-		Fumaric acid, hexadecyl propagyl ester	1.38
15	-		-		Octadecanedioic acid	4.21
16	-		-		3-methoxy,-3,4-dimethyl-1-heptyne	1.30
17	-		-		2-oxa-spiro(4.5) dec-8-ene-1,7,-dione, 4,6-dihydroxy-3,10-dimethyl	0.86

Key: - absent

The main constituents in the n-hexane extract of the leaves plucked in the morning and afternoon include: 9,12,15-octadecatrienoic acid; 3,7,11,15-Tetramethyl-2-hexadecen-1-ol; Squalene and n-Hexadecanoic acid while Phytol, α -linolenic acid, Pinane, Palmitic acid and 5-nonadecen-1-ol are the major constituents in the evening extract (Table 5). Furthermore, main constituents present in the ethanol extract of the morning, afternoon and evening samples include; n-Hexadecanoic acid, phytol, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol and 9,12-Octadecadienoyl chloride, (Z,Z)- in the morning sample, campesterol, 9,12,15-octadecatrienoic acid, and ethyl ester in the afternoon sample and Linolenic acid, Nonadecyl cyclohexane and 9,12,15-octadecatrienoate in the evening sample (Table 6).

Result of Biological Activities

These compounds possess many biological properties; however, the influence of the time of collection of the leaves on the composition affects the biological activity and pharmacological applications of the plant. The variation in the constituents in the samples could be attributed to sunlight effect, since all other conditions are the same. The biological activities of

some of the compounds identified as published by a researcher are as summarized in Table 2.

Table 7 shows the effects of ethanol and n-hexane extracts of *P. alliacea* collected at three different times of the day on average weight of rats over two weeks of administration (treatment) compared to acclimatization weeks (pre-treatment) average weight respectively. In the first week of acclimatization, the experimental rats in Groups B to G (186.1, 191.8, 189.5, 191.6, 178.2 and 182.3 g respectively) when compared with the Positive control Group A (182.3 g) shows no significant difference ($P > 0.05$). In the second week, the rats in Groups B to G recorded significant difference in weight across the groups. As the plant extracts administration was introduced to the experimental rats in the third week, all *P. alliacea* administered groups recorded further slight decrease in weights across the groups, which continued into the fourth week.

Table 7 shows there were no significant decrease in the average weight of rats administered with ethanol and n-hexane extracts of *P. alliacea* over two weeks of administration. The slight weight reductions recorded across the weeks of administration per group were not significant enough to cause any harm in the experimental rats. This suggests that the n-hexane and ethanol extracts of *P. alliacea* did not cause any form of damage, swelling or inflation (Sulaiman *et al.*, 2014a).

Table 7: Effects of Ethanol and n-hexane Extracts of *P. alliacea* collected at three different times of the day on Average Weight of Rats over Two Weeks of Treatment vs Pre-treatment.

Groups	Week one	Week two	Week three	Week four
A	182.3 $\pm$ 19.43 ^a	174.4 $\pm$ 17.63 ^{ab}	176.4 $\pm$ 16.57 ^{ab}	169.7 $\pm$ 18.89 ^{ac}
B	186.1 $\pm$ 19.48 ^a	177.6 $\pm$ 18.39 ^{ab}	179.3 $\pm$ 17.17 ^{ab}	173.3 $\pm$ 19.03 ^{ab}
C	191.8 $\pm$ 19.48 ^a	182.1 $\pm$ 19.10 ^{ab}	183.6 $\pm$ 17.90 ^{ab}	178.1 $\pm$ 20.19 ^{ac}
D	189.5 $\pm$ 17.71 ^a	178.5 $\pm$ 16.50 ^{ab}	180.2 $\pm$ 15.97 ^{ab}	173.2 $\pm$ 19.41 ^{ab}
E	191.6 $\pm$ 20.53 ^a	175.7 $\pm$ 18.66 ^b	176.8 $\pm$ 18.21 ^b	168.2 $\pm$ 21.97 ^c
F	178.2 $\pm$ 04.96 ^a	171.6 $\pm$ 05.37 ^a	172.9 $\pm$ 09.95 ^a	168.0 $\pm$ 20.47 ^{ab}
G	182.3 $\pm$ 19.43 ^a	174.4 $\pm$ 17.63 ^{ab}	176.4 $\pm$ 16.57 ^{ab}	169.7 $\pm$ 18.89 ^b

Values are expressed as mean of three replicates $\pm$ S.E.M and those with different superscripts across the group are significantly different at ($P < 0.05$). (A): Control; (B): Morning Ethanol *P. alliacea*; (C): Morning n-hexane *P. alliacea*; (D): Afternoon Ethanol *P. alliacea*; (E): Afternoon n-hexane *P. alliacea*; (F): Evening Ethanol *P. alliacea*; (G): Evening n-hexane *P. alliacea*)

Table 8 shows the effects of ethanol and n-hexane extracts of *P. alliacea* collected at three different times of the day on weight of organs. For the liver, Kidney and Heart weights, there was no significant difference ($P > 0.05$) between the three control groups (5.62), (0.58), (1.15) and all the treated groups.

Table 8: Effects of Ethanol and n-hexane Extracts of *P. alliacea* on weight of organs

Groups	Liver	Heart	Kidney
A	5.62 $\pm$ 0.62 ^a	0.58 $\pm$ 0.06 ^a	1.15 $\pm$ 0.09 ^a
B	5.56 $\pm$ 0.72 ^a	0.61 $\pm$ 0.07 ^a	1.19 $\pm$ 0.06 ^a
C	5.60 $\pm$ 0.72 ^a	0.62 $\pm$ 0.08 ^a	1.17 $\pm$ 0.06 ^a
D	5.75 $\pm$ 0.74 ^a	0.65 $\pm$ 0.08 ^b	1.18 $\pm$ 0.07 ^a
E	5.67 $\pm$ 0.71 ^a	0.68 $\pm$ 0.08 ^b	1.19 $\pm$ 0.06 ^a
F	5.50 $\pm$ 0.80 ^a	0.70 $\pm$ 0.08 ^b	1.20 $\pm$ 0.07 ^a
G	6.33 $\pm$ 0.33 ^b	0.57 $\pm$ 0.01 ^a	1.10 $\pm$ 0.01 ^a

Values are expressed as mean of three replicates $\pm$ S.E.M and those with different superscripts down the group are significantly different at ($P < 0.05$). (A): Control; (B): Morning Ethanol *P. alliacea*; (C): Morning n-hexane *P. alliacea*; (D): Afternoon Ethanol *P. alliacea*; (E): Afternoon n-hexane *P. alliacea*; (F): Evening Ethanol *P. alliacea*; (G): Evening n-hexane *P. alliacea*)

Organ-body weight ratio is a useful marker of cellular swelling, atrophy or hypertrophy (Amresh *et al.*, 2008; Sulaiman *et al.*, 2017). Therefore, the absence of an increase in the computed organ-body weight ratios (Table 8) and lack of significant decrease in organ weights recorded (Table 9) suggests that the n-hexane and ethanol extracts of *P. alliacea* did not cause any form of swelling, atrophy and hypertrophy on the organs of interest within the experimental rats as previous studies (Sulaiman *et al.*, 2015; Oluyomi *et al.*, 2016) have associated changes to body weight with toxicity of the drugs, chemicals or extracts administered.

Table 9 shows the effects of ethanol and n-hexane extracts of *P. alliacea* collected at three different times of the day on Organ/Body-Weight ratio. For the liver, heart and kidney, there was no significant difference ($P > 0.05$) between the three Control groups (3.45), (3.59), (0.71) respectively and all the other treated groups (B to G down the group).

Table 9: Effects of Ethanol and n-hexane Extracts of *P. alliacea* collected at three different times of the day on Organ Body-Weight ratio

Groups	Liver	Heart	Kidney
A	3.50 ± 0.52^a	0.36 ± 0.05^a	0.71 ± 0.09^a
B	3.43 ± 0.65^a	0.38 ± 0.06^a	0.73 ± 0.27^a
C	3.34 ± 0.52^a	0.37 ± 0.07^a	0.70 ± 0.09^a
D	3.36 ± 0.56^a	0.38 ± 0.07^a	0.69 ± 0.10^a
E	3.39 ± 0.54^a	0.41 ± 0.08^a	0.71 ± 0.11^a
F	3.44 ± 0.59^a	0.44 ± 0.09^a	0.75 ± 0.12^a
G	3.97 ± 0.37^b	0.35 ± 0.03^a	0.66 ± 0.09^a

Values are expressed as mean of three replicates $\pm$ S.E.M and those with different superscripts down the group are significantly different at ($P < 0.05$). (A): Control; (B): Morning Ethanol *P. alliacea*; (C): Morning n-hexane *P. alliacea*; (D): Afternoon Ethanol *P. alliacea*; (E): Afternoon n-hexane *P. alliacea*; (F): Evening Ethanol *P. alliacea*; (G): Evening n-hexane *P. alliacea*).

Aspartate Transaminases (AST, E.C. 2.6.1.1)

Figure 1 shows the AST Activity (U/I) in the serum of rats administered with n-hexane and ethanol extracts of *Petiveria alliacea*. The AST Activity was significantly higher ($P < 0.05$) in the serum of rats administered with Afternoon n-hexane, Evening Ethanol extracts and Evening n-hexane extracts of *P. alliacea* in Groups E, F and G with a

concurrent decrease ($P < 0.05$) in AST activity in the hearts of rats in the same groups.

The AST activity in the heart of rats administered with three of the extracts in Groups B, C and D when compared with the control Group A shows a significant increase ($P < 0.05$) as shown in figure 1.

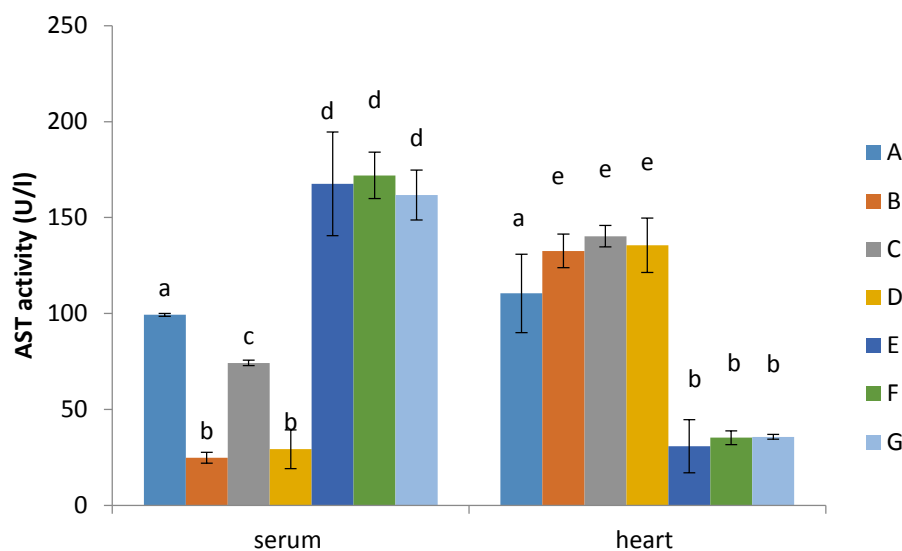


Figure 1: Effects of ethanol and n-hexane extract of *Petiveria alliacea* leaf on AST activity in Wistar rats. Values are mean $\pm$ SEM of 3 replicates and those with different alphabets on the bars are significantly different at ($P < 0.05$). (A): Control; (B): Morning Ethanol *P. alliacea* C): Morning n-hexane *P. alliacea*; (D): Afternoon Ethanol *P. alliacea*; (E): Afternoon n-hexane *P. alliacea*; (F): Evening Ethanol *P. alliacea*; (G): Evening n-hexane *P. alliacea*.

However, the AST activity was significantly reduced ($P < 0.05$) in the serum of rats administered, Morning Ethanol *P. alliacea* (Group B): Morning n-hexane *P. alliacea*; (Group C) and Afternoon Ethanol *P. alliacea* (Group D) when compared with the control (Group A).

Alanine Transaminases

Figure 2 shows the ALT Activity (U/I) in the serum of rats administered with the six leaf extracts of *Petiveria alliacea*. No significant difference (P

> 0.05) in ALT Activity was recorded in the serum of all rats administered with various extracts of *P. alliacea* in Groups B to G when compared to the control (Group A). Similarly, The ALT activity in the liver of rats administered with the six extracts in Groups B to G when compared with the control Group A shows no significant increase ($P > 0.05$).

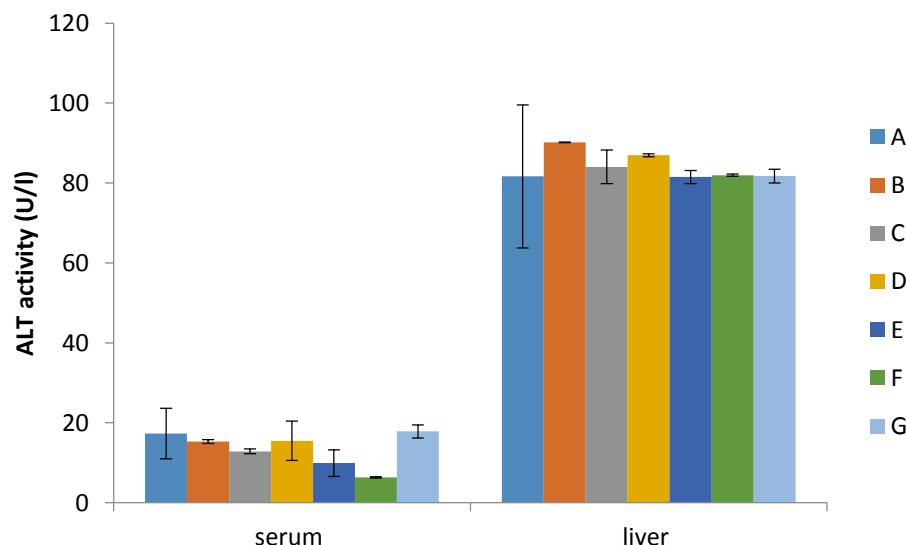


Figure 2: Effects of ethanol and n-hexane extract of *Petiveria alliacea* leaf on ALT activity in Wistar rats. Values are mean $\pm$ SEM of 3 replicates and those with different alphabets on the bars are significantly different at ($P < 0.05$). (A): Control; (B): Morning Ethanol *P. alliacea* (C): Morning n-hexane *P. alliacea*; (D): Afternoon Ethanol *P. alliacea*; (E): Afternoon n-hexane *P. alliacea*; (F): Evening Ethanol *P. alliacea*; (G): Evening n-hexane *P. alliacea*.

Alkaline Phosphatase (ALP, E.C. 3.1.3.1)

Figure 3 shows the ALP Activity (U/I) in the serum, liver and kidney of rats administered with n-hexane and ethanol extracts of *Petiveria alliacea*. The ALP activity in the serum of rats administered with the six extracts were significantly lower ($P < 0.05$) in all the groups than the control. However, the ALP activity was significantly reduced ($P < 0.05$) in the serum of rats administered with the last three extracts in Groups E, F, and G than the first three groups; B, C and D.

The ALP Activity in the liver of rats administered with extracts in Groups B, C, D, and E when compared with the control Group A shows no significant difference ($P > 0.05$) (figure 3). However, the ALP Activity was significantly reduced ($P < 0.05$) in the liver of rats administered with the last two extracts in Groups F and G when compared with the control group.

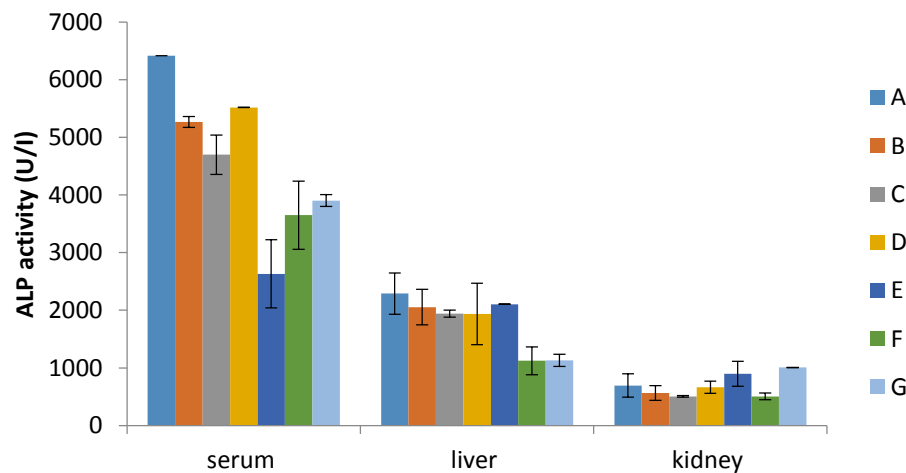


Figure 3: Effects of ethanol and n-hexane extract of *Petiveria alliacea* leaf on ALP activity in Wistar rats. Values are mean $\pm$ SEM of 3 replicates and those with different alphabets on the bars are significantly different at ($P < 0.05$). (A): Control; (B): Morning Ethanol *P. alliacea* (C): Morning n-hexane *P. alliacea*; (D): Afternoon Ethanol *P. alliacea*; (E): Afternoon n-hexane *P. alliacea*; (F): Evening Ethanol *P. alliacea*; (G): Evening n-hexane *P. alliacea*.

Also, the ALP activity in the kidney of rats administered with extracts in Groups B-G (666, 687, 694, 684, 681 and 690) when compared with the control Group A(668) shows no significant difference ($P > 0.05$) (figure 3).

Albumin

Figure 4 shows the albumin concentration (mg/dl) in the serum of rats administered with n-hexane and ethanol extracts of *Petiveria alliacea*. The albumin concentration (mg/dl) in the serum of rats administered with

extracts in Groups B, C, D when compared with the control Group A shows no significant difference ($P > 0.05$). However, the albumin concentration was significantly higher ($P < 0.05$) in the serum of rats administered with extracts in Groups E, F and G when compared with the control (group A).

The albumin concentration (mg/dl) in the liver of rats administered with extracts in Groups C and F when compared with the control Group A are significantly higher ($P < 0.05$), while no

significant difference ($P > 0.05$) was recorded in the albumin concentration of the liver of rats administered with extracts in Groups B, D, E and G when compared with the control (group A).

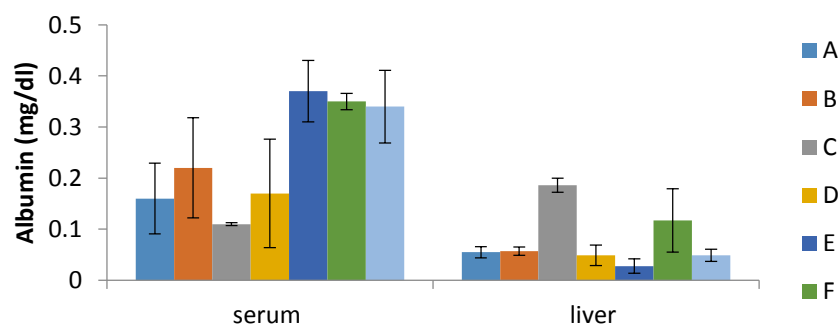


Figure 4: Effects of ethanol and n-hexane extract of *Petiveria alliacea* leaf on albumin concentration in serum and liver of Wistar rats. Values are mean $\pm$ SEM of 3 replicates and those with different alphabets on the bars are significantly different at ($P < 0.05$). (A): Control; (B): Morning Ethanol *P. alliacea* (C): Morning N-hexane *P. alliacea*; (D): Afternoon Ethanol *P. alliacea*; (E): Afternoon n-hexane *P. alliacea* F): Evening Ethanol *P. alliacea*; (G): Evening n-hexane *P. alliacea*.

Urea

Figure 5 shows the urea concentration (mg/dl) in the serum and kidney of rats administered with n-hexane and ethanol extracts of *Petiveria alliacea*. The urea concentration (mg/dl) in the serum of rats administered with the two extracts in Groups C to G (12.1, 12.6, 13.5 11.9 and 13.5) were

significantly reduced ($P < 0.05$) but no significant decrease in group B (24.9) when compared with the control Group A (28.5).

The urea concentration (mg/dl) in the kidney of rats administered with extracts in Groups B to F (13.2, 12.7, 15.6, 14.9, 16.7) when compared to control Group A (34.3) showed a significant decrease ($P < 0.05$) while no significant decrease was recorded in the kidney of rats in Group G.

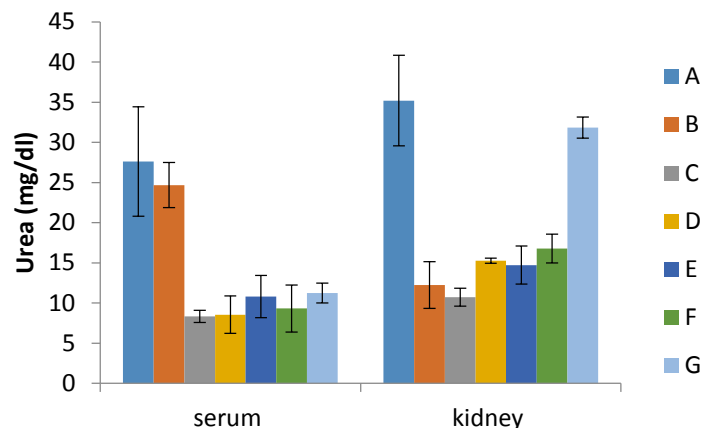


Figure 5: Effects of ethanol and n-hexane extract of *Petiveria alliacea* leaf on urea concentration in serum and kidney of Wistar rats. Values are mean $\pm$ SEM of 3 replicates and those with different alphabets on the bars are significantly different at ($P < 0.05$). (A): Control; (B): Morning Ethanol *P. alliacea* (C): Morning n-hexane *P. alliacea*; (D): Afternoon Ethanol *P. alliacea*; (E): Afternoon n-hexane *P. alliacea*; (F): Evening Ethanol *P. alliacea*; (G): Evening n-hexane *P. alliacea*

Total Protein Concentration

Figure 6 shows the protein concentration (mg/ml) in the liver of rats administered with the extracts in all the Groups were significantly reduced ($P < 0.05$) when compared with the control Group A. However the protein concentration (mg/ml) ($P < 0.05$) in the liver of rats administered with extracts in Groups D, E and G were significantly higher ($P < 0.05$) than those recorded in Groups B, C and F, when compared with the control A.

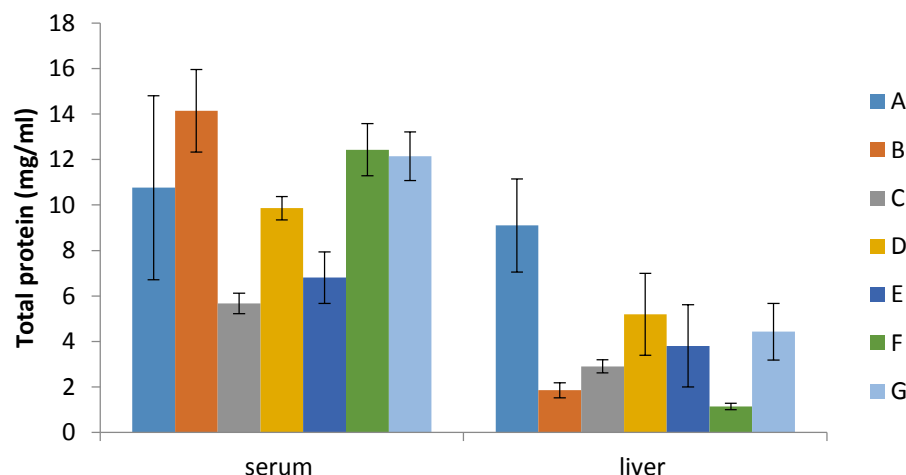


Figure 6: Effects of ethanol and n-hexane extract of *Petiveria alliacea* leaf on total protein concentration in serum and liver of Wistar rats. Values are mean $\pm$ SEM of 3 replicates and those with different alphabets on the bars are significantly different at ($P < 0.05$). (A): Control; (B): Morning Ethanol *P. alliacea* (C): Morning n-hexane *P. alliacea*; (D): Afternoon Ethanol *P. alliacea*; (E): Afternoon n-hexane *P. alliacea*; (F): Evening Ethanol *P. alliacea*; (G): Evening n-hexane *P. alliacea*.

Creatinine Concentration

Figure 7 shows the creatinine concentration (mg/dl) in the kidney of rats administered with n-hexane and ethanol extracts of *Petiveria alliacea*. The creatinine concentration (g/dl) in the kidney of rats administered with extracts in Groups B-G (8.3, 8.1, 7.9, 7.5, 6.9, 7.2) when compared with the control Group A (8.9) was significantly reduced ($P < 0.05$).

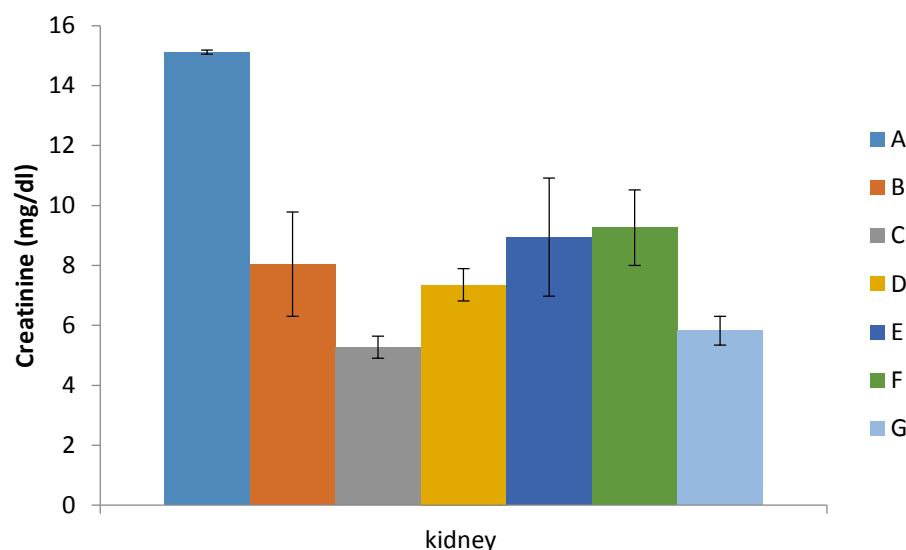


Figure 7: Effects of ethanol and n-hexane extract of *Petiveria alliacea* leaf on creatinine concentration in kidney of Wistar rats. Values are mean $\pm$ SEM of 3 replicates and those with different alphabets on the bars are significantly different at ($P < 0.05$). (A): Control; (B): Morning Ethanol *P. alliacea* (C): Morning n-hexane *P. alliacea*; (D): Afternoon Ethanol *P. alliacea*; (E): Afternoon n-hexane *P. alliacea*; (F): Evening Ethanol *P. alliacea*; (G): Evening n-hexane *P. alliacea*.

Discussion

Aspartate Transaminase (AST) Activity (U/I) in the Serum and Heart of Rats Administered Aqueous Extracts of *Petiveria alliacea* collected at three different times of the day.

The aminotransferases (ALT and AST) are ‘markers’ of liver and heart damage and can thus be used to assess liver and heart cytolysis (Pramyothin *et al.*, 2006; Oluyomi *et al.*, 2016). The high AST activity recorded in the serum of rats administered with Afternoon n-hexane, Evening Ethanol extracts and Evening N-hexane extracts of *P. alliacea* in Groups E, F and G with a concurrent decrease ($P < 0.05$) in AST activity of their hearts may be as a result of the rats’ adaptive mechanism in order to offset the stress imposed by the extracts’ administration (Sulaiman *et al.*, 2015).

The reduced AST activity in the serum of rats administered Morning Ethanol *P. alliacea* (Group B): Morning N-hexane *P. alliacea*; (Group C) and Afternoon Ethanol *P. alliacea* (Group D) when compared with the control (Group A, which shows that AST may not have leaked from the heart into the serum, implying no damage to the cardiac membranes no toxicity.

Alanine Aminotransferase Activity (ALT) (U/I) in the Serum and Liver of Rats Administered Aqueous Extracts of *Petiveria alliacea* collected at three different times of the day.

The measurement of ALT Activity in human serum has proven to be a valuable indicator of liver function in clinical settings (Huang *et al.*, 2006). The aminotransferases (ALT and AST) are ‘markers’ of liver damage and can thus be used to assess liver cytolysis with ALT being a more sensitive biomarker of hepatotoxicity than AST (Pramyothin *et al.*, 2006). The insignificant increase recorded in ALT Activity in the serum of all rats administered the various extracts of *P. alliacea* in Groups B to G as well as the no significant reduction in hepatic ALT activity implies that the integrity of the hepatic membranes were preserved (Oluyomi *et al.*, 2016; Sulaiman *et al.*, 2017) as no leakage has occurred from the liver into the serum.

Alkaline Phosphatase Activities of the Serum and Selected Tissues of Rats Administered Aqueous Extracts of *Petiveria alliacea* collected at three different times of the day.

ALP is present in all tissues throughout the body, but is particularly concentrated in liver, bile duct, kidney, bone, intestinal mucosa and the placenta (Tamas *et al.*, 2002). ALP is a ‘marker’ enzyme for the plasma membrane and endoplasmic reticulum (Wright and Plummer, 1972) and is often used to assess the integrity of the plasma membrane and endoplasmic reticulum (Akanji *et al.*, 1993; Sulaiman *et al.*, 2015; Oluyomi *et al.*, 2016)

The reduced ALP activity in the serum of rats administered with the six extracts in all the groups is a positive indication of little or no toxicity as an increase may have been as a result of membrane compromise which would have allowed ALP’s leakage from other tissues into the serum. The

last three extracts administered to Groups E, F, and G are therefore, even safer for consumption than the first three extracts in Groups B, C and D.

A reduced trend was recorded in the liver with no significant difference recorded in the ALP activity in the liver of rats administered with extracts in Groups B, C, D, and E as well as more reduction in the ALP Activity recorded in the liver of rats administered with the last two extracts in Groups F and G. Also, the low ALP activity in the kidney of rats administered with extracts in Groups B-G may not be unconnected with leakage of ALP from the kidney into the serum,

resulting in the high ALP activity initially recorded in the serum, which still queries the level of safety achievable with the consumption of the extracts.

Albumin Concentration (g/dl) in the Serum and Liver of Rats Administered Aqueous Extracts of *Petiveria alliacea* collected at three different times of the day.

The level of albumin has been associated with the synthetic function of the liver (Adeyemi *et al.* 2010, 2012). Albumin is the major plasma protein with molecular weight of approximately 66.3kDa. It is synthesized exclusively in the liver. The concentration of albumin is a useful 'marker' of secretory, synthetic and excretory functioning of the liver and kidney (Yakubu and Musa, 2012; Sulaiman *et al.*, 2014a). A falling concentration in chronic liver disease suggests a clinically significant deterioration in liver function 'decompensation'.

There was no significant albumin concentration (g/dl) recorded in the serum of rats administered with extracts in Groups B, C, D when compared with the control Group A shows that these three extracts are safe for consumption as they do not alter significantly, the serum albumin concentration of the host.

However, the increased albumin concentration recorded in the serum of the remaining rats administered with extracts in Groups E, F and G when compared with the control (group A) may imply that the membranes of the liver have been ruptured. Similarly, the albumin concentration in the liver of rats administered with extracts in Groups C and F is high, which may

be an indication that more plasma protein has been synthesized (Yakubu and Musa, 2012; Sulaiman *et al.*, 2017a).

Urea Concentration in the Serum and Kidney of Rats Administered Aqueous Extracts of *Petiveria alliacea* collected at three different times of the day.

Serum urea is one of many useful parameters for evaluating the status of renal function. An increase in the level of serum urea may imply renal excretion (Adeyemi and Akanji, 2012). The significance of the enzyme urease includes: to serve as a virulence factor in human and animal infections of the urinary and gastrointestinal tracts, play role in recycling of nitrogenous wastes in the rumens of domestic livestock (Mobley and Hausinger, 1989; Sulaiman *et al.*, 2017b). In this study, the reduced urea concentration (mg/dl) recorded in the serum of rats administered with the two extracts in Groups C-G after the administration of the first five extracts may be attributed to decreased amino acid degradation by the liver. A similar reduction in urea concentration (g/dl) in the kidney of rats administered with extracts in Groups B-G was also recorded except for Group G where no significant increase was recorded.

Protein Concentration in the Serum and Liver of Rats Administered Aqueous Extracts of *Petiveria alliacea* collected at three different times of the day.

The concentration of total protein is a useful 'marker' of secretory, synthetic and excretory functioning of the liver (Sulaiman *et al.*, 2017; Yakubu and Musa, 2012). The total protein is composed of albumin and globulin and reflects the balance of protein biosynthesis and catabolism.

The no significant difference recorded for protein concentration (mg/ml) in the serum of rats administered with extracts in Groups B, D, F and G is a positive safety indication, while the reduced protein concentration (mg/ml) recorded in the serum of rats administered with two of the extracts in Groups C and E may be as a result of decreased protein synthesis, increased protein loss, increased catabolism or protein malabsorption, consequent upon the extracts' administration.

The protein concentration (mg/ml) in the liver of rats administered with the extracts in all the Groups were significantly reduced ($P < 0.05$) when

compared with the control Group A. However, the protein concentration (mg/ml) ($P < 0.05$) in the liver of rats administered with extracts in Groups D, E and G were significantly higher ($P < 0.05$) than those recorded in Groups B, C and F, when compared with the control. Improved protein synthesis with concomitant reduction in loss of protein may have contributed to this.

Creatinine Concentration in the Kidney of Rats Administered Aqueous Extracts of *Petiveria alliacea* collected at three different times of the day.

Creatine is derived from creatine and creatine phosphate in muscle tissue and may be defined as a nitrogenous waste product. Creatinine is not reutilized but is excreted from the body in the urine via the kidney. During the reaction involving creatine and phosphocreatine, catalyzed by creatine kinase, spontaneous conversion to creatine might occur (Allen, 2012). A rise in blood creatinine levels is observed only with marked damage to functioning nephrons. The reduction in creatinine concentration (mg/dl) in the kidney of rats administered with extracts in Groups B to G when compared with the control Group A suggests an enhanced glomerular clearance rather than an impaired muscle metabolism.

Conclusion

It can be concluded that *Petiveria alliacea* leaves contain biologically active components that may be responsible for the folklore and scientifically documented medicinal effect of the plant and also that the time of collection of the leaves have significant effect on the chemical composition and hence the biological activity of the plant from the results generated in this research. Biochemical results obtained generally suggest that, the aqueous extracts of *Petiveria alliacea* leaves collected at three different times of the day showed alterations, both positive and negative, toxicological effects. Conclusively, it can be inferred that leaf extract of *P. alliacea* has the tendency to rupture the tissues' membrane in their hosts if taken in excess. Hence, care must be taken by the public from the excessive or erratic consumption of leaf extracts of *P. alliacea* for agricultural and medicinal purposes.

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References

- Adeyemi OS, Sulaiman FA. (2012). Biochemical and Morphological changes in trypanosoma brucei brucei infected rats treated with homidium chloride and diminazene aceturate. *Journal of Basic & clinical physiology & pharmacology*. Walter de Gruyter. Germany 23(4): 179-183.
- Akanji MA, Olagoke OA, Oloyede OB. (1993). Effect of chronic consumption of metabisulphite on the integrity of the rat kidney cellular system. *Toxicol.* 81: 173-179.
- Akinmoladun AC, Ibukun EO, Afor E, Obuotor EM, Farombi EO. (2007). Phytochemical constituent and antioxidant activity of extract from the leaves of *Ocimum gratissimum*. *Sci. Res. Essay*. 2: 163-166.
- Alegre J.C, and Clavo M. (2007). *Petiveria alliacea* L. Record from PROTA (Plant Resources of Tropical Africa). <http://www.prota4u.org/search.asp>.
- Amresh, G.R., Singh, P.N. and Rao, C.V. (2008). Toxicological screening of traditional medicine Laghupatha (*Cissampelos pareira*) in experimental animals. *Journal of Ethnopharmacology*. 116: 454-460.
- Beloin, N., Gbeassor, M., Akpagana, K., Hudson, J., de Soussa, K., Koumaglo, K., and Arnason, J. (2005). Ethnomedicinal uses of *Momordica charantia* (Cucurbitaceae) in Togo and relation to its phytochemistry and biological activity. *J Ethnopharmacol*, 96:49-55.
- Blank, A. F. (2005). Harvest time of influence and leaves drying in the essential oil of lemon balm (*Melissa officinalis* L.) grown in two. *Journal of Medicinal Plants*. 8(1): 73-78.
- De Lima T. C., Morato G. S., and Takahashi R. N., (1991). *Mem. Inst. O. Cruz*. 86:153-158.

De Lima TC, Morato GS, and Takahashi RN (1991). Evaluation of antinociceptive effect of *Petiveria alliacea* (Guine) in animals. Mem. Inst. Oswaldo Cruz, 86(2):153-158.

Djeridane A.T., Yousfi M., Nadjemi B., Boutassounna D., Stocker P. and Vidal N. (2006). Antioxidant Activities of Some Algerian Medicinal Plant Extracts Containing Phenolic Compounds, *Food Chemistry*, 97: 654-660.

Duke Jim. (2016). Dr. Duke's Phytochemical and Ethnobotanical Databases. <https://phytochem.nal.usda.gov/phytochem/search>;
http://www.researchmoringa.com/uploads/4Dr._Dukes_539_activities_of_Moringa.pdf

Galm U., and Shen B. (2007). “Natural Product Drug Discovery: the time has never been better”, *Chemical Biology*.19: 1-16.

García-González M, Morales TC, Ocampo R, and Pazos L. (2006). Subchronic and acute preclinic toxicity and some pharmacological effects of the water extract from leaves of *Petiveria alliacea* (*Phytolaccaceae*). *Int J Trop Biol* 54: 1323–6.

Grover, S., and Yadav, J. (2004). Pharmacological actions and potential uses of *Momordica charantia* activity, *A Rev. J Ethnopharmacol*, 93(1):123-132.

Harborne, J.B. (1984). *Phytochemical Methods: A guide to modern techniques of plant analysis*. London: Chapman and Hall.p.277.

Hernández J.F, Urueña C.P, and Cifuentes M.C. (2014) “A *Petiveria alliacea* standardized fraction induces breast adenocarcinoma cell death by modulating glycolytic metabolism”. *Journal of Ethno-pharmacology*. 153: 641-649.

Huang PR, Tsai ST, Hsieh KH, and Wang TC. (2006). Heterogeneous nuclear ribonucleoprotein a3 binds single-stranded telomeric dna and inhibits telomerase extension in vitro. *biochim biophys acta*.;1783:193–202.

Illnait J. (2007). Principales referencias etnomédicas sobre el anamú (*Petiveria alliacea* Linn) y principios activos encontrados en la planta. Un acercamiento al tema. *Rev CENIC Cienc Biol*. 38: 27-30.

- Johnson M, Aparna J.S, Jeeva S, Sukumaran S. and Anantham B. (2012). Preliminary phytochemical studies on the methanolic flower extracts of some selected medicinal plants from India. *Asian Pac J Trop Biomed.*; 1(S1): S79-S82
- Kim S., Kubec R., and Musah R.A. (2006) “Anti-bacterial and anti-fungal activity of sulfur-containing compounds from *Petiveria alliacea*”, *Journal of Ethnopharmacology*. 104:188-92
- Mendes J. (1986). Cote Cote la: Trinidad and Tobago Dictionary, Arima, Trinidad, p. 95.
- Mobley, H. L. T., and Hausinger, R. P. (1989). Microbial urease, significance, regulation, and molecular characterization. *Microbiological Reviews*, 53: 85-108.
- Pramyothin, P., Samosorn, P., Pongshompoo, S. and Chaichantipyuth, C. (2006). The protective effects of *Phyllanthus emblica* Linn. extract on ethanol induced rat hepatic injury. *Journal of Ethnopharmacology*, 107(3): 361–364.
- Sarker S.D, and Nahar L. (2007). Chemistry for Pharmacy Students General, Organic and Natural Product Chemistry. England: John Wiley and Sons. Pp: 283-359.
- Shmelzer G.H; and Gurib-Fakim A. (2008). “Medicinal Plants”, *Plant Resources of Tropical Africa*. pp. 412–415. ISBN 978-90-5782-204-9.
- Shoge, M. (2011). Quality of Soaps Using Different Oil Blends. *Journal of Microbiology and Biotechnology Research*, 1: 29-34.
- Singh V and Garg A.N. (1997). Availability of essential trace elements in Ayurvedic Indian medicinal herbs using instrumental neutron activation analysis. *Appl. Radiat. Isot.*; 48(1):97–101.
- Sofowora, A., (1996). Medicinal plants and Traditional Medicines in Africa. Spectrum Books LTD, Sunshine House 1, Emmanuel Alayande Street, P.M.B 5612, Ibadan, Nigeria.
- Sulaiman FA, and Adeyemi OS. (2010). Changes in haematological indices and protein concentrations in *Trypanosoma* infected-rats treated

with homidium chloride and diminazene aceturate. *EXCLI J.* 2010;9:39e45.

Sulaiman, A. F., Kazeem, M. O., Waheed, A. M., Temowo, S. O., Azeez, I. O., Zubair, F. I., Adeyemi, T. A., Nyang, A. and Adeyemi O.S. (2014a). Antimicrobial and toxic potentials of the aqueous extracts of *Allium sativum*, *Hibiscus sabdariffa* and *Zingiber officinale* in wistar rats. *Journal of Taibah University for Science (JTUS-Elsevier)* 8: 315-322. Available online at; http://elsevier.thomsondigital.com/authorproofs/JTUSCI_75/CD5CB7453793BF726B19EE1967539DC6Please

Sulaiman A.F., Ahmed El-Imam, A.M., Adeyemo A.A., Muhammed R.B., Sulaiman A.M., Aliyu A. O. and Adeyemi O.S. (2014b). *Aspergillus niger*-fermented *Jatropha curcas* seed cake: Proximate composition and effects on biochemical indices in Wistar rats. *Biological Letters*. 51(1):37-46. Available online at: <http://www.versita.com/science/lifesciences/bl> Doi:10.2478/biolet-2013-0017.

Sulaiman A.F., Oloyede H.O.B., Akanji M. A., Akinyele T.J. and Dosunmu K.O. (2017a). GC-MS analysis of bioactive fractions of *Terminalia avicennoides* and *Bombax buopodezense* bark and lipid profile of *Trypanosoma brucei* infected wistar rats. *African Scientist*. A NISEB Publication. Volume 17:4 pg.

Sulaiman Adenike Faoziyat, Iyiola Oluyinka Ajibola, Ahmed Olatunde, Tejidini Taibat Tosin., Badrudeen Oluwafemi; Efuntoye Ayolekan Fiyin (2017b). Toxicological effects of chlorpyrifos on *clarias gariepinus* (African catfish) and the ameliorative effects of *Blighia sapida* (Ackee apple) seed on mopping up the toxicity. *International Journal of Phytofuels and Allied Sciences*. Accepted for publication on the 13th of April 2017. <http://www.phytofuelsciences.com> ISSN 2354 1784.

Tamás L, Huttová J, Mistrk I, Kogan G. (2002). Effect of Carboxymethyl Chitin-Glucan on the Activity of Some Hydrolytic Enzymes in Maize Plants" (PDF). *Chem. Pap.* 56(5): 326–329.

Teow, C., Van-Den Truong, V., McFeeters, R., Thompson, R., Pecota, K., and Yencho, G. (2007). Antioxidant activities, phenolic and beta-carotene contents of sweet potato genotypes with varying flesh colours. . *Food Chemistry*, 103: 829–838.

Tonthubthimthong P, Chuaprasert S, Douglas P, Luewisutthichat W. (2001). Supercritical CO₂ extraction of nimbin from neem seeds an experimental study. *J. Food Eng.* 47: 289-293.

Trease G.E., and Evans W.C. (1996). Trease and Evans' Pharmacognosy, 4th edition s.820-835, WB Saunders, USA.

Wright, P.J. and Plummer, D.T. (1974). The use of urinary enzyme measurement to detect renal damages caused by nephrotoxic compounds. *Biochem Pharmacol.*,12:65.

Yakubu, M. T. and Musa, I. F. (2012). Liver and Kidney Functional Indices of Pregnant Rats Following the Administration of the Crude Alkaloids from *Senna alata* (Linn.Roxb) Leaves. *Iranian Journal of Toxicology*, 6(16): 615-625.

Unsteady Non-Newtonian Magnetohydrodynamic Radiative Thin Film Flow in a Porous Medium Past an Oscillating Vertical Belt

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Abstract

This study examines the unsteady non-Newtonian magnetohydrodynamic radiative thin film flow in a porous medium past an oscillating vertical belt. Based on the physical model of the problem, the governing momentum and energy equations are non dimensionalized and solved analytically using Optimal Homotopy Asymptotic Method (OHAM). The velocity and temperature profiles are studied for different physical parameters such as the magnetic field, radiation parameter, Grashof number, Prandtl number, and Brinkman number for both lift and drainage problems. The analytical results are presented graphically and discussed.

Keywords: OHAM, Thin film, Unsteady, Second grade fluid, Buoyancy, Viscous dissipation.

Introduction

The study of non-Newtonian fluids of thin film flow has aroused the interest of researchers and received considerable attention because of its numerous applications in various branches of science, engineering and technology: particularly in chemical and nuclear industries, material processing, geophysics and bio-engineering. The importance of non-Newtonian fluid flow especially in a porous medium has led to the development of mathematical models in an extensive range. Its areas of application include petrochemical, bio-medical and environmental engineering such as polypropylene coalescence sintering, blood, geological and debris flows.

Literature Review

Several researchers have discussed the flows of second grade fluid in different dimensions, Amir and Gul (2015), studied unsteady magnetohydrodynamic flow of second grade fluid due to impulsive motion of plate. Ali, *et al.* (2014) investigated the closed form solution for unsteady second grade fluid near vertical oscillating belt. Samiulhaq, *et al.* (2014) studied unsteady free convection flow of a second grade fluid in a porous medium with ramped wall temperature. Kamran, *et al.* (2012) investigated the thin-film flow of Johnson-Segalman fluids for lifting and drainage problems.

Due to the non-linearity of the equations governing the flow, several approximate methods have been developed in Mathematics and Engineering sciences to solve the equations. Some of the common methods are Homotopy Analysis Method (HAM) by Liao, (2003) and Optimal Homotopy Asymptotic Method (OHAM). These methods deal with the nonlinear problems effectively. OHAM was built on convergence criteria similar to HAM but it differs from it in that, it does not require an initial guess as successfully applied by Marinca, *et al.* (2008) to problems in mechanics and has also showed its effectiveness and accuracy. Gul *et al.* (2014), used ADM and OHAM for solution of heat transfer analysis of magnetohydrodynamic (MHD) thin film flow of an unsteady second grade fluid past a vertical oscillating belt. An application of OHAM for solving non-linear equations arising in heat transfer was investigated by Marinca, *et al.* (2009) who applied OHAM to solve steady flow of a fourth-grade fluid past a porous plate. Gul, *et al.* (2013) investigated MHD thin film flow of a third grade fluid on a vertical belt with slip boundary conditions. Mabood *et al.* (2013) used OHAM for flow and heat transfer of a viscoelastic fluid in an axisymmetric channel with a porous wall.

The above studies neglected the combined effects of radiation, buoyancy force and porous medium on dissipative fluids. In the light of this, the interaction of radiation with heat transfer of a MHD thin film flow in a porous medium past an oscillating vertical belt is presented in this study. The governing momentum equation includes porosity and buoyancy force while the effect of radiation is taken into account in the energy equation.

Methodology

The problem of unsteady non-Newtonian magnetohydrodynamic radiative thin film flow of a second grade fluid in a porous medium past an oscillating vertical belt is considered. It is assumed that the flow is unsteady and laminar after a small distance above the liquid surface layer and the film thickness remains constant for both lift and drainage problems. The flow is only in y-direction (vertical flow) in the presence of magnetic field and heat dissipation and the problem is fully developed laminar flow. By using the Rosseland approximation, the radiative heat flux q_r for the given problem is

$$q_r = -\frac{4\sigma_s}{3k_e} \frac{\partial T'^4}{\partial x} \quad (1)$$

and the flow is governed by the following equations.

Continuity equation:

$$\frac{\partial u}{\partial x} + \frac{\partial v}{\partial y} = 0$$

$$0 \quad (2)$$

$$\rho \frac{\partial u}{\partial t} = -\frac{\partial p}{\partial y} + \mu \frac{\partial^2 u}{\partial x^2} + \alpha_1 \frac{\partial}{\partial t} \left(\frac{\partial^2 u}{\partial x^2} \right) - \rho g \beta_T (T - T_\delta) - \frac{\mu u}{k} - \sigma B_0^2 u \quad (3)$$

Energy equation:

$$\rho c_p \frac{\partial T}{\partial t} = k \frac{\partial^2 T}{\partial x^2} + \mu \left(\frac{\partial u}{\partial x} \right)^2 - \frac{\partial q_r}{\partial x} + \alpha_1 \left(\frac{\partial u}{\partial x} \right) \frac{\partial}{\partial t} \left(\frac{\partial u}{\partial x} \right) \quad (4)$$

In the formulation of the lift problem in this second grade fluid flow, a wide flat belt is considered to move vertically at time $t = 0^+$ and a layer of liquid of constant thickness is been carried by the belt. The belt oscillated and translated with constant speed u_o in the presence of

magnetic field which is placed transverse to the belt. Heat is applied to the fluid and atmospheric pressure is negligible. The y-axis is taken parallel to the belt while x-axis is perpendicular to it for the coordinate system. Velocity and temperature fields are defined as:

$$\mathbf{V} = (0, u(x, t), 0) \quad , \quad T = T(x, t) \quad (5)$$

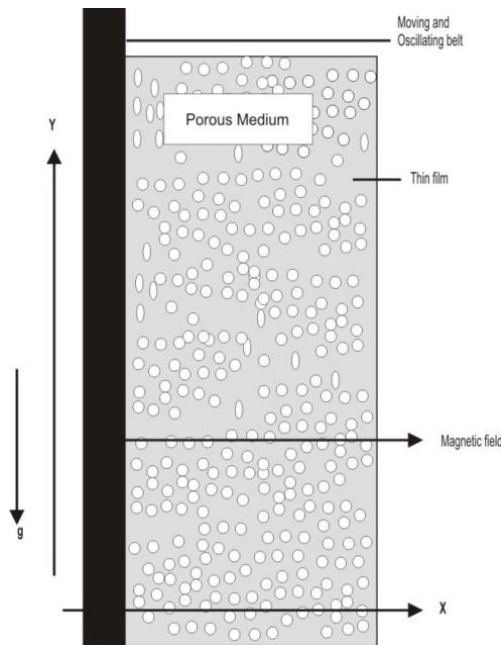


Figure 1: Geometry of the lift problem.

Oscillating boundary conditions are:

$$u(0, t) = U(1 + \xi \cos \omega t), \quad u(\delta, t) = 0 \quad (6)$$

$$T(0, t) = T_o, \quad T(\delta, t) = T_\delta \quad (7)$$

where U , ξ and δ are the flow mean velocity, amplitude and boundary layer parameter respectively. Substituting equation (5) into equation (2), continuity equation is satisfied.

In order to present the governing equations in dimensionless forms, the following non-dimensional variables are introduced.

$$\begin{aligned} \bar{u} = \frac{u}{u_o}, \quad \bar{x} = \frac{x}{\delta}, \quad \theta = \frac{(T-T_o)}{(T_\delta-T_o)}, \quad \alpha = \frac{\alpha_1}{\rho\delta^2}, \quad \frac{\partial p}{\partial y} = 0, \quad S = \frac{\delta^2}{k}, \quad M = \\ \frac{\sigma B_o^2 \delta^2}{\mu}, \quad G_r = \frac{\rho\delta^2 g \beta_T (T_\delta-T_o)}{\mu u_o}, \quad H = \left[1 + \frac{4}{3N}\right], \quad N = \frac{k_e k}{4\sigma_s T_\infty'^3}, \quad p_r = \frac{\mu c_p}{k}, \quad B_r = \\ \frac{\mu u^2}{k(T_\delta-T_o)}, \quad \bar{t} = \frac{\mu t}{\rho\delta^2} \end{aligned} \quad (8)$$

Inserting these non-dimensional variables in equations (3) and (4) and dropping bars, the momentum and energy equations becomes:

$$\begin{aligned} \frac{\partial u}{\partial t} = \frac{\partial^2 u}{\partial x^2} + \alpha \frac{\partial}{\partial t} \left(\frac{\partial^2 u}{\partial x^2} \right) - Gu \\ - G_r \theta \end{aligned} \quad (9)$$

$$\begin{aligned} p_r \frac{\partial \theta}{\partial t} = \left[1 + \frac{4}{3N}\right] \frac{\partial^2 \theta}{\partial x^2} + B_r \left(\frac{\partial u}{\partial x} \right)^2 \\ + B_r \alpha \left(\frac{\partial u}{\partial x} \right) \left(\frac{\partial^2 u}{\partial t \partial x} \right) \end{aligned} \quad (10)$$

The non-dimensional boundary conditions of equations (6) and (7) are:

$$\begin{aligned} u(0, t) = (1 + \xi \cos \omega t), \quad u(1, t) \\ = 0 \end{aligned} \quad (11)$$

$$\theta(0, t) = 0, \quad \theta(1, t) = 1 \quad (12)$$

In the formulation of the drainage problem, coordinate system of the geometry and assumptions of the problem are the same as in the lift problem. But in drainage problem, the belt is oscillating only. The fluid drains down the oscillating belt due to gravity and the gravity is opposing the lift. Therefore, the term with Grashof number is positive in this model as follows:

$$\frac{\partial u}{\partial t} = \frac{\partial^2 u}{\partial x^2} + \alpha \frac{\partial}{\partial t} \left(\frac{\partial^2 u}{\partial x^2} \right) - Gu + G_r \theta \quad (13)$$

$$p_r \frac{\partial \theta}{\partial t} = \left[1 + \frac{4}{3N} \right] \frac{\partial^2 \theta}{\partial x^2} + B_r \left(\frac{\partial u}{\partial x} \right)^2 + B_r \alpha \left(\frac{\partial u}{\partial x} \right) \left(\frac{\partial^2 u}{\partial t \partial x} \right) \quad (14)$$

Dimensionless boundary conditions for drainage problem when the belt is only oscillating are:

$$u(0, t) = \xi \cos \omega t, \quad u(1, t) = 0 \quad (15)$$

$$\theta(0, t) = 0, \quad \theta(1, t) = 1 \quad (16)$$

By means of OHAM which is the method of solution adopted, a family of equations is given as (Marinca *et al.* 2008):

$$(1 - p)[L\phi(x, p, c_i)] + g(x) = h(x, p, c_i)[L\phi(x, p, c_i) + g(x) + N(\phi(x, p, c_i))] \quad (17)$$

where p is an embedding parameter, $g(x)$ is a known function, L is a linear operator, N is a non linear operator, $\phi(x, p, c_i)$ is an unknown function which is expanded in series form with respect to the parameter p , $h(x, p, c_i)$ is a non-zero auxiliary function chosen as

$$h(p, c_i) = pc_1 + p^2c_2 + \dots \quad (18)$$

where c_1, c_2, c_3, c_4 are called convergence control parameters that will be determined from the series solution of velocity and temperature profiles using the method of least squares.

Adopting least squares method, the option is to minimize the square residual error (R)

where residual is defined as

$$R(x, c_i) = L\phi(x, c_i) + g(x) + N\phi(x, c_i), i = 1, 2, 3, \dots \quad (19)$$

Minimizing the squared residual error, we have

$$J(c_1, c_2, \dots c_m) = \int_a^b R^2(x, c_1, c_2, \dots c_m) dx \quad (20)$$

where a and b are real numbers depending on the given problem.

The convergence parameters $c_1, c_2, \dots c_m$ can be identified from the conditions

$$\frac{\partial J}{\partial c_1} = \frac{\partial J}{\partial c_2} = \frac{\partial J}{\partial c_m} = 0 \quad (21)$$

Finally, from these auxiliary constants, the series solution of velocity and temperature profiles is well-determined.

OHAM Solution of Lifting Problem

Constructing a homotopy for equations (9) and (10), using the standard form of OHAM in equation (17), the zero (p^0), first (p^1) and second (p^2) components problems are obtained respectively as:

$$\begin{aligned} p^0: & \frac{\partial^2 u_o}{\partial x^2} \\ & = 0, \end{aligned} \quad (22)$$

$$\begin{aligned} & \left[1 + \frac{4}{3N} \right] \frac{\partial^2 \theta_o}{\partial x^2} \\ & = 0, \end{aligned} \quad (23)$$

$$\begin{aligned}
 p^1: & \quad \frac{\partial^2 u_1}{\partial x^2} \\
 & = \frac{\partial^2 u_o}{\partial x^2} + c_1 \frac{\partial^2 u_o}{\partial x^2} - c_1 \frac{\partial u_o}{\partial t} + \alpha c_1 \frac{\partial}{\partial t} \left(\frac{\partial^2 u_o}{\partial x^2} \right) - c_1 S u_o - c_1 M u_o \\
 & - c_1 G_r \theta_o
 \end{aligned} \tag{24}$$

$$\begin{aligned}
 & H \frac{\partial^2 \theta_1}{\partial x^2} \\
 & = H \frac{\partial^2 \theta_o}{\partial x^2} + c_3 H \frac{\partial^2 \theta_o}{\partial x^2} - p_r c_3 \frac{\partial \theta_o}{\partial t} + c_3 B_r \left(\frac{\partial u_o}{\partial x} \right)^2 \\
 & + c_3 B_r \alpha \left(\frac{\partial u_o}{\partial x} \right) \left(\frac{\partial^2 u_o}{\partial x \partial t} \right),
 \end{aligned} \tag{25}$$

$$\begin{aligned}
 p^2: \quad \frac{\partial^2 u_2}{\partial x^2} & = \frac{\partial^2 u_1}{\partial x^2} + c_1 \frac{\partial^2 u_1}{\partial x^2} - c_1 \frac{\partial u_1}{\partial t} + c_2 \frac{\partial^2 u_o}{\partial x^2} - c_2 \frac{\partial u_o}{\partial t} \\
 & + \alpha c_1 \frac{\partial}{\partial t} \left(\frac{\partial^2 u_1}{\partial x^2} \right) - c_1 S u_1 - c_1 M u_1 - c_1 G_r \theta_1 \\
 & + \alpha c_2 \frac{\partial}{\partial t} \left(\frac{\partial^2 u_o}{\partial x^2} \right) - c_2 S u_o - c_2 M u_o \\
 & - c_2 G_r \theta_o
 \end{aligned} \tag{26}$$

$$\begin{aligned}
 & H \frac{\partial^2 \theta_2}{\partial x^2} \\
 & = H \frac{\partial^2 \theta_1}{\partial x^2} + c_3 H \frac{\partial^2 \theta_1}{\partial x^2} - p_r c_3 \frac{\partial \theta_1}{\partial t} + c_3 B_r \alpha \left(\frac{\partial u_1}{\partial x} \right) \left(\frac{\partial^2 u_1}{\partial x \partial t} \right) \\
 & + c_4 H \frac{\partial^2 \theta_o}{\partial x^2} - p_r c_4 \frac{\partial \theta_o}{\partial t} + c_4 B_r \alpha \left(\frac{\partial u_o}{\partial x} \right) \left(\frac{\partial^2 u_o}{\partial x \partial t} \right) + c_3 B_r \left(\frac{\partial u_1}{\partial x} \right)^2 \\
 & + c_3 B_r \alpha \left(\frac{\partial u_1}{\partial x} \right) \left(\frac{\partial^2 u_1}{\partial x \partial t} \right)
 \end{aligned} \tag{27}$$

By solving equations (22) - (25) subject to the boundary conditions given in equations (11) and (12), the solutions are:

$$\begin{aligned}
 & u_o[x, t] \\
 & = -(-1 + x)(1 \\
 & + \xi \text{Cos}[t\omega]),
 \end{aligned} \tag{28}$$

$$\begin{aligned}\theta_0[x, t] \\ &= x,\end{aligned}\tag{29}$$

$$\begin{aligned}u_1[x, t] \\ &= 1 + \xi \text{Cos}[t\omega] \\ &+ x \left(-1 - \xi \text{Cos}[t\omega] + c_1 \left(\frac{2M}{3} + \frac{S}{3} + \frac{2}{3} M \xi \text{Cos}[t\omega] + \frac{1}{3} S \xi \text{Cos}[t\omega] \right) \right. \\ &\quad \left. - \frac{1}{3} \xi \omega \text{Sin}[t\omega] + \frac{G_r}{6} \right) \\ &+ x^2 \left(-M - \frac{S}{2} - M \xi \text{Cos}[t\omega] - \frac{1}{2} S \xi \text{Cos}[t\omega] + \frac{1}{2} \xi \omega \text{Sin}[t\omega] \right) c_1 \\ &+ x^3 c_1 \left(\frac{M}{3} + \frac{S}{6} + \frac{1}{3} M \xi \text{Cos}[t\omega] + \frac{1}{6} S \xi \text{Cos}[t\omega] - \frac{1}{6} \xi \omega \text{Sin}[t\omega] \right. \\ &\quad \left. - \frac{G_r}{6} \right)\end{aligned}\tag{30}$$

$$\begin{aligned}\theta_1[x, t] \\ &= \frac{1}{2(3 + 4N)} (6x + 8Nx - 3x B_r c_3 + 3x^2 B_r c_3 - 6x \xi \text{Cos}[t\omega] B_r c_3 \\ &+ 6x^2 \xi \text{Cos}[t\omega] B_r c_3 - 3x \xi^2 \text{Cos}[t\omega]^2 B_r c_3 + 3x^2 \xi^2 \text{Cos}[t\omega]^2 B_r c_3 \\ &+ 3x \alpha \xi \omega \text{Sin}[t\omega] B_r c_3 - 3x^2 \alpha \xi \omega \text{Sin}[t\omega] B_r c_3 \\ &+ 3x \alpha \xi^2 \omega \text{Cos}[t\omega] \text{Sin}[t\omega] B_r c_3 \\ &- 3x^2 \alpha \xi^2 \omega \text{Cos}[t\omega] \text{Sin}[t\omega] B_r c_3)\end{aligned}\tag{31}$$

Equations (28)-(31) are substituted into eqns. (26) and (27) and solved for u_2 and θ_2 .

The series solutions of velocity and temperature for lift problem are obtained as

$$\begin{aligned}u[x, t] \\ &= u_0[x, t] + u_1[x, t] \\ &+ u_2[x, t]\end{aligned}\tag{32}$$

$$\begin{aligned}\theta[x, t] \\ &= \theta_0[x, t] + \theta_1[x, t] \\ &+ \theta_2[x, t]\end{aligned}\tag{33}$$

The arbitrary constants c_1, c_2, c_3 , and c_4 for both equations (32) and (33) are found by using the procedures in equations (19) - (21).

The arbitrary constants in velocity solution (equation. (32)) are obtained as

$$c_1 = -0.00028480877852541417, c_2 = -2.9977468464837203, \\ c_3 = 0.9864541962047597.$$

For temperature solution (equation. (33)), the arbitrary constants are

$$c_1 = -0.04264932656376229, c_2 = 0.15164362549144306, \\ c_3 = -0.8683837554484702, c_4 = -5.921227467947385.$$

OHAM Solution of Drainage Problem

Constructing a homotopy for equations (13) and (14), using the standard form of OHAM in equation (17), the zero p^0 , first (p^1) and second (p^2) components problems, respectively, are obtained as:

$$p^0: \frac{\partial^2 u_o}{\partial x^2} \\ = 0 \quad (34)$$

$$\left[1 + \frac{4}{3N}\right] \frac{\partial^2 \theta_o}{\partial x^2} \\ = 0 \quad (35)$$

$$p^1: \frac{\partial^2 u_1}{\partial x^2} \\ = \frac{\partial^2 u_o}{\partial x^2} + c_1 \frac{\partial^2 u_o}{\partial x^2} - c_1 \frac{\partial u_o}{\partial t} + \alpha c_1 \frac{\partial}{\partial t} \left(\frac{\partial^2 u_o}{\partial x^2} \right) - c_1 S u_o - c_1 M u_o \\ + c_1 G_r \theta_o \quad (36)$$

$$\begin{aligned}
 & H \frac{\partial^2 \theta_1}{\partial x^2} \\
 &= H \frac{\partial^2 \theta_o}{\partial x^2} + c_3 H \frac{\partial^2 \theta_o}{\partial x^2} - p_r c_3 \frac{\partial \theta_o}{\partial t} + c_3 B_r \left(\frac{\partial u_o}{\partial x} \right)^2 \\
 &+ c_3 B_r \alpha \left(\frac{\partial u_o}{\partial x} \right) \left(\frac{\partial^2 u_o}{\partial x \partial t} \right)
 \end{aligned} \tag{37}$$

$$\begin{aligned}
 p^2: \frac{\partial^2 u_2}{\partial x^2} &= \frac{\partial^2 u_1}{\partial x^2} + c_1 \frac{\partial^2 u_1}{\partial x^2} - c_1 \frac{\partial u_1}{\partial t} + c_2 \frac{\partial^2 u_o}{\partial x^2} - c_2 \frac{\partial u_o}{\partial t} \\
 &+ \alpha c_1 \frac{\partial}{\partial t} \left(\frac{\partial^2 u_1}{\partial x^2} \right) - c_1 S u_1 - c_1 M u_1 + c_1 G_r \theta_1 \\
 &+ \alpha c_2 \frac{\partial}{\partial t} \left(\frac{\partial^2 u_o}{\partial x^2} \right) - c_2 S u_o - c_2 M u_o \\
 &+ c_2 G_r \theta_o
 \end{aligned} \tag{38}$$

$$\begin{aligned}
 & H \frac{\partial^2 \theta_2}{\partial x^2} \\
 &= H \frac{\partial^2 \theta_1}{\partial x^2} + c_3 H \frac{\partial^2 \theta_1}{\partial x^2} - p_r c_3 \frac{\partial \theta_1}{\partial t} + c_3 B_r \alpha \left(\frac{\partial u_1}{\partial x} \right) \left(\frac{\partial^2 u_1}{\partial x \partial t} \right) \\
 &+ c_4 H \frac{\partial^2 \theta_o}{\partial x^2} - p_r c_4 \frac{\partial \theta_o}{\partial t} + c_4 B_r \alpha \left(\frac{\partial u_o}{\partial x} \right) \left(\frac{\partial^2 u_o}{\partial x \partial t} \right) + c_3 B_r \left(\frac{\partial u_1}{\partial x} \right)^2 \\
 &+ c_3 B_r \alpha \left(\frac{\partial u_1}{\partial x} \right) \left(\frac{\partial^2 u_1}{\partial x \partial t} \right)
 \end{aligned} \tag{39}$$

Solving equations (34)-(37) subject to the boundary conditions given in equations (15) and (16), the solutions are:

$$\begin{aligned}
 & u_0[x, t] \\
 &= -(-1 + x) \xi \text{Cos}[t\omega]
 \end{aligned} \tag{40}$$

$$\begin{aligned}
 & \theta_0[x, t] \\
 &= x
 \end{aligned} \tag{41}$$

$$\begin{aligned}
 & u_1[x, t] \\
 &= \xi \cos[t\omega] + x^2 \left(-M\xi \cos[t\omega] - \frac{1}{2}S\xi \cos[t\omega] + \frac{1}{2}\xi\omega \sin[t\omega] \right) c_1 \\
 &+ x \left(-\xi \cos[t\omega] + c_1 \left(\frac{2}{3}M\xi \cos[t\omega] + \frac{1}{3}S\xi \cos[t\omega] \right) \right. \\
 &\quad \left. - \frac{1}{3}\xi\omega \sin[t\omega] - \frac{G_r}{6} \right) \\
 &+ x^3 c_1 \left(\frac{1}{3}M\xi \cos[t\omega] + \frac{1}{6}S\xi \cos[t\omega] - \frac{1}{6}\xi\omega \sin[t\omega] \right. \\
 &\quad \left. + \frac{G_r}{6} \right) \tag{42}
 \end{aligned}$$

$$\begin{aligned}
 & \theta_1[x, t] \\
 &= \frac{1}{2(3+4N)} \left(6x + 8Nx - 3x\xi^2 \cos[t\omega]^2 B_r c_3 + 3x^2 \xi^2 \cos[t\omega]^2 B_r c_3 + \right. \\
 &\quad \left. 3x\alpha \xi^2 \omega \cos[t\omega] \sin[t\omega] B_r c_3 - 3x^2 \alpha \xi^2 \omega \cos[t\omega] \sin[t\omega] B_r c_3 \right) \tag{43}
 \end{aligned}$$

The series solutions of velocity and temperature for drainage problem are expressed respectively as:

$$\begin{aligned}
 u[x, t] &= u_0[x, t] + u_1[x, t] \\
 &\quad + u_2[x, t] \tag{44}
 \end{aligned}$$

$$\begin{aligned}
 \theta[x, t] &= \theta_0[x, t] + \theta_1[x, t] \\
 &\quad + \theta_2[x, t] \tag{45}
 \end{aligned}$$

The arbitrary constants c_1, c_2, c_3 , and c_4 for both equations (44) and (45) are found by using the procedures in equations (19) - (21).

The arbitrary constants in velocity solution (equation (44)) are obtained as

$$\begin{aligned}
 c_1 &= -0.9684126046268788, \quad c_2 = -0.9479219250666509, \\
 c_3 &= -38.323428491839174.
 \end{aligned}$$

For temperature solution (equation (45)), the calculated arbitrary constants are

$$\begin{aligned}
 c_1 &= -0.09152659686668704, \quad c_2 = 0.09581570123493369, \\
 c_3 &= -3.777664639888141, \quad c_4 = -5.352328033713726.
 \end{aligned}$$

Results and Findings

If the parameters that were introduced to the governing equations are relaxed in these problems, a plot of 3-dimensional graphs with $\alpha = 0.02$, $\xi = 0.4$, $\omega = 0.2$, $M = 0.5$, $Gr = 0.5$, $Br = 4$, $pr = 0.6$, $S = 0$, $N \rightarrow \infty$ obtained in Figures (2) - (4) are in agreement with the work of Gul *et al.* (2014).

The numerical results for velocity and temperature profile are presented with different values of radiation parameters, heat source parameter, Grashof number, Prandtl number, Hartman number and Brinkman number. The 3-dimensional graphs are produced with a fixed Porous medium shape factor (S) = 0.3, radiation parameter (N) = 2, prandtl number (p_r) = 0.6, brinkman number (B_r) = 4, Grashof number G_r = 0.5, Hartman number (M) = 0.5, frequency of oscillating belt (ω) = 0.2, amplitude of oscillation (ξ) = 0.4, and non-Newtonian effect (α) = 0.02.

The 3-dimensional lift and drainage velocity profiles of time level variation were plotted in Figures (5) and (6). It was observed that lift and drainage velocity profiles are oscillating with time. The wavelengths for drainage and lift velocity profiles appear constant with time. It could be due to the fact that the belt is oscillating. Figures (7) and (8) show that there is a sharper variation in drainage temperature with time when compared with lift temperature: it is due to the fact that a layer of fluid with zero temperature was being carried by the moving belt in lift problem.

Figures (9) and (10) present lift and drainage velocity profiles plotted against frequency parameter (ω) for different values of M and fixed values of $\alpha = 0.02$, $\xi = 0.4$, $G_r = 0.5$, $B_r = 4$, $p_r = 0.6$, $S = 0.6$, $K = 2$, $x = 0.7$, $t = 5$. An increase in magnetic parameter M , decreases the velocity profile of the fluid in lift problem while the velocity profile in drainage problem increases within the intervals [0.3, 1.0], [1.75, 2.0] and decreases within the intervals [0, 0.3], [1.0, 1.75] with an increase in M . Figures (11) and (12) show the influence of Brinkman number B_r with a fixed values of $\alpha = 0.02$, $\xi = 0.6$, $\omega = 0.4$, $M = 0.5$, $G_r = 0.5$, $p_r = 0.6$, $S = 2$, $t = 10$, $K = 0.4$ for both lift and drainage temperature profiles. The

temperature distribution increases with an increase in B_r for both lift and drainage temperature problems.

Increasing Grashof number (G_r) decreases the velocity in lift flow while a rise in Grashof number increases the velocity in drainage problem as shown in Figures (13) and (14). An increase in Radiation parameter N decreases the temperature profiles in lift and drainage problems as shown in Figures (15) and (16). Figures (17) and (18) present the temperature profiles of lift and drainage problems. Increasing Prandtl number (p_r) accelerates the temperature distributions in lift and drainage problems. Figures (19) and (20) present the effect of Porosity on lift and drainage velocity profiles. Increasing the porous medium shape factor decreases the velocity distributions in both lift and drainage problems.

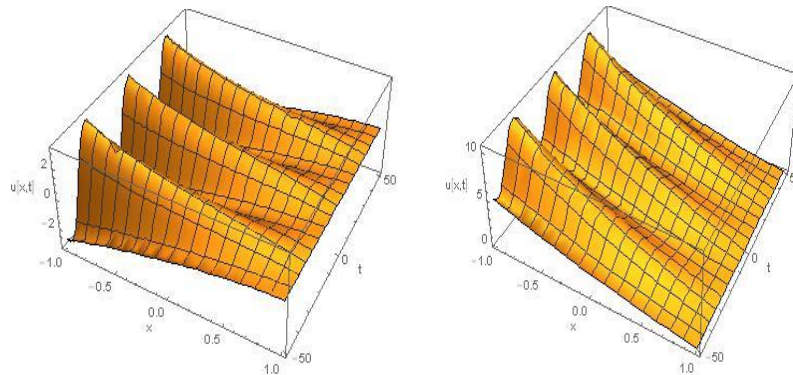


Figure 2: Influence of different time level on drainage velocity profile.
Figure 3: Influence of different time level on lift velocity profile

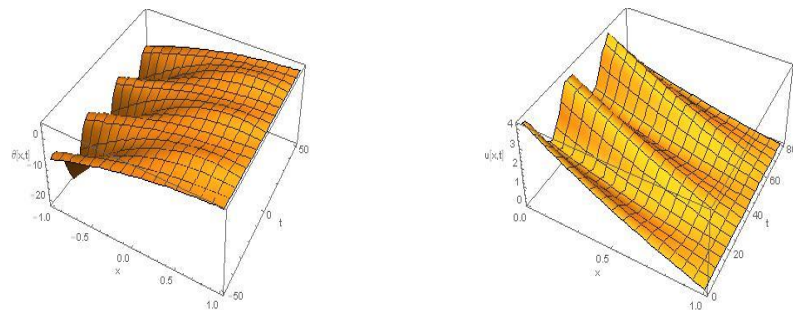


Figure 4: Influence of different time level on lift temperature profile.
Figure 5: Time level variation on lift velocity profile.

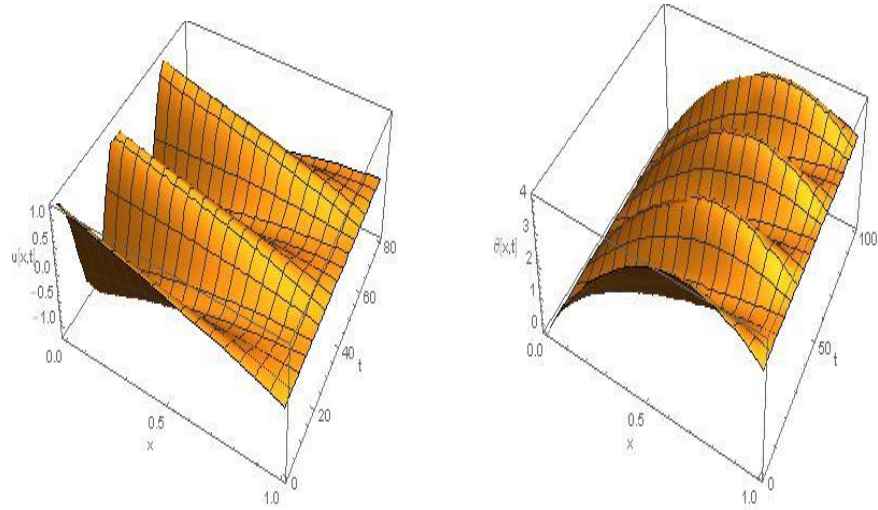


Figure 6: Time level variation on drainage velocity profile.

Figure 7: Time level variation on lift temperature.

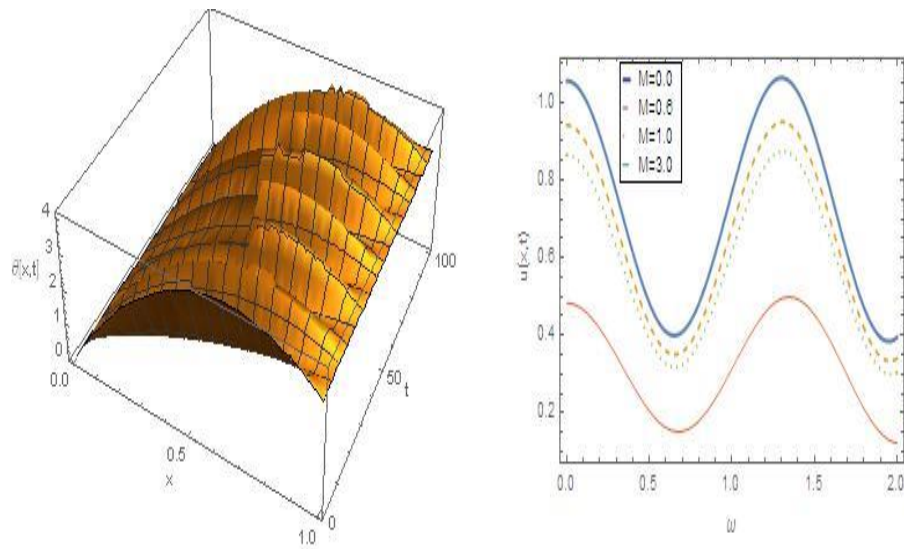


Figure 8: Time level variation on drainage temperature profile.

Figure 9: The effect of M and ω on lift velocity.

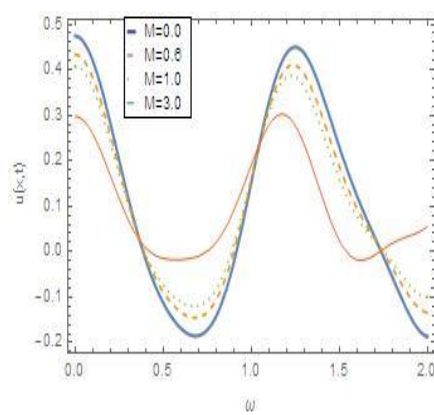


Figure 10: The effect of M and ω on drainage velocity.

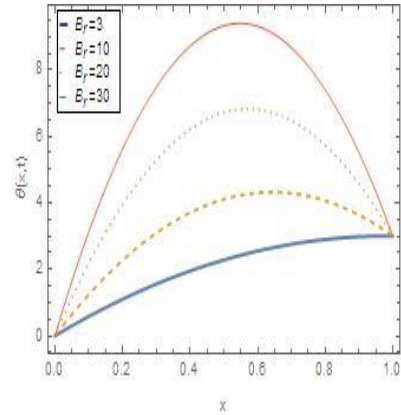


Figure 11: Influence of B_r on lift temperature profile.

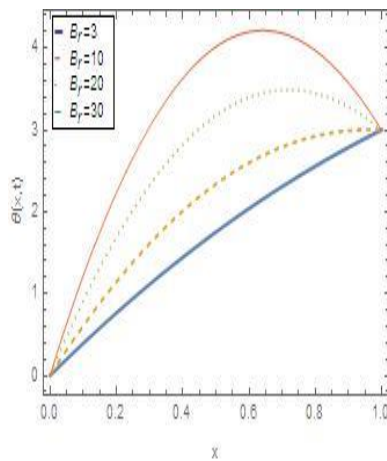


Figure 12: Influence of B_r on drainage temperature profile.

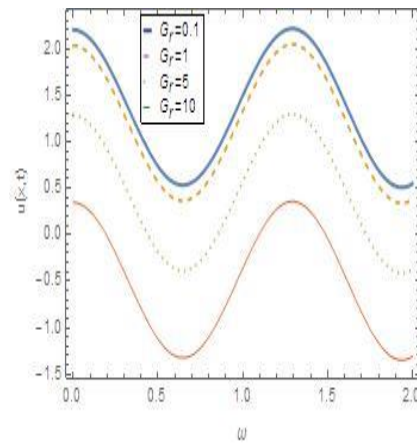


Figure 13: Effect of G_r and ω on lift velocity profile.

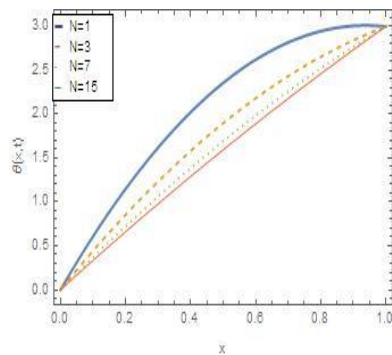
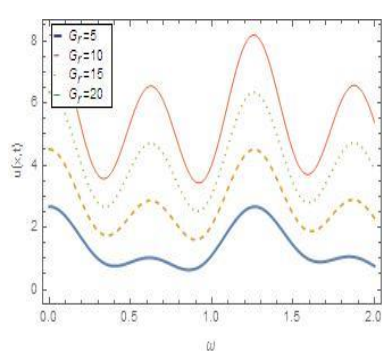


Figure 14: Effect of G_r and ω on drainage velocity.

Figure 15: Influence of N on lift temperature.

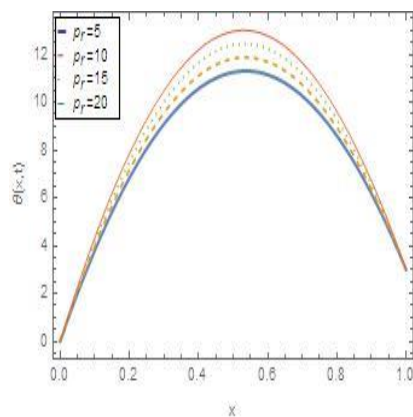
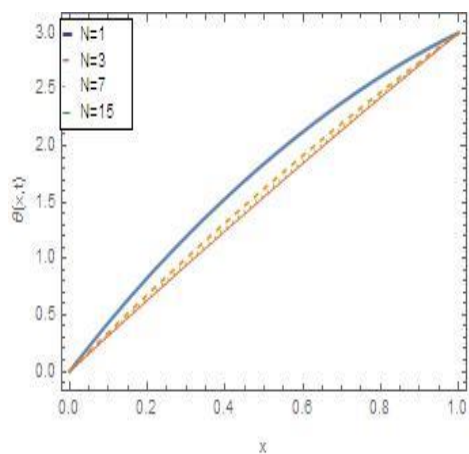


Figure 16: Influence of N on drainage temperature.

Figure 17: Influence of p_r on lift temperature.

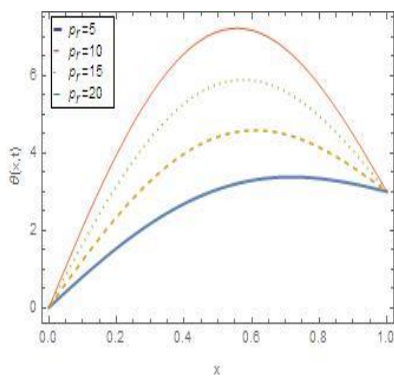


Figure 18: Influence of p_r on drainage temperature.

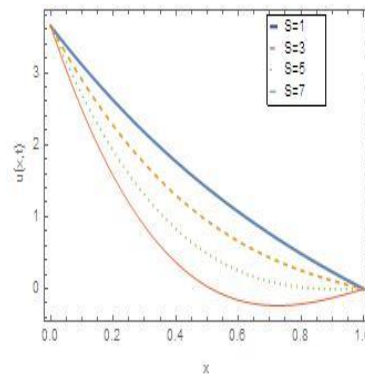


Figure 19: Effects of S on lift velocity.

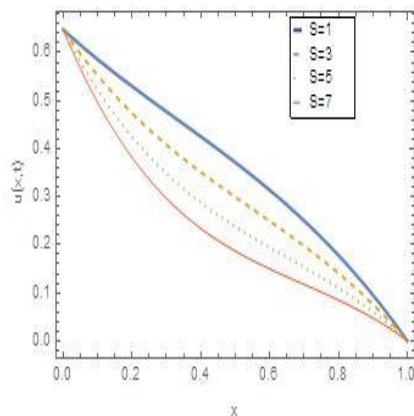


Figure 20: Effects of S on drainage velocity.

Conclusion

In this paper, the unsteady non-Newtonian magnetohydrodynamic radiative thin film flow in a porous medium past an oscillating vertical belt has been investigated. An Optimal Homotopy Asymptotic Method was employed in solving the resulting coupled partial differential equations. The effects of different physical parameters have been observed. The graphical results of the present work compared well with the work of Gul *et al.* (2014). From the study, it is concluded that:

the fluid temperature decreases with an increase in radiation parameter N in both cases of lift and drainage problems;

the magnitude of the velocity profile decreases as the porous medium shape factor (S) increases in lift velocity profile and drainage velocity profile increases with an increase in S ;

an increase in magnetic parameter M for lift flow, increases the velocity profile.

Nomenclature

T	Fluid temperature	σ_e	Stefan bolzmann-constant
α_1	Non-Newtonian effect	k_e	Conductivity of the fluid
T_o	Wall temperature	θ	Fluid temperature
U	Flow mean velocity	ξ	Amplitude
T	Time	ω	Frequency of oscillation
P	Pressure	α	Non-Newtonian effect
Q	Radiative heat flux	β_T	Coefficient of thermal expansion
S	Porous medium factor	T_δ	Temperature at boundary layer
K	Thermal conductivity	M	Hartman number
B_o	Electro magnetic induction	$\bar{u}$	Axial velocity
p_r	Prandtl number	μ	Fluid viscosity
G	Gravitational force	δ	Boundary layer
B_r	Brinkman number	ρ	Fluid density
G_r	Grashof number	μ_e	Magnetic permeability
N	Radiation parameter	ν	Kinematic viscosity

References

- Ali F, Khan I, Shafie S, (2014). Closed Form Solutions for Unsteady Free Convection Flow of a Second Grade Fluid Over an Oscillating Vertical Plate. *plos one* 9(2): e85099.doi:10.1371/journal.pone.0085099.
- Amir, K. and Gul, Z. (2015). Unsteady magnetohydrodynamic flow of a second grade fluid due to impulsive motion of plate. *Electronic Journal of Mathematical Analsis and Applications*. 3(1): 215-227.
- Griffiths R.W. (2000). The Dynamics of Lava Flows. *Annu. Rev. Fluid Mech*. 32: 477–518.
- Gul T, Shah, R.A, Islam, S. Arif, M. (2013). MHD Thin Film Flows of a Third Grade Fluid on a Vertical Belt With Slip Boundary Conditions. *Journal of Applied Mathematics* Article ID 707286, <http://dx.doi.org/10.1155/2013/707286>
- Gul T, Islam S, Shah R.A, Khan I, Shafie S, (2014). Thin Film Flow in MHD Third Grade Fluid on a Vertical Belt with Temperature Dependent Viscosity. *Plos One* 9(6). e97552. doi:10.1371/journal.pone.0097552.
- Gul, T., Islam S., Shah, R.A., Khan, I, Khalid A., Shafie S. (2014). Heat Transfer analysis of MHD Thin Film Flow of an Unsteady Second Grade Fluid Past a Vertical Oscillating Belt. *Plos One* 9(11): e103843.doi:10.1371/journal.pone.0103843.
- Gul, T., Islam, S., Shah, R.A., Khalid A., Khan, I., Shafie S, (2015). Unsteady MHD Thin Film Flow of an Oldroyd-B Fluid over an Oscillating Inclined Belt. *Plos One* 10(7): e0126698. doi:10.1371/journal.pone.0126698.
- Kamran, A.M., Rahim, M.T., Haroon, T., Islam S., Siddiqui, A.M. (2012). Thin-Film Flow of Johnson-Segalman Fluids for Lifting and Drainage Problems. *Appl. Math. Comput* 218: 10413–10428.
- Liao, S.J. (2003). Beyond Perturbation. Introduction to Homotopy Analysis Method. Chapman and Hall/CRC Press, Boca Raton.

Mabood, F., Khan, W.A., Ismail, A.I. (2013). Flow and heat transfer of a viscoelastic fluid in an axisymmetric channel with a porous medium. Plos One 8 (12): e83581. doi:10.1371/journal.pone.0083581.

Marinca, V. Herisanu, N., Nemes, I. (2008). Optimal homotopy asymptotic method with application to thin film flow. Cent. Eur. J. Phys. 6: 648–653.

Marinca, V., Herisanu, N. (2008). Application of Optimal Homotopy Asymptotic Method for Solving Non-Linear Equations Arising in Heat Transfer, Int.Communications in heat and mass.Trans 35: 710-715.

Marinca, V., Herisanu, N., Constantin, B., Bogdan, M. (2009). An Optimal Homotopy Asymptotic Method Applied to the Steady Flow of a Fourth Grad Fluid Past a Porous Plate. Appl. Math. Lett. 22: 245-251.

Samiulhaq, Sohail A., Dumitru V, Ilyas K, Sharidan S. (2014).Unsteady magnetohydrodynamic free convection flow of a second grade fluid in a porous medium with ramped wall temperature. Plos One 9(5):: e88766. doi: 10.1371/journal.pone.0088766

Magnetohydrodynamic free convection flow of viscoelastic fluid past a vertical porous plate with thermal conductivity and Chemical reaction

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Abstract

Unsteady heat and mass transfer of magneto-hydrodynamic (MHD) of viscoelastic fluid flow past a vertical porous plate with thermal conductivity and chemical reaction in the presence of heat generation and variable suction was studied in this work. The governing partial differential equations were solved numerically by implicit finite difference scheme of Crank-Nicolson. Computations were performed for a wide range of the governing flow parameters such as thermal Grashof number (Gr), solutal Grashof number (Gc), magnetic field parameter (M), Prandtl number (Pr), Dufour number (Du), Soret number (Sr), thermal conductivity and Schmidt number (Sc). The effects of these flow parameters on the velocity, temperature and concentration were shown graphically. It was observed that the velocity increases with the increase in the flow parameters such as Gr, Gc, Du, Da and λ while it decreases with the increase in M, Fs and Pr. Temperature decreases with an increase in λ , R and Pr and concentration decreases with the increase in Sr. Finally, the effects of various parameters on the skin friction coefficient, Nusselt number and Sherwood number were shown on Tables of results.

Introduction

In the past decades, the study of MHD heat and mass transfer of viscoelastic fluid past a vertical porous plate with thermal conductivity and chemical reactions effects has attracted the interest of a considerable

number of researchers because of its numerous applications in several branches of science and engineering. These applications include the industrial processes like drying, cooling of nuclear reactors and chemical deposition on surfaces, magneto hydrodynamic (MHD) power generators, solar physics, astrophysical studies and polymer technology. Heat and mass transfer problem in a porous medium has important applications in geothermal reservoir, geothermal extraction and heat exchangers. In the category of non-Newtonian fluids, investigation on viscoelastic of Kuvshinshiki flow is one of the very few studies have been investigated on the heat and mass transfer characteristics in the presence of uniform magnetic field. Also, this type of flow is common in the petroleum engineering which is primarily concerned with the movement of oil, gas and water through reservoir of oil or gas field or hydrologist in the study of migration of underground water and in chemical engineering for the purification and filtration processes. It is also useful in industries which deal with determination of drug permeation through human skin. Magneto-hydrodynamic principle is also engaged in recovering water for drinking and in agriculture for irrigation purposes. Finite difference method was applied to an unsteady mixed convection flow past a semi-infinite vertical permeable moving plate with heat and mass transfer in the presence of radiation and viscous dissipation in separate study by Latha, *et al.* (2012). Also, Rushi, *et al.* (2012) considered the impact of MHD viscoelastic non-Darcy fluid flow along vertical cone using perturbation analysis. Sarada, *et al.* (2013) examined the effects of Soret and Dufour on an unsteady MHD free convection flow past a vertical porous plate in the presence of suction or injection with the aid of analytical method of perturbation techniques. Mohan, *et al.* (2013) considered the effects of chemical reaction and radiation on MHD free convection flow of Kuvshinshiki fluid through a vertical porous plate with heat sources by using perturbation method. Also, Idowu, *et al.* (2013) applied the analytical method to solve the heat and mass transfer of magneto-hydrodynamics (MHD) and dissipative fluid flow pass a moving vertical porous plate with variable suction.

Attention has been focused in recent years by various scientists and engineers in on the study of problem involving the phenomena of heat and mass transfer with radiation effect. This interest is due to the fact that the effect of radiation on convection is quite important in the context of many practical applications such as in cooling and heating of channels, nuclear

power plant, fire research, electrical power generation, gas turbines and nuclear waste disposal. Uwanta and Halima (2014) have has discussed the convective heat and mass transfer flow over a vertical plate with n th-order chemical reaction in a porous medium. The results were obtained using Crank-Nicolson method. Vidya *et al.* (2014) considered, unsteady MHD free convection boundary layer flow of radiation absorbing Kuvshinshiki fluid through porous medium. Unsteady MHD free convection flow of a Kuvshinski fluid past a vertical porous plate in the presence of chemical reaction and heat source/sink was studied by Harinath, *et al.* in (2015). Uwanta and Halima (2015) solved the problem of finite difference solutions of magneto-hydrodynamic free convective flow with constant suction and variable thermal conductivity in a Darcy-Forchheimer porous medium. Halima *et al.* (2016) presented the effect of magneto-hydrodynamics free convection flow with thermal radiation and chemical reaction effects in the presence of variable suction. Heat and mass transfer on MHD free convection flow of viscoelastic Kuvshinshiki fluid through porous medium past an infinite vertical porous plate was studied by Raju and Siva-Prasad (2016). In their studies numerical solutions were generated using an implicit finite-difference scheme.

It can be concluded from the previous studies that the effects of Soret number, heat source and viscoelastic parameters were not considered. Hence, inspired by several applications of the effects of these parameters on MHD free convection flow, such as in the design of temperature control devices in satellites industry and filtration processes in the chemical industries.(recast) The current work investigated the unsteady MHD free convection flow of viscoelastic fluid past a vertical porous plate with thermal conductivity and chemical reaction.

Formulation of the Problem

Consider an unsteady viscoelastic of kuvshinshiki fluid of MHD heat and mass transfer flow of an electrically conducting, viscous incompressible and radiating fluid over a porous vertical infinite plate with chemical reaction and thermal conductivity. In the Cartesian coordinate, the x_- - axis is taking along the vertical infinite plate in the upward direction of the flow and the y_- - axis is taken normal to the plate since the plate was considered infinite in x_- - direction. A magnetic field B_0 of uniform strength is applied transversely to the direction of the flow as shown in

figure1. Initially the plate and the fluid are at the same temperature T_{∞} in a stationary condition with concentration level C_{∞} at all points. For $t > 0$, the temperature and concentration level at the plate $y=0$ is raised to T_w and C_w respectively and that of $y \rightarrow \infty = 0$ is lowered to T_{∞} and C_{∞} . It is assumed that the induced magnetic field is negligible in comparison with the applied one which is corresponding to a very small magnetic Reynold number. The effects of

Dufour, variable suction, thermal radiation and viscoelastic parameters were taken into consideration. By applying Boussinesq's application, the flow field is governed by the following equations:

Continuity equation

$$\frac{\partial u}{\partial y} = 0 \quad (1)$$

Momentum equation

$$\left(1 + \lambda^* \frac{\partial}{\partial t}\right) \frac{\partial u^*}{\partial t^*} + v^* \frac{\partial u^*}{\partial y^*} = \frac{\partial^2 u^*}{\partial y^{*2}} + g\beta(T^* - T_{\infty}^*) + g\beta(C^* - C_{\infty}^*) - \left(1 + \lambda^* \frac{\partial}{\partial t}\right) \left(\frac{\sigma B_0^2}{\rho} + \frac{\nu}{k^*}\right) u^* + \frac{bu^{*2}}{\rho k}. \quad (2)$$

Temperature equation

$$\begin{aligned} & \left(1 + \lambda^* \frac{\partial}{\partial t}\right) \frac{\partial T^*}{\partial t^*} + v^* \frac{\partial T^*}{\partial y^*} \\ &= \frac{k}{\rho C_p} \frac{\partial}{\partial y^*} \left[1 + m(T^* - T_0) \frac{\partial T^*}{\partial y^*}\right] + \frac{D_m K_T \partial^2 C^*}{C_s C_p \partial y^{*2}} - \frac{1}{\rho C_p} \frac{\partial q_r}{\partial y^*} \\ &+ \frac{\alpha T}{\rho C_p} (T^* - T_{\infty}^*) \dots \dots \dots 3 \end{aligned}$$

Concentration equation

$$\frac{\partial C^*}{\partial t^*} + v^* \frac{\partial C^*}{\partial y^*} = D_m \frac{\partial^2 C^*}{\partial y^{*2}} + \frac{D_m K_T}{T_m} \frac{\partial^2 T^*}{\partial y^{*2}} - K_r (C^* - C_{\infty}^*) \quad (4)$$

The boundary conditions for the velocity, temperature and concentration fields are

$$t < 0, u^* = 0, T^* \rightarrow T_{\infty}^*, \text{ for as } y^* = 0 \quad (5)$$

$$t \leq 0, u^* = 0, T^* \rightarrow T_\infty^*, \text{ as } y^* = 0 \quad (6)$$

From the continuity equation, it can be seen that v_- is either a constant or a function of time. Hence, on integrating equation (1), the suction velocity normal to the plate is assumed in the form: $v = -v_0^*$ where v_0 is the mean suction velocity which is non-zero positive constant. The negative sign indicates that the suction velocity is directed towards the plate, ν is the kinematic viscosity, g is the gravitational force, β is the coefficient of thermal expansion, ρ the density of the fluid, σ is the electrical conductivity of the fluid, β is the coefficient of concentration expansion, C_p is the specific heat capacity at constant pressure, K^* is the permeability of the porous medium, k is the thermal conductivity of the fluid, α is a constant depending on

the nature of the fluid, R is the dimensionless chemical reaction, n is the order of chemical reaction, D is the coefficient of mass diffusivity, (some terms defined are not in the equations and some terms in the equations are not defined ie $m, D_m, \dots$) q_r is the radiative heat flux in the y - direction, B_0 is the electromagnetic induction. T_∞^* is the free stream temperature, C_∞^* is the free stream concentration, T_w and C_w are wall dimensional temperature and concentration respectively, n^* is a constant.

$$v^* = -V_0 (1 + \varepsilon A e^{-nt})$$

where A is a real positive constant, ε and εA are small values less than unity and V_0 is a scale of suction velocity normal to the plate and is assumed as a function of time only surface. In order to write conservative equations and the boundary conditions in dimensionless form, the transformation model are introduced. The thermal conductivity depends on temperature. It was used by Molla et al. (2005), and defined as:

$$K(T) = k_0 \{1 + m(T^* - T_\infty^*)\} \quad (7)$$

where k_0 is the thermal conductivity of the ambient fluid, m is a constant depending on the nature of the fluid and m is defined by

$$m = \frac{1}{K(T)} \left(\frac{\partial K(T)}{\partial T} \right) \quad (8)$$

The radiative heat flux q_r under Rosseland approximation has the form of:

$$q_r = \frac{4\sigma}{3k_e} \frac{\partial T^4}{\partial y^*} \quad (9)$$

With greater interest in the application of the Roseland diffusion approximation as reported in Halima et al. We simplify the radiative heat flux by using with σ as the stefan-Boltzman constant and k_e as the mean absorption coefficient. Using the Roseland model, the fluids in this paper are optically thick. Assuming that the temperature difference within the flow is sufficiently small, Equation (9) is linearized by the expansion of T^4 using Taylor's series about T_∞ as describe below

$$m(x) = m(x_0) + (x - x_0)m'(x_0) + \frac{(x - x_0)^2}{2!}m''(x_0) + \dots + \frac{(x - x_0)^n}{n!}m^{(n)}(x_0) \quad (10)$$

(m here is different from the previous m , use another symbol.)

Now, setting $m(x) = T^4$, $m(x_0) = T_\infty^4$ and $(x - x_0) = (T - T_\infty)$ in equation 10 above and neglecting the higher order terms to give:

$$T^4 = 4T_\infty^3 - 3T_\infty^4 \quad (11)$$

Put Equation (11) on equation (9) and substituting the result into the last term in the RHS of equation (3) to obtain

$$\frac{-1}{\rho C_\rho} \frac{\partial q_r}{\partial y^{*2}} = \frac{16\sigma_s T_\infty^3 \partial^2 T}{3\rho C_\rho k_e \partial y^{*2}} \quad (12)$$

In order to simplify expressed the governing and boundary condition equations in a dimensionless form, the following non-dimensional quantities are introduced

Transformation of the Model

In order to solve the governing equations in dimensionless form, we introduce

the following non-dimensional quantities into (2), (3) and (4):

$$\begin{aligned} u &= \frac{u^*}{U_0}, \quad y = \frac{U_0 y^*}{\nu}, \quad t = \frac{U_0^2 t^*}{\nu}, \quad \theta = \frac{T' - T_\infty'}{T_w' - T_\infty'}, \quad C = \frac{C' - C_\infty'}{C_w' - C_\infty'}, \\ \text{Pr} &= \frac{\nu \rho C \rho}{k} = \frac{\nu}{\alpha}, \quad \text{Gr} = \frac{g \beta \nu (T_w' - T_\infty')}{U_0^2}, \quad \text{Gc} = \frac{g \beta \nu (C_w' - C_\infty')}{U_0^2}, \\ M &= \frac{\sigma B_0^2 \nu}{\rho U_0^2}, \quad R = \frac{4 I_1 \nu}{k U_0^2}, \quad \text{Sc} = \frac{\nu}{Dm}, \quad \text{Sr} = \frac{Dm K_T (T^* - T_\infty^*)}{T_m \nu (C^* - C_\infty^*)}, \quad \text{and } KC = \frac{k_r \nu}{U_0^2}, \\ \gamma &= \frac{\nu_0}{U_0^2}, \quad \tau = m = (T' - T_\infty'), \quad K = \frac{\nu}{k^* U_0^2}, \quad Du = \frac{Dm K_T (C^* - C_\infty^*)}{C_s C_p (T^* - T_\infty^*)}, \quad (13) \end{aligned}$$

where u and v are dimensionless velocities, t is the dimensionless time, T is the dimensionless temperature function, θ is the dimensionless temperature function. F is the conduction-radiation heat transfer parameter, Pr is the Prandtl number, Gr is the thermal Grashof number, Gc is the mass Grashof number, M is the Magnetic field, K is the permeability parameter, α_T is heat source parameter, Sc is Schmidt number, Kr is the chemical reaction, n is the order reaction and k is the thermal conductivity.

$$\left(1 + \lambda \frac{\partial}{\partial t}\right) \frac{\partial u}{\partial t} - \left(1 + \varepsilon A e^m\right) \frac{\partial u}{\partial y} = \frac{\partial^2 u}{\partial y^2} - \left(1 + \lambda \frac{\partial}{\partial t}\right) \left(M + \frac{Fs}{Da}\right) u^2 - \frac{1}{Da} + \text{Gr} \theta + \text{Gc} C \quad (14)$$

$$\left(1 + \lambda \frac{\partial}{\partial t}\right) \frac{\partial \theta}{\partial t} - \left(1 + \varepsilon A e^m\right) \frac{\partial T}{\partial y} = \frac{1}{\text{Pr}} (1 + \lambda \theta) \frac{\partial^2 T}{\partial y^2} + \frac{\lambda}{\text{Pr}} \left(\frac{\partial \theta}{\partial y}\right)^2 - \frac{1}{\text{Pr}} (R - S) \theta + Du \frac{\partial^2 C}{\partial y^2} \quad (15)$$

$$\frac{\partial C}{\partial t} - \left(1 + \varepsilon A e^{i\omega t}\right) \frac{\partial C}{\partial y} = \frac{1}{\text{Sc}} \frac{\partial^2 T}{\partial y^2} - K_r C \quad (16)$$

With the boundary conditions

$$u = 0, \theta = 1, \quad C = 1 \quad \text{for} \quad y = 0 \quad (17)$$

$$u = 0, \theta = 0, \quad C = 0 \quad \text{as} \quad y \rightarrow \infty \quad (18)$$

(There is fundamental problem with eqns 16 and 17)

NUMERICAL SOLUTION

The system of transformed coupled non-linear partial differential equations (14), (15) and (16) together with the initial and boundary conditions (17 and 18) have been solved numerically using a finite difference technique of implicit type namely Crank-Nicolson finite difference method. The equivalent finite difference approximations corresponding to equations (14)-(16) are given as follows:

$$\left(1 + \frac{\lambda}{\Delta t}\right) \frac{(U_i^{j+1} - U_i^j)}{\Delta t} - (1 + \varepsilon A e^{\pi}) \frac{U_{i+1}^{j+1} - U_{i-1}^{j+1}}{2\Delta y} = \frac{U_{i+1}^{j+1} - 2U_i^{j+1} + U_{i-1}^{j+1} + U_{i+1}^j - 2U_i^j + U_{i-1}^j}{2(\Delta y)^2} + Gr(\theta_i^j) + Gc(C_i^j) \quad (19)$$

$$- \left(1 + \frac{\lambda}{\Delta t}\right) \left(M + \frac{Fs}{Da}\right) (U_i^j)^2 - \frac{1}{Da} (U_i^j)$$

$$\left(1 + \frac{\lambda}{\Delta t}\right) \frac{Pr(\theta_{i+1}^{j+1} - \theta_i^j)}{\Delta t} - (1 + \varepsilon A e^{\pi}) \frac{\theta_{i+1}^{j+1} - \theta_{i-1}^{j+1}}{2\Delta y} = \frac{q(\theta_{i+1}^{j+1} - 2\theta_i^{j+1} + \theta_{i-1}^{j+1} + \theta_{i+1}^j - 2\theta_i^j + \theta_{i-1}^j)}{2(\Delta y)^2} - (R - Q) \left(\frac{\theta_{i+1}^j - \theta_{i-1}^j}{2}\right) \quad (20)$$

$$+ \frac{Du \left(C_{i+1}^{j+1} - 2C_i^{j+1} + C_{i-1}^{j+1} + C_{i+1}^j - 2C_i^j + C_{i-1}^j\right)}{2(\Delta y)^2}$$

$$\left[\frac{ScSr(C_i^{j+1} - C_i^j)}{\Delta t} - ScSr(1 + \varepsilon A e^{\pi}) \frac{C_{i+1}^{j+1} - C_{i-1}^{j+1}}{2\Delta y} = \frac{Sr(C_{i+1}^{j+1} - 2C_i^{j+1} + C_{i-1}^{j+1} + C_{i+1}^j - 2C_i^j + C_{i-1}^j)}{2(\Delta y)^2} - Kr(C_i^j) \right] \quad (21)$$

$$+ \left[\frac{Sc(\theta_{i+1}^{j+1} - 2\theta_i^{j+1} + \theta_{i-1}^{j+1} + \theta_{i+1}^j - 2\theta_i^j + \theta_{i-1}^j)}{2(\Delta y)^2} \right]$$

$$- A_1 U_{i-1}^{j+1} + A_2 U_i^{j+1} - A_1 U_{i+1}^{j+1} = A_3 U_{i-1}^j + A_4 U_i^j + A_6 U_{i+1}^j - A_5 U_i^j + A_7 \theta_i^j + A_8 C_i^j$$

$$- B_1 \theta_{i-1}^{j+1} + B_2 \theta_i^{j+1} - B_1 \theta_{i+1}^{j+1} = B_3 \theta_{i-1}^j + B_4 \theta_i^j + B_5 \theta_{i+1}^j - B_6 (\theta_{i+1}^j - \theta_{i-1}^j)^2$$

$$+ B_7 (C_{i+1}^{j+1} - 2C_i^{j+1} + C_{i-1}^{j+1} + C_{i+1}^j - 2C_i^j + C_{i-1}^j)$$

$$- D_1 C_{i-1}^{j+1} + D_2 C_i^{j+1} - D_1 C_{i+1}^{j+1} = D_3 C_{i-1}^j + D_4 C_i^j + D_5 C_{i+1}^j + D_6 C_i^j$$

With the following boundary conditions

$t^* \leq 0, u = 0, \theta = 0, C = 0$ for ally

$t^* > 0, u = 0, \theta = 1, C = 1$ for $y = 0$ (22)

$u = 0, \theta = 0, C = 0$ as $y \rightarrow \infty$

$$\begin{aligned} r_1 &= \frac{\Delta t}{\Delta y}, r_2 = \frac{\Delta t}{(\Delta y)^2}, r_3 = 2\Delta t, r_4 = r_3 GM, r_5 = r_3 G \frac{Fs}{Da}, r_6 = r_3 Gr, r_7 = r_3 Gc, r_8 = 2r_2 \lambda, r_9 = r_3 (R - S), \\ r_{10} &= r_2 \text{Pr} Du, r_{11} = r_3 ScKr, A_1 = r_2, A_2 = 2(G + r_2), A_3 = (r_2 - r_1 B), A_4 = (2G - 2r_2 - r_4), \\ A_5 &= r_5, A_6 = (r_1 B + r_2), A_7 = r_6, A_8 = r_7, B_1 = Hr_2, B_2 = 2(\text{Pr} G + r_2 H), B_3 = (Hr_2 - r_1 B \text{Pr}), B_4 = (2 \text{Pr} G - 2r_2 H), \\ B_5 &= (Hr_2 + r_1 B \text{Pr}), B_6 = 2\tau r_2 a_2, B_7 = r_2 r_{10}, C_1 = r_2, C_2 = 2(Sc + r_2), C_3 = (r_2 - r_1 BSc), C_4 = (2Sc - r_2), \\ C_5 &= (r_2 + r_1 BSc), C_6 = r_{11}, B = (1 + \varepsilon Ae^{i\omega t}), G = \left(1 + \lambda \frac{\partial}{\partial t}\right), H = (1 + \tau \theta_i^j). \end{aligned} \quad (23)$$

(Eqn 20 is not an equivalent finite difference approximations of eqn 15)

The index i corresponds to space y and j corresponds to time. Δy and Δt are the mesh sizes along y -direction and time t -direction respectively. The numerical computations are carried out for different values of physical parameters involved in the problem. The finite difference equations (14)-(16) at every internal nodal point on a particular n -level constitute a tri-diagonal system of equations which are solved by using the Thomas Algorithm.

In each time step, the concentration and temperature profiles have been computed first from equations 15 and 16 and then the computed values are used to obtain the velocity profile which meets the convergence criteria. The skin-friction, rate of heat and mass transfer in terms of Nusselt number and Sherwood number respectively are given by the dimensionless local value of skin friction (τ), Nusselt number (Nu) and the Sherwood number (Sh) can be characterized in order by

$$\tau_x = -\left(\frac{\partial U}{\partial y}\right)_{y=0} \quad (24)$$

$$Nu_x = -X \left[\frac{\partial T}{\partial y}\right]_{y=0} \quad (25)$$

$$Sh_x = -X \left[\frac{\partial C}{\partial y} \right]_{y=0} \quad (26)$$

The average of skin friction (τ); Nusselt number (Nu); and Sherwood number (Sh) can be written in dimensionless form as

$$(\bar{\tau})_x = - \int_0^1 \left[\frac{\partial u}{\partial y} \right]_{y=0} dX \quad (27)$$

$$(\overline{Nu})_x = - \int_0^1 \left[\frac{\partial T}{\partial y} \right]_{y=0} dX \quad (28)$$

$$(\overline{Sh})_x = - \int_0^1 \left[\frac{\partial C}{\partial y} \right]_{y=0} dX \quad (29)$$

Results and Discussion

In order to get a physical view of the present work, an efficient numerical method called Crank-Nicolson method has been used to solved the transformed equations (14)-(16) subject to the boundary conditions (17). Numerical results for velocity profiles (u), temperature profiles (T), concentration profiles (C), skin friction (Cf), rate of heat transfer in terms of Nusselt number (Nu) and rate of mass transfer in term of sherwood number (Sh) have been computed graphically and in tabular form by assigning numerical values to different values of pertinent parameters. The following values are used for the investigation: Gr=1.0, Gc=1.0, M=1, K=0.1, Pr=0.71, Sc=0.60, R=1, Kr=0.5, $\tau = 0:01$, $\lambda = 0:0002$, Sr=0.5, Da=0.1.

Velocity Profiles

Figures 2 and 3 represents different values of thermal Grashof number on the velocity, it is noted that, the velocity rises with increasing thermal Grashof number. The effect of mass Grashof number on the velocity was presented. It was observed that, the velocity increases with increase in mass Grashof number. It is noted that greater cooling of surface (i.e Gr and Gc) gives an increase in the velocity for air. This is because, increase in the values of thermal Grashof number and mass Grasshof number has a tendency of increasing the thermal and mass buoyancy effect. This gives rise to an increase in the induced flow. It is seen that the dimensionless velocity increases with increase in Darcy number. This is due to the fact

that larger values of D parameter correspond to higher permeability porous media, which implies that, less porous media less porous media which implies that, less porous fiber resistance to the flow and therefore acceleration in transport (figure 4). It is noticed in figure 5 that an increase in F_s parameter increases the resistance to the flow and so a decrease in the velocity profile. The influence of viscoelastic parameter on the velocity is given in figure 5. The viscoelastic parameter represents the normal stress coefficient on the flow. It is noticeable in figure 6 that at a given point on the flow domain, the velocity is increased with an increase in the viscoelastic parameter. It can be seen in Fig. 7 that increase in the magnetic parameter (M) causes a fall in the velocity of the fluid. The effect of transverse magnetic field on an electrically conducting fluid slows down the motion of the fluid. This is because of the application of transverse magnetic field which result to a resistive force (Lorentz force). This force is similar to drag force which ends to resist the fluid flow and however reducing the velocity of the fluid. Figure 8 exhibits the velocity profiles for various values of Prandtl number. From this figure, it is observed that velocity decreases with an increase in Prandtl number. This is physically true because, the Prandtl number is a dimensionless number which is the ratio of momentum diffusivity (kinematic viscosity) to thermal diffusivity. In many of the heat transfer problems, the Prandtl number controls the relative thickness of the momentum and thermal boundary layers. When Pr is small, it means that the heat diffuses very quickly compared to the velocity (momentum). This means that for liquid metals the thickness of the thermal boundary layer is much bigger than the velocity boundary layer.

This figure 9 represents the velocity for different values of R , it is observed that the velocity profile decreases with an increase of R . This is because larger value of R correspond to an increased dominance of conduction over radiation, thereby decreasing the buoyancy force and the thickness of the momentum boundary layer. It was observed in figure (10) that as velocity profile decreases, Sc increases. Also, as the velocity decreases with an increase in suction parameter α (figure 11). This is because suction parameter decelerates fluid particles through porous wall thereby reducing the growth of the fluid boundary layers.

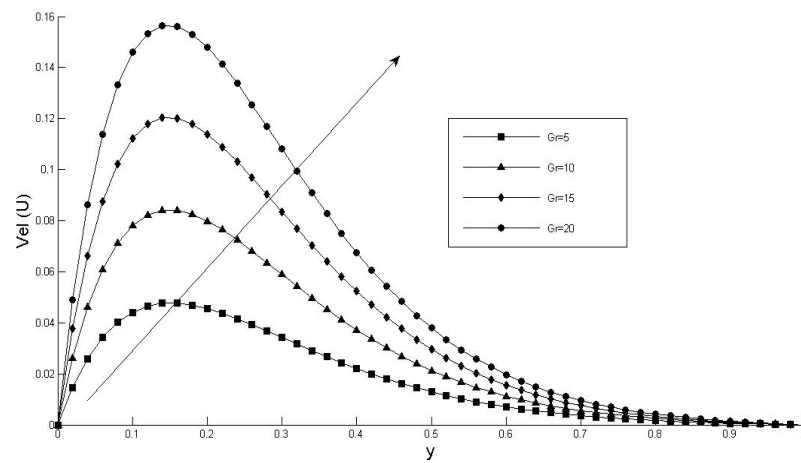


Figure 2: Effect of Gr on velocity

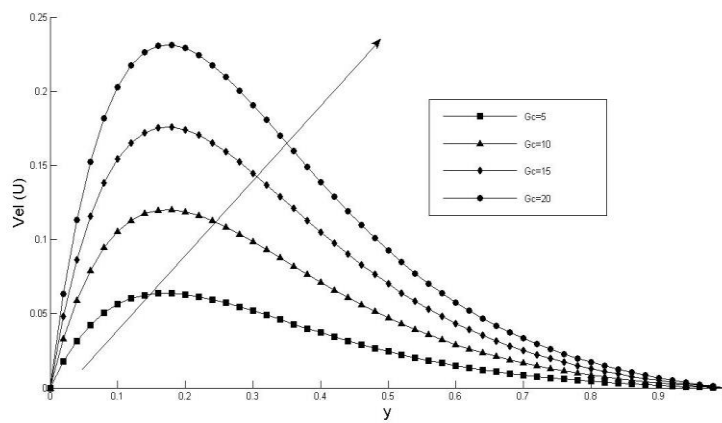


Figure 3: Effect of Gc on velocity.

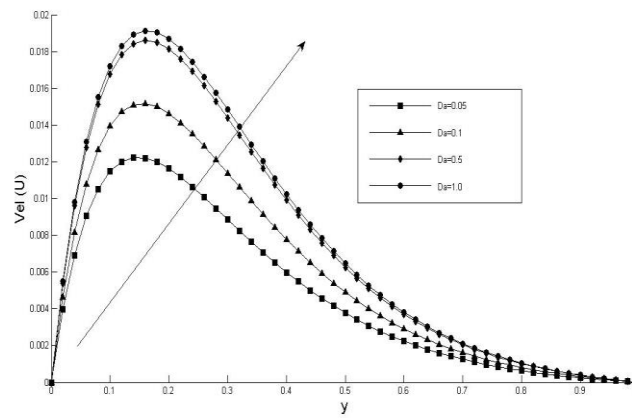


Figure 4: Effect of Da on velocity.

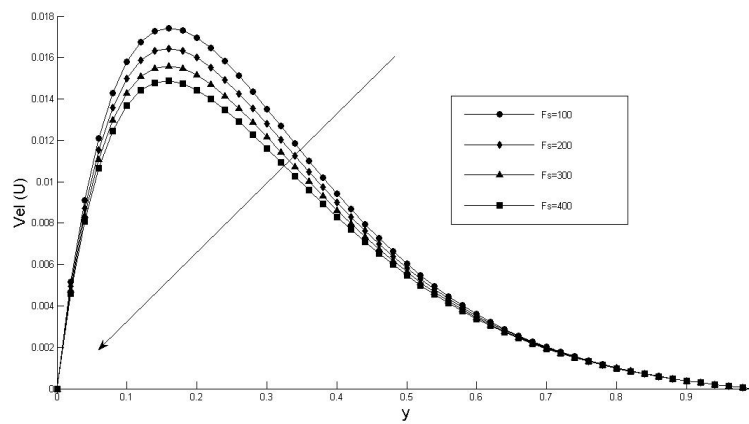


Figure 5: Effect of F_s on velocity.

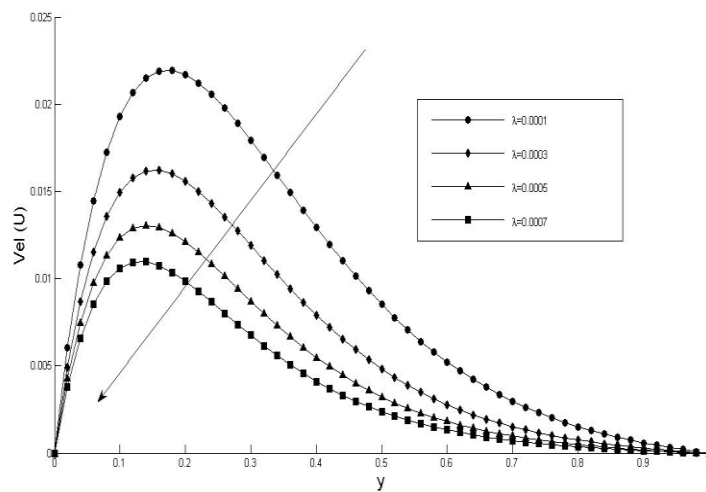


Figure 6: Effect of λ on velocity.

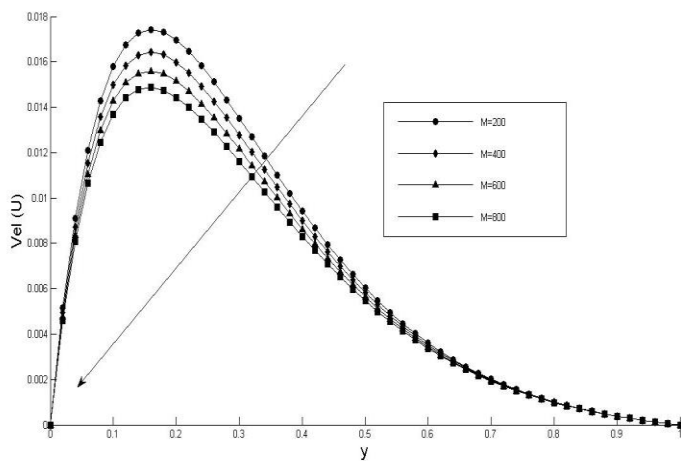


Figure 7: Effect of M on velocity.

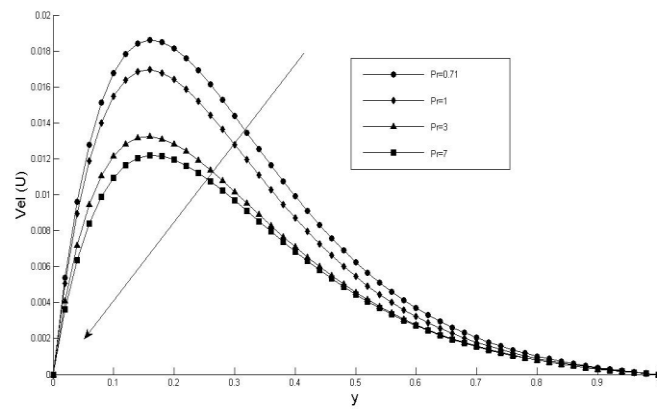


Figure 8: Effect of Pr on velocity.

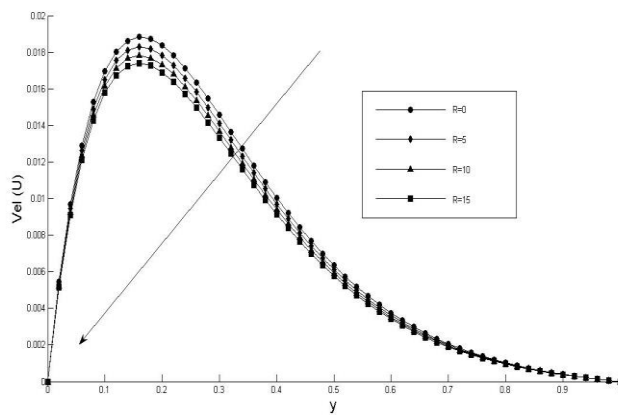


Figure9: Effect of R on velocity.

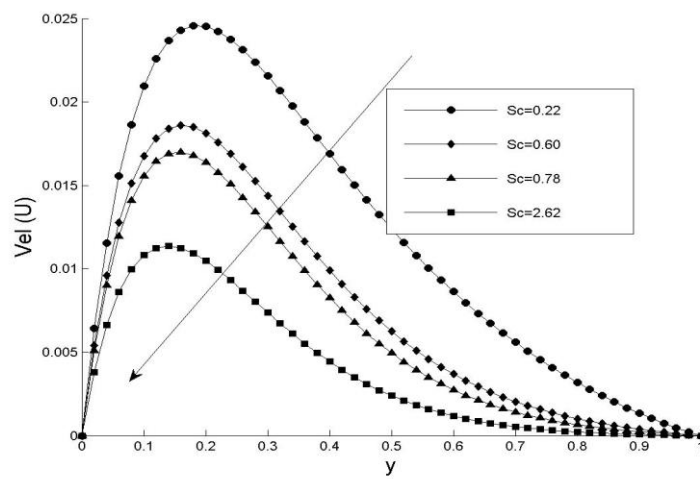


Figure10: Effect of Sc on velocity.

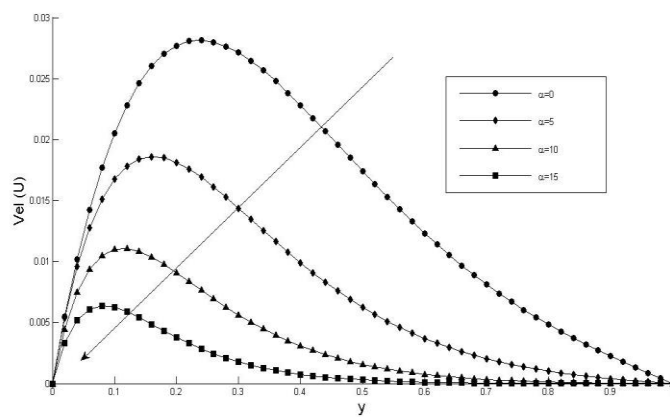


Figure11: Effect of α on velocity.

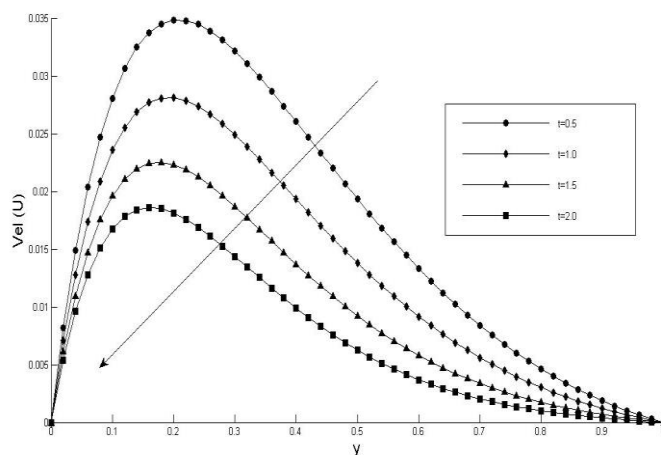


Figure 12: Effect of t on velocity.

Temperature Profiles

Figure 13 Shows the effect of Dufour number (Du) on temperature profiles. It is observed that temperature increases as Dufour number increases. The effect of the λ on the temperature profile has been shown in figure14. It is observed that the temperature profile decreases as λ increases. Figure 15 shows the effects of Pr on temperature profile. It is noticed that temperature decreases with the increase in Pr . It is observed in Figure 16 that temperature increases with increasing Q . Figure 17 presents the variation of temperature component for various values of R . The result shows that the effect of increasing values of R results in an decreasing the temperature profile. Figure.18 displays the effect of variable suction parameter on temperature profile.

We discovered that as temperature profile decreases the suction parameter α increases. In figure 19. The effect of thermal conductivity parameter τ increases along the temperature profile.

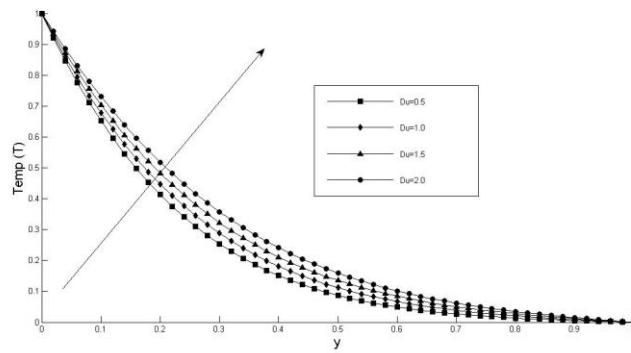


Figure13: Effect of Du on temperature

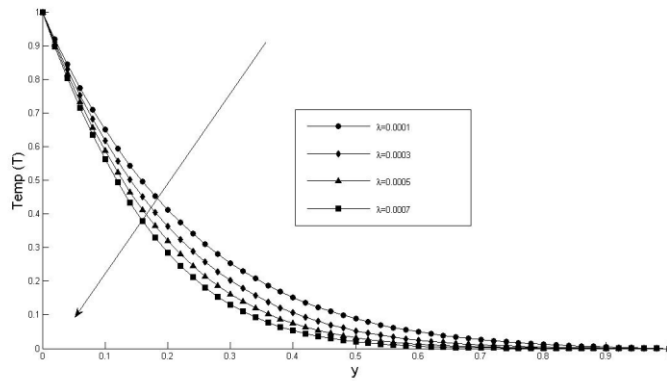


Figure14: Effect of λ on temperature

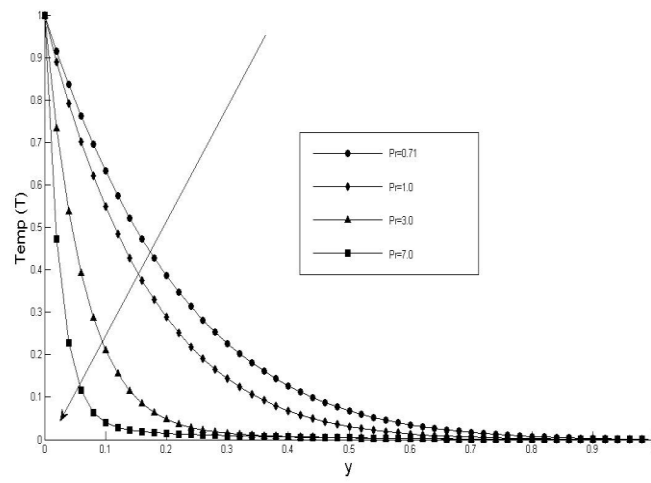


Figure15: Effect of Pr on temperature

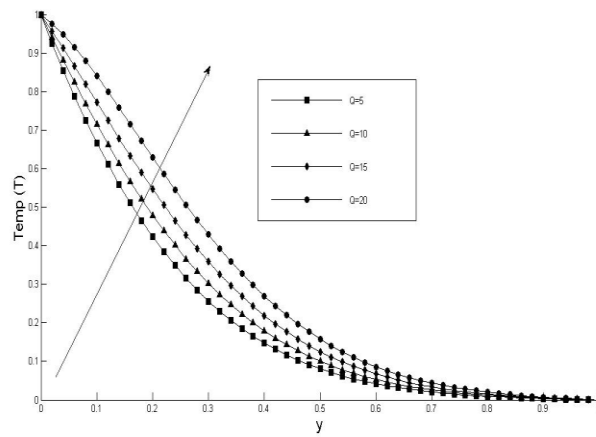


Figure16: Effect of Q on temperature

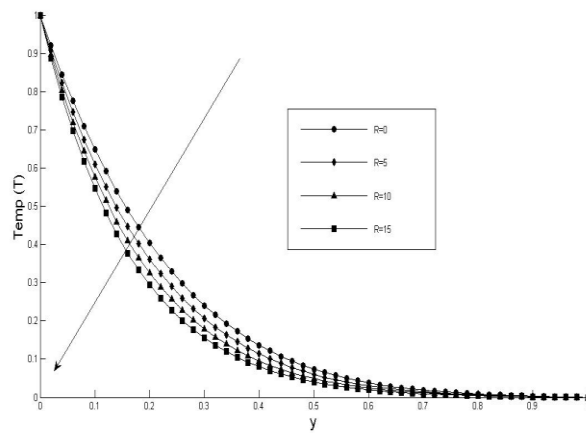


Figure17: Effect of R on temperature

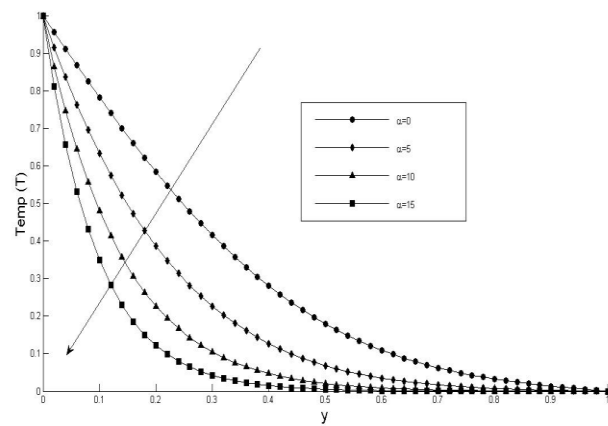


Figure18: Effect of α on temperature

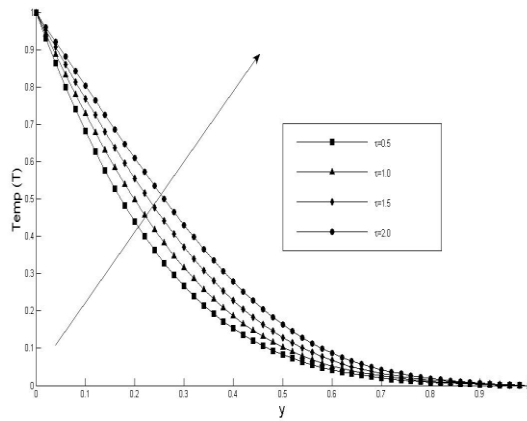


Figure19: Effect of τ on temperature

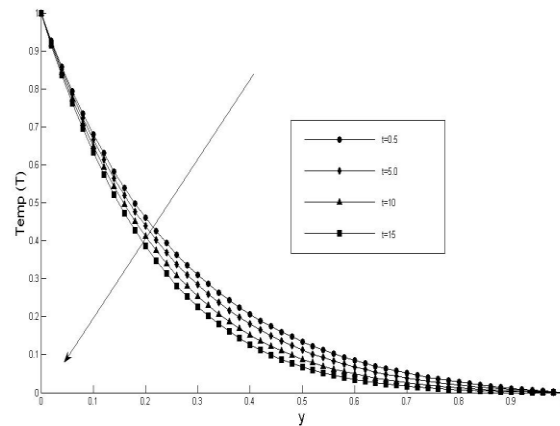


Figure20: Effect of t on temperature

Concentration Profiles

The concentration profiles are illustrated in figures 21, 22 and 23 for different values of Schmidt number (Sc), (Sr) and Chemical reaction (Kr). Figures 21 and 22 depict the influence of Schmidt number Sc and chemical reaction on concentration. As the Schmidt number increases, the concentration profile decreases. Also, it is noted that the concentration boundary layer becomes thin as the Schmidt number increases. This causes the concentration buoyancy effect to decrease yielding a reduction

in the fluid concentration. Physically, increase of Sc means decrease in molecular diffusivity. While the concentration decreases with increasing chemical reaction Kr . In the same vein, the concentration boundary layer becomes thin as the chemical reaction parameter increases. The influence of (Sr) also observed in figure 23, when the solet parameter increase the concentration profile also increased.

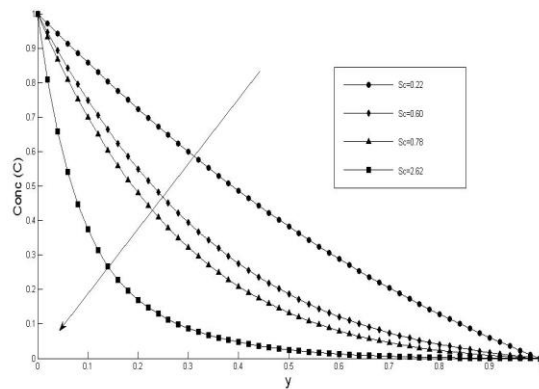


Figure21: Effect of Sc on Concentration

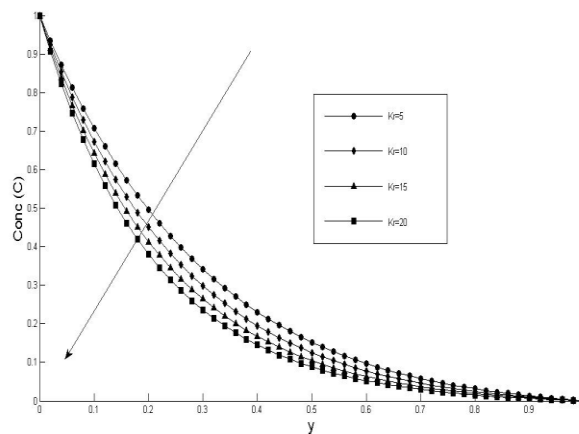


Figure22: Effect of Kr on Concentration

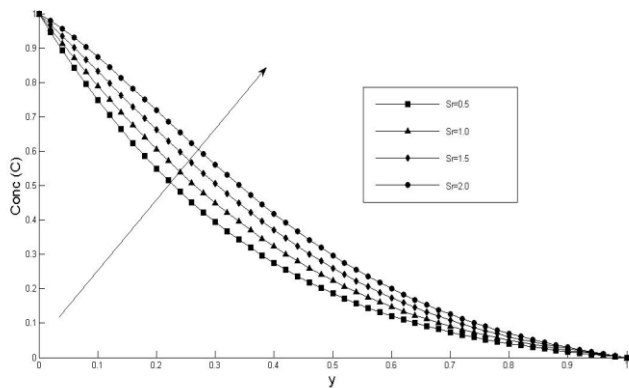


Figure 23: Effect of Sr on Concentration

Table of Values

Tables 1 and 2 shows the comparison between the present work and that of Uwanta and Halima work. The effects of thermal conductivity parameter on Skin Friction and Nusselt number was observed. It is observed that an increase in λ parameter increases the Skin friction coefficient for (Pr=0.71 and 7.0). Whereas, heat transfer rate increased with an increase in λ . The effects of various thermo-physical parameters on skin friction, Nusselt number and Sherwood number were shown in Tables 3-7. It is observed that the Skin friction increases with an increase in Gr, Gc, Da and α why the (M, λ , Fs, R, Pr) dropped. But it is noticed that there is no significant change in the Nusselt number when the (λ , R, Pr, τ , α) increased. Whereas Q and Du increase as Nusselt number increasing. The Numerical values for Sc, Kr and Sr are displayed in Table 7. The values of Sc and Kr increasing the Sherwood number decreases. There is a significant change in Sherwood number when the Sr parameter increased. It is seen that the agreement between the present work and Uwanta and Halima work are excellent because of the various numerical values for λ when the Pr=0.71 and 7.0 presented in tables 1 and 2.

Table-1: Numerical values for skin-friction coefficient C_f and Nusselt number Nu for various values of thermal conductivity compared with Uwanta and Halima (2015); Gr = Gc = 1:0; Pr = 0:71; Sc = 0:60; α = 0:5;

$n = 0:1; M = 1:0; \varepsilon = 0:02; R = 0:1; Da = 0:5; Fs = 1:0; Du = 0:1; _ = 0; Sr = 0; Q = 0$

Fluid Parameter τ	Present Work τ Nu		Uwanta and Halima (2015) Cf Nu	
$\tau = 0.0$	0.2774	1.5634	0.2820	1.5656
$\tau = 0.005$	0.2779	1.5264	0.2826	1.5276
$\tau = 0.10$	0.2785	1.4905	0.2832	1.4908
$\tau = 0.20$	0.2796	1.4218	0.2843	1.4207
$\tau = 0.25$	0.2801	1.3890	0.2848	1.3872

Fluid Parameter	Velocity gradients	Nusselt numbers	Sherwood numbers
R=0	0.3022	-4.1213	-2.8360
R=5	0.2960	-4.7998	-2.7378
R=10	0.2906	-5.4102	-2.6488
M=200	0.2866	-4.4018	-2.7955
M=400	0.2756	-4.4018	-2.7955
M=600	0.2661	-4.4018	-2.7955
Gr=5	0.8168	-4.4018	-2.7955
Gr=10	1.4617	-4.4018	-2.7955
Gr=6	2.1052	-4.4018	-2.7955
Gc=5	0.9785	-4.4018	-2.7955
Gc=10	1.8228	-4.4018	-2.7955
Gc=15	2.6635	-4.4018	-2.7955
Da=0.05	0.2239	-4.4018	-2.7955
Da=0.1	0.2594	-4.4018	-2.7955
Da=0.5	0.2996	-4.4018	-2.7955
Fs=100	0.2866	-4.4018	-2.7955
Fs=200	0.2756	-4.4018	-2.7955
Fs=300	0.2662	-4.4018	-2.7955
$\lambda = 0.0001$	0.3329	-4.1929	-2.8293
$\lambda = 0.0003$	0.2749	-4.5999	-2.7642
$\lambda = 0.0005$	0.2399	-4.9655	-2.7073
Pr=0.71	0.2996	-4.4018	-2.7955
Pr=1	0.2817	-5.7652	-2.5914
Pr=7	-0.2035	-33.5102	-1.1894
Q=5	0.3049	-3.8275	-2.8782
Q=10	0.3123	-3.0267	-2.9923
Q=15	0.3209	-2.1131	--3.1208
Sr=1.0	0.3120	-4.4219	-2.2007

$S_r=1.5$	0.3244	-4.4422	-1.5999
$S_r=2.0$	0.3368	-4.4627	-0.9928
$S_c=0.22$	0.3533	-4.4469	-1.4647
$S_c=0.60$	0.2996	-4.4018	-2.7955
$S_c=0.78$	0.2836	-4.3791	-3.4830
$K_r=5$	0.2905	-4.3830	-3.4039
$K_r=10$	0.2825	-4.3658	-4.9447
$K_r=15$	0.2755	-4.3503	-4.4233
$Du=0.5$	0.3050	-4.1275	-2.8365
$Du=1.0$	0.3117	-3.7679	-2.8902
$Du=1.5$	0.3186	-3.3883	-2.9469
$\tau =0.5$	0.3092	-3.5497	-2.9234
$\tau =1.0$	0.3094	-2.8764	-3.0246
$\tau =1.5$	0.3298	-2.3638	-3.1019
$\alpha =0$	0.2923	-2.2519	-1.1962
$\alpha =5$	0.2996	-4.4018	-2.7955
$\alpha =10$	0.2583	-7.2204	-4.9955

Conclusion

Magneto-hydrodynamics free convection flow of viscoelastic fluid past a vertical porous plate with thermal conductivity and chemical reaction has been analyzed numerically. The resulting governing equations of the problem are non-dimensionalized, simplified and solved by Crank Nicolson type of implicit finite difference method. It reveals that the velocity decreased due to decreases in either of the viscoelastic parameter, magnetic parameter, or radiation parameter. An increase in the fluid temperature and the thermal boundary layer thickness is a function of an increase in thermal conductivity parameter, Dufour parameter, heat generation parameter why the radiation parameter, viscoelastic parameter and Prandtl number fall as Dufour increases. In addition, the concentration decreased, as either of the Schmidt number or chemical reaction parameter was increased. The viscoelastic parameter and Prandtl number decreases the skin friction coefficient, whereas reverse trend is seen in the rate of heat transfer increasing with increase in thermal conductivity, heat generation parameter and Dufour parameter why Prandtl number, viscoelastic parameter, radiation parameter and suction decreases. Also, the increase in Schmidt number and chemical reaction parameter leads to decrease in the Sherwood number. It is hoped that solutions presented in this work for various thermo physical effects will serve as a tool for

understanding more general problems in heat and mass transfer in the satellite industry, chemical industry and in metallic materials processing.

References

Harinath, S. Reddy, M. C., Raju, M.C. and Keshava, R. E. (2015). Unsteady MHD free convection flow of a Kuvshinski fluid past a vertical porous plate in the presence of chemical reaction and heat source/sink, *International Journal of Engineering Research in Africa* ,14:13-27.

Idowu, A.S., Dada, M.S. and Jimoh .A. (2013). Heat and mass transfer of Magnetohydrodynamic (MHD) and dissipative fluid flow pass a moving vertical porous plate with variable suction. *Mathematical Theory and Modeling*. ISSN 2224-5804 (paper). 3.

Latha, M. D., Haritha, C. and N. Kishan,(2012). Finite difference analysis on an unsteady mixed convection flow past a semi-infinite vertical permeable moving plate with and mass transfer with radiation and viscous dissipation, *Pellagra Research Library. Advances in Applied Science Research* 3 (4):2266-2279.

Mohan, K. P., Sugunamma, V. and Sandeep, N. (2013). Effect of chemical reaction and radiation on MHD free convection flow of Kuvshinshiki fluid through a vertical porous plate with heat sources, *American Eurasian Journal of science Research* 8(3): 135-143.

Rachna, K. (2013). Unsteady MHD flow, heat and mass transfer along an accelerated vertical porous plate in the influence of viscous dissipation, heat source and variable suction, *International Journal of Mathematics and Computer Applications Research*, 3(1): 229-236.

Raju .G. and Siva Prasad .R. (2016). Heat and Mass Transfer on MHD Free Convection Flow of Visco-Elastic Kuvshinshiki fluid through porous medium past an infinite vertical porous plate. *IOSR Journal of Mathematics (IOSR-JM)* ,ISSN: 2278-5728 36-45.

Rushi, B. K. and Sivaraj.R.(2012). MHD viscoelastic non-Darcy fluid flow along vertical cone, *int. J. of Appl.Math.*8 (11): 69-81.

Uwanta, I.J. and Murtala, S.(2013). Heat and mass transfer flow past an infinite vertical plate with variable thermal conductivity, *International Journal of Applied Information Systems* 6 (1):S.

Uwanta, I. J. and Halima, U. (2014). Convective heat and mass transfer flow over a vertical plate with Nth order chemical reaction in a porous medium, *International Journal of Scientific Engineering and technology*, 3(2): 172-185.

Uwanta, I. J. and Halima, U. (2015). Finite difference solutions of magnetohydrodynamic free convective flow with constant suction and variable thermal conductivity in a Darcy-Forchheimer porous medium, *IOSR Journal of Mathematics*,(11):2278-5728.

Halima .U.(2016). Magneto-hydrodynamic Free Convection Flow with Thermal Radiation and Chemical Reaction Effects in the Presence of Variable Suction, *MATEC Web of Conferences*,(64),

Vidyasagar, G. and Ramana, B. (2013). Radiation effect on MHD free convection flow of Kuvshinshiki fluid with mass transfer past a vertical porous plate through porous medium, *Asian Journal of current Engineering and Maths* 2(3): 170-174.

Vidya, B., Raju, M.C., Varma, S.V.K., Venkataraman, S. (2014). Unsteady MHD free convection boundary layer on of radiation absorbing Kuvshinshiki fluid through porous medium, *Review of Advances in Physics Theories and Applications* 3 (1).page number missing

Toxicological studies of *Terminalia glaucescens* using an animal model

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Abstract

Terminalia glaucescens is a plant widely known for its therapeutic value. It is frequently used by un-orthodox medical practitioners in Nigeria for the treatment of infections. However, majority of the studies on *T. glaucescens* are *in vitro*. Thus this study was carried out to investigate the toxicological effects of *T. glaucescens* *in vivo*. Male albino Wister mice were infected with pathogenic *Escherichia coli* and treated with ethanolic extracts of roots of *T. glaucescens*. Toxicological evaluation of the plant was determined by examining changes in haematological parameters of the mice, liver, kidney function indices, enzyme activities and histopathological analyses. Results showed that administration of varying concentration of the ethanolic extract of *T. glaucescens* *in vivo* did not produce significant effect on the liver and kidney of the mice when compared to the reference drug (augmentin). Increased bacterial count of *E. coli* from 2.6×10^2 in day 0 to 4.8×10^3 in day five was subsequently reversed to 3.8×10^2 using the ethanolic extract at 600 mg/kg body weight. Gross degeneration was noticed in the histoarchitectural structure of the liver of infected mice. These changes were significantly reversed in mice treated with reference drug and extracts of *T. glaucescens*. Furthermore, the extracts of *T. glaucescens* had similar effects to the liver and kidney function indices of the mice as did the reference drug. This study revealed that the ethanolic extract of the root of *T. glaucescens* did not pose adverse effect when administered *in vivo* in mice.

Keywords: Toxicology, Liver, Kidney, *Terminalia glaucescens*, Mice

Introduction

The rate at which bacteria are developing resistance to antibiotics has left scientists in constant search for antibacterial agents with ability to inhibit the growth of these organisms (Zhou *et al.*, 2015). A constant source of these antibacterial agents is often derived from nature (Hosseinzadeh *et al.*, 2015). Medicinal plants especially those that are consumed in their natural forms (as herbs), play a critical role in the management of diseases in Nigeria, especially among the traditional medical practitioners (Nwachukwu *et al.*, 2010). The recognition of traditional medicine as an alternative form of health care has led researches to further investigate into antimicrobial activity of medicinal plants (Borokini and Lawal, 2014). Other countries in Africa, Asia, and Latin America use traditional medicine to help meet some of their primary health care needs (Hosseinzadeh *et al.*, 2015; Raphael, 2011). Although it requires more scientific approach in determining the

efficacy and safety for traditional medicine to gain global acceptance, there is a general belief that modern/synthetic medicines have higher toxicity and side-effects (Vahdana *et al.*, 2015). Herbal medicine, usually regarded as complementary and alternative medicine (CAM) has a unique and essential attribute which is the harmony or synergy exhibited by plant constituents (McIntyre, 2012). Despite a plethora of literature existing on the *in vitro* activities of medicinal plants, there is a dearth of literature accessing the toxicological effects of *T. glaucescens*.

T. glaucescens is a commonly used plant in Nigeria, explored for its numerous health benefits. The plant has been shown to possess ability to lower cholesterol (Njomen *et al.*, 2008), bactericidal action against pathogenic bacteria (Silver and Serrano, 2015) and active against multidrug resistant microorganisms (Gbadamosi and Ogunsuyi, 2014). *T. glaucescens* has been reported to contain different phytochemicals which include: anthraquinones, alkaloids, triterpenes, flavonoids, saponins, tannins and phenols. Some studies have reported these phytochemicals more abundant in the leaves and stem than the roots of the plant (Bulama *et al.*, 2014; Adebayo and Ishola, 2009).

The high usage of *T. glaucescens* as a medicinal plant in Nigeria, coupled with the lack of sufficient information on its toxicological effect necessitated this study. This study was carried out to determine the effect of the plant *in vivo* utilizing an animal model.

Materials and Methods

Experimental animals

A total of 42 Male albino Wistar mice weighing an average of 28.17g were used for this study. They were obtained from animal house of Biochemistry Department, University of Ilorin, Ilorin. They were bred and maintained in the animal house of the Department and housed in plastic cages. The mice were divided into 6 groups and allowed to acclimatize for two weeks under standard laboratory conditions before the commencement of the study. They were fed with rat pellets and tap water *ad libitum*. The cages were cleaned daily.

Animal grouping

A total of 42 (forty-two) mice were randomly divided into 6 groups (A, B, C, D, E and F) of 7 animals each. Group A was positive control (not infected and not administered). Group B was negative control (infected but not administered). Group C was infected and administered with reference drug (augmentin: Glaxo SmithKline Nigeria). Groups D – F were infected and treated with different concentrations of the ethanolic extract of *T. glaucescens*.

Inoculation

Clinical isolates of *Escherichia coli* were isolated using standard microbiology techniques and identified through biochemical reactions and Polymerase chain reactions. Each mouse in 5 of the groups were inoculated with 0.5 ml of 1.5×10^8 bacterial cells of *Escherichia coli* obtained by McFarland standardization method except the 6th group which

served as positive control. The mice were inoculated by artificially contaminating their feed (0.5 ml of 1.5×10^8 bacterial cells in 10g of feeds) and water (0.5 ml of 1.5×10^8 bacterial cells in 20ml of water). Infection was allowed to manifest over a period of 5 days and clinical symptoms of the infection were observed. The faeces of the mice were analyzed on daily basis for *E. coli* count, by culturing in Eosin Methylene Blue agar, and identified using the Microbat system of identification

The technique described by (Banso and Ayodele, 2001) was used to prepare the plant extract. The root of the plant was washed under running tap water to remove the debris. They were cut into smaller pieces and allowed to dry at room temperature for about two weeks until a constant weight was obtained. The dried parts were separately pre-crushed manually using mortar and pestle. The materials were further processed into powder by using a mechanical grinder (Marlex, model IS 4250, India). The powdered samples were soaked into ethanol in the ratio of 1:10 w/v for about 48 hours. The suspension was filtered using Whatman No.1 filter paper. The filtrate was later concentrated to dry mass by using vacuum rotary evaporator (Bibby RE200) and the crude extract obtained was weighed and recorded. The extract was stored in a sample bottle at 4°C prior to use.

Extract administration

The treatment of the infected mice was orally administered as follows: Group A (positive control) which was not infected but only given distilled water; Group B (negative control), infected but administered with only distilled water. Group C was administered with 300mg/kg body weight of reference drug (augmentin). Groups D, E and F were treated with 150mg/kg, 300mg/kg and 600mg/kg body weight of ethanolic extract of the root of *Terminalia glaucescens* respectively. The administration was done daily using cannula for a period of 5 days and the recovery rate of the mice was noted. The faeces of the mice were analyzed on daily basis during treatment for *E. coli* count. After treatment, the mice were sacrificed for toxicological study.

Evaluation of toxicological effect of *Terminalia glaucescens*

Serum was collected and prepared as described by Yakubu *et al.* (2003). The dissected mice liver and kidney were removed, blotted with cleaned tissue paper and weighed. They were transferred into ice-cold 0.25M sucrose solution and homogenized at $1\text{g}/5\text{cm}^3$ (1:5w/v), using mortar and pestle placed on ice blocks. The homogenate was further centrifuged at 3000rpm for fifteen minutes to obtain supernatant which was kept in specimen bottles and maintained frozen until required for enzyme activity assay (Yakubu *et al.*, 2003). Parts of the organs were transferred into specimen bottles containing 10% formalin for histopathological examination. Liver function test and kidney function test were carried out using standard procedures as described by Tietz *et al.* (1994).

Results

Toxicological studies of Terminalia glaucescens using an animal model

The effect of the inoculation of *E. coli* into the male albino Wistar mice for 5 days is described in Table 1. From the result, the bacterial count of *E. coli* from the faeces showed significant increase from 2.7×10^2 cfu/g in day 0 to 4.7×10^3 cfu/g in day 5 from group D and from 2.6×10^2 cfu/g in day 0 to 4.8×10^3 cfu/g in day 5 from group F. While group A (positive control) which was not inoculated with 0.5 ml of 1.5×10^8 of bacterial cells of *E. coli* did not show any significant increase in the bacterial count of *E. coli*.

The result of the administration of different concentrations of ethanolic extract of *Terminalia glaucescens* on male albino Wistar mice for 5 days is presented in Table 2. From the Table, the *E. coli* counts of the faeces showed a decrease from 3.1×10^3 CFU/g in day 1 to 3.8×10^2 CFU/g in day 5 from group F administered with 600mg/ml of the extract and from 3.1×10^3 CFU/g in day 1 to 3.6×10^2 CFU/g in day 5 from group C administered with augmentin. While the *E. coli* counts showed significant increase from 4.7×10^3 in day 1 to 6.1×10^3 in day 5 from group B which was infected but not administered with any form of extract or reference drug. Group A (positive control) which was not infected or administered with any form of extract or reference drug did not show any significant change in the bacterial count of *E. coli* from day 1 to day 5.

From the result, the administration of varying concentrations of the extract (150, 300 and 600 mg/kg body weight) and 300 mg/kg body weight of reference drug did not produce any significant effect on the liver and kidney organ-body weight ratio when compared with the control animal (Group A) (Table 3). Only group B (infected but not treated) produced significant effect on the liver and kidney organ-body weight ratio.

Table 4 shows that there was no significant difference in total protein when compared with the control animal (Group A). Also there was no significant difference in globulin except for the negative control which was not administered (Group B). But there was significant difference in albumin except for group F which was administered with 600 mg/kg body weight of the extract. There were significant differences in ALT, AST and ALP of the serum and liver when compared with the control animal except serum AST which had no significant difference when compared with the control animal.

Results further showed that WBC and MCH showed significant difference (increase) when compared with the control animal except group B (not administered with extract or reference drug) which showed significant difference (decrease) when compared with the control animal. But RBC, HB, PCV, MCV, PLT, LYM and MCHC did not show any significant difference when compared with the control animal except group B (not administered with extract or reference drug) which showed significant difference (decrease) when compared with the control animal (Table 5 and 6).

The histoarchitectural structure of the liver of uninfected mice administered with distilled water only showed normal hepatic tissues with portal tracts and venules (Figure 1). Contrarily, the photomicrograph of the liver of *E. coli* infected mice administered with distilled water showed gross degeneration of hepatic tissues with infiltration (Figure 2) while the *E. coli* infected mice administered with reference drug (Augmentin) showed slightly distorted hepatic tissues (Figure 3). The histoarchitectural changes shown in mice

administered with 150, 300 and 600 mg/kg body weight of the extract were normal hepatic tissues, mild and moderate hepatic degeneration respectively (Figures 4, 5 and 6).

Table 1: *E. coli* count of faecal samples in male albino Wistar mice after inoculation for 5 days

Group	Day 0 (CFU/g)	Day 1 (CFU/g)	Day 2 (CFU/g)	Day 3 (CFU/g)	Day 4 (CFU/g)	Day 5 (CFU/g)
A	$2.5 \times 10^2 \pm 0.03$	$2.6 \times 10^2 \pm 1.35$	$2.4 \times 10^2 \pm 3.26$	$2.7 \times 10^2 \pm 1.89$	$2.6 \times 10^2 \pm 5.47$	$2.5 \times 10^2 \pm 4.73$
B	$2.4 \times 10^2 \pm 0.57$	$8.5 \times 10^2 \pm 7.12$	$1.2 \times 10^3 \pm 7.86$	$1.8 \times 10^3 \pm 7.45$	$2.9 \times 10^3 \pm 0.67$	$4.6 \times 10^3 \pm 6.21$
C	$2.6 \times 10^2 \pm 1.98$	$9.4 \times 10^2 \pm 2.67$	$1.2 \times 10^3 \pm 4.00$	$1.9 \times 10^3 \pm 2.11$	$3.1 \times 10^3 \pm 3.46$	$4.2 \times 10^3 \pm 7.39$
D	$2.7 \times 10^2 \pm 0.09$	$9.3 \times 10^2 \pm 0.67$	$1.3 \times 10^3 \pm 8.21$	$1.9 \times 10^3 \pm 4.22$	$3.2 \times 10^3 \pm 8.00$	$4.7 \times 10^3 \pm 2.89$
E	$2.3 \times 10^2 \pm 4.57$	$9.2 \times 10^2 \pm 4.21$	$1.1 \times 10^3 \pm 3.67$	$1.7 \times 10^3 \pm 2.00$	$2.9 \times 10^3 \pm 2.31$	$4.5 \times 10^3 \pm 1.04$
F	$2.6 \times 10^2 \pm 5.27$	$8.8 \times 10^2 \pm 4.78$	$1.2 \times 10^3 \pm 1.12$	$1.7 \times 10^3 \pm 7.54$	$3.3 \times 10^3 \pm 5.17$	$4.8 \times 10^3 \pm 0.46$

Key: A (positive control) not inoculated with 0.5 ml of 1.5×10^8 of bacterial cells of *E. coli*, B, C, D, E and F were inoculated with 0.5 ml of 1.5×10^8 of bacterial cells of *E. coli*

Table 2: *E. coli* count of faecal samples in male albino Wistar mice after administration of various concentrations of ethanolic extracts of the roots of *T. glaucescens* for a period of 5 days

Group	Day 1 (CFU/g)	Day 2 (CFU/g)	Day 3 (CFU/g)	Day 4 (CFU/g)	Day 5 (CFU/g)
A	$2.6 \times 10^2 \pm 5.76$	$2.7 \times 10^2 \pm 2.78$	$2.5 \times 10^2 \pm 3.33$	$2.6 \times 10^2 \pm 5.77$	$2.7 \times 10^2 \pm 0.89$
B	$4.7 \times 10^3 \pm 2.14$	$4.9 \times 10^3 \pm 1.09$	$5.2 \times 10^3 \pm 3.78$	$5.8 \times 10^3 \pm 1.46$	$6.1 \times 10^3 \pm 3.46$
C	$3.1 \times 10^3 \pm 0.90$	$2.4 \times 10^3 \pm 0.45$	$1.3 \times 10^3 \pm 8.42$	$8.6 \times 10^2 \pm 8.00$	$3.6 \times 10^2 \pm 1.47$
D	$3.8 \times 10^3 \pm 3.96$	$2.9 \times 10^3 \pm 1.47$	$1.8 \times 10^3 \pm 1.77$	$1.0 \times 10^3 \pm 5.11$	$5.1 \times 10^2 \pm 0.57$
E	$3.4 \times 10^3 \pm 1.56$	$2.5 \times 10^3 \pm 9.45$	$1.3 \times 10^3 \pm 0.47$	$9.8 \times 10^2 \pm 3.77$	$4.2 \times 10^2 \pm 4.90$
F	$3.1 \times 10^3 \pm 0.57$	$2.2 \times 10^3 \pm 7.49$	$1.0 \times 10^3 \pm 2.66$	$7.4 \times 10^2 \pm 2.07$	$3.8 \times 10^2 \pm 1.04$

Key: A (positive control) not infected or administered with extract or reference drug, B (negative control) infected but not administered with extract or reference drug, C administered with 300 mg/kg of the reference drug (augmentin), D administered with 150 mg/kg of the extract, E administered with 300 mg/kg of the extract, F administered with 600 mg/kg body weight of the extract

Toxicological studies of Terminalia glaucescens using an animal model

Table 3: Organ weights of male albino Wister mice infected with *E. coli* and administered with varying concentrations of ethanolic extracts of *T. glaucescens* for a period of 5 days

Treatment groups (mg/kg body weight)	Liver g/L	Kidneyg/L
A (not infected or treated with extract),	2.76 ± 0.11 ^a	0.55 ± 0.02 ^a
B (Infected but not treated with extract)	3.87 ± 0.21 ^b	0.32 ± 0.03 ^b
C (Infected & treated with 300mg/kg of antibiotic)	2.93 ± 0.31 ^a	0.44 ± 0.05 ^a
D (Infected & treated with 150mg/kg of extract)	2.85 ± 0.07 ^a	0.49 ± 0.03 ^a
E (Infected & treated with 300mg/kg of extract)	2.70 ± 0.17 ^a	0.47 ± 0.08 ^a
F (Infected & treated with 600mg/kg of extract)	2.50 ± 0.27 ^a	0.54 ± 0.02 ^a

Values are mean of 7 replicates ± SEM, values carrying different superscripts along the column for each parameter are significantly (P <0.05) different.

Table 4: Effect of administration of varying concentrations of ethanolic extract of the root of *Terminalia glaucescens* and reference drug on selected enzymes and liver function indices on male albino Wistar mice for 5 days.

Treatment groups	Parameters								
	Total Protein (g/L)	Albumin (g/L)	Globulin (g/L)	Liver ALT (U/L)	Serum ALT (U/L)	Liver AST (U/L)	Serum AST (U/L)	Liver ALP (U/L)	Serum ALP (U/L)
A	57.50 ± 1.32 ^a	31.25 ± 0.75 ^a	26.25 ± 0.63 ^a	710.75 ± 22.96 ^a	5.50 ± 0.65 ^a	833.75 ± 14.43 ^a	17.00 ± 0.41 ^a	132.2 ± 4.77 ^a	27.51 ± 1.19 ^a
B	59.50 ± 0.65 ^a	12.75 ± 0.48 ^b	46.25 ± 0.44 ^b	1298.75 ± 6.85 ^b	16.75 ± 0.45 ^b	1932.50 ± 21.34 ^b	6.75 ± 1.11 ^b	83.27 ± 1.47 ^b	62.75 ± 1.04 ^b
C	60.50 ± 2.65 ^a	33.00 ± 0.00 ^c	27.50 ± 0.65 ^a	941.00 ± 19.78 ^c	9.75 ± 0.25 ^c	1038.25 ± 8.23 ^c	17.50 ± 0.65 ^a	102.0 ± 1.08 ^c	41.50 ± 1.32 ^c
D	62.50 ± 1.95 ^a	33.00 ± 0.41 ^c	29.50 ± 1.32 ^a	923.75 ± 13.92 ^c	10.50 ± 0.65 ^c	1051.00 ± 17.56 ^c	17.25 ± 0.75 ^a	109.2 ± 2.17 ^d	41.50 ± 1.32 ^c
E	61.75 ± 1.95 ^a	33.75 ± 0.85 ^c	28.00 ± 1.22 ^a	853.57 ± 17.12 ^d	9.75 ± 0.63 ^c	1048.00 ± 8.09 ^c	16.75 ± 1.11 ^a	118.2 ± 2.46 ^c	29.75 ± 1.65 ^a
F	61.13 ± 0.17 ^a	31.04 ± 0.87 ^a	28.50 ± 1.05 ^a	808.75 ± 14.85 ^c	9.25 ± 0.85 ^c	1068.10 ± 17.08 ^c	16.75 ± 0.95 ^a	120.0 ± 1.08 ^c	24.75 ± 2.63 ^a

Values are mean of 7 replicates $\pm$ SEM, values carrying different superscript across the rows for each parameter are significantly ($P < 0.05$) different.

Key: A (positive control) not inoculated or administered with extract or reference drug, B (negative control) infected but not administered with extract or reference drug, C, infected and administered with 300 mg/kg of the reference drug (augmentin), D infected and administered with 150 mg/kg of the extract, E infected and administered with 300 mg/kg of the extract, F infected and administered with 600 mg/kg body weight of the extract

Table 5: Effect of administration of varying concentrations of ethanolic extract of the root of *Terminalia glaucescens* and reference drug on selected serum electrolytes and kidney function indices on male albino Wistar mice for 5 days

Treatment Groups	Urea (mmol/L)	Parameters						
		Creatinine (μ mol/ml)	Na ⁺ (mmol/L)	K ⁺ (mmol/L)	Cl ⁻ (mmol/L)	ALT (U/L)	AST (U/L)	ALP (U/L)
A	3.28 $\pm$ 0.11 ^a	53.75 $\pm$ 0.85 ^a	131.00 $\pm$ 0.41 ^c	3.70 $\pm$ 0.04 ^a	91.27 $\pm$ 3.41 ^a	615.00 $\pm$ 13.23 ^a	737.75 $\pm$ 16.33 ^a	889.25 $\pm$ 14.04 ^b
B	2.67 $\pm$ 0.11 ^b	72.75 $\pm$ 1.03 ^b	101.25 $\pm$ 0.48 ^a	2.63 $\pm$ 0.09 ^b	93.50 $\pm$ 2.16 ^a	1677.50 $\pm$ 30.23 ^b	1827.00 $\pm$ 14.77 ^b	676.25 $\pm$ 8.38 ^a
C	3.03 $\pm$ 0.09 ^c	57.50 $\pm$ 1.04 ^c	122.50 $\pm$ 0.65 ^b	3.15 $\pm$ 0.06 ^c	90.11 $\pm$ 0.94 ^a	744.50 $\pm$ 18.97 ^c	935.85 $\pm$ 19.42 ^c	933.75 $\pm$ 13.04 ^c
D	3.48 $\pm$ 0.05 ^d	56.25 $\pm$ 0.24 ^c	124.75 $\pm$ 0.85 ^b	3.18 $\pm$ 0.05 ^a	89.70 $\pm$ 4.20 ^a	770.00 $\pm$ 31.33 ^c	932.75 $\pm$ 8.33 ^c	923.75 $\pm$ 5.02 ^c
E	3.27 $\pm$ 0.09 ^a	52.50 $\pm$ 0.65 ^a	133.50 $\pm$ 1.64 ^c	3.63 $\pm$ 0.09 ^a	92.97 $\pm$ 1.05 ^a	746.50 $\pm$ 17.03 ^c	927.51 $\pm$ 4.59 ^c	916.20 $\pm$ 7.88 ^c
F	3.30 $\pm$ 0.09 ^a	53.00 $\pm$ 1.47 ^a	132.50 $\pm$ 3.30 ^c	3.72 $\pm$ 0.10 ^a	91.70 $\pm$ 2.06 ^a	710.15 $\pm$ 14.09 ^d	894.75 $\pm$ 8.23 ^d	907.25 $\pm$ 20.14 ^{bc}

Values are mean of 7 replicates $\pm$ SEM, values carrying different superscript across the rows for each parameter are significantly ($P < 0.05$) different.

Key: A (positive control) not infected or administered with extract or reference drug, B (negative control) infected but not administered with extract or reference drug, C infected and administered with 300 mg/kg of the reference drug (augmentin), D infected and administered with 150 mg/kg of the extract, E infected and administered with 300 mg/kg of the extract, F infected and administered with 600 mg/kg body weight of the extract,

Toxicological studies of Terminalia glaucescens using an animal model

ALP- Alkaline phosphatase activity, AST-Aspartate aminotransferase activity, ALT- Alanine aminotransferase activity

Table 6: Effect of administration of varying concentrations of ethanolic extract of the root of *Terminalia glaucescens* and reference drug on selected haematological parameters on male albino Wistar mice for 5 days.

Treatment groups	Parameters								
	WBC ($\times 10^9/l$)	RBC ($\times 10^{12}/l$)	HB (g/L)	% PCV (l/l)	MCV (fl)	MCH (pg)	PLT ($\times 10^9/l$)	% LYM	MCHC (g/dl)
A	8.09 $\pm$ 0.33 ^a	2.46 $\pm$ 0.12 ^a	40.73 $\pm$ 2.10 ^a	5.52 $\pm$ 0.21 ^a	2.24 $\pm$ 0.10 ^a	16.56 $\pm$ 1.36 ^{ac}	93.41 $\pm$ 5.33 ^c	85.01 $\pm$ 2.77 ^b	7.38 $\pm$ 1.15 ^a
B	5.36 $\pm$ 1.23 ^b	1.65 $\pm$ 0.33 ^b	17.31 $\pm$ 0.35 ^b	3.76 $\pm$ 0.13 ^b	2.28 $\pm$ 0.05 ^a	10.49 $\pm$ 0.14 ^b	53.00 $\pm$ 7.51 ^a	78.70 $\pm$ 1.07 ^a	5.15 $\pm$ 0.50 ^b
C	10.05 $\pm$ 1.15 ^c	2.49 $\pm$ 0.17 ^a	37.19 $\pm$ 1.76 ^c	5.73 $\pm$ 0.19 ^a	2.30 $\pm$ 0.08 ^a	14.94 $\pm$ 1.33 ^c	77.12 $\pm$ 3.15 ^b	83.00 $\pm$ 1.52 ^b	6.49 $\pm$ 1.00 ^a
D	9.30 $\pm$ 0.78 ^c	2.22 $\pm$ 0.20 ^a	41.03 $\pm$ 3.45 ^a	5.11 $\pm$ 0.72 ^a	2.30 $\pm$ 0.03 ^a	18.48 $\pm$ 2.10 ^a	93.10 $\pm$ 2.54 ^c	84.10 $\pm$ 2.70 ^b	8.03 $\pm$ 1.44
E	9.94 $\pm$ 0.01 ^c	2.35 $\pm$ 0.14 ^a	43.00 $\pm$ 2.48 ^a	6.06 $\pm$ 1.00 ^a	2.58 $\pm$ 0.27 ^a	18.30 $\pm$ 1.74 ^a	96.04 $\pm$ 3.01 ^c	86.20 $\pm$ 1.82 ^b	7.10 $\pm$ 0.89 ^a
F	9.74 $\pm$ 0.55 ^c	2.38 $\pm$ 0.09 ^a	45.22 $\pm$ 4.80 ^a	5.44 $\pm$ 0.08 ^a	2.29 $\pm$ 0.02 ^a	19.00 $\pm$ 2.41 ^a	103.50 $\pm$ 8.15 ^c	83.60 $\pm$ 2.91 ^b	8.31 $\pm$ 1.24 ^a

Values are mean of 7 replicates $\pm$ SEM, values carrying different superscript across the rows for each parameter are significantly (P <0.05) different.

Key: A (positive control) not infected or administered with extract or reference drug, B (negative control) infected but not administered with extract or reference drug, C infected and administered with 300 mg/kg of the reference drug (augmentin), D infected and administered with 150 mg/kg of the extract, E infected and administered with 300 mg/kg of the extract, F infected and administered with 600 mg/kg body weight of the extract, haemoglobin (Hb), red blood cell (RBC), white blood cell (WBC), lymphocytes (LYM), platelets (PLT), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), packed cell volume (PCV) and mean corpuscular volume (MCV)

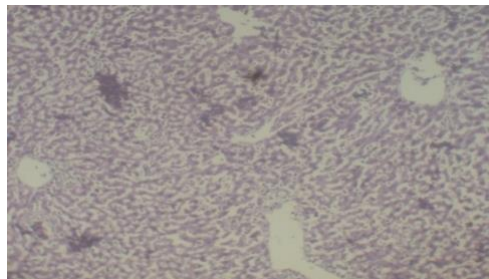


Plate 1: Cross section of the liver of mice not infected but administered with distilled water showing normal hepatic tissues with portal tracts and venules ($\times 400$).

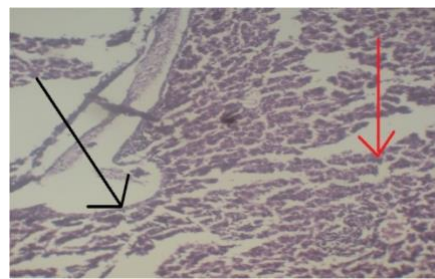


Plate 2: Cross section of the liver of *E. coli* infected mice administered with distilled water showing gross degeneration of hepatic tissues with infiltration ($\times 400$).

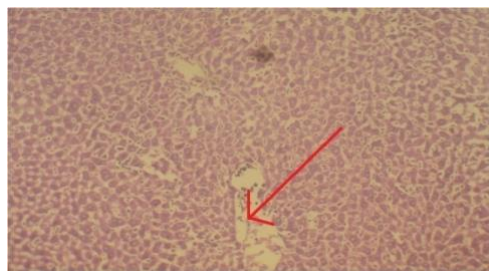


Plate 3: Cross section of the liver of *E. coli* infected mice administered with 300 mg/kg body weight of reference drug (augmentin) showing slightly distorted hepatic tissues ($\times 400$).

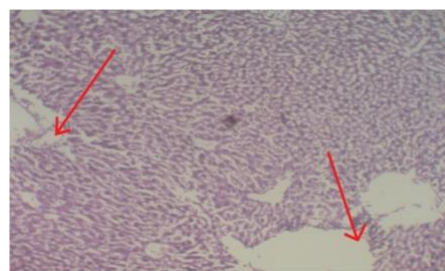


Plate 4: Cross section of the liver of *E. coli* infected mice administered with 300 mg/kg body weight of the extract showing mild hepatic degeneration ($\times 400$).

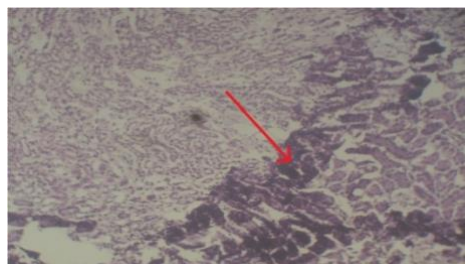


Plate 5: Cross section of the kidney of *E. coli* infected mice administered with distilled water showing glomerular shrinkage with distorted renal cortex ($\times 400$).



Plate 6: Cross section of the kidney of *E. coli* infected mice administered with 600 mg/kg body weight of the extract with mildly distorted renal cortex ($\times 400$).

Discussion

Despite the potentials of *T. glaucescens* in the treatment of diseases, its toxicological activities *in vivo*, have not been fully explored. There is no doubt that *T. glaucescens* is of great health importance which are not limited to their therapeutic effects

in diabetes, cardiac pathologies, malaria, liver pathologies, obesity and dysentery (Mustofa *et al.*, 2000; Sote and Wilson, 1995). However, the potentials of this plant to have negative toxicological effects have not been explored. The result of inoculation of 0.5 ml of 1.5×10^8 of bacterial cells of *E. coli* on male albino Wistar mice for 5 days showed an increase in the bacterial count of *E. coli* in all the groups except group A. This was specially observed in group F where the bacterial count of *E. coli* increased from 2.6×10^2 in day 0 to 4.8×10^3 in day 5 and this resulted in very soft faeces and sluggishness of the mice. This confirmed that increased bacterial count of *E. coli* has the ability to induce diseased condition in mice with clinical symptoms of very soft feces. Increase in the count and recovery of *E. coli* was expected as seen in this study when standardized *E. coli* inocula was used as shown by Johnson *et al.* (2006). However, the reverse was the case in group A (positive control) which did not show any significant increase in the bacterial count of *E. coli* and no diseased condition was observed.

The *in vivo* determination of the efficacy of the administration of ethanolic extract of the root of *Terminalia glaucescens* on male albino Wistar mice for 5 days revealed that the extract produced significant healing effect on the infected mice through reduction in the bacterial count of *E. coli* in all the groups except groups A & B. This was specially observed in group F where the bacterial count of *E. coli* reduced from 3.1×10^3 in day 1 to 3.8×10^2 in day 5.

No significant difference was obtained between the effects of ethanolic extract of *T. glaucescens* and reference drug (augmentin) in treating the mice infected with *E. coli* *in vivo*. With most *E. coli* strains now showing resistance to augmentin and other beta-lactams, works from Fenique *et al.* (2015) showed that ethanolic extract of *T. glaucescens* was very effective against *E. coli* which supported the findings from this study. Furthermore, Gbadamosi and Ogunsuyi, (2014) showed that *T. glaucescens* could be used in the management of *E. coli* related infections.

Organ-body weight ratios usually done to see if there is a considerable change in the organ body weight of a test animal after continuous administration of an experimental drug. Results from this study clearly showed that the administration of varying concentrations of the extract (150, 300 and 600 mg/kg body weight and 300 mg/kg body weight of the reference drug) did not produce any significant effect on the liver and kidney organ-body weight ratio when compared with the control animal (group A). Only group B (infected but not treated) produced significant effect on the liver and kidney organ-body weight ratio. Various reports have shown that extracts of *T. glaucescens* have little or no effect on the organ body weight of mice especially when taken at lower concentrations. This indicates that the extracts of *T. glaucescens* have no adverse effect on the organ body weight of the mice. Dominique *et al.* (2013) showed that after administering a high dosage of 1,390 mg/kg body weight of the extracts of *T. glaucescens*, no significant changes occurred in the organ body weight of the experimental mice. However, they pegged the lethal dose for the extracts at concentration higher than 2500 mg/kg body weight. However, Njomen *et al.* (2009) showed that extracts of *T. glaucescens*, had a considerable effect on the weight of a diabetes-induced mice, although their study did not show if this reduction

in weight (weight loss) of the mice had any effect on the organ body weight of the animal. Furthermore, Dominique *et al.* (2013) noticed a therapeutic effect on the body weight of mice without affecting the organ body weight.

Liver and kidney function test are generally used to access changes in the liver or kidney as a result of the consumption of a particular substance. Results from this study further revealed that there was no significant difference in total protein when compared with the control animal (Group A). Also there was no significant difference in globulin except for the negative control which was not administered (Group B). But there was significant difference in albumin except for group F which was administered with 600 mg/kg body weight of the extract. Changes in albumin could be attributed to hepatoprotection by the extracts of *T. glaucescens* (Dominique *et al.*, 2013). There were significant changes in ALT and ALP of the mice which could be attributed to none liver cells involving in leucopenia (Dominique *et al.* (2013). From the result, urea, creatinine, Na^+ , and K^+ showed significant difference when compared with the control animal except for groups E and F administered with (300 mg/kg and 600 mg/kg body weight ratio) respectively that did not show any significant difference when compared with control animal due to higher concentration of the extract. Also kidney ALT, AST and ALP showed significant difference when compared with the control animal. But the result of Cl^- did not show any significant difference when compared with the control animal. These changes in the kidney function indices could be as a result of manifestations of nephropathy and kidney malfunction (Dasofunjo *et al.*, 2013). Furthermore, change in the activity of enzymes and serum indices would indicate likely damage to the external boundary of the cells (Yakubu *et al.*, 2008).

The result of the effect of administration of varying concentrations of ethanolic extract of the root *T. glaucescens* and reference drug on selected haematological parameters on male albino wistar mice showed that WBC and MCH had significant difference (increase) when compared with the control animal except group B (not administered with extract or antibiotic) which showed significant difference (decrease) when compared with the control animal. But RBC, HB, PCV, MCV, PLT, LYM and MCHC did not show any significant difference when compared with the control animal except group B (not administered with extract or antibiotic) which showed significant difference (decrease) when compared with the control animal. Similar results for haematological parameters were obtained by Dominique *et al.* (2013).

Histology reports clearly showed that no changes occurred to the liver of the uninfected mice, with the hepatic tissues, portal tracts all normal. However, gross degeneration of hepatic tissues with infiltration was seen in the liver of the mice infected with *E. coli* but was untreated. Gradual changes were seen in the histo-architecture of the liver of the infected mice as various concentrations of the extracts of *T. glaucescens* were administered. This restoration could be due to the antibacterial activities of the bioactive components of extracts of *T. glaucescens* used in the treatment of the *E. coli* infected mice. This is supported by the work of Adeeyo *et al.* (2016) which showed that aqueous extract

of *T. glaucescens* has no toxic effect on the histology of the cerebrum but enhances its development as shown by the numerous pyramidal neurons.

Conclusion

This study clearly established that *T. glaucescens* was not toxic to the experimental mice *in vivo* while showing healing effects, thus this plant can be recommended as an impressive and promising natural herbal source to be used in pharmaceutical industry especially in this era of drug resistance by many pathogenic organisms

References

- Adebayo, E.A. and Ishola, O.R. (2009). Phytochemical and antimicrobial screening of crude extracts from the root, stem bark, and leaves of *Terminalia glaucescens*. *African Journal of Pharmacy and Pharmacology* 3(5): 217-221
- Adeeyo O.A., Adegoke A.A., Adenowo T.K., Yusuf U.A. and Dare B.J.(2016). Some Effects of Aqueous Leaf Extract of *Terminalia glaucescens* On the Micro-Architecture of The Histology of Cerebrum of Wistar Rats. *International Journal of Advanced Academic Research | Sciences and Technology* 2(1):1-10
- Borokini, T.I. and Lawal, I.O. (2014). Traditional medicine practices among the Yoruba people of Nigeria: a historical perspective. *Journal of Medicinal Plants Studies* 2(6): 20-33.
- Bulama, J.S., Dangoggo, S.M., Bwala, Y.A., Abah, J.O. (2014). Phytochemicals and Antibacterial Evaluation of Root Bark Extract of *Terminalia glaucescens*. *J App Pharm Sci*. 4(2): 129-132
- Dominique, T.K., Halbin, K.J., William, Y., Joseph, D.A. and Hervé, E.B.I. (2013). Toxic effect study of methanol extract of *Terminalia glaucescens* leaves following single or short-term repetitive oral administration in Swiss mice. *American Journal of BioScience*, 1(4): 85-90.
- Fernique, K.K., Souleymane, M., Nathalie, G.K., Calixte, B., Djaman, A., Adama, C. and Mireille, D. (2015). Antioxidant and Antibacterial Activity of the Extracts of Stem Bark of *Terminalia glaucescens* Planch Ex Benth (Combretaceae) on Enterobacteriaceae Extended-Spectrum Beta-Lactamase Producing (ESBL). *Ijppr.Human*, 2015; Vol. 4 (4): 148-160.
- Gbadamosi, I.T. and Ogunsuyi, A.O. (2014). An appraisal on the potency of roots of *Anogeissus leiocarpus* (DC.) Guill. & Perr. And *Terminalia glaucescens* Benth. in management of *E. coli* related infections. *Journal of Applied Biosciences* 78:6646 – 6653
- Hosseinzadeh, S., Jafarikukhdan, A., Hosseini, A. and Armand, R. (2015). The Application of Medicinal Plants in Traditional and Modern Medicine: A Review of *Thymus vulgaris*. *International Journal of Clinical Medicine*, 6:635-642.
- Mustofa, Valentin, A., Benoit-Vical, F., Pélissier, Y., Koné-Bamba, D., Mallié, M. (2000) Antiplasmodial activity of plant extracts used in west African traditional medicine. *Journal of Ethnopharmacology* 73(1-2):145-51

- Njomen, B.S., Kamgang, R., Soua, R., Oyono, L.E., Njikam, N. (2009) Protective effect of methanol-methylene chloride extract of *Terminalia glaucescens* leaves on streptozotocin-induced diabetes in mice. *Tropical Journal of Pharmaceutical Research* 8 (1): 19-26
- Nwachukwu, C.U., Umeh, C.N., Kalu, I.G., Okere, S. and Nwoko, M.C. (2010). Identification And Traditional Uses Of Some Common Medicinal Plants In Ezinihitte Mbaise L.G.A., Of Imo State, Nigeria. *Report and opinion* 2(6): 1-8.
- Raphael, E.C. (2011). Traditional medicine in Nigeria: Current status and the future. *Research Journal of Pharmacology* 5 (6): 90-94.
- Silva, O. and Serrano, R. (2015). *Terminalia* genus as source of antimicrobial agents. The Battle Against Microbial Pathogens: Basic Science, Technological Advances and Educational Programs (A. Méndez-Vilas, Ed.)
- Sote, E.O. and Wilson, M. (1995). In-vitro antibacterial effects of extracts of Nigerian tooth-cleaning sticks on periodontopathic bacteria, *Afr. Dent. J*, 9:15-19.
- Tietz, N. W., Prude, E. L and Sirgard Anderson, O (1994) In: Tietz textbook of Clinical Chemistry. (Burtis CA and Ashwood ER, eds). W.B. Saunders Company: London. Pp. 1354-1374.
- Yakubu, M.T., Bilbis, L.S., Lawal, M. and Akanji, M.A. (2003). Evaluation of selected parameters of rat liver and kidney function following repeated administration of yohimbine. *BIOKEMISTRI*, 15(2): 50-56
- Yakubu M.T., Akanji M.A., Oladiji A.T. J. (2008). *Ethano. Pharmacol.*, 11(2), 288-292.
- Zhou, G., Shi, Q., Huang, X. and Xie, X. (2015). The Three Bacterial Lines of Defense against Antimicrobial Agents. *Int. J. Mol. Sci.*, 16, 21711-21733.