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**Serial Exhaustive Extraction: Influence of Solvent Polarity on Antibacterial Activity of
Extracts of Leaves of *Tithonia diversifolia***

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Abstract

Recently there has been a growing interest in the need for more efficient and reliable extraction methods for isolation of active components from plants without damaging/modifying their chemical constituent and biological activity. One of such is serial exhaustive extraction utilizing solvents of increasing polarity in the extraction of a wide array of active components from plants. This study investigated effect of serial exhaustive extraction on antibacterial activity of extract from *Tithonia diversifolia* leaves using the following solvents: toluene, acetone, ethanol and water. The presence and quantity of phytochemicals were evaluated. Activities of crude extracts were determined against three pathogenic bacterial strains using agar well diffusion technique. Minimum inhibitory and bactericidal concentrations (MIC & MBC) were evaluated by agar plate method. Efficacy of crude extracts was compared with those of conventional antibiotics by antibiotic sensitivity assay. Yields obtained upon extraction of crude extracts were: 7.40g, 14.60g, 8.5g and 32.70g for toluene, acetone, ethanol and aqueous extracts respectively. Phytochemicals present in the extracts were saponins, phenols, flavonoids and glycosides. Quantitative phytochemical assay revealed alkaloids to possess highest concentration (18.2mg/l) in ethanolic extract while flavonoids was least (2.3mg/l) in the aqueous. Only ethanolic extract exhibited antibacterial activity at 300mg/ml against one test strain (*Escherichia coli* ST2747), while MIC and MBC were observed also for ethanolic extract at 175mg/ml against the strain. Ethanolic extract demonstrated a better efficacy compared to reference antibiotics, showing 14mm inhibition zone while gentamicin, 3mm against *E. coli* ST2747. Tetracycline exhibited inhibition zone of 5.5mm against *Salmonella typhimurium* 08-1736. From this study, ethanol has proven to be a solvent of choice for extraction of phytoconstituents from leaves of *T. diversifolia* which has translated to the activity obtained. Thus this extract can serve as an alternative to tetracycline and gentamicin in the treatment of infections caused by *E. coli* ST2747.

Keywords: *Tithonia diversifolia*, Successive extraction, *Escherichia coli* ST2747, bacterial susceptibility, phytochemistry, gentamicin, tetracycline

Introduction

The recent global emergence of microorganisms that present resistance to most of the available antibiotics has become one of the gravest threats to public health in the 21st century (Woolhouse *et al.*, 2016). Recently, WHO released a global report on the extent of antimicrobial resistance. It also observed that there are certain limitations to effective treatment options for some common community-acquired infections in several countries (WHO, 2014). Furthermore, affordability and availability of existing treatment options are major concerns. It is feared that if current trends in antimicrobial resistance continue in this state, treatments of common bacterial infections may become dangerously limited in the coming years (Ventora, 2015).

These challenges have necessitated the search for new antimicrobial substances to which these microorganisms have not developed resistance. Therefore, recent advances in drug production processes are directed at natural sources, especially active compounds of plant origin due to observations of plants' efficacy against certain diseases in traditional medicine (Mohammed *et al.*, 2014). Thus the use of phytochemicals as alternative remedies for cure and prevention of illnesses has attracted the interest of scientists globally (Sofowora, 1982).

With the current global interest in natural products as drug remedies, there has been the need to seek the best extraction technologies capable of producing quality extract with intact pharmacological properties. One of such extraction methods is the serial exhaustive extraction technique. This is usually carried out with different solvents of increasing polarity in order to extract a wide array of active components with the highest possible efficacy while conserving their chemical and biological properties. Mathew and Benny (2016) investigated the effect of serial exhaustive extraction of 10 common flowers and observed that the order of efficacy of

extracts based on solvent of extraction to be methanol > acetone > aqueous > petroleum ether. According to several other literatures, methanol has been suggested as a solvent of choice for extraction of most secondary metabolites from plant materials (Parekh *et al.*, 2006; Iloki-Assanga *et al.*, 2015).

Tithonia diversifolia is a succulent shrub belonging to the family, *Asteraceae*, which can reach a height of 2 to 3m (Gualberto *et al.*, 2011). It is native to Central America and the West Indies but it has become naturalized in many tropical countries including Nigeria (Obafemi *et al.*, 2006). An oral decoction of the leaves and stem is used for the treatment of hepatitis in Taiwan and gastrointestinal disorders in Kenya and Thailand (Johns *et al.*, 1995). In Costa Rica, the dried leaves are applied externally on wounds (Kuo *et al.*, 1997) while in Cameroon, an infusion of the leaves is used for the treatment of measles (Kandem *et al.*, 1986). In Nigeria, the decoctions of its various parts are used for the treatment of malaria, diabetes mellitus, sore throat, liver and menstrual pains (Owoyele *et al.*, 2004). Currently, information on the use of serial exhaustive extraction for the extraction of secondary metabolites from the leaves of *T. diversifolia* is scarce, thus necessitating the present study.

Materials and Methods

Collection and identification of plant materials

Fresh leaves of *T. diversifolia* were obtained from Oke-Odo, Ilorin, Kwara state, Nigeria (with coordinates 8.480266 N, 4.627828 E) and authenticated at the herbarium unit of the Department of Plant Biology, University of Ilorin, with voucher specimen number UILH/001/1129.

Chemicals

Toluene, acetone, and ethanol used for the extraction were obtained from BDH

Chemicals Ltd, Poole, England. They were of analytical grade.

Collection and maintenance of test strains

Bacterial strains (*Pseudomonas aeruginosa* VRFPA04, *Escherichia coli* ST2747 and *Salmonella enterica* subspecies *enterica* sv. Typhimurium 08-1736) were obtained from the culture collection unit of Department of Microbiology, University of Ilorin. They were maintained on appropriate agar slants, routinely sub-cultured for the purpose of purity and refrigerated at 4 °C.

Plant preparation and extraction

Leaves were rinsed and air-dried at room temperature for a few days and pulverized into fine powder using an electric blender (Model: Binatone BLG 650). A serial exhaustive extraction was carried out using toluene, acetone, ethanol and water as extractants according to their order of increasing polarity as described by Fajimi *et al.* (2001) with slight modifications. One hundred grams (100 g) of powdered leaves were soaked into 1000 ml of the first solvent (toluene), left in a dark cupboard for 48hrs after which it was filtered through a Whatman No 1. filter paper. The residue was squeezed using a muslin cloth and air-dried in order to free it of any residual toluene. This was subsequently soaked in the second solvent (acetone) and the procedure was observed up till the last solvent (water) to obtain the respective extracts.

Qualitative phytochemical screening

The presence of the following phytochemicals were determined as described by the respective authors: flavonoids (Murthy *et al.*, 2005), saponins (Ifemeje *et al.*, 2014), tannins (Pradhan *et al.*, 2010), terpenoids (Sharker and Shahid, 2010), cardiac glycosides, phenols and alkaloids (Joshi *et al.*, 2013).

Quantitative phytochemical screening

Quantification of the following phytochemicals were determined as described by the following authors: flavonoid (Chang *et al.*, 2002), alkaloids

(Obdoni and Ochuko, 2001; Joshi *et al.*, 2013; Ifemeje *et al.*, 2014), cardiac glycosides and saponins (Obdoni and Ochuko, 2001; Ifemeje *et al.*, 2014), terpenoids (Ladan *et al.*, 2014), tannin and phenolic contents (Vijay and Rajendra, 2014).

Antibacterial susceptibility assay

Agar well diffusion technique

The agar well-diffusion technique was adopted in determining the antibacterial activity of extracts as described by Kowti *et al.* (2010). A day old inoculum of each test strain (0.1ml) (as compared with the McFarland standard) was separately streaked onto sterile Mueller-Hinton agar plates and allowed to dry for few minutes. Sterile cork borer was used to make wells at the centre of the agar plates. One millilitre (1ml) each of the extracts at 150 mg/ml and 300 mg/ml was introduced separately into the wells and allowed to diffuse at room temperature and incubated at 37 °C for 24 hours. Plates were observed for presence of clear zones around agar wells and diameter of zones of inhibition measured to the nearest millimetres.

Minimum Inhibitory Concentration (MIC)

Agar plate method was adopted. Extracts were reconstituted into various concentrations (275, 250, 225, 200, and 175 mg/ml), from an initial stock concentration of 500 mg/ml. Solidified agar plates were streaked with bacterial strains and agar wells (6mm) were created at the centre of the plates. The wells were filled with 1ml of extracts and incubated at 37 °C for 24 hours after which zones of inhibition were detected and recorded (Ugoh and Haruna, 2013).

Minimum bactericidal concentration (MBC)

The MBC was determined by measuring 9.7 ml of peptone water into different test tubes and autoclaving for 15 min. This was allowed to cool for some minutes after which 0.2 ml of each extracts and 0.1 ml of

test strains were introduced. This was incubated at 37 °C for 24 hours. About 0.2ml of the broth culture was introduced into sterile petri dish by pour plate method. Plates were incubated invertedly at 37 °C for 24 hours and observed for possible growth. Concentrations of extracts that showed no growth after incubation were regarded as MBC (Doughari, 2006).

Antibiotic sensitivity assay

The method of Sen and Batra (2012) was adopted with slight modifications using two reference antibiotics (ampicillin and tetracycline). Day old inoculums were streaked on nutrient agar plate using sterile swab stick. The plate was allowed to dry and antibiotic discs were placed on the surface of inoculated agar plate with the aid of sterile forceps. These were incubated and the diameter of zones of inhibition measured and recorded after 24 hours. Experiments were carried out in triplicates and the mean values were recorded.

Results

Recovery of extracts from leaves of *T. diversifolia*

The yields obtained after serial exhaustive extraction using the four solvents were 7.40 g, 14.60 g, 8.5 g and 32.70 g for toluene, acetone, ethanol and aqueous extracts respectively.

Phytochemical analysis of extracts of *T. diversifolia* leaves

The results of phytochemical screening of extracts of leaves of *T. diversifolia* are presented in Table 1 and Figure 2. From the qualitative screening, it was observed that ethanolic and acetone extracts demonstrated the presence of the seven tested phytochemicals (Table 1). The composition of alkaloids (18.2 mg/l) was highest in ethanolic extract while flavonoids was least in the aqueous extract (2.3 mg/l) as shown in Fig. 2.

Table 1: Qualitative phytochemical profile of various extracts of *Tithonia diversifolia*

Bioactive compounds	Toluene	Acetone	Ethanol	Aqueous
Alkaloids	-	+	++	+
Saponins	+	+	++	+
Phenols	+	+	+	+
Flavonoids	+	+	++	+
Terpenoids	+	+	+	-
Tannins	-	+	++	+
Glycosides	+	+	+	+

Keys

+ = Slightly present, ++ = Abundantly present, - = Absent

Antibacterial activity of various concentrations of extracts of *T. diversifolia*

The evaluation of antibacterial activities of extracts of *T. diversifolia* at different concentrations is presented in Fig. 2. Only ethanolic extract demonstrated antibacterial activity against one of the test strains (*E. coli* ST2747) at 300mg/ml.

Evaluation of MIC and MBC of ethanolic extract of *T. diversifolia*

The results of the MIC and MBC of ethanolic extract of *T. diversifolia* against *E. coli* ST2747 is shown in Table 2. It was observed that there was minimum inhibition at 175mg/ml. Also bactericidal activity was observed at the same concentration with MIC i.e. 175mg/ml.

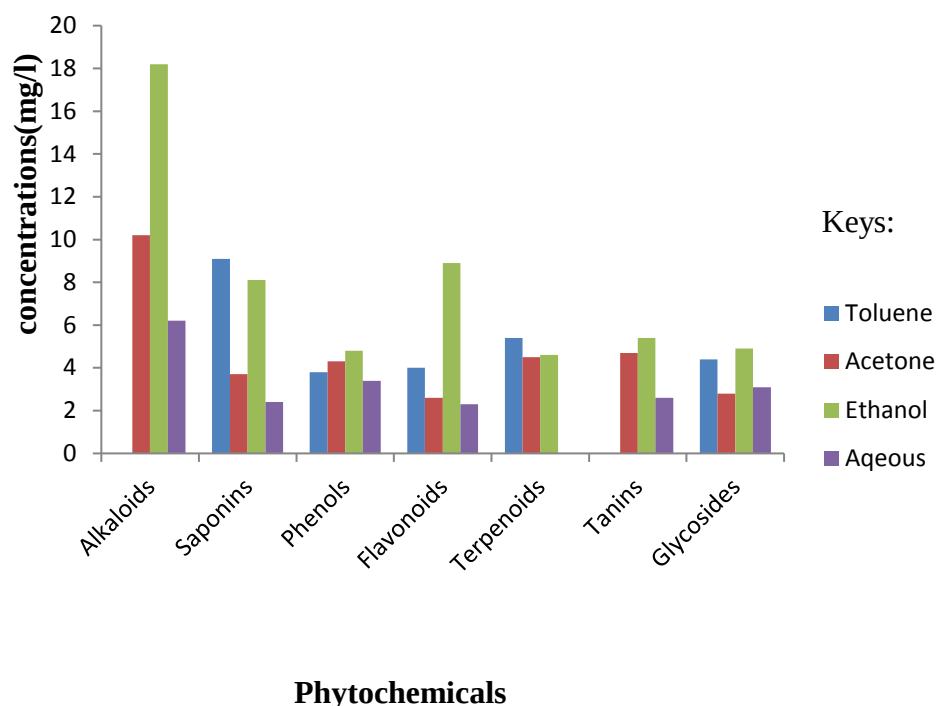


Figure 1: Quantitative phytochemical profile of various extracts of *Tithonia diversifolia*

Table 2: Susceptibility pattern of test strains to extracts of *T. diversifolia*

Test strains	TO (150 mg/ml)	TO (300 mg/ml)	AT (150 mg/ml)	AT (300 mg/ml)	ET (150 mg/ml)	ET (300 mg/ml)	AQ (150 mg/ml)	AQ (300 mg/ml)
P.a	0	0	0	0	0	0	0	0
E.c	0	0	0	0	0	14	0	0
S.t	0	0	0	0	0	0	0	0

KEYS:

TO = Toluene

AT = Acetone

ET = Ethanol

AQ = Aqueous

P.a = *Pseudomonas aeruginosa* VRFPA04,

E.c = *Escherichia coli* ST27477

S. t = *Salmonella enterica* subspecies *enterica* sv. Typhimurium 08-1736

Table 3: Minimum inhibitory and bactericidal concentrations of ethanolic extract of *T. diversifolia*

Extract (mg/ml)	concentrations	MIC (mg/ml)	MBC (mg/ml)
275		-	-
250		-	-
225		-	-
200		-	-
175		+	+

Key:

+ = MIC , MBC - = No MIC, MBC

Comparison of antibacterial activity of ethanolic extract of *T. diversifolia* with standard antibiotics *Escherichia coli* ST2747. The result is presented in Table 3.

Ethanolic extract compared favourably with reference antibiotics demonstrating better activity than the two antibiotics against

Table 4: Comparison of antibacterial activities of ethanolic extracts of *T. diversifolia* with standard antibiotics

Test Strains	Ethanol	Tetracycline	Gentamicin
<i>Escherichia coli</i> ST2747.	14mm	NI	3mm
<i>Salmonella typhimurium</i> 08-1736.	NI	5.5mm	NI
<i>Pseudomonas aeruginosa</i> VRFPA04.	NI	NI	NI

Keys: NI = No inhibition, mm = millimetres.

Discussion

With the growing interest in the search for alternative remedies for the treatment of microbial infections threatening human existence, it is evident that secondary plant metabolites have become very promising in proffering solutions to this effect. This work has demonstrated that ethanol used for the extraction of leaves of *T. diversifolia* in a serial exhaustive extraction system is a solvent of choice. Mathew and Benny (2016) demonstrated that methanol and acetone are

solvents of choice in the serial exhaustive extraction of flowers. This is similar to this study since ethanol and methanol are solvents of similar polarity range.

The yield obtained from the serial exhaustive extraction was adequate for use in the assays investigated, implying that the solvent system and technology used in this study were effective for the solubilization of secondary plant metabolites from the leaves of *T. diversifolia*.

The phytochemicals present in the crude extracts are in line with some past literatures (Goffin *et al.*, 2002; Owoyele *et al.*, 2004; Obafemi *et al.*, 2006; Oyewole and Ibidapo, 2008). However, most authors have demonstrated sole extraction with individual solvent at a time unlike the present research which carried out a successive extraction on the leaves of *T. diversifolia*. The presence of these active components may have been responsible for the antibacterial activity demonstrated by the ethanolic extract. Variations in activity of different extraction solvents have earlier been reported by Falodun *et al.* (2006). Variance in polarity of solvents has been reported to be responsible for the differences in solubility of plant active principles, and therefore different levels of efficacy (El-Mahmood *et al.*, 2008).

This study focused on the influence of serial extraction on the antibacterial activity of the crude extracts. The activity of ethanolic extract observed in this study is similar to that reported by Ogunfolakan *et al.* (2010). The presence of several hydroxyl groups in this solvent enhances the formation of hydrogen bonds with other molecules, which could result in their binding with microbial proteins (Ivanovska *et al.*, 1996) thereby leading to their inactivation.

The lack of activity of the other extracts from this study could be as a result of the differences in the polarity of the solvents. The exceptional antibacterial activity demonstrated by ethanol is due to the fact that it is a highly polar molecule due to its hydroxyl (OH) group, with the high electronegativity of oxygen allowing hydrogen bonding to take place with other molecules. The ethyl group in ethanol is non-polar. Thus, ethanol can dissolve both polar and non-polar substances which may be present in the leaves of the plant (Pin *et al.*, 2010). However, the strain types used in the assay may have contributed to the limited activity observed with extracts from ethanol as well as the other solvents. According to literature it has been established that the genetic traits of microbial cells are resident in the different

strains of microorganisms (Castillo and Ling, 2014). Hence, strains have often become more important in determining the specific behaviour of microorganisms. This implies that the types of strains used in this study may possess some resistance plasmids in their genome which have contributed to reduced susceptibility as established by Beceiro *et al.* (2013). For example, *Pseudomonas aeruginosa* is a multidrug-resistant pathogen with an advanced inherent antibiotic resistance (Poole, 2004).

The assay of the minimum inhibitory and minimum bactericidal concentrations also buttresses the fact that ethanolic extract had the highest antibacterial effect. This suggests that the extract, even at a lower concentration, has therapeutic properties and therefore justifies its uses in traditional medicine.

It was observed that the activity of ethanolic extract of *T. diversifolia* was higher than those of gentamicin and tetracycline. This may be due to the synergy of several classes of active compounds present in ethanolic extract unlike conventional antibiotics which are made up of few chemical compounds usually targeted towards specific microorganisms.

Conclusion

This work has provided a novel baseline information in terms of serial extraction of leaves of *T. diversifolia* for activity against *Pseudomonas aeruginosa* VRFP04, *Escherichia coli* ST2747 and *Salmonella enterica* subspecies *enterica* sv. Typhimurium 08-1736. From this study, serial exhaustion extraction of leaves of *T. diversifolia* suggests ethanolic extract as a solvent of choice. Thus this extract can serve as a replacement for the use of tetracycline and gentamicin in the treatment of infections caused by *E. coli* ST2747. However, isolation and structural elucidation of the active components as well as toxicology of the extract is imperative.

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Conflict of Interests

Authors declare no conflict of interest.

References

- Beceiro, A., Tomás, M. and Bou, G. (2013). Antimicrobial resistance and virulence: a successful or deleterious association in the bacterial world? *Clin Microbiol Rev.* 26(2): 185–230.
- Castillo, C.F.G. and Ling, M.H.T. (2014). Resistant traits in digital organisms do not revert preselection status despite extended deselection: implications to microbial antibiotics resistance. *BioMed Research Intl.* 648389:1-10.
- Chang, C., Yang, M. and Wen, H. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis* 10:178-182.
- Doughari, J.H. (2006). Antimicrobial activity of *Tamarandus indica*. *Tropical Journal of Pharmaceutical Research.* 5(2):597-603.
- Elmahmood AM, Doughari JH, Ladan N (2008). Antimicrobial screening of stem bark extracts of *Vitellaria paradoxa* against some enteric pathogenic microorganisms. *Afri. J. Pharm. Pharm.* 2(5): 089-094.
- Fajimi, A.K.A.A, Ayodoji, H., Adebawale E.A and Ogundola F.L. (2001). Therapeutic trials on gastrointestinal helminth parasite of goats using pawpaw seeds as a drench. Proceedings of the international conference on sustainable crop. Livestock prod. for improved livelihoods and national resources Management, West Africa held at the International Livestock Research Institute (ILRI) in partnership with International

Institute of Tropical Agriculture between Nov. 9–23, 2001.

- Goffin, E., Ziemons, E., De Mol, P., Madureira, M.C., Martins, A.P., Da Cunha, A.P, Philippe, G., Tits, M., Angenot, L. and Fredrich, M. (2002). In vitro antiplasmodial activity of *Tithonia diversifolia* and identification of its main active constituent: Tagitinin C. *Planta Medica.* 68:543-545.
- Gualberto, R., Souza, O.F., Costa, N.R., Bracialli, C.D., Gaion, L.A. (2011). Influence of spacing and plant development stadium in biomass yield and nutritional value of *Tithonia diversifolia* (Hemsl.) Gray. *Nucleus.* 8 (1):242-256.
- Ifemeje, J.C., Egbuna, C., Eziokwudiaso, J.O. and Ezebuo F.C. (2014). Determination of the Anti-nutrient composition of *Ocimum gratissimum*, *Corchorus olitorius*, *Murraya koenii* Spreng and *Cucurbita maxima*. *International Journal of Innovation and Scientific Research.* 3:127- 133.
- Iloki-Assanga, S.B., Lewis-Luján, L.M., Lara-Espinoza, C.L., Gil-Salido, A.A., Fernandez-Angulo, D., Rubio-Pino, J.L. and Haines, D.D. (2015). Solvent effects on phytochemical constituent profiles and antioxidant activities, using four different extraction formulations for analysis of *Bucida buceras* L. and *Phoradendron californicum*. *BMC research notes.* 8(1): 1. 35.
- Ivanovska, N., Philipov, S., Istatkova, R. and Georgieva, P. (1996). Antimicrobial and immunological activity of ethanol extracts and fractions from *Isopyrum thalictroides*. *J. Ethnopharmacol.* 54:143–151.
- Johns, T., Faubert, G.M., Kokwaro, J.O., Mahunnah, R.L.A. and Kimanani, E.K. (1995). Anti-giardial activity of gastrointestinal remedies of the LUO of East Africa. *J. Ethnopharmacol.* 46: 17 – 23.

Joshi, A., Bhobe, M. and Saatarkar, A. (2013). Phytochemical investigation of the roots of *Grewia microcos* Linn. *J. Chem. Pharm. Res.* 5: 80–87.

Kamdem, L., Messi, H.M., Ndongo, N.A., Mbi, C., Njikam, A.P. and Elobo, S. (1986). Ethnobotanical investigations carried out in Mouloundou (Eastern province) and Zoetele (Southern province). *Rev. Sci. Technol. (Health SCI SER)*, 3/4: 59 – 68.

Kowti, R., Harsha, R., Ahmed, M.G., Hareesh, A.R., ThammanaGowda, S.S., Dinesha, R., Kumar, B.P. and Irfan, A.M. (2010). *Res. J. of Pharmaceut., Biol. and Chem. Sci.* 1(3):691-692.

Kuo, Y.H. and Chen, C.H. (1997). Diversifolol, a novel rearranged Eudesmane sesquiterpenes from the leaves of *Tithonia diversifolia*. *Chem. Pharm. Bull.* 45: 1223 – 1224.

Ladan, Z., Amupitan, J.O., Oyewale, O.A., Ayo, R.G., Temple, E. and Ladan, E.O. (2014). Phytochemical screening of the leaf extract of *Hyptis spicigera* plant. *African Journal of Pure and Applied Chemistry* 8(5): 83-85.

Mathew, T.K. and Benny, P.J. (2016) Antibacterial activities of petroleum ether, acetone, methanol and aqueous extracts of 10 common flowers. *World Journal of Pharmacy and Pharmaceutical Sciences* 5(12):1244-1257.

Mohammed, M., Musa, A. M., Garba, M.A., Adeiza, A.A. and Hanwa, U.A. (2014). Phytochemical and antimicrobial study on the leaf extracts of *Erythrophleum africanum* (Caesalpiniaceae). *African Journal of Biotechnology*. 13(4):598-603.

Murthy, M.M., Subramanyam, M., Bindu, M.H. and Annapurna, J. (2005). Antimicrobial activity of clerodane diterpenoids from *Polyalthia longifolia* seeds. *Fitoterapia* 3:336-339.

Obafemi, C.A., Sulaimon, T.O., Akinpelu, D.A. and Olugbade, T.A. (2006).

Antimicrobial activity of extracts and a germacranolidetype sesquiterpene lactone from *Tithonia diversifolia* leaf extract. *Afr. J. Biotech.* 5(12):1254-1258.

Obdoni, B. and Ochuko, P. (2001). Phytochemical studies and comparative efficacy of the crude extracts of some homostatic plants in Edo and Delta States of Nigeria. *Global Journal of Pure and Applied Sciences* 8: 203–208.

Ogunfolakan, O., Kolawole, O.S., Olowe, A.O. (2010). In vitro antimicrobial activity of *T. diversifolia* leaf extracts on bacterial isolated from wound infection from Nigeria Hospital. *Research Journal of Medical Sciences.* 4:305-308.

Owoyele, V.B., Wuraola, C.O., Soladoye, A.O. and Olaleye, B.S. (2004). Studies on the anti-inflammatory and analgesic properties of *Tithonia diversifolia* leaf extract. *J. Ethnopharmacol.* 90(2-3):317-321.

Owoyele, V.B., Wuraola, C.O., Soladoye, A.O. and Olaleye, B.S. (2004). Studies on the anti-inflammatory and analgesic properties of *Tithonia diversifolia* leaf extract. *J. Ethnopharmacol.* 90(2-3):317-321.

Oyewole, I.O. and Ibidapo, C.A. (2008). Anti-malaria and repellent activities of *Tithonia diversifolia* (Hemsely) leaf extracts. *Journal of Medicinal Plants Research.* 2(8):171-5.

Parekh, J., Jadeja, D. and Chanda, S. (2006). Efficacy of aqueous and methanol extracts of some medicinal plants for potential antibacterial activity. *Turkish Journal of Biology* 29(4): 203-10.

Pi n, K.Y., Chuah, A.L., Rashih, A.A., Mazura, M.P., Fadzureena, J., Vimala, S. and Rasadah, M.A. (2010). Antioxidant and anti-inflammatory activities of extracts of betel leaves (*Piper betle*) from solvents with different polarities. *J. Trop. For. Sci.*, 22: 448-455.

Poole K. (2004). Efflux-mediated multiresistance in Gram-negative bacteria. *Clin Microbiol Infect.* 10(1):12–26.

Pradhan, P., Joseph, L., George, M., Kaushik, N. and Chulet, R. (2010). Pharmacognostical, phytochemical and quantitative investigation of *Saraca asoca* leaves. *Journal of Pharmacy Research* 3(4): 776-780.

Sen, A. and Batra, A. (2012). Evaluation of antimicrobial activity of different solvents extracts of medicinal plant: *Melia azadarach* L. *International Journal of Current Pharmaceutical Research* 4(2): 67-73.

Sharker, S.M. and Shahid, I.J. (2010). Assessment of antibacterial and cytotoxic activity of some locally used medicinal plants in Sundarban mangrove forest region. *African Journal of Pharmacy and Pharmacology* 4(2): 066-069.

Sofowora, A. (1982). Medicinal Plants and Traditional medicine in Africa. Published by John Wiley and Sons Ltd. 1st edition 131: 168 –171.

Ugoh, S.C. and Haruna, M. (2013). Phytochemical screening and antibacterial activity of the fruit and leaf extracts of *Tamarindus indica* (Linn). *Rep Opinion.* 5(8):18-27.

Ventola, C.L. (2015). The Antibiotic Resistance Crisis, Part 1: Causes and Threats. *P T.* 40(4): 277–283.

Vijay, J.D. and Rajendra, S.B. (2014). Estimation of total phenol, tannin, alkaloid and flavonoid in *Hibiscus tiliaceus* Linn. wood extracts. *Journal of Pharmacognosy and Phytochemistry* 2(4): 41-47.

Woolhouse, M., Waugh, C., Perry, M.R. and Nair, H. (2016). Global disease burden due to antibiotic resistance – state of the evidence. *Journal of Global Health.* 6(1):010306.

World Health Organisation (2014). Antimicrobial resistance: Global report on surveillance 2014. Available: <http://>

apps.who.int/iris/bitstream/10665/112642/1/9789241564748_eng.pdf?ua=1. Accessed: 23 January 2017.