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Sharaibi OJ
Department of Botany, Faculty of Science, Lagos State University, Nigeria

Omolokun KT
Department of Botany, Faculty of Science, Lagos State University, Nigeria

Oluwa OK
Department of Botany, Faculty of Science, Lagos State University, Nigeria

Ogbe AA
Department of Botany, Faculty of Science, Lagos State University, Nigeria

Adebayo AO
Department of Botany, Faculty of Science, Lagos State University, Nigeria

Corresponding Author:
Sharaibi OJ
Department of Botany, Faculty of Science, Lagos State University, Nigeria

Comparative studies of chemical compositions and antioxidant potentials of *Dysphania ambrosioides* L (Amaranthaceae) and *Hybanthus enneaspermus* (L) F. Muell (Violaceae)

Sharaibi OJ, Omolokun KT, Oluwa OK, Ogbe AA and Adebayo AO

Abstract
Nigeria and Africa are good repositories of great diversity of therapeutic plants which are traditionally used to treat myriads of ailments and diseases. *Dysphania ambrosioides* is one of the valuable medicinal plants in African folk medicine known for its anti-inflammatory, antifungal, anti-diarrhoea, anti-diabetic, and purgative properties. *Hybanthus enneaspermus* is an annual herb or shrub plant that contains a variety of phytoconstituents for the treatment of various acute or chronic disorders. Recent research has revealed that these plants are extensively used for therapeutic purposes in the Badagry community of Lagos State, and more research on their phytochemistry and antioxidant potentials is generally required. Thus, this present study aimed at comparing the quantities of phytochemicals and antioxidant activities of *Dysphania ambrosioides* L and *Hybanthus enneaspermus* (L) F. Muell. Different fractions of *H. enneaspermus* and *D. ambrosioides* were screened for their phytochemicals including total phenolic content, tannins content, total flavonoid content, alkaloids content and total saponins content. Also, *in vitro* antioxidant activity was assessed using DPPH radical scavenging, reducing power and nitric oxide assays. Other phytoconstituents were evaluated through GC-MS determination. Results showed that the ethanol fraction was found to have high levels of alkaloids (189.43 mg/g) and flavonoids (135.07 mg/g) content in both *D. Ambrosioides* and *H. Enneaspermus* respectively. At 25µg/mL and 50µg/mL, the extracts of *H. Enneaspermus* exhibited higher antioxidant activities than *D. Ambrosioides* while at 75 µg/mL and 100 µg/mL, *D. Ambrosioides* exhibited higher antioxidant than *H. Enneaspermus*. Significant phytoconstituents were discovered in both plants through GCMS determination.

Keywords: *Hybanthus enneaspermus*, *Dysphania ambrosioides*, GCMS, Nigeria

Introduction
The use of therapeutic properties in plants for the treatment of diseases and ailments is as old as man. Early men used varieties of plants to prevent and cure diseases through trial and error; in which the safe and potent ones were retained, and the poisonous ones were discarded. According to WHO (2005) [16], most people in developing nations depend on medicinal plants for their basic health care. Nigeria and Africa are good repositories of great diversity of therapeutic plants which are traditionally used to treat myriads of ailments and diseases (Sofowora, 1983) [12]. Nigeria is blessed with a wide range of habitats and ecosystems, as well as a vast array of domesticated and wild animals and plants. This heritage meets the population's needs for clothing, food, shelter, healthcare, recreation, and other things (Odugbemi and Akinsulire, 2006) [11]. Additionally, this biodiversity maintains the fundamental ecological processes that support life, such as nutrients cycling and the upkeep of the soil. It is the treasure site where future food requirements, medical treatments, and components for knowledge and technology will be found. Plants have served as the foundation for conventional treatments for various ailments and continue to offer a vast potential supply of new therapeutic molecules (Adewunmi *et al*., 2001) [1]. This calls for the extraction of the bioactive compounds of pharmacological significance after purification, identification, and toxicity assessments. Therefore, it is crucial to maintain and record traditional knowledge of medicinal plants for the sake of drug discovery; in most cases, this is dependent on local practitioners and field research (Anita, 2004) [17]. To address this issue, however, it may be possible to use plant extracts and phytochemicals, which are highly significant in therapeutic treatments (Erdogrul, 2002, Acharya and Shrivastava, 2008) [18, 19]. Olowokudejo *et al*. (2008) [20] stated that, in Nigeria, the ratio of people visiting herbal medicine practitioner is 110:1 compared to visits to orthodox medicine practitioner of 1:9. Among the significant medicinal plants used in Nigeria are *Dysphania ambrosioides* L. *and Hybanthus enneaspermus* (L) F. Muell used in this study.

Dysphania ambrosioides formerly known as *Chenopodium ambrosioides* commonly called Mexican tea or Jesuit's tea belongs to the family Amaranthaceae. It originated from Southern Mexico and is widely distributed in some African countries like Nigeria, Ghana, and Cameroon. It is also found in Central America and South America (Clemantis and Mosyakin, 2003) [3]. *D. Ambrosioides* is a cosmopolitan, polymorphic annual and perennial weed (Correa *et al*., 2004) [4]. According to reports, it is one of the valuable medicinal plants in African folk medicine. Due to its strong fragrance and pungent flavour, it is taken as herbal tea and leaf vegetable to expel worms (Nyerges, 2016). The pesticidal, analgesic, anti-inflammatory, antifungal, anti-diahorrea, anti-diabetic and purgative properties have been reported (Isman, 2020 [6]; Laferrière, 1990 [8]; Ngbolua *et al*., 2014 [9]).

Hybanthus enneaspermus is an annual herb of the family Violaceae, commonly called spade flower, or pink ladies slipper. It is widely distributed in India, Sri-Lanka, Africa, Madagascar, and Tropical Australia. It is also a perennial herb with an erect stem up to 30cm-60 cm tall. The leaves are sessile up to 1 mm long with lamina of 16-60x2x 3-9 mm. The leaf is hairy and stipulated. H. *enneaspermus* is significantly used traditionally to treat cholera, inflammation, leucorrhoea, diarrhoea, dysuria, sterility and urinary infections. Recent ethnobotanical studies of the medicinal plants used in Badagry community of Lagos State showed that *Dysphania ambrosioides* L. and *Hybanthus enneaspermus* (L) F. Muell were frequently mentioned as the most used plants in the research area (Sharaibi *et al.*, 2022) [13]. This necessitated the need to evaluate their phytochemistry and the antioxidant potentials. This study, therefore, aims at comparing the quantities of phytochemicals and antioxidant activities of *Dysphania ambrosioides* L. and *Hybanthus enneaspermus* (L) F. Muell.

Materials and Methods

Collection of Plants

Fresh leaves of *D. ambrosioides* and *H. enneaspermus* were collected from the wild in the Badagry local community and identified by the curator of the Department of Botany, Lagos State University. The voucher specimens (LSH/SHA 234 and 235) were placed in the herbarium for reference purposes.

Preparation of the Plant Extracts

Fresh leaves of *D. ambrosioides* and *H. enneaspermus* were carefully rinsed under running tap water in the laboratory and then oven dried at 40 ºC using Carbolite AX60 laboratory oven (Equipnet, MA, USA). The dried leaves were pulverized to expand their surface area for the absorption of the solvents. 200 g of each pulverized plant samples were dissolved in 1000 ml of absolute ethanol, acetone, and distilled water in separate conical flasks. They were placed on orbital shaker for 24 hours for homogenous mixing of the solvents and the solutes. The mixtures were later filtered using Whatman No. 1 filter paper with cotton wool. Then, the result filtrates of acetone and ethanol were undiluted using rotary evaporator (REV-2000AX) while the aqueous filtrate was freeze dried with benchtop freeze drier (LYO60BB). The extracts were stored in air-tight containers for future use.

Estimation of Extract Yields

The extract yield was calculated using this formula:

$$\text{Extract yield (\%)} = \frac{\text{Weight of dried extract X 100}}{\text{Weight of dried plant sample}}$$

Estimation of Phytochemicals in *D. ambrosioides* and *H. enneaspermus*

Estimation of Tannins: 200 mg of each extract was dissolved completely in 50 ml of distilled water, and the mixture was shaken for 1 hour. A 5 ml portion of the filtrate was carefully mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1 M HCl and 0.008 M potassium Ferro cyanide. Within 10 minutes, the absorbance at 720 nm was measured.

Estimation of Total Phenolic Compound: Here, 0.5g sample of each extract was dissolved in 50 ml of distilled water. The mixture was then mixed with 0.5 ml of 0.1 ml Folin-Ciocalteu reagent (0.5 N) and was incubated at room temperature for 15 mins. 2.5 ml sodium carbonate solution (7.5% w/v) was further added and also incubated for 30 mins at room temperature. At 760 nm, the solution absorbance was observed. Gallic acid equivalent (GAE) (mg/g of dry mass), a widely used reference value, was used to express the concentration of total phenol.

Total flavonoid content estimation: 3 ml of methanol, 5.6 ml of distilled water, 0.2 ml of 10% Aluminum chloride, and 0.2 ml of 1 M potassium acetate were combined with 1 ml of each sample solution (100 g/ ml). The resulting mixture was then incubated at room temperature for 30 minutes and the reaction mixture absorbance was measured at 415 nm. The calibration curve was created by making methanol solutions of different quercetin concentrations.

Determination of Alkaloids

In a separate 250 mL beaker, 5 g of each extract were combined with a total of 200 mL of 20% acetic acid and allowed to stand for 4 hours. The mixture was filtered well, and the volume was reduced to one quarter by placing it in a water bath. Until the precipitate was fully formed, concentrated ammonium hydroxide was gradually added to the mixture. The entire solution was given time to settle, then the precipitate was filtered out and weighed. Thus, the percentage of the total amount of alkaloid was calculated as:

Percentage of total alkaloids (%) = Weight of residue×100/ Weight of sample taken

Estimation of total saponins content

Modified method of Makkar *et al.* (2007) [21] approach based on vanillin-sulphuric acid colorimetric reaction was used to evaluate the total saponin contents of the extracts of *D. ambrosioides* and *H. Enneaspermus*. About 50 µL of plant extract and 250 µL of distilled water was mixed together. The mixture was then added to a volume of 250 µL of vanillin reagent (800 mg of vanillin in 10 mL of 99.5% ethanol). Afterward, 2.5 mL of 72% sulfuric acid solution was included. For ten minutes, this solution was maintained in a water bath at 60 °C. The absorbance was read at 544 nm after it had been cooled in ice-cold water. The values were represented as diosgenin equivalents (mg DE/g extract), which were generated using a standard curve.

Calculation of Reducing Sugar Content

The 3, 5 dinitrosalicylic acid (DNSA) technique was used to calculate the reducing sugar content (RSC). The DNSA reagent was made by dissolving 1 g of DNSA and 30 g of

sodium-potassium tartaric acid in 80 mL of 0.5 N NaOH at 45 °C. After dissolving, the solution was cooled to room temperature and diluted with distilled water to a volume of 100 mL. 2 mL of DNSA reagent was pipetted into a test tube containing 1 mL of plant extract (1 mg/mL) for the measurement, which was then kept at 95 °C for 5 minutes. A UV-VIS spectrophotometer (Shimadzu UV-1800) was used to test the solution's absorbance at 540 nm after it had cooled and 7 mL of distilled water had been added. The RSC was estimated using the standard D-glucose calibration curve (200-1000 mg/L), and the results were represented as mg D-glucose equivalent (GE) per gram dry extract weight.

Assessment of *in vitro* Antioxidant Activities of *H. enneaspermus* and *D. ambrosioides*

DPPH radical scavenging activity assay

2.0 ml of the reagent solution (0.004 g of DPPH in 100 ml methanol) was combined with an aliquot of 0.5 ml of each extract in ethanol (95%) at various concentrations (25, 50, 75, and 100 g/ml). Methanol was used as the blank and the control contained only DPPH solution in the place of the sample. The mixture was strongly shaken and the mixture was allowed to stand for 30 minutes at room temperature. Then, the absorbance was read at 517 nm.

The formula below was used to determine the scavenging effect: % inhibition = $[A_0\text{-}A_1] \times 100/A_0$

Where A_1 is the absorption of the extract and A_0 is the absorption of the blank sample.

Nitric oxide scavenging activity assay

The method described by Alisi *et al*. (2008) [22] was used to determine the NO scavenging activities of the plant extracts. A volume of 4 ml sample of each plant extract of different concentrations (25, 50, 75, 100 µg/ml) were taken in different test tubes and 1 ml of Sodium nitroprusside (5 mM in phosphate buffered saline) solution was added into the test tubes. They were incubated for 2 h at 30 °C to complete the reaction. A 2 ml sample was withdrawn from the mixture and mixed with 1.2 ml of Griess reagent (1% Sulphanilamide, 0.1% Naphthylethylene diamine dihydrochloride in 2% H_3PO_4). The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and its subsequent coupling with Naphthylethylene diamine was measured at 550 nm. Ascorbic acid was used as standard, and the percentage (%) inhibition activity was calculated from the following equation: $[(A_0 – A_1)/A_0] \times 100$.

Where, A_0 is the absorbance of the Control and A_1 is the absorbance of the extract or standard.

Reducing Power Assay

Different extract concentrations (20 to 100 g/ml) in 1.0 ml of deionized water were combined with phosphate buffer (2.5 ml) and potassium ferricyanide. The mixture was incubated for 20 minutes at 50 ºC. After centrifuging the mixture at 3000 rpm for 10 minutes, aliquots of trichloroacetic acid (2.5 ml) were added. The upper layer of the solution (2.5 ml) was then combined with distilled water (2.5 ml) and a freshly made ferric chloride solution (0.5 ml). The absorbance was measured at 700 nm. Without adding extract, a blank was created and ascorbic acid at different range of concentrations (1 to 16 µg/ml) was used as standard.

GC-MS Determination

The MS was automatically tuned to perfluorotributylamine (PFTBA) prior to analysis using pre-established criteria to check the abundance of m/z 69, 219, and 502 as well as other instrument optimum & sensitivity parameters. Using GC-MS, the amounts of phytochemicals in the sample were determined. To achieve complete detection of the target constituents, MSD was operated in Scan mode.

The Agilent 7820A gas chromatograph and 5975C inert mass spectrometer (with triple axis detector) with electron-impact source (Agilent Technologies) were utilized. The HP-5 capillary column (30 m length x 0.32 mm diameter x 0.25µm film thickness) coated with 5% phenyl methyl siloxane served as the stationary phase for the separation of the compounds (Agilent Technologies). A steady flow of 1.4871 mL/min of helium was employed as the carrier gas, with a starting nominal pressure of 1.4902 psi and an average velocity of 44.22 cm/sec. At an injection temperature of 300 °C, 1 µL of the samples were injected in split-less mode. A total of 16.654 mL/min of purge flow was sent to the spilled vent, with gas saver mode turned off, at a rate of 15 mL/min at 0.75 minutes. The oven was set to run at 40 °C for one minute before ramping up to 300 °C (10 min) at a rate of 12 °C/min. Runtime was 32.667 minutes, and the solvent delay was 5 minutes. The mass spectrometer was used in electron-impact ionization mode at 70eV with 230 °C for the ion source, 150 °C for the quadrupole, and 280 °C for the transfer line. Scan mode was used for ion acquisition (scanning from m/z 45 to 550 amu at 2.0s/scan rate).

Analysis

The one-way analysis of variance was applied to all the collected data

Results and Discussion

Extract Yield: The yields of the acetone are shown in Table 1, aqueous and ethanol extracts of *Dysphania ambrosioides* and *Hybanthus enneaspermus*. The extracts yield in ethanol was higher than the yields in acetone and distilled water for both plants. However, distilled water extracted more *D. ambrosioides* extract than acetone.

Table 1: Extract yields of *D. Ambrosioides* and *H. Enneaspermus* in different Solvents

Solvents/ Extract Yields	H *Enneaspermus* (g)	D *Ambrosioides* (g)
Acetone	9.07	8.15
Aqueous	7.06	8.20
Ethanol	9.87	9.17

The extracts yield in ethanol was the highest followed by acetone while distilled water extracts yield was the lowest. Ethanol is a very strong polar solvent and has been used in extraction for ages. The ability of ethanol to extract more yields may be explained by the fact that the plant extracts contained phytoconstituents that were of high polarity and solubility.

Table 2: Quantification of Phytochemicals in *D Ambrosioides* and *H Enneaspermus* Extracts

	Phytochemicals (mg/100 g)					
Extracts	**Phenols**	**Flavonoids**	**Reducing sugar**	**Saponin**	**Alkaloids**	**Tannins**
Da Acetone extract	38.76	111.53	80.26	36.76	163.54	37.48
Da Aqueous extract	32.71	90.67	79.62	31.31	109.16	37.42
Da Ethanol extract	41.12	110.67	109.62	66.32	189.43	67.43
He Acetone extract	53.09	114.99	95.20	37.54	140.97	51.33
He Aqueous extract	53.41	125.07	79.75	37.56	143.48	51.64
He ethanol extract	53.41	135.07	99.75	37.56	143.19	61.64

All the extracts possessed high number of alkaloids with highest amount of 189.43 mg/g in the ethanol extract of *D. ambrosioides.* The quantities of flavonoids were also high in all the extracts with highest amount (135.07 mg/g) recorded in the ethanol extract of *H. enneaspermus*. Phenols, saponins, reducing sugars and tannins were also reported in all the extracts. These phytochemicals may be the source of the important biological activities of the plants which in turn account for their frequent usage in traditional medicine. Alkaloids are secondary metabolites that exert important function in human medicine and natural defence in living organisms. [Kaur and Arora, 2015) [7]. In plants, alkaloids control plant growth and defend it against predators (Chik *et al.*, 2013) [2]. Alkaloids are widely known for their anaesthetic, cardio protective, anti-sedative, analgesic, antimalarial, aphrodisiac and inflammatory properties. Flavonoids are class of compounds present in fruits and vegetables and are possesses high antioxidant capacity. Flavonoids have anti-viral, anti-inflammatory, anticancer, and antioxidant effects. Additionally, they have cardio protective and neuroprotective properties. (Ullah *et al*., 2020) [15].

Table 3: Chemical constituents of *Dysphania ambrosioides*

S/N	**RT**	**Compound**	**Area** %
1	3.451	1-Butanol	30.72
2	4.721	1-Butanol, 2-methyl-	0.16
3	5.085	Butanoic acid	4.66
4	5.316	1-Nonylcycloheptane	0.47
5	5.489	Pentane, 1-methoxy-	3.92
6	5.715	2-Oxazolidinone, 3-methyl-	0.29
7	5.813	Butanoic acid, butyl ester	1.31
8	5.911	Succinic acid, di(2-methylallyl) ester	0.62
9	6.223	Butyl lactate	0.54
10	6.391	1,3-Dioxane	1.04
11	6.552	Thiophene, 2-methoxy-5-methyl-	0.17
12	6.685	Bioallethrin	0.16
13	6.887	Succinic acid, butyl 10-chlorodecy1 ester	0.12
14	7.014	Butane, 1,1'-[ethylidenebis(oxy)] bis	0.29
15	7.158	Dibutoxy(dimethyl)silane	1.57
16	7.343	Morpholine, 2,6-dimethyl-	0.06
17	7.511	Hexanoic acid, 2-ethyl-	0.22
18	7.597	Heptane, 3,5-dimethyl-	0.04
19	7.834	N-tert-Butoxycarbonyl-D-alanine	0.49
20	8.077	2-Propenoic acid, 2-(acetylamino)-	0.33
21	8.251	Tetrahydropyran	0.13
22	8.377	Piperazine	0.03
23	8.521	Benzothiazole	0.18
24	8.631	m-Guaiacol	0.72
25	8.787	Butane, 1,1-dibutoxy-	17.28
26	8.983	1,2-Benzisothiazole, 3-butoxy-	0.14
27	9.197	9,12,15-Octadecatrienoic acid, 2,3-bis(acetyloxy)propyl ester	0.2
28	9.486	Butyric acid, crotyl ester	0.6
29	9.688	3,6,9,12-Tetraoxatetradecane-1,14-diyl bis(2-methylbutanoate)	3.58
30	9.879	Phenol, 2,6-dimethoxy-	0.3
31	10.075	alpha.-D-Galactofuranoside, methyl 2,3,5,6-tetra-O-methyl-	0.38
32	10.3	2-Propanone, 1,1-dibutoxy-	1.01
33	10.404	Ribitol	0.15
34	10.589	Acetic acid, 1-(2-hydroxy-1-methyl-ethyl)-3-methoxymethoxy-2-methylpropyl ester	0.36
35	10.733	Oxalic acid, isobutyl octadecyl ester	0.06
36	10.941	trans-Isoeugenol	0.12
37	11.149	Succinic acid, butyl 2-decyl ester	4.85
38	11.265	3,4-Difluoroaniline	0.24
39	11.386	Heptyl tiglate, 4-	0.07
40	11.502	Morpholine, 4-methyl-, 4-oxide	0.09
41	11.756	dl-Isocitric acid lactone	0.33
42	12.171	cis-3-Hexenoic acid	0.44

43	12.35	2-Decen-1-ol, (E)-	0.7
44	13.02	4(1H)-Isobenzofuranone, hexahydro-3a,7a-dimethyl-, cis-	0.48
45	13.199	(1. Alpha, 2. Alpha, 3. Alpha)-Dimethyl 1,3-dimethyl-4-cyclohexene-1, 2-dicarboxylate	0.27
46	13.419	(E)-2, 6-Dimethoxy-4-(prop-1-en-1-yl)phenol	0.13
47	13.598	3(2H)-Isothiazolone, 2-methyl-	0.05
48	13.765	Benzeneacetic acid, alpha.-hydroxy-3-methoxy-, methyl ester	0.24
49	13.916	4-Ethoxy-3-methoxyphenethyl alcohol	1.24
50	14.077	Silane, dimethoxymethylphenyl-	0.26
51	14.314	Guaifenesin	0.22
52	14.637	Benzoic acid, 4-hydroxy-3,5-dimethoxy	0.46
53	14.817	2,8-Nonadienoic acid, methyl ester	0.04
54	14.932	1,5-Dimethyl-6-oxa-bicyclo[3.1.0]hexane	0.1
55	15.094	p-Menth-8(10)-en-9-ol, cis-	0.16
56	15.492	1-(4-Methoxyphenyl)-1,4-butanediol	0.22
57	15.619	n-Hexadecanoic acid	0.29
58	15.845	4-Chlorobutyric acid, eicosyl ester	0.11
59	17.005	Oleic Acid	0.55
60	17.173	Octadecanoic acid	0.25
61	17.375	9-Octadecenoic acid (Z)-, methyl ester	0.16
62	17.635	(+)-Valeranone	0.04
63	17.901	2-Dodecen-1-yl(-)succinic anhydride	0.19
64	18.062	Cycloheptane, 1-bromo-3-iodo-	0.08
65	18.322	Cimetidine	0.05
66	18.524	Cyclotriacontane	0.1
67	18.611	Eicosanoic acid	0.04
68	18.755	Bacchotricuneatin c	0.03
69	19.212	Spiro(1,3-dithiolane)-2,2'-(naphthalene)-1'-acetic acid, 1',2',3',4' -tetrahydro-, methyl ester	0.12
70	19.292	Cycloeucalenol acetate	0.05
71	19.419	5,16-Pregnadiene, 20-acetoxy-3-oxo	0.04
72	19.523	Medroxyprogesterone acetate	0.11
73	19.858	Phthalic acid, decyl 2-methoxybenzyl ester	0.08
74	20.754	Hentriacontane	0.52
75	20.858	Pregna-5, 16-dien-20-one, 3-hydroxy -, (3.beta.)-	1.48
76	21.008	Phthalic acid, bis(7-methyloctyl) ester	1.35
77	21.452	Isophthalic acid, decyl 3,5-dichlorophenyl ester	0.49
78	21.551	2,4,7,14-Tetramethyl-4-vinyl-tricyclo[5.4.3.0(1,8)]tetradecan-6-ol	0.05
79	21.689	Phthalic acid, decyl 3-iodobenzyl ester	0.64
80	22.024	Furosemide tri-methyl derivative	0.51
81	22.163	Cyclopentanecarboxamide, 3-ethenyl-2-(3-pentenylidene)-N-phenyl-, [1. Alpha, 2 Z(E), 3. Alpha]	0.48
82	22.492	Thiocarbamic acid, N,N-dimethyl, S-1,3-diphenyl-2-butenyl ester	0.78
83	22.602	1,4-Benzenediol, 2,5-bis(1,1-dimethylethyl)-	1.05
84	23.214	Octadecanoic acid, 2-propenyl ester	4.00
85	23.774	Demecolcine	0.33
86	24.045	Benzo[h]quinoline, 2,4-dimethyl-	0.29
87	24.762	2-Ethylacridine	0.87
88	24.906	2-Bromo-4, 5-dimethoxycinnamic acid	0.04
89	26.477	Olean-12-ene, 3-methoxy-, (3.beta.)-	0.23
90	28.978	Cyclopenteno[4.3-b]tetrahydrofuran, 3-[(4-methyl-5-oxo-3-phenylthio)tetrahydrofuran-2-yloxymethylene]-	0.06
91	29.237	Anthracene, 9,10-dihydro-9,9,10-trimethyl-	0.04
92	30.156	6-(Adamantan-1-yl)-2-chloropyridine-3,4-dicarbonitrile	1.23
93	30.652	1H-Indole, 1-methyl-2-phenyl-	0.03
94	31.103	2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene	0.19
95	31.605	Arsenous acid, tris(trimethylsilyl) ester	0.03
		Total	99.99

Table 4: Chemical constituents of phytochemicals in *Hybanthus enneaspermus*

S/N	RT	Compounds	Area%
1	3.208	2-Imidazolidinethione	0.01
2	3.422	Cyclotrisiloxane, hexamethyl-	0.03
3	3.618	Ethanethiol, 2,2'-thiobis-	0.05
4	3.728	Benzeneethanol, 2-hydroxy-	0.01
5	3.918	Benzaldehyde, 3-pentafluorophenoxymethyl-4-methoxy-	2.21
6	4.201	4-Cyclopentene-1,3-dione	0.1
7	4.38	Oxime-, methoxy-phenyl-_	0.01
8	4.692	2(3H)-Furanone, 5-methyl-	0.02

9	4.819	3-Furanmethanol	0.02
10	5.045	Thiourea, methyl-	0.01
11	5.224	Indolizine	0.03
12	5.356	Ethyl(dimethyl)isopropoxysilane	0.07
13	5.634	α-Thujene	0.05
14	5.795	Diglycerol	0.02
15	5.899	o-Cymene	0.18
16	6.257	1,2,3,4-Butanetetrol, [S-(R*,R*)]-	0.05
17	6.477	Thiodiglycol	0.02
18	6.747	Linalool	1.06
20	7.025	1,3,8-p-Menthatriene	0.03
21	7.164	p-Menth-1(7)-en-9-ol	0.01
22	7.28	Bornane	0.02
23	7.464	(+)-2-Bornanone	0.02
24	7.632	Isoborneol	0.04
25	7.857	E,E-Farnesal	0.1
26	8.03	(Z),(Z)-2,4-Hexadiene	0.14
27	8.181	(-)-cis-Sabinol	0.06
28	8.435	m-Fluorobenzyl alcohol	0.02
29	8.799	Piperitone	0.14
30	8.914	3,5-Dimethoxytoluene	0.09
31	9.024	Isoneral	0.09
32	9.255	Phenol, 2,3,5,6-tetramethyl-	0.05
33	9.388	Phenol, 2-methyl-5-(1-methylethyl)	0.04
34	9.63	Germacrene B	0.2
35	9.746	δ-EIemene	0.46
36	9.884	alpha.-Cubebene	0.26
37	9.982	Eugenol	0.1
38	10.196	alpha-Copaene	0.94
39	10.375	(-)-β-Elemene	2.33
40	10.473	Methyleugenol	0.25
41	10.577	α-Gurjunene	0.56
42	10.704	Beta-ylangene	4.24
43	10.808	Germacrene D	5.4
44	11.034	(E)-.beta.-Famesene	4.88
45	11.184	Beta-Humulene	1.01
47	11.496	Valencene	2.32
48	11.594	beta.-Bisabolene	3.12
49	11.767	β-Sesquiphellandrene	4.77
50	11.917	(E)-α-Bisabolene	1.68
51	12.05	Longifolene	3.45
52	12.166	Nerolidol 2	3.35
53	12.42	Eremophilene	2.5
54	12.529	Guaiol	1.47
55	12.668	cis-Z-.alpha.-Bisabolene epoxide	1.24
56	12.783	Apiol	4.42
57	12.957	β-Patchoulene	1.07
58	13.095	γ-Gurjunene	2.43
59	13.199	Viridiflorol	1.02
60	13.396	alpha-Bisabolol	7.27
61	13.667	(-)-Spathulenol	1.72
62	13.863	Oplopanone	0.65
63	13.973	Tetradecanoic acid	0.65
64	14.129	9-epi-(E)-Caryophyllene	1.4
65	14.441	Isoaromadendrene epoxide	1.42
66	14.528	Thunbergol	0.77
68	14.747	(-)-Neoclovene-(I), dihydro-	0.55
69	14.938	Calarene epoxide	1.46
70	15.128	Thujopsenal	0.65
71	15.359	Hexadecanoic acid, methyl ester	0.79
72	15.492	2,4-Decadienamide, N-isobutyl-,E	0.62
73	15.758	n-Hexadecanoic acid	2.34
74	15.914	Hexadecanoic acid, ethyl ester	0.56
75	16.104	2-Octen-1-ol, 7-ethoxy-3,7-dimethyl-,1- (E)-	0.12
76	16.197	Alloaromadendrene oxide-(1)	0.07
77	16.393	Cyclohexene, 2-ethenyl-1,3,3-trimethyl	0.15
78	16.618	Cyclotetracosane	0.08
79	16.716	9,12-Octadecadienoic acid (Z,Z)-,methyl ester	0.17
80	16.855	Phytol	0.03
81	16.947	Methyl stearate	0.06
82	17.086	Linoleic	2.44
83	17.225	Linoleic acid ethyl ester	0.88
84	17.398	Hexadecanamide	0.34

86	17.999	Disparlure	0.01
87	18.154	Caparratriene	0.02
88	18.235	8-(2-Nitrophenoxy)octan-1-ol	0.02
89	18.409	Farnesol	0.01
90	18.524	Cyclopropaneoctanal,2-octyl-	0.05
91	18.709	9-Octadecenamide, (Z)-	1.14
93	19.061	Dihydropiperlonguminine	0.33
94	19.321	3-(Benzylthio)acrylic acid, methyl ester	0.04
95	19.408	5-Methyl-3,7-diphenyl-1-azaadamantane-4,6-dione	0.05
96	19.569	Dimethylcarbamodithioic acid, 3-phenylallyl ester	0.03
97	19.679	Glycerol 1-palmitate	0.11
98	19.928	Bis(2-ethylhexyl) phthalate	0.04
99	20.037	11-Hexadecyn-1-ol	0.12
100	20.257	2-Hydroxycyclopentadecanone	0.01
101	20.407	Methyl palmitoleate	0.01
102	20.522	Piperanin	0.18
103	20.673	Piperlonguminine	0.09
104	20.817	Cyclopentadecanone, 2-hydroxy-	0.04
105	20.984	Fumaric acid, 4-cyanophenyl dodecy- 1-ester	0.26
106	21.198	4-Caranone	0.16
107	21.319	(2E,4E)-N-Isobutyloctadeca-2,4-dienamide	0.36
108	21.487	(2E,4E)-1-(Piperidin-1-yl) hexadeca-2,4-dien-1-one	0.04
109	21.672	Piperonyl alcohol, 3-methylbutyl ether	0.83
110	21.805	Thymol	0.16
111	21.914	2(1H)-Naphthalenone, octahydro-4a-methyl-7-(1-methylethyl)-. (4aalpha,7beta,8abeta)-	0.13
112	22.035	Supraene	0.31
113	22.209	1-Adamantanecarboxamide, N-(4-methoxyphenyl)-	0.22
114	22.33	Methyl 2-hydroxy-pentadecanoate	0.15
115	22.509	trans-Decalin, 2-methyl-	0.22
117	22.729	[1,1'-Biphenyl]-4-carboxaldehyde	0.51
118	22.89	Cedr-8-en-15-ol	0.1
119	22.983	gamma.-Tocopherol	0.15
120	23.167	2(3H)-Furanone, 3,4-bis(1,3-benzodioxol-5-ylmethyl)dihydro-, (3R-trans)-	1.11
121	23.3	N-Methyl-1-adamantaneacetamide	0.53
123	23.768	(+)-Sesamin	3.76
124	23.895	Geranyl palmitoleate	0.3
126	24.19	Dihydroanhydropodorhizol	1.23
127	24.409	4,5-Diphenyl-1,3-dioxol-2-one	0.42
128	24.588	Benzo[h]quinoline, 2,4-dimethyl-	0.09
129	24.842	1,4-Bis(trimethylsilyl)benzene	0.16
130	24.946	gamma.-Sitosterol	0.22
131	25.148	Cyclopropa [5, 6] cholestan-3-ol, 3',6-dihydro-, (3.beta.,5.beta.,6.alpha.)-	1.29
132	25.241	alpha.-Ergostenol	0.06
134	25.501	beta.-Tocopherol	0.7
135	26.049	Arsenous acid, tris(trimethylsilyl) ester	0.13
136	26.217	Acetamide, N-[4-(trimethylsilyl)phenyl]-	0.07
137	26.355	Thymol, TMS derivative	0.23
138	26.529	Tetrasiloxane, decamethyl-	0.04
141	27.089	4-tert-Butylphenol, TMS derivative	0.06
143	27.297	4-(4-Hydroxyphenyl)-4-methyl-2-pentanone, TMS derivative	0.09
145	27.736	Methyltris(trimethylsiloxy)silane	0.02
148	28.388	4-tert-Octylphenol, TMS derivative	0.02
149	28.637	Tris(tert-butyldimethylsilyloxy)arsane	0.02
			99.91

Table 3 showed the chemical constituents separated and identified in the extract of *Dysphania ambrosioides*. A total of 95 components constituting 99.99% of all the constituents. These constituents were characterized by high amount of non-terpenoic components, but a generalized representation of hydrocarbons, steroids, phenols, aldehydes, terpenoids, and fatty acids. Butanol (30.72%), Butane, 1, 1-dibutoxy- (17.28%), and Octadecanoic acid, 2-propenyl ester (4%) are the major components reported from this extract. Fatty acids like oleic acid and palmitic acid were only reported in trace amount of < 1.00%. The components observed in the GCMS results corroborate with the phytochemical screening and its quantification. Butanol is an alcohol class of compounds, which could result from metabolic processes in the plant material. These constituents acting in synergy can lead to the pharmacological properties of the plant material.

The GCMS analysis of the extracts of *Hybanthus enneaspermus* showed the presence of 149 compounds accounting for 99.91% of the total constituents as shown in table 4. The major components were alpha-Bisabolol (7.27%), Germacrene D (5.40%), and (E)-. beta. -Farnesene (4.88%). The component of the extract comprised of sesquiterpenoids, steroids, and sulphur containing compounds. Compounds of the terpenoids classes were majorly essential oils component. Generally, these terpenoids possesses different pharmacological properties, which includes, analgesics, anti-inflammatory, anticancer, and antioxidants.

Antioxidant Evaluations of *D. ambrosioides and H. enneaspermus* Extracts

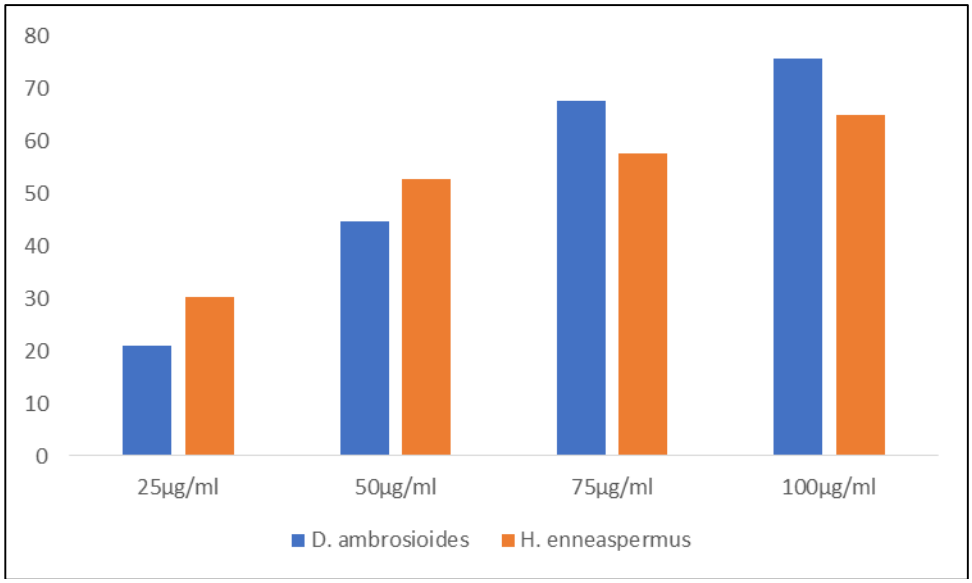

Fig 1: Nitric oxide scavenging activities of the plant extracts

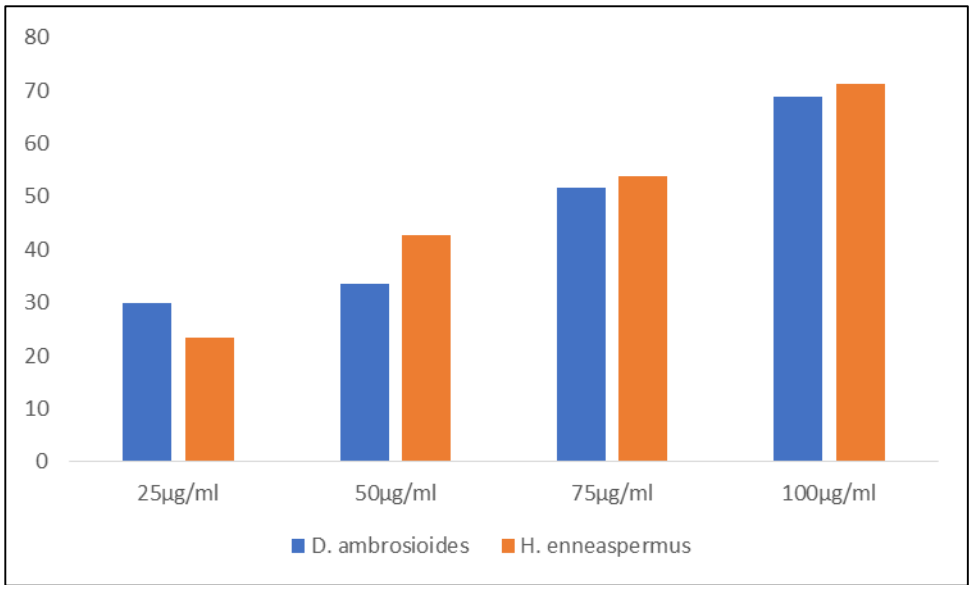

Fig 2: DPPH scavenging activity of plant extracts

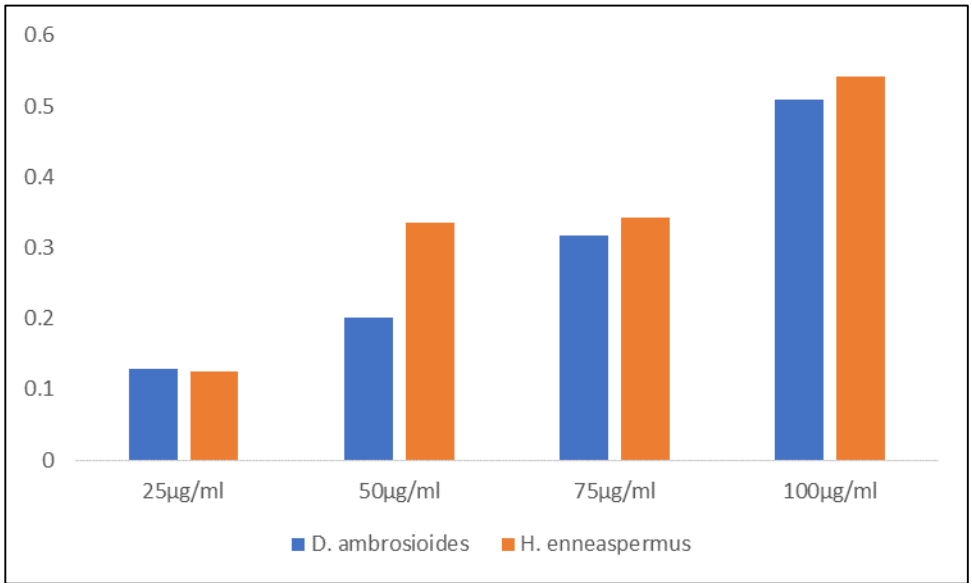

Fig 3: Ferric Reducing Power of plant extracts

At 25 µg/mL and 50 µg/mL, the extracts of *H. Enneaspermus* exhibited higher antioxidant activities than *D. Ambrosioides* while at 75 µg/mL and 100 µg/mL, *D. Ambrosioides* exhibited higher antioxidant than *H. Enneaspermus* nitric oxide scavenging assay. This indicated that *D. Ambrosioides* can only scavenge nitric oxide radicals at higher concentrations while *H. Enneaspermus* can only scavenge at low concentrations.

In DPPH scavenging activities of the extracts, there was no observable difference in the antioxidant effects of both *D. Ambrosioides* and *H. Enneaspermus*. This showed that both plants may have similar DPPH radicals scavenging potentials. The ferric reducing powers of both plants showed no significant difference as observed in nitric oxide radicals scavenging activities. This might be a strong indication of both plants having similar antioxidant potentials which may be of the reasons for their high therapeutic activities.

Conclusion

D. Ambrosioides and *H. Enneaspermus* contained significant phytoconstituents and exhibited similar antioxidant activities. These phytochemicals may be the cause of their effective therapeutic activities in humans; hence both plants are commonly used to prevent and treat different ailments in the study area.

References

1. Adewunmi CO, Agbedahunsi J, Adebajo AC, Aladesanmi AJ, Murphy NB, Wando J. Ethno-veterinary medicine: screening of medicinal plants for trypanocidal properties. Journal of Ethnopharmacology. 2001;77(1):19-24.
2. Chik SCC, Or TCT, Luo D, Yang CLH, Lau ASY. Pharmacological Effects of Active Compounds on Neurodegenerative Disease with Gastrodia and Uncaria Decoction, a Commonly Used Poststroke Decoction. Sci. World J; c2013. p. 1-22.
3. Clemantis SE, Mosyakin SL. Dysphania sect. Adenois - online. In: Flora of North America Editorial Committee (ed.): Flora of North America North of Mexico. Volume 4: Magnoliophyta: Caryophyllidae, part 1. Oxford University Press, New York, ISBN: 0-19-517389-9; c2003. p. 269.
4. Correa A, Galdames MDC, Stapf MNS. Catalogue of vascular plants of Panama (Catalogo de Plantas Vasculares de Panama.), Panama: Smithsonian Tropical Research Institute; c2004. p. 599.
5. Harborne JB. Phytochemical methods - a guide to modern techniques of plant analysis. 2nd ed. London: Chapman and Hall; c1984. p. 4-16.
6. Isman MB. Commercial development of plant essential oils and their constituents as active ingredients in bio insecticides. Phytochemistry Reviews. 2020;19(2):235-241.
   DOI: 10.1007/s11101-019-09653-9. ISSN 1572-980X.
7. Kaur R, Arora S. Alkaloids-Important Therapeutic Secondary Metabolites of Plant Origin. J Crit. Rev. 2015;2:1-8.
8. Laferrière JE. Nutritional and pharmacological properties of yerbaníz, epazote, and Mountain Pima oregano Seed head News; c1990. p. 29.
9. Ngbolua KN, Mpiana PT, Mudogo V, Ngombe NK, Tshibangu DST, Ekutsu E. Ethno-pharmacological survey and Floristical study of some medicinal plants traditionally used to treat infectious and parasitic pathologies in the Democratic Republic of Congo. International Journal of Medicinal Plants. 2014;106:454-467.
10. Nyerges C. Foraging Wild Edible Plants of North America: More than 150 delicious recipes using Nature's Edibles. Rowman & Littlefield; c2016. p. 73-75.
11. Odugbemi A, Akinsulire C. Medicinal plants by species names. University of Lagos; c2006. p. 95-156.
12. Sofowora A. Medicinal Plants and Traditional Medicine in Africa. John Wiley and Sons, Chichester; c1993.
13. Sharaibi OJ, Oluwa OK, Omolokun KT, Hundeyin CS, Ogbe AA, Banwo HO. Ethnobotany of Medicinal Plants commonly used by Eegun Tribe in Badagry Local Community of Lagos State, Nigeria. Research & Reviews: Journal of Botanical Sciences; c2022. ISSN: 2320-0189, DOI: 10.4172/2320-0189.11.7.001
14. Trease G, Evans SM. Pharmacognosy. 15th ed. London: Bailer Tindal; c2002. p. 23-27.
15. Ullah A, Munir S, Badshah SL, Khan N, Ghani L, Poulson BG, *et al*. Important Flavonoids and Their Role as a Therapeutic Agent. Molecules. 2020 Nov 11;25(22):5243. DOI: 10.3390/molecules25225243. PMID: 33187049; PMCID: PMC7697716.
16. WHO. National policy on traditional medicine and regulation of herbal medicines: Report of a WHO Global Survey. World Health Organization, Geneva, Switzerland; c2005.
17. Ainseba BE, Anita S. Internal exact controllability of the linear population dynamics with diffusion. Electronic Journal of Differential Equations (EJDE)[electronic only]. Paper-No; c2004.
18. Erdogrul ÖT. Antibacterial activities of some plant extracts used in folk medicine. Pharmaceutical Biology. 2002 Jan 1;40(4):269-73.
19. Acharya D, Shrivastava A. Indigenous herbal medicines. Aavishkar Publishers, Distributors; c2008.
20. Olowokudejo JD, Kadiri AB, Travih VA. An ethnobotanical survey of herbal markets and medicinal plants in Lagos State of Nigeria. Ethnobotanical leaflets. 2008;2008(1):116.
21. Makkar HP, Siddhuraju P, Becker K. Plant secondary metabolites. Totowa, NJ, USA: Humana Press; c2007 Jan 1.
22. Nobili V, Alisi A, Panera N, Agostoni C. Low birth weight and catch-up-growth associated with metabolic syndrome: a ten year systematic review. Pediatric endocrinology reviews: PER. 2008 Dec 1;6(2):241-7.