

Epipremnum aureum (Linden and André) G. S. Bunting (Araceae): Free Radicals Scavenging, Cell Growth Inhibition and Cytotoxic effects on Rhabdomyosarcoma and Laryngeal Carcinoma Cancer Cells

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Abstract

Patients with chronic health conditions such as cancer often benefit from plant based herbal recipes with minimal adverse effects. There also exists growing worries over the safety and efficacy of most of the currently used conventional chemotherapeutic agents. This study examined the radical scavenging and cytotoxicity of *E. aureum* traditionally employed for the management of cancerous diseases.

The aerial part of *Epipremnum aureum* was collected from the University of Ibadan botanical garden and authenticated at the Forest Herbarium Ibadan. Air-dried aerial part of *Epipremnum aureum* was pulverized and extracted with 70% aq. methanol and concentrated in vacuo. A portion the methanol extract was fractionated and crude alkaloidal extract was prepared from another portion of the methanol extract. The extracts and fractions were subjected to; antioxidant DPPH radical scavenging assay and cytotoxicity of the extracts and fractions against rhabdomyosarcoma (Rd) and laryngeal carcinoma (Hep-2C) cancer and non-cancerous (Vero) cell lines were determined.

Ethyl acetate fraction and alkaloidal extract displayed better DPPH radical scavenging activity than the methanol extract and other fractions. The alkaloidal extract (CC₅₀=0.17±0.03µg/mL) and dichloromethane fraction (CC₅₀=0.28±0.05 µg/mL) displayed the highest cytotoxicity against Rd cancer cell lines compared to cyclophosphamide (CC₅₀=2.23±0.33 µg/mL). However, the alkaloidal extract displayed the least cytotoxicity to Vero cell line and it is about 14.25 and 22.18 times more cytotoxic to Rd and Hep-2C cancer cells, respectively.

This present study demonstrated that *E. aureum* possess antioxidant and cytotoxic activities with less cytotoxicity on healthy cells.

Key words: Radical Scavenging, Cytotoxicity, GC-MS Analysis, *Epipremnum aureum*.

Introduction

Cancer remains a leading cause of death and about 19.3 million new cancer cases and 10.0 million cancer related deaths were reported recently in the year 2020 alone (1). The emergence of chemoresistant cancers is posing new challenges to cancer chemotherapy with many anticancer drugs become ineffective for management of metastasised cancers (2). The narratives of anticancer drug discovery is tightly woven with medicinal plants and many chemotherapeutic drugs including paclitaxel, vincristine, podophylotoxin own their origin to medicinal plants (3). Cancer such as rhabdomyosarcoma and laryngeal carcinoma has been reported to sometimes arise from activity of free radicals generated from sources such as tobacco smoke and alcohol (4). Rhabdomyosarcoma type of cancer arises from soft tissues of the skeletal muscle tissue or hollow organs such as the bladder and uterus (5). While, laryngeal carcinoma is form in the tissues of the larynx and like the rhabdomyosarcoma are influenced by environmental and lifestyle-related factors, such as tobacco use, consumption of alcohol, and exposure to toxic substances (6). Antioxidants have been reported to reduced the risk of some forms of cancer, including skeletal muscle, larynx, prostate, colon and breast cancer risk (7). The process of new anticancer drug discovery from medicinal plants often commence with *in vitro* cytotoxicity screening of medicinal plants followed by isolation of anticancer compounds from active plants (8).

Epipremnum aureum (Linden and André) G.S. Bunting, belongs to araceae family (9) is an evergreen climber (10, 11). It is commonly grown indoors where is used for beautification and purification of polluted air (12-14).

Epipremnum aureum possess antibacterial, anti-termite and antioxidant properties and traditionally in Malaysia and Singapore it is popularly used as a major ingredient in the preparation of traditional anticancer remedy (15, 16). The aim of this study was to assess the radical scavenging and anticancer potential of *E. aureum* aerial part and identify phyto-constituent present in the plant.

Methodology

Plant Material

The aerial part of *Epipremnum aureum* was collected from the University of Ibadan botanical garden and authenticated at the Forest Herbarium Ibadan (FHI 112371).

Preparation of extracts and fractions: Air-dried *E. aureum* (2000g) powder was extracted exhaustively into 70% aqueous methanol (3L). The extract was concentrated in vacuo at 45°C, labelled as crude extract and stored in refrigerator at 4°C until it was needed. Fifty grams (50g) of crude extract obtained above was partitioned into n-hexane, dichloromethane (DCM), ethyl acetate (EtoAc) and butanol soluble fractions. All fractions obtained were concentrated in vacuo at 45°C and stored in the refrigerator at 4°C until they were needed.

Alkaloidal Extraction: One hundred gram (100 g) of crude extract obtained above was dissolved in 10% HCl solution (500 mL) and defatted with 500 mL each of n-hexane (5X) to remove chlorophyll, lipids and other non-polar constituents. The aqueous solution was made basic to pH 10 with dilute NH₄OH and extracted (4 X) each with chloroform (500 mL) as shown in Figure 1. The combined chloroform phase was concentrated and yielded 12 g of crude alkaloid extract which was stored in the refrigerator (4 °C) until further use (17). The percentage yield of extract and fraction (% w/w) was calculated using the formula below;

$$\% \text{ yield of extract} = \frac{\text{weight of of extract}}{\text{weight of powdered plant material}} \times 100\%$$

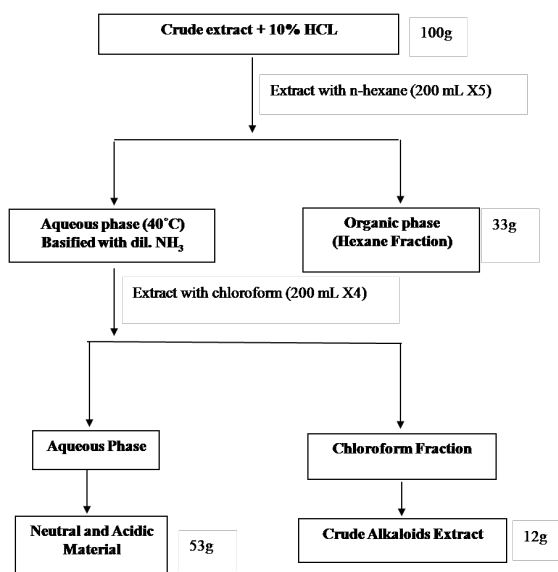


Figure 1. Extraction of Crude Alkaloids from *E. aureum* Crude Extract.

1, 1-diphenyl-2-picryl hydrazyl (DPPH) scavenging activity

The DPPH radicals scavenging ability of the extracts and fractions was assessed by a method previously reported (18). From a stock solution (1000 mg/mL) of the extracts and fractions, serial dilutions of 200, 100, 50, 25, 12.5 and 6.25 µg/mL were prepared in the 96 well-plate and 150 µL of 0.04 µg/mL DPPH in methanol was added to each well and the microplate plate was incubated for 30 minutes in the darkness at room temperature (25 °C). The UV absorbance (Absorbance sample) of the resulting solution was measured at 517 nm using the Ultra-violet spectrophotometer (Titertek Uniskan). Control simple contained DPPH and solvent (methanol) without the extract. Ascorbic acid was used as positive controls while methanol as negative control. This experiment was carried out in triplicate. The results obtained were expressed as percentage inhibition and calculated by the formula below.

$$\text{Inhibition (\%)} = \frac{\text{Absorbance control} - \text{Absorbance sample}}{\text{Absorbance control}} \times 100\%$$

Growth inhibitory activity

The extracts and fractions were subjected to growth inhibitory assays as described below;

Sorghum bicolor radicle growth inhibitory assay: Fresh seeds of *Sorghum bicolor* were purchased from *Ipata* market in Ilorin. Viable seeds were selected by introducing a 100 g of the seeds in distilled water. The floating seeds and husk were decanting off while the submerged seeds were collected aired and washed with 95% ethanol for a minute and later rinsed with distilled water before they were dried and stored in dried dark place until needed. Only viable seeds were used for *Sorghum bicolor* radicle growth (SBRG) inhibitory assay. Ten milliliters of 39.06, 156.25, 625, 2500 and 10000 µg/mL were prepared by a fourfold dilution of the stock solution (40 mg/mL) prepared in 5% DMSO. Cyclophosphamide was used as the positive control at similar concentration with the extract/fractions. A volume of 10 mL different concentrations of the extracts (39.06 - 10000 µg/mL) were transferred into pre-labelled petri-dish of 10 cm lined with filter paper underlie with wool, thereafter ten (10) seeds were placed on each filter paper in the petri-dishes. The petri-dishes were incubated in a dark cupboard at ambient temperature (32°C) and the lengths of emerging radicle were measured after 48 and 96 h of incubation. Seeds of the negative control group were treated with 10mL of 5% DMSO in distilled water. This experiment was performed in triplicates. With the aid of a scaled ruler changes in radicle length were measured. The mean change in radicle length per concentration was calculated. The percentage radicle growth inhibition at 48 and 96 h and IC₅₀ were determined (19).

Percentage inhibition of the root growth was calculated using formula below; (next page)

$$\text{inhibition (\%)} = \frac{A - B}{A} \times 100\%$$

Where; A = length of untreated root.
B = length of treated root with plant extracts/
cyclophosphamide

Allium cepa root growth inhibitory assay: *Allium cepa* root growth (ACRG) inhibitory assay was conducted using method described in our earlier work (19). Moderate size onion bulbs (50±10 g) were obtained from *Ipata* market. *Allium cepa* bulbs (50±10 g) were rinsed with water (distilled) and grown with the root submerged in water in the dark at room temperature (32°C) for 24-48 h until the roots have grown up to 3cm length.

Twenty (20) millilitre of different concentrations (39.06, 156.25, 625, 2500 and 10000µg/mL) of each extract was prepared by four fold dilution of the stock solution (40mg/mL) prepared in 5% DMSO. Twenty (20mL) of various concentration were transferred into the petri-dish of 10cm diameter and the roots of each of onion bulbs submerged in the extract/fraction in the petri-dishes containing each extract/fraction (39.06-10000 µg/mL). Cyclophosphamide was used as the positive control at similar concentration with the extract/fractions, while 5% DMSO in water was applied as the negative control. This experiment was performed in triplicates. New root growth lengths were measured at 0, 48, 96h and the percentage root growth inhibition were estimated by the formula below;

Percentage inhibition was calculated as:

$$\text{inhibition (\%)} = \frac{A - B}{A} \times 100\%$$

Where; A = length of untreated radicle.
B = length of treated radicle with plant extracts/
cyclophosphamide

Cytotoxicity assays

Cytotoxic potential of the extracts and fraction of *E. aureum* were determined by the cytotoxic potential of the test samples on the nauphill of *Artemia salina* and malignant cell lines as described below.

Brine shrimp lethality (BSL) assay: The eggs of *Artemia salina* were incubation in seawater for 48 h to hatch the eggs. Ten milligrams of the extract was dissolved in 2mL 5% DMSO in sea water and resulted in 5mg/mL stock solution. Further dilutions of 1, 10, 100, 500 and 1000 µg/mL were made in microplates in triplicates. A 250 µL suspension of nauplii in the extract was added to each well. The plates were incubated at room temperature (RT = 25-33°C) for 24 h. 0.5% DMSO in sea water was used as the negative control and thereafter the number of dead nauplii in each well was counted(20).

The percentage cytotoxicity was estimated by the formula below;

$$\% \text{ inhibition (\%)} = \frac{A - B}{A} \times 100\%$$

Where; A = the mean number of shrimp in negative control wells.
B = the mean number of shrimp in the wells with plant extracts/cyclophosphamide.

Extract(s) or fraction(s) with LC₅₀ values <100µg/mL are regarded as active and are selected for further cytotoxicity studies. The method was only employed for preliminary screening fractions and compounds obtained were not tested using this model. Extracts that demonstrate strong brine shrimp lethality are regarded as promising candidate for anticancer drug discovery.

Cytotoxicity of extracts and fractions on Rd and Hep-2C cancer cell lines:

Cytotoxicity of the extracts and fractions were determined by MTT (3-(4, 5-dimethyl thiazole-2yl)-2, 5-diphenyl tetrazolium bromide) assay using the method described by (21). Solutions of extracts for MTT assay were prepared by dissolving 10 mg of extract/fraction in 1 mL of DMSO separately to give a concentration of 10mg/mL. Stock (0.1mL) solution prepared above was added to 0.9 mL of medium to obtain a dilution of 1000µg/mL, there after ten-fold serial dilutions of the samples were prepared from stock solution to obtain graded concentrations (0.01, 0.1, 1, 10, 100 and 1000 µg/mL). Thereafter, 50µL of each sample solution was dispensed into 96-well microtitre plates already seeded with monolayer of cells in triplicates. The plates were incubated at 37°C in a carbon-dioxide environment and the cells observed under microscope after 72 h. Supernatants were removed from all wells after 72h incubation at 37°C and 25µL of 2 mg/mL MTT (sigma) solution in phosphate buffer saline (PBS) was added to each well and the plates were incubated for 2h at 37°C. Thereafter, 125µL of DMSO was added to the wells to dissolve the MTT crystals formed. The UV absorbance of resulting solution was measured using microplate reader (Titertek Uniskan) at wavelength of 570nm against a background control as blank. The UV absorbance of each plate directly correlates to the number of viable cells in each well.

Percentage inhibition of cancer cell proliferation was calculated by the formula below;

$$\% \text{ inhibition (\%)} = \frac{A - B}{A} \times 100\%$$

Where; A = the mean optical density of untreated cells.
B = the optical density of cells treated with plant extracts/fractions (22)

The IC₅₀ was determined from a non-linear regression curve plotted using Graphpad prism version 6.0

The selective index (SI) of the two extracts and cyclophosphamide were estimated using the Vero cells. Data were expressed as the means ± SEM. When SI is greater than 1, it is indicative of selectivity (20).

$$\text{Selective index (SI)} = \frac{\text{CC}_{50} \text{ on Vero cells}}{\text{CC}_{50} \text{ on cancer cells}}$$

Phytochemical analysis

Preliminary phytochemical screening: The extracts and fractions of *E. aureum* were screened for the presence of

secondary plant metabolites as described below using the method previously reported (23).

Gas chromatography mass spectrometry (GC-MS) analysis: The phytochemical investigation of the n-hexane fraction of *E. aureum* was performed using a GC-MS (Thermo Scientific Co.) equipment. The GC-MS instrument controlled parameters are as follows: Injection source (PAL Sampler), injection volume (2 μ L), column (Agilent technologies-HP-5MS), column dimension (360°C: 30m $\times$ 250 μ m $\times$ 0.25 μ m), initial temperature (50°C), program temperature (260°C), initial pressure (9.05psi), average velocity (38.724cm/sec), holdup time (1.2912min), run time (82.286min). Sample dissolved in HPLC grade hexane was run full and compared by using Database- Spectrum MS-NW-1798. Suggestions with the highest confidence interval greater than 80% were selected. Compounds were identified based on comparison of their retention and mass spectra data to those generated under identical experimental conditions by applying a two-dimensional search algorithm, considering the retention index, as well as mass spectral similarity.

Statistical analysis

Data obtained were expressed as means $\pm$ SEM of values obtained in triplicates from three independent experiments. Statistical differences between treated groups and standards were evaluated using one way analysis of variance (ANOVA). A p value <0.05 was considered to be significantly. Data were analysed using GraphPad Prism Version 6.0 Software.

Results

Extracts preparation and radical scavenging activity:

The plant material yielded 10.78% w/w of dark green extract. Among the fractions obtained, n-hexane (2.96% $_{w/w}$) had the highest yield, while dichloromethane (0.34 % $_{w/w}$) had the lowest yield. Alkaloid extract (0.12g) was obtained per 1g of the crude extract as shown in the **Table 1** below. The ethyl acetate fraction (109 $\pm$ 4.04 μ g/mL) and alkaloidal extract (57.43 $\pm$ 3.33 μ g/mL) displayed better DPPH radical scavenging activity than the crude extract (IC₅₀ of 157.06 $\pm$ 3.62 μ g/mL) and other fractions as shown in **Table 1**.

Growth inhibitory and cytotoxic activities: The crude extracts (GI₅₀ of 60.00 $\pm$ 2.73 and 356.00 $\pm$ 6.24 μ g/mL) displayed growth inhibitory effect in SBRG and ACRG inhibitory assays. The alkaloidal extract (GI₅₀=45 $\pm$ 3.26 and 215 $\pm$ 9.92 μ g/mL) demonstrated better growth inhibitory effects to cyclophosphamide (GI₅₀=705.33 $\pm$ 11.69 and 151.50 $\pm$ 0.52 μ g/mL) in SBRG and ACRG inhibitory models while the other fraction all weaker growth inhibitory effects (**Table 1**). Similarly, the alkaloids extract (LC₅₀=82 $\pm$ 4.26 μ g/mL) and dichloromethane fraction (LC₅₀=99 $\pm$ 5.57 μ g/mL) displayed cytotoxic effect on the nauplii of *Artemia salinathat* was comparable to cyclophosphamide (LC₅₀ of 98 $\pm$ 3.33 μ g/mL) and better than the methanol extract and other fractions (**Table 1**).

Interestingly, alkaloids extract (CC₅₀=0.17 $\pm$ 0.03 and 0.21 $\pm$ 0.03 μ g/mL) and dichloromethane fraction (CC₅₀=0.28 $\pm$ 0.05 and 0.43 $\pm$ 0.06 μ g/mL) displayed better cytotoxic effects cyclophosphamide (CC₅₀=2.23 $\pm$ 0.33 and 2.66 $\pm$ 0.78 μ g/mL), methanol extract (CC₅₀ of 2.92 $\pm$ 1.06 and 2.51 $\pm$ 1.80 μ g/mL) and other fractions against Rd and Hep-2C cancer cell lines (**Table 1**). However, the crude extract (10 $\pm$ 1.76 μ g/mL), dichloromethane fraction (2.99 $\pm$ 0.33 μ g/mL) and alkaloidal extract (2.44 $\pm$ 0.54 μ g/mL) all displayed lesser cytotoxicity on the normal non-cancerous (Vero) cells and the estimated selectivity index of the crude extract (3.43 and 3.98), dichloromethane fraction (10.68 and 6.95) and alkaloids extract (14.35 and 22.18) for Rd and Hep-2C cancer cell lines (**Table 1**).

Phytochemical constituents: Preliminary phytochemical analysis of the extracts and fractions revealed the presences of alkaloids, flavonoids, tannins, saponins, anthraquinones and steroidal terpenoids (**Table 2**). However, the GC-MS analysis of the n-hexane fraction led to identification of 49 bioactive compounds (**Figure 2 and Table 3**). The most abundant constituents of the fraction included; phytol (21.51% occurrence), toluene (21.44% occurrence) and n-Hexadecanoic acid (16.17% occurrence). Other compounds include; hexadecanoic acid, methyl ester, 9,12-Octadecadienoic acid and 9,12,15-Octadecatrienoic acid.

Table 1. Yield (% $_{w/w}$), antioxidant, growth inhibitory and cytotoxic activities of *E. aureus* Extracts and Fractions.

Samples	DPPH Radical scavenging IC ₅₀ $\pm$ SEM (μ g/mL)	DPPH Radical scavenging IC ₅₀ $\pm$ SEM (μ g/mL)	Growth inhibition (μ g/mL)		Cytotoxicity (μ g/mL)				Cancer Cell Selective Index (SI)	
			SBRGI GI ₅₀ $\pm$ SE-M	ACRGI GI ₅₀ $\pm$ SEM	BSL LC ₅₀ $\pm$ SEM	Rd Cell Line CC ₅₀ $\pm$ SEM	Hep-2c Cell Line CC ₅₀ $\pm$ SEM	Vero Cell Line+++ CC ₅₀ $\pm$ SEM	Rd Cell Line	Hep-2C Cell Line
Crude Extract	10.78	157.06 $\pm$ 3.62*	60.00 $\pm$ 2.73*	356.00 $\pm$ 6.24*	200 $\pm$ 2.16*	2.92 $\pm$ 1.06	2.51 $\pm$ 1.80	10 $\pm$ 1.76	3.43	3.98
n-Hexane	2.96	> 400*	620.80 $\pm$ 42.41	2156.00 $\pm$ 77.05*	453 $\pm$ 42.73*	6.98 $\pm$ 2.65*	1.00 $\pm$ 0.54*	1.24 $\pm$ 0.67*	0.18	1.24
Dichloromethane	0.34	320 $\pm$ 19.74*	1126.40 $\pm$ 37.96*	1571.00 $\pm$ 17.01*	99 $\pm$ 5.57	0.28 $\pm$ 0.05*	0.43 $\pm$ 0.06*	2.99 $\pm$ 0.33	10.68	6.95
Ethyl Acetate	0.72	109 $\pm$ 4.04*	193.90 $\pm$ 9.20*	310.70 $\pm$ 13.89*	250 $\pm$ 8.69*	11.19 $\pm$ 0.96*	1.94 $\pm$ 0.67	0.67 $\pm$ 0.59*	0.06	0.35
Butanol	1	348 $\pm$ 16.48*	1412.64 $\pm$ 53.95*	2388 $\pm$ 0.13*	633 $\pm$ 33.53*	7.77 $\pm$ 1.33*	2.63 $\pm$ 0.33	0.38 $\pm$ 0.04*	0.05	0.14
Aqueous	0.83	> 400*	1078 $\pm$ 31.73*	4501.00 $\pm$ 61.60*	658 $\pm$ 15.62*	5.18 $\pm$ 8.69*	4.92 $\pm$ 0.56*	0.22 $\pm$ 0.09*	0.04	0.04
Crude Alkaloids	0.12	57.43 $\pm$ 3.33*	45 $\pm$ 3.26	215 $\pm$ 9.92	82 $\pm$ 4.26	0.17 $\pm$ 0.03	0.21 $\pm$ 0.03	2.44 $\pm$ 0.54	14.35	22.18
Standard Drug		9.26 $\pm$ 0.00+	705.33 $\pm$ 11.69++	151.50 $\pm$ 0.52++	98 $\pm$ 3.33++	2.23 $\pm$ 0.33++	2.66 $\pm$ 0.78++	8.71 $\pm$ 0.56++	3.91++	3.27++

Key:

SBRGI = Sorghum bicolor radicle growth inhibition; ACRGI = Allium cepa root growth inhibition; * = Significantly Different from Standard Drug (P <0.05); + = IC₅₀ of Ascorbic acid; ++ = IC₅₀ of Cyclophosphamide; +++ = Normal non-cancerous cell line.

Table 2. Phytochemical Examination of Selected Plant Extract.

Bioactive constituent	Chemical Test	Crude Extract	n-hexane fraction	Dichloro-methane fraction	Ethyl acetate fraction	Butanol fraction	Aqueous fraction	Alkaloid extract
Alkaloid	Drangendorff's	++	-	++	-	-	-	+++
	Wagner's	++	-	++	-	-	-	+++
	Meyer's	++	-	++	-	-	-	+++
Flavonoid	Shinoda's test	++	++	+	+++	++	++	-
	Lead ace tate	+	-	+	+++	++	++	-
Tannins	Fec13	+	-	+	+	++	+++	-
Saponins	Frothing	+++	-	-	+	++	+++	-
	Emulsifying	++	-	-	-	++	+++	-
Anthraquinone	Combined	++	-	-	-	+	++	-
	Free	+	-	-	-	+	++	-
Cardiac glycoside	Keller killiani	-	-	-	-	-	-	-
	Kedde	-	-	-	-	-	-	-
Steroidal terpenes	Salkowski	++	+++	+	-	-	-	+

Table 3. Chemical composition of the hexane fraction of *E. aureum* aerial part

No	Compounds	RT	% Occurrence
1	Toluene	6.46	21.44
2	Ethanone	19.79	0.02
3	Decanoic acid	22.5	0.02
4	2,5,5,6,8a-Pentamethyl-trans-4a,5,6,7,8,8a-hexahydro-gamma-chromene	24.08	0.02
5	Dodecanoic acid	25.16	0.08
6	Dihydroactinidiolide	25.21	0.05
7	Fumaric acid, ethyl 2-propylphenyl ester	25.46	0.11
8	Tricyclo[4.3.1.1(3,8)]undecane, 1-methoxy-	26.58	0.03
9	1-(2,6,6-Trimethyl-2-cyclohexen-1-yl)acetone	27.43	0.03
10	Methyl 12-methyl-tridecanoate	27.81	0.06
11	Tetradecanoic acid	28.47	0.31
12	Thiazolo[4,5-b]pyridin-2(3H)-one, 5-hydroxy-7-methyl-6-propyl-	28.92	0.22
13	Pentadecanoic acid, methyl ester	29.1	0.05
14	Methyl 12-methyltetradecanoate	29.3	0.1
15	Pentadecanoic acid	29.87	0.11
16	Pentadecanoic acid, methyl ester	29.95	0.14
17	Propyl 12-methyltetradecanoate	30.11	0.25
18	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	30.36	0.37
19	2-Pentadecanone, 6,10,14-trimethyl-	30.52	0.24
20	Pentadecanoic acid	30.83	0.59
21	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	31.01	0.04
22	Phthalic acid, butyl tetradecyl ester	31.32	0.09
23	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	31.54	0.1
24	Hexadecanoic acid, methyl ester	31.67	0.07
25	Hexadecanoic acid, methyl ester	32.86	8.36
26	n-Hexadecanoic acid	34.59	16.17
27	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	40.47	4.8
28	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	40.89	5.04
29	Phytol	41.73	21.51
30	Heptadecanoic acid, 16-methyl-, methyl ester	42.33	2.12
31	9,12-Octadecadienoic acid (Z,Z)-	43.71	5.87
32	Linolenic acid	44.2	3.46
33	Stearic acid	45.11	0.43
34	Eicosanoic acid, methyl ester	50.97	0.87
35	cis-Vaccenic acid	51.61	0.21
36	(2,2-Dimethyl-6-methylene-cyclohexyl)-3-methyl-pent-2-enyl]-[1,4]benzoquinone	52.35	0.16
37	Methyl 11-(3-pentyl-2-oxiranyl)undecanoate	53.27	0.04
38	Methyl 14-methyl-eicosanoate	55.12	0.3
39	Benzenedicarboxylic acid, diisooctyl ester	55.47	0.84
40	Methyl-9-tetradecen-1-ol acetate	57.63	0.05
41	Oleic acid	57.76	0.03
42	Oleic acid	58.73	0.09
43	Heptatriacotanol	59.27	0.04
44	Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol	59.38	0.51
45	γ-Tocopherol	62.8	0.2
46	α-Tocopherol	64.26	0.72
47	Stigmasterol	67.29	0.76
48	γ-Sitosterol	68.71	0.51
49	β-Sitosterol	71.48	1.27

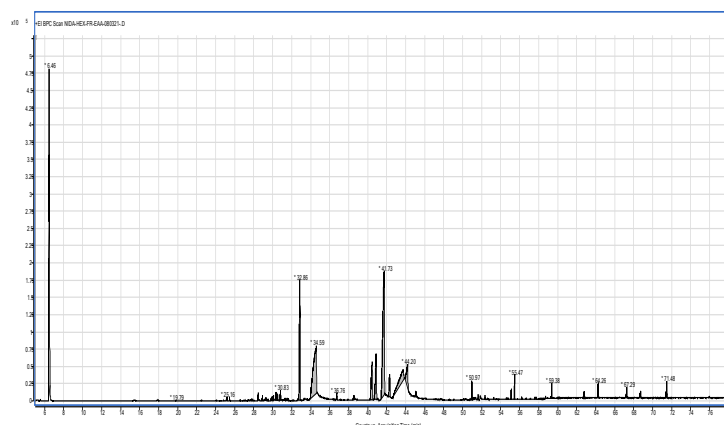


Figure 2. GC-MS Chromatogram of the hexane fraction of *E. aureum* aerial part.

Discussion

About 80% of the global population utilize medicine that are derived or inspired from plants (24). It is reported that patients with chronic health conditions such as diabetes, hypertension and cancer often benefit tremendously from plant based herbal recipes and they often experience minimal adverse effects (25), however because of the growing worries over the safety and efficacy of most of the currently used chemotherapeutic agents. There is need to explore natural products for identification of new leads with cytotoxic and antioxidant potential. This study examined the radical scavenging, growth inhibitory and cytotoxic potential of *E. aureum* extracts with a focus to identify the class of phytochemical present in the plant and identify compounds present in the plant.

The crude extract displayed some radical scavenging, growth inhibitory and cytotoxic activities. However, the fractions and alkaloids extract displayed different activities across the different test models. The n-hexane fraction was found to be rich in steroidal terpenes and flavonoids and the apolar fraction was most active against Hep-2C cancer cells ($1.00 \pm 0.54 \mu\text{g/mL}$) and the GC-MS analysis of the fraction lead to identification of 49 compounds and the most abundant constitutes include; phytol, n-hexadecanoic acid, hexadecanoic acid, 9,12-octadecadienoic acid and

9,12,15-Octadecatrienoic acid. Other important bioactive compounds identified in the fraction include; dihydroactinidiolide, Linolenic acid, Vaccenic acid (an omega-7 fatty acid), Oleic acid, (γ & α -Tocopherols), Stigmasterol and sitosterol (γ & β -Sitosterol). Interestingly, phytol has been reported to demonstrate potent cytotoxic effect against lungs cancer cell by induces apoptosis in A549 cells by depolarizing the mitochondrial membrane potential(26). Similarly n-hexadecanoic has been reported to possess some antiproliferative activities (27). Dihydroactinidiolide is a powerful anti-proliferative compound that had been reported to regulates Nrf2/HO-1 expression and inhibits caspase-3/Bax pathway to protect SH-SY5Y human neuroblastoma cells from oxidative stress induced neuronal apoptosis (28). Other notable compounds with antiproliferative activities include both γ & α -Tocopherols. Tocopherols have been reported to possess cytotoxic effect on prostate cancer cell lines (29). Stigmasterol and sitosterol have been reported to possess cytotoxic effects on MCF-7 breast cancer cell lines (30).

On the other hand the dichloromethane fraction and alkaloidal extract contain similar chemical contents and the displayed similar cytotoxicity against nauplii of *Artemia salina* and cancer cell lines. This may infer that the alkaloidal content of the dichloromethane fraction and those present in the alkaloidal extract maybe responsible for the observed cytotoxicity. Interesting earlier report of the GC-MS analysis of the alkaloidal extract of *E. aureum* led to identification of twenty six structurally different alkaloids among which includes known cytotoxic alkaloids such as; Catharanthine that is clinically used in the treatment of Hodgkin's lymphoma, nonsmall-cell lung, bladder, brain, and testicular cancers (17, 31).

Conclusion

This present study demonstrated that *E. aureum* possess antioxidant and cytotoxic activities and the plant contain alkaloids that display potent cytotoxic activities against human rhabdomyosarcoma and laryngeal carcinoma. It is recommended that the cytotoxic activity of the plant should be examined in vivo and the plant extract should be explored for the isolation of the anticancer compounds.

Conflict of interest

There is no conflict of interest with the data contained in this manuscript.

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