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PHYTOCHEMICAL PROFILING AND *IN VITRO* ANTI-PROLIFERATIVE ACTIVITY OF EXTRACT AND FRACTIONS OF *ANTHONOTHA MACROPHYLLA* (FABACEAE) P. BEAUVOIS AGAINST *SORGHUM BICOLOR* RADICLES

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ABSTRACT

Anthonotha macrophylla (P. Beauvois) is used in folkloric medicine for treating boil, malaria, tumor, diarrhoea, gonorrhea and skin infections. Its use in the treatment of tumor led to the determination of the anti-proliferative potential in relation to the identified phytoconstituents from the extract of the leaves. Phytochemical screening, Gas Chromatography-Mass Spectrometry (GC-MS) and High Pressure Liquid Chromatography (HPLC) analytical methods were used in the identification and quantification of the phytochemical constituents. Effects on *Sorghum bicolor* radicles were utilized to ascertain the anti-proliferative potentials of the macerated crude extract and partitioned fractions of *A. macrophylla.* Screening of the powdered leaves for phytochemicals revealed alkaloids, tannins, flavonoids, steroids, glycosides and saponins. Analysis by HPLC of the extract (methanol) displayed kaempferol, narigenin, quercetin and catechin, while analysis of similar extract by GC-MS revealed 3-aminopyrrolidine (96.44%). Methanol leaf extract of *A. macrophylla* (1 mg/mL) suppressed the growing radicles by 1.55% within 24 hours, this increased to 35.97% in 96 hours. Also 16 mg/mL of the extract of methanol significantly ($p<0.05$) suppressed the emerging radicles by 31.78% in 24 hours; this increased to 57.30% after 96 hour. Ethylacetate fraction (1 mg/mL) of *A. macrophylla* significantly ($p<0.05$) suppressed the emerging radicles by 13.44% in 48 hours, which increased to 25.09% in 96 hours. At a higher dose of 16 mg/mL, ethylacetate fraction significantly ($p<0.05$) suppressed the emerging radicles by 69.60% in 48 hours, which increased to 77.41% after 96 hour. From our research findings, the methanol leaf extract and ethylacetate fraction of *A. macrophylla* possess phyto-constituents that have anti-proliferative potentials.

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INTRODUCTION

One genetic disease that has affected so much people in the world is cancer. It is the second major cause of mortality among the non-communicable diseases. In 2018, it accounted for over nine million death globally [1]. In 2023, the United State of America recorded over 600 thousand death with over 1.95

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million reported cases [2]. Cancer burden continues to grow worldwide affecting individuals and families physically, emotionally and financially [3]. These have adversely affected the healthcare system negatively and in under-developed country settings, the facilities are unable to manage the patients with such ailments. Also, the development of tolerance and resistance in the affected cells to chemotherapeutic drugs have prompted the search for compounds that will control or treat the individual with such conditions. Thus, the need to research phytoconstituents from plants that are believed to be safe, efficacious and cheap.

Anthonotha macrophylla (P. Beauvois) belongs to family Fabaceae; it was formerly known as *Macrolobium macrophyllum*. It occurs as a shrub or tree that is evergreen year round with a broad disseminating crown, also can grow to 20 m in height on an abandoned land. The leaves are peri-pinnately compound organized, with 2-4 obovate to elliptical leaflet per pinnae [4]. *Anthonotha crassifolia* is synonym with *Anthonotha macrophylla* [5]. It is endemic to the rainforest zone of tropical Africa and widely distributed in Senegal through to Nigeria, Cameroon to southern Central Africa Republic, south to northern Democratic Republic of Congo [4]. *A. macrophylla* is known in English as African rosewood and locally it is called Nya (Uyo, Akwa-Ibom State).

Groups of phytochemicals isolated from the leaf of *A. macrophylla* are methyl alkane, sterol-glycoside, sugars, fatty acids, esters, diamine and alcohol. Specific examples include bergenine, tri-O-methylnorbergenin, α-D-glucopyranosyl(1→2')-β-D-sorbopyranoside, 2,3-dihdroxypropylheptacosanoate and 4-C-β-D-glucosyl(1,1-bis(N-E),N'-(E)-diferuloylethanamide)buta-1,3-diene [6]. Also, 9-octadecenoic acid (Z)-methyl ester, 11-octadecenoic acid, methylester, 2-propenyl ester propanoic acid, methyl stearate, 2-hydroxypropanamide carbamic acid, 1-methyl-4-(1-methylethenyl-,trans)cyclohexane, diglycolamine, 3-cyclohexyl-4-hydroxy-4-methyl-1-oxo-3-azaspiro[4,5]decan-2-one were identified from Gas Chromatography coupled to a Mass Spectrometer [7].

The leaves are used traditionally in the tending of tumor, boil, malaria, yellow fever, diarrhea, gonorrhea, dysentery and skin infection. It is administered as an antidote in the treatment of venomous bite and sting [8,9]. Leaf decoction are used to treat jaundices [10] and relieve tooth arches [11]. Pharmacologically, *A. macrophylla* has been reported with the following activities: anti-oestrogenic [7], anti-malarial [12], antibacterial and toxicological [13], analgesic, aphrodisiac[10], cytotoxicity [14], hematological effect [15], anti-inflammatory[16] and antitrypanosomal[17]. This study aims to identify and quantify the phytoconstituents in relation to the anti-proliferative potential of the leaf extract and fractions of *A. macrophylla*.

MATERIALS AND METHODS

Plant Collection and Identification of *Anthonotha macrophylla*

Anthonotha macrophylla plant was collected in November, 2023 from Akwa-Ibom State, Ibesikpo Local Government Area, Afaha village. Identification was carried out by Professor Akinnibosun H.A. of Plant Biology and Biotechnology Department. Voucher sample number UBH333 was issued following the deposition of the specimen in the herbarium.

Preparation and Extraction

The leaves of *A. macrophylla* were detached from the stem and dried under shade for 14 days. These were pulverized using electric milling machine and the powdered leaf (200 g) was macerated with methanol (1L) (99.98%) at room temperature for 3 days. Extract (crude) was obtained following decantation of the solvent mix, filtration through filter paper (size 1) and concentration *in-vacuo* utilizing a rotary evaporator set at 40^{o}C. The extract obtained (crude extract) (20.00 g) was kept in a refrigerator maintained at 4^{o}C when not in use.

Solvent Partitioning of the Crude

Ten gram (10 g) of the crude extract was solubilized in methanol (20 mL), distilled water (80 mL) and placed in a separating funnel. *n*-Hexane (4 x 50 ml) was used to partition the mixture; the portions were collected and concentrated *in vacuo*. This process was repeated for dichloromethane and ethylacetate. The different fractions were combined in various beakers and concentrated to obtain the *n*-hexane, dichloromethane and ethylacetate fractions of *A. macrophylla.*

Phytochemical Screening of the powdered leaves

Powdered leaf of *A. macrophylla* was tested for alkaloids, cyanogenic glycosides, cardiac glycosides, flavonoids, saponins, steroids, terpenoids and tannins [18,19].

Analysis of the Methanol Leaf Extract *A. macrophylla* Using High Pressure Liquid Chromatography

Determination of the methanol leaf extract of *A. macrophylla* was conducted utilizing twofold binary pumps, column oven and Ultra-violet/Visible detector (Shimadzu: LC-10AD; CTO-10AS; SPD-20A were used respectively). Normal phase column C-12 with measurement (5 µ thickness x 200 mm length x 4.8 mm internal diameter) was used for analysis. Acetic acid-acidified deionized water (pH 2.8) was used as part of the mobile phase (A), while acetonitrile with a flow rate of 0.8 mL/min was used as the other mobile phase (B). The column was balanced by five percent of solvent B for 20 min after each sample injection. Column temperature was set at 38^{o}C; 20 µL was used as injection volume while wavelength was fixed at 280 nm. Identification and quantification was based on peak areas and retention times comparison by external standards calibration plot. The solvent mix for elution is as shown in Table 1.

Table 1: Gradient elution

SN	Time (min)	Solvent B %	Solvent A%
1	0-5	5-9	91-95
2	5-15	9	91
3	15-22	9-11	89-91
4	22-38	11-18	82-89
5	38-43	18-23	77-82
6	43-44	23-90	10-77
7	44-45	90-80	10-20
8	45-55	100	0

Standards include kaempferol, anthocyanidine, steroids, catechin, anthocyanin, cyanogenic glycoside, naringenin, dihydrocytisine,aphylidine, quercetin, ammodendrine, tannin, cardiac glycoside, spartein, flavone, ribalinide, phytate and sapogenin [20].

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of methanol leaf extract of *A. macrophylla*

Gas Chromatography - Mass Spectrometry (Agilent USA 7890A GC system, 5675C Inert MSD) utilized in this analysis is a triple axis detector, outfitted with a 10 µL auto injector syringe. The carrier gas was helium gas and capillary column used in the separation was an Agilent 19091-433HP-5Ms with 5% phenyl polymethyl siloxane, with the following specification of length, internal diameter and thickness: 30 m, 0.2 um and 250 um respectively. Operating conditions associated with this determination include a 250^{o}C as temperature of the source of the ion (EI), 300^{o}C interface temperature; 16.2 psia pressure, Injector of 1uL was at split mode, with 1:50 split ratio, at injection temperature of 280^{o}C. Temperature of the column was set at 50^{o}C for 2 minutes and vary to 100^{o}C at the rate of 20 ^{o}C/min. Temperature was then raised to 250^{o}C at the rate of 20^{o}C/minutes and held for 5 minutes, the total elution was 19 minutes. The system was controlled by the supplier's software solution of the mass spectrometry and in the process data were acquired. Phyto-compounds identification was done by comparing the mass spectra of the individual compounds and those of standard from library of NIST [21].

Anti-proliferative Effect of Methanol Extract and Fractions of *A. macrophylla* Using *Sorghum bicolor*

The growth-suppression effect of *A. macrophylla* methanol extract and fractions wascarried out using a previously described method. Glass Petri dishes were laid with cotton wool and Whatman filter paper of size 1. Ten millilitre (10 ml) of 1, 2, 4, 8 and 16 mg/mL of the methanol extract and each fractions were added to the individual Petri dishes, including distilled water and 2.5% dimethylsulphoxide (DMSO) that were used as controls. Twenty viable seeds of *Sorghum bicolor* were spread on each plate and kept in the dark. The length of emerging radicles was measured at 24, 48, 72 and 96 hours. The experiment was conducted in triplicate [22].

Statistical Analysis

In vitro anti-proliferative test was in triplicate and results expressed as mean ± standard error of mean. Analysis of the data was done by one way ANOVA, *posthoc* test was done on the means obtained and level of significance set at P<0.05. Data were plotted and analyzed using 16.0 Graphpad version.

RESULTS

Qualitative Phytochemical Screening

Alkaloid, flavonoid, glycoside, saponin, steroid and tannin were the phytochemicals observed from the screening of the powdered leaf (Table 2).

HPLC Analysis

The HPLC analysis revealed the following class of compounds: kaempferol, narigenin, steroid, catechin, cyanogenic glycoside, dihydrocytisine, quercetin, aphyllidine, ammodendrine, tannin, flavonones, cardiac glycoside, spartein, flavone, ribalinidine, phytate, oxalate, epehdrine and sapogenin (Table 3). The prominent phytocompounds (5.0000 to 11.0000 µg/ml) include steroid (7.8880 µg/ml), cyanogenic glycoside (6.0167 µg/ml), dihydrocytisine (8.3477 µg/ml), flavonones (5.0720 µg/ml), cardiac glycoside (6.8916 µg/ml), spartein (5.3031 µg/ml), flavone (5.0956 µg/ml), ribalinidine (9.2765 µg/ml), phytate (10.8675 µg/ml) and sapogenin (7.2987 µg/ml).

GC-MS Analysis

Phytochemical composition of *A. macrophylla* leaf extract was assessed by GC-MS analysis. The identified suggested compounds (48) are contained in Table 4 and the main compounds as 3-aminopyrrolidine.

Anti-proliferative Activity of the Methanol Leaf Extract of *Anthonotha macrophylla*

Methanol leaf extract of *A. macrophylla* evoked concentration-dependent suppression of the length of the emerging radicles. The 1 mg/mL concentration suppressed the emerging radicles by 1.55% in 24 hours, which increased to 35.97% in 96 hours. The 16 mg/mL concentration significantly (p<0.05) suppressed the emerging radicles by 31.78% in 24 hours that increased to 57.30% after 96 hour. (Figure 1)

Anti-proliferative Activity of the Ethyl Acetate Fraction of *Anthonotha macrophylla* Crude Extract

From the anti-proliferative experiment conducted, only ethylacetate fraction showed anti-proliferative activity from the various fractions. Ethyl acetate fraction of *A. macrophylla* evoked concentration-dependent suppression of the length of the emerging radicles. The 1 mg/mL concentration significantly (p<0.05) suppressed the emerging radicles by 13.44% in 48 hours, which increased to 25.09% in 96 hours. The 16 mg/mL concentration significantly (p<0.05) suppressed the emerging radicles by 69.60% in 48 hours that increased to 77.41% after 96 hour (Figure 2).

Table 2: Phytochemical screening of the powdered leaf of *Anthonotha macrophylla*

S/N	Phytochemical	Inference
1	Alkaloids	+
2	Flavonoids	+
3	Tannins	+
4	Glycosides	+
5	Steroids	+
6	Saponins	+
7	Terpinoids	-

+ = present, - = absent

Table 3: Phytochemicals from the methanol leaf extract of *A. macrophylla* from HPLC analysis.

S/N	Compound	Retention time (min.)	% Area	Concentration (µg/ml)
1.	Kaempferol	0.140	3.61	4.7443
2.	Naringenin	3.423	3.62	2.8339
3.	Steroid	4.613	5.62	7.8880
4.	Catechin	9.480	4.30	3.4056
5.	Cyanogenic glycoside	10.866	4.13	6.0167
6.	Dihydrocytisine	13.126	10.17	8.3477
7.	Quercetin	16.656	2.97	0.2195
8.	Aphyllidine	19.440	4.05	3.1075
9.	Ammodendrine	22.883	7.28	4.0347
10.	Tannin	25.713	7.02	4.3193
11.	Flavonones	28.606	2.62	5.0720
12.	Cardiac glycoside	30.280	4.91	6.8916
13.	Spartein	32.730	3.92	5.5031
14.	Flavone	34.633	3.63	5.0956
15.	Ribalinidine	36.896	6.61	9.2765
16.	Phytate	39.326	11.68	10.8675
17.	Oxalate	40.326	5.48	2.3389
18.	Epehidrine	40.953	3.16	4.4295
19.	Sapogenin	41.816	5.20	7.2987

Table 4 :Chemical Composition of methanol leaf extract of *Anthonotha macrophylla* from GC-MS

S/N	Compound	Retention time (min)	% Area	Molecular formula	Molecular weight
1.	3-aminopyrrolidine	2.369	96.44	$C_4H_{10}N_2$	86.14
2.	2-Oxo-1-oxa-spiro[4.5]decane-4-carboxylic acid (furan-2-ylmethyl)-amide	4.426	-0.01	$C_{16}H_{20}O_5N$	306.27
3.	3,9-Epoxypregnane-11.beta.,20-diol, 3.alpha.-methoxy-18-[N-methyl-N-(2',14-epoxyethyl)amino]-	4.764	0.01	$C_{25}H_{41}NO_5$	435.298
4.	3,3-Dimethyl-2-butanone oxime, o-[(pentafluorophenyl)methyl]-	4.877	0.01	$C_{13}H_{15}F_5NO$	385.10402
5.	2-ethoxy-2-methylbutane	5.102	0.04	$C_7H_{16}O$	116.2013
6.	dodecamethylcyclohexasiloxane	5.440	0.05	$C_{12}H_{36}O_6Si_6$	444.93
7.	N-[4-bromo-n-butyl]-2-piperidone	5.806	0.02	$C_9H_{16}BrNO$	234.13
8.	2-methyl-4-oxo-5-(2,3,4,5-tetrahydro-5-methyl[2,3'-bifuran]-5-yl)-2-pentenal	6.003	0.03	$C_{15}H_{18}O_4$	262.30
9.	4,6-diamine quinoline	6.172	0.08	$C_9H_9N_3$	159.19
10.	3-(3-fluorophenylcarbamoyl)-1,2,2-trimethylcyclopentanecarboxylic acid,	6.257	0.06	$C_{16}H_{20}FNO_3$	293.33
11.	Ethylidenecyclopentane	6.426	0.32	C_7H_{12}	96.17
12.	8-Methyl-3-phenyl-5-quinolinecarboxylic acid	6.905	0.17	$C_{17}H_{13}NO_2$	264.32

13.	Octadecanoic acid, 2-(octadecyloxy)ethyl ester	7.412	0.37	$C_{38}H_{76}O_3$	581.14
14.	3-[(6-deoxy-3,4-O-methylenehexopyranos-2-ulos-1-yl)oxy]-7,8-epoxy-11,14-dihydroxy-12-oxo-, (3.beta.,5.beta.,7.beta.,11.alpha.)carda-16,20(22)-dienolide,	7.722	0.04	$C_{30}H_{38}O_{11}$	574.6
15.	3-cyclopropyl,-7-carbethoxy,cis-bicyclo[4.1.0]heptane	8.032	0.01	$C_{13}H_{20}O_2$	208.3
16.	N-(4-chlorobenzyl)furan-2-carboxamide	8.144	0.05	$C_{12}H_{10}ClNO_2$	235.66
17.	1-(5-iodo-2,4-dimethylpyrazol-3-yl)ethanone	8.511	0.01	$C_8H_{10}INO$	263.08
18.	2-(chloromethyl)-3-(1-ethylethenyl)oxirane	8.595	0.01	C_6H_7ClO	130.63
19.	N-(aminocarbonyl)-2-ethyl-,(Z)-2-butenamide	9.694	0.17	$C_7H_{12}N_2O_2$	156.1824
20.	3-methyl-1-nitrosopiperidine	9.919	0,17	$C_6H_{12}N_2O$	128.18
21.	2-cyclopropyl-2-methyl-N-(1-cyclopropylethyl)cyclopropane carboxamide,	10.144	0.11	$C_{13}H_{21}NO$	207.31
22.	3,12-Oleandione	10.285	0.51	$C_{30}H_{48}O_2$	440.7
23.	N-(4-chlorobenzyl)furan-2-carboxamide	11.412	0.02	$C_{12}H_{10}ClNO_2$	235.66
24.	2-Dodecanol	11.553	0.01	$C_{12}H_{26}O$	186.33
25.	Tetrazolo[1,5-b]pyridazine	11.666	0.02	$C_4H_3N_5$	121.10
26.	3-Butyl-6-methyl-4-prop-2-en-1-yl-2,6-dioxo-4,5,6,7-tetrahydro-1,2,3-triazolo[4,5-d]pyrimidine	11.919	0.01	$C_{12}H_{17}N_5O_2$	263.29
27.	1,9-Dichlorophenazine 5-oxide	12.116	0.02	$C_{10}H_6Cl_2N_2O$	265.095
28.	N-Methylmaleimide	12.313	0.01	$C_5H_5NO_2$	111.10
29.	4-(4-Ethoxy-2-hydroxybenzylidenehydrazino)-2-methyl-6-morpholinopyrimidine	12.596	0.02	$C_{18}H_{23}N_5O_3$	357.4
30.	d-Mannitol, 1-O-methyl-, pentaacetate	12.794	0.02	$C_{17}H_{26}O_{11}$	406.38
31.	1,2-Benzenediol,4-(2-amino-1-hydroxypropyl)-	12.877	0.03	$C_9H_{17}NO_3$	183.2044
32.	4-(2,4-Dimethylcyclohex-3-enyl)but-3-en-2-one	13.215	0.03	$C_{12}H_{18}O$	178.27
33.	Oleyl oleate	13.497	0.01	$C_{36}H_{68}O_2$	532.92
34.	Tridecanoic acid, 4,8,12-trimethyl methyl ester	13.637	0.02	$C_{17}H_{34}O_2$	270.5
35.	1,2-didehydroaspidospermidin-20-one	13.806	0.02	$C_{19}H_{22}N_2O$	294.1732
36.	6-Hydroxy-1-oxogermacr-4,10(15),11(13)-trien-12,8-olide	13.919	0.02	$C_{16}H_{22}O_3$	262.38
37.	9,10-dihydro-9,9,10-trimethylanthracene	14.116	0.04	$C_{17}H_{18}$	222.32
38.	2,4,6,8-Tetrathiatricyclo[3.3.1.1(3,7)]decane-1-thiol, 3,5,7-trimethyl-	14.286	0.01	$C_9H_{14}S_5$	282.5
39.	2-Thiopheneacetic acid, 2-ethylcyclohexyl ester	14.398	0.01	$C_{14}H_{20}O_2S$	252.37
40.	1-[2-(Heptylmethylcarbamoyl)acetyl]pyrrolidine-2-carboxylic acid, heptylmethylamide	14.567	0.03	$C_{24}H_{45}N_3O_3$	423.6
41.	Ethyl 5-bromonicotinate	14.821	0.03	$C_8H_8BrNO_2$	230.059
42.	2,2'-Bi(bicyclo[2.2.1]heptane)	15.074	0.02	C_7H_{10}	94.15
43.	6-Methyl-4-propan-2-on-3-propyl-2,6-dioxo-4,5,6,7-tetrahydro-1,2,3-triazolo[4,5-d]pyrimidine	15.871	0.01	$C_{11}H_{15}N_5O_3$	265.27
44.	4-phenylpyrido[2,3-d]pyrimidine	15.581	0.01	$C_{13}H_9N_3$	207.23
45.	4-allyl-5-furan-2-yl-2,4-dihydro-[1,2,4]triazole-3-thione	15.750	0.01	$C_9H_9N_3OS$	207.25
46.	Ethanone, 2-(5H-indeno[1,2-b]pyridinylidene)-1-phenyl-,oxime	16.004	0.01	$C_{20}H_{14}N_2O$	298.11
47.	Hexamethylcyclosiloxane	18.342	0.61	$C_6H_{18}Si_3O_3$	222.46
48.	(9-Oxo-9,10-dihydroacridin-4-yl)acetic acid	18.680	0.24	$C_{15}H_{11}NO_3$	253.25

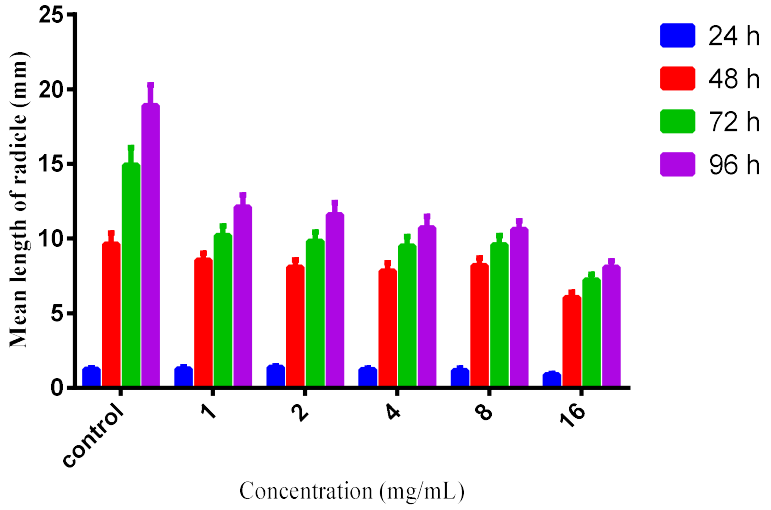

Figure 1: Antiproliferative effect of the methanol extract of *A. macrophylla* on *S. bicolor* radicle.
Each bar represents mean ± SEM. Data were considered to be significant at p<0.05. n=20

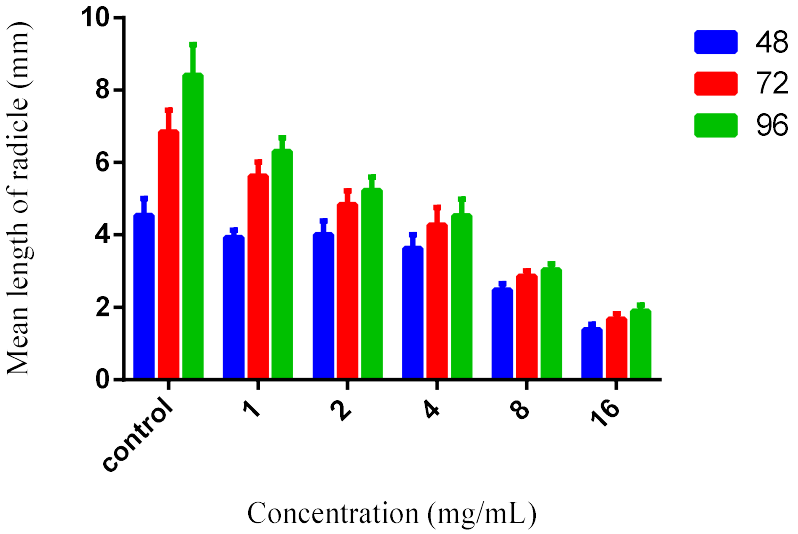

Figure 2: Anti-proliferative effect of the ethyl acetate fraction of *A. macrophylla* on *S. bicolor* radicle.
Each bar represents mean ± SEM. Data were considered to be significant at p<0.05. n=20

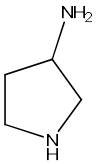

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Figure 3 Structure of 3-aminopyrrolidine

DISCUSSION

The leaves of *A. macrophylla* have been reported to possess alkaloids, saponins, glycosides, flavonoids, tannins and steroids [6,13,17], which are in agreement with this study. Phytochemicals are important plant compounds that are met for the protection of the plant against stress or predators, however these substances have been discovered to possess valuable pharmacological potentials [23], which could be linked to difference in their chemical make-up and functional groups.

HPLC analysis is a well-known method used by researcher for analysis of plant extract [24], it provides information for compounds that are non-volatile in nature, in addition to the identification and quantitative nature of the phyto-compounds. The technique used in this study, has the advantage of suggesting of compounds with diverse polarity and molecular weight [25]. Most of the compounds identified have been reported already from the phytochemical screening results (Table 2). They include alkaloids (ephedrine, dihydrocytisine, aphyllidine, sparteine, ribalinidine and ammodendrine), flavonoids (catechin, kaempferol, quercetin and naringenin), steroid, tannin and glycoside. Thus, the HPLC analysis emphasize the presence of phytochemicals in the leaves of *A. macrophylla*, though sub-classes of the phytochemicals were identified flavone, flavonone, sapogenin, cyanogenic and cardiac glycosides. Phytochemicals such as catechin and quercetin are utilized as standard in protocol that involve anti-oxidant potential, total flavonoid and total phenolic contents determination[26].

Flavonoids are bioactive polyphenolic compounds in plant with varying medicinal properties [27]. They are the third most abundant phytochemicals beside terpenoids and alkaloids [28]. It has a basic skeleton of fifteen carbon atoms distributed between two six membered ring and one three carbon unit linking both six membered ring. Flavonoids are broadly classified into bio-flavonoid, iso-flavonoid and neo-flavonoid [29]. Individually, flavonoids have shown activity in relation to anti-proliferative potential [30]. Kaempferol has been reported to possess significant anti-proliferative and increased cell cytotoxicity in urinary tract carcinoma which may be due to significant cell growth suppression by down-regulating the c-Met/p38 signaling pathways [31]. Naringenin exerts its cytotoxic effect by inhibition of the enzymes topoisomerase and kinase, and also from its pro-oxidant effect. Its anti-proliferative effect could be linked to the inhibition of radical oxidative species which is responsible for mediating cell death due to their ability to act as important signaling molecules [32]. Quercetin induces anti-proliferative effect through the activation of apoptotic signal pathway such as MAPKs pathway in colon 26 cells and prevent viability of colon 26 and 38 cells [33]. Also, quercetin has been shown to prevent the activity of protein kinase that is dependent on phospholipids and calcium for its action [34]. Literature has shown that selected sapogenin possesses more anti-proliferative activity than cytotoxic potential [35].

3-Aminopyrrolidine (Figure 3) suggested as the major compound from the GC-MS analysis, is a five member pyrrolidine ring with a versatile scaffold for different biological activities [36]. It has been identified as a novel scaffold used in the synthesis of compounds that act as dual inhibitor for both Abelson (Abl) kinase and P13K. These compounds have been shown to be promoting cytotoxicity against chronic myeloid leukaemia, leukaemia cell line (K562) and as moderate inhibitor against Abl and P13K kinases [37].

Figures 1 and 2 show that the *Sorghum bicolor* radicles experienced unhindered proliferation for both controls. However, there was growth suppression when the growing radicles were tested against the methanol extract and ethylacetate fraction of *A. macrophylla*. These were observed to be significant ($p<0.05$) as the time of contact with the radicles increased from 48 hours to 76 hours and also as the concentration increased from 1 mg/mL to 16 mg/mL. The effect produced at 96 hours of probing with 16 mg/mL concentration by the ethylacetate fraction (77.41%) supersedes that produced by methanol extract (57.30%), indicating 21.11% difference; this shows that the methanol extract may possess phytoconstituents responsible for the difference in activity.

Fractionation of the methanol extract with *n*-hexane helps to defatten the extract, while partitioning into dichloromethane and ethylacetate partially separate the constituents into semi-polar and polar fractions. These also have the advantage of partitioning the various phytochemicals into these solvents. Thus, the effect shown by the ethylacetate fraction, indicate that a particular group of phytochemicals are responsible for this increased effect observed. Flavonoids are common occurrence in the ethylacetate fractions, which has initially been reported by the HPLC analysis.

CONCLUSION

The leaves of *A. macrophylla* are rich in phytochemicals such as alkaloids, flavonoids, tannins, saponins, steroids and glycosides. This study was able to suggest major compounds with antioxidant potential which could be responsible for the anti-proliferative potential, such an activity have been linked to compounds such narigenin, catechin, kaempferol and quercetin. The GC-MS analysis suggested 3-aminopyrrolidine an important scaffold with wide activity. The methanol extract and ethylacetate fraction showed significant levels of anti-proliferation. This study has paved the way for further search into the exact compounds responsible for the anti-proliferative activity of the leaf extract of *A. macrophylla* through isolation and characterization.

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AUTHORS' CONTRIBUTION

Conceptualization, data curation and formal analysis was carried out by Emmanuel Eimiomodebheki Odion (EEO), Daniel Akpe-efiak Ambe (DAA), and James Oghomwen Aghahowa

(JOA). Investigation, and methodology was carried out by JOA, Eravweroso Congrat Odiete (ECO), Project supervision was done by EEO, writing original draft of the manuscript was archived by EEO, DAA, JOA, ECO, Chinyelu Clementina Osigwe (CCO) while writing-review and editing were done by EEO, DAA, JOA, ECO, and CCO.

CONFLICT OF INTEREST

None

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