

Phytochemical profile and in vitro antioxidant evaluation of the root of *Dennetia tripetala* BAK. F. (Annonaceae)

Enema O. J^{1*}, Umoh U. F¹ and Johnny I. I¹

¹ Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria.
Received 01 June 2024; Accepted 13 June 2024

Abstract

Dennetia tripetala BAK. F. is a plant traditionally used in Nigeria for its therapeutic properties including the treatment of inflammations. This study aims to investigate the biochemical profile and in vitro antioxidant properties of the root of *D. tripetala*. The research utilized Gas Chromatography-Mass Spectrometry (GC-MS) to identify bioactive compounds and employed quantitative phytochemical screening to measure the concentrations of key phytochemicals. The antioxidant activity of the root of *D. tripetala* and their fractions was determined using methods such as total phenolic content determination, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, nitric oxide (NO) scavenging assay and ferric reducing antioxidant power assay, respectively. Quantitative phytochemical screening showed a total phenolic content of 231 mg GAE/g and a total flavonoid content of 313.8 µg QE/g. Among the fractions tested, the dichloromethane fraction exhibited the highest free radical inhibition. The GC-MS analysis revealed the presence of 22 compounds in the methanol extract of the root. These findings suggest that the root of *D. tripetala* has substantial antioxidant potential due to its high phenolic and flavonoid content, supporting its traditional medicinal use and indicating its potential for developing natural antioxidant therapies.

Keywords: *D. tripetala*, antioxidant properties of root of *D. tripetala*, chemical profile, GC-MS analysis, quantitative phytochemical profile.

I. Introduction

In recent years, there has been a growing demand for natural antioxidants from plant sources due to the growing awareness of the adverse effects associated with synthetic antioxidants. This interest can be linked to the traditional use of plants across the world as sources of food and medicine [1].

D. tripetala, commonly known as "pepper fruit," is Indigenous to the tropical regions of West Africa, this plant is revered not only for the culinary applications of the fruit but also for its medicinal properties. *D. tripetala* belongs to the family Annonaceae, a group known for its rich array of chemically diverse and biologically active species. In Nigeria, *D. tripetala* is traditionally used for the treatment of diabetes, fever, cough, catarrh, asthma, diarrhoea, as well as pains. The fruits are also used to clear throat, check excessive saliva and enhance appetite. Traditionally, the fruits are used in women's diets after birth like the fruits of *Tetrapleura tetraptera* to enhance contraction of the uterus [2].

Research has shown that *D. tripetala* is an important source of medicine as different parts of the plant have been evaluated for various pharmacological uses including antioxidants, antidiabetic, antimicrobial, analgesic, antiinflammatory, as well as an anticancer agent. Qualitative phytochemical screening of water and methanol extract of the root of *D. tripetala* revealed the presence of alkaloid, cardiac glycosides, carbohydrates, phenols and tannin. 70% ethanol and acetone extracts had alkaloid, cardiac glycoside, carbohydrates, phenol, tannin and terpenoids. The hexane extract of the root also showed the presence of alkaloid, cardiac glycosides, sterols and terpenoids [3].

Antioxidants are crucial in combating oxidative stress, a condition implicated in the pathogenesis of numerous chronic diseases, including but not limited to, cardiovascular diseases, diabetes, and cancer. The exploration of natural antioxidants is pivotal not only in the context of therapeutic interventions but also in food preservation, providing safer alternatives to synthetic antioxidants, which are increasingly being scrutinized for their potential health risks [4][5].

Previous researches on *D. tripetala* focused on the chemical and nutritive properties of different parts of *D. tripetala*, including the fruit, peel, whole fruit, and seed. Gas chromatography-mass spectroscopy studies have identified major constituents in the essential oils of pepper fruit. Some identified bioactive agents include alkaloids, tannins, saponins, flavonoids, terpenoids, glycosides, and phenol. These compounds are present in fruits and seeds and thus their huge therapeutic uses [6]. However, no research has been reported on the quantitative phytochemical profile, GCMS studies of the methanol extract, and the antioxidant studies of the root extract and partitioned fractions of *D. tripetala* hence, the novelty of this research. The objective of this study is to evaluate the chemical profile of the root extract of *D. tripetala* and the antioxidant properties of the root extracts and various partitioned fractions.

II. Materials And Methods

Plant collection and Identification

The root of *D. tripetala* was collected from Itak Ikot Akap village in Ikono local government, Akwa Ibom. The plant was identified by Imeh Imoh Johnny (Ph.D) of the Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Uyo and authenticated by Prof Mrs. M.E Bassey of the department of Botany and Ecological Studies, Faculty of Science, University of Uyo, Uyo and a voucher specimen deposited in the herbarium.

Preparation of Extract

D. tripetala root was air-dried and coarsely powdered with hammer mill. About 1 kg of the powdered plant material was extracted in 70% methanol. The extract was dried and weighed.

Quantitative phytochemical Screening of extracts

Determination of tannin Content

The tannin constituents of *D. tripetala* root extract was determined by Folin - Ciocalteu method described by [7]. About 0.1 ml of the sample extract was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteuphenol reagent, 1 ml of 35 % Na₂CO₃ solution and dilute to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of gallic acid (20, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. Absorbance for test and standard solutions were measured against the blank at 725 nm with an UV/Visible spectrophotometer. The tannin content was expressed in terms of µg of QE /g of extract.

Determination of total phenolic content

The total phenolic content of the fractions was determined spectrophotometrically with folin – ciocalteu reagent 0.5 ml (1 mg/ml) of the fractions was mixed with 2.5 ml of 10% folin ciocalteu reagent and 2ml of Na₂CO₃ (7%). The resulting mixture was vortexed for 15 seconds and incubated at 40°C for 30 minutes for colour development. The absorbance of the samples was measured at 765 nm wavelength. 2.5 ml of water was added to different concentrations for the calibration curve of gallic acid. The total phenolic content was calculated from the calibration curve and the results were expressed as mg of gallic acid equivalent per gram dry weight. These were performed in triplicates [8].

Determination of Alkaloid

The root extract (1mg) was dissolved in dimethyl sulphoxide (DMSO), added 1ml of 2 N HCl and filtered. This solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3 and 4 ml chloroform by vigorous shaking and collected in a 10-ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (20, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer. The total alkaloid content was expressed as mg of AE/g of extract [9].

Determination of Total flavonoid content

The total flavonoid content was determined by the method described by [10]. Total flavonoid content was measured by the aluminium chloride colorimetric assay. The reaction mixture consists of 1 ml of extract and 4 ml of distilled water was taken in a 10 ml volumetric flask. To the flask, 0.30 ml of 5 % sodium nitrite was treated and after 5 minutes, 0.3 ml of 10 % aluminium chloride was mixed. After 5 minutes, 2 ml of 1M Sodium hydroxide was treated and diluted to 10 ml with distilled water. A set of reference standard solutions of quercetin (20, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 510 nm with an UV/Visible spectrophotometer. The total flavonoid content was expressed as mg of QE/g of extract.

Determination of Saponins

The total saponins of the root extract was determined by the method described by [11]. 1g of the dried sample of the root and stem extract of *D. tripetala* that had passed through 125 mm sieve was transferred into a 250 ml beaker, and 100 ml of isobutyl alcohol was added. The mixture was swirled for 5 minutes and filtered. The filtrate was transferred into a 100 ml beaker containing 20 ml of 40% saturated solution of MgCO₃. About 1 ml of the colourless filtrate was pipette into a 50 ml volumetric flask and 2 ml of 5% FeCl₃ solution was added and made up to the marked level with distilled water. This was then allowed to stand for 30 minutes for a blood red colour to develop. Percentage saponin was calculated using the formula:

$$\% \text{ Saponin} = \text{AS} \times \text{AG} \times \text{DF} / \text{Wt of sample} \times 10,000$$

Where AS =Absorbance of sample; AG = Average gradient; DF = Dilution factor and Wt of sample = Weight of sample

Total Terpenoid determination

The total terpenoid was determined by the method described by [12]. Stock standard solution of each terpene [α -pinene, (-)- β -pinene, myrcene, (R)- (+)-limonene, terpinolene, linalool, α -terpineol, β -caryophyllene, α -humulene, caryophyllene oxide] was prepared in ethyl acetate. The standard terpenes were mixed and the concentration of each terpene was adjusted to be 1.0 mg/mL from which serial dilutions were made to prepare the individual points of the calibration curves. Internal standard preparation: n-Tridecane (C₁₃ hydrocarbon) was selected as the IS, and its concentration was kept at 100 μ g/ mL, which was added to all of the calibration and sample solutions.

Calibration curves:

Nine calibration points ranging from 0.75 – 100 μ g/mL were prepared from the previously mentioned stock standard solutions (0.75, 1.0, 2.0, 5.0, 10, 25, 50, 70, and 100 μ g/mL) and IS. The concentration of the IS at each calibration point was 100 μ g/mL. These solutions were used to construct individual terpene calibration curves (Fig. 1S, Supporting Information).

Sample solution preparation:

The root extracts of *D. tripetala* were dried for 24 h at 40 °C in a ventilated oven and then ground in a stainless steel coffee grinder. Triplicates (1.0 g each) of the powdered samples were weighed in a 15-mL centrifuge tube and each were extracted with 10 mL of the extraction solution (100 μ g/mL of the IS in ethyl acetate) by sonication for 15 min. The mixture was centrifuged for 5 min at 1252 \times g and the supernatants (without filtration) were used for the GC/MS analysis.

Antioxidant evaluation of extracts and fractions

The antioxidant evaluation was carried out using ferric reducing antioxidant power (FRAP) assay and 2,2 – diphenyl – 1 – picrylhydrazyl (DPPH) radical scavenging activity models, nitric oxide (NO) scavenging assay and determination of total phenolic content.

Ferric reducing antioxidant power (FRAP) assay

The FRAP activity of the methanol root extract and fractions were determined by the method described by [13]. Various concentrations (20, 40, 60, 80, 100 μ g/ml) of the methanol extract and fractions of root of *D. tripetala* (2.5 ml) were mixed individually with the mixture containing 2.5 ml of 0.2 m sodium phosphate buffer (pH 6.6) and 2.5 ml of Potassium Ferricyanide (K₂Fe(CN)₆) (1% w/v). The resulting mixture was incubated at 50°C for 20 min., 2.5 ml of trichloroacetic acid (10% w/v) was added. The resulting mixture was centrifuged at 650 rpm for 10 minutes. The upper layer of the solution (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of ferric chloride (0.1% w/v). The absorbance was measured at wavelength 700 nm against a blank sample. Ascorbic acid was used as the reference compound.

Determination of 2,2 – Diphenly – 1 – picrylhydrazyl (DPPH) radical scavenging Activity)

The DPPH free radical scavenging activity of methanol extract and fractions of root of *D. tripetala* and ascorbic acid prepared in methanol at various concentrations (20, 40, 60, 80, 100 μ g/ml) was evaluated according to the method of [14]. 2,2 – Diphenyl – 1 – Picrylhydrazyl (0.1 mm, 1 ml) was added to 3ml of the solutions prepared with the extracts, fractions and ascorbic acid and stirred for 1 minute. Each mixture was incubated in the dark for 30 minutes and absorbance (A_s) was measured at 517 nm. The assays were carried out in triplicates and the results expressed as mean values \pm standard error of mean. The percentage DPPH scavenging effect was calculated using the following equation:

DPPH Scavenging effect (%) or percentage exhibition

$$= [(A_0 - A_s)/A_0] \times 100$$

Where A_0 is the absorbance of control reaction and A_s is the absorbance of the test samples or standard sample (ascorbic acid).

Nitric oxide (NO) scavenging assay

Nitric Oxide generated from sodium nitroprusside (SNP) was measured according to the modified method of [15]. 3.0 ml of 10 μ M of SNP in phosphate buffered saline (PH 7.4) was added to 2ml of different concentrations of methanol extract and fractions of root of *D. tripetala* and ascorbic acid (20, 40, 60, 80, 100 μ g/ml). The resulting solutions were incubated at 25°C for 60 minutes. A similar procedure was repeated with methanol as blank which served as control to 30 ml of Griess reagent (1% sulfanillamide in 2% phosphoric diamene dihydrochloride). The absorbance formed during the diazotization of nitric ions with sulphamylamide and subsequent coupling with ethylenediamine dihydrochloride was measured at 540 nm. The assays were carried out in triplicate and the results expressed as mean values \pm standard deviation.

Determination of total phenolic content

The total phenolic content of the fractions was determined spectrophotometrically with folin – ciocalteu reagent 0.5 ml (1 mg/ml) of the fractions was mixed with 2.5 ml of 10% folin ciocalteu reagent and 2ml of Na_2CO_3 (7%). The resulting mixture was vortexed for 15 seconds and incubated at 40°C for 30 minutes for colour development. The absorbance of the samples was measured at 765 nm wavelength. 2.5 ml of water was added to different concentrations for the calibration curve of gallic acid. The total phenolic content was calculated from the calibration curve and the results were expressed as mg of gallic acid equivalent per gram dry weight. These were performed in triplicates [8].

Gas Chromatography Mass Spectrum (GC-MS) Analysis

GC-MS analysis was carried out using 7890A GC system, 5675C Inert MSD with triple-Axis detector. The column has a length of 30m with an internal diameter of 0.2 μ m and a thickness of 250 μ m, treated with phenyl methyl silox. Other GC-MS conditions are ion source temperature (EI), 2500C, interface temperature; 3000C, pressure; 16.2 psia, out time, 1.8mm, 1 μ l injector in split mode with split ratio 1:50 with injection temperature of 3000C, the column temperature started at 350C for 5minutes and changed to 1500C at the rate of 40C/min, the temperature was raised to 2500C at the 162 rate of 200C/min and held for 5minutes. The total elution was 47.5 minutes. Ms Solution software provided by supplier was used to control the system and to acquire the data; identification of the compounds was carried out by comparing the mass spectra obtained with those of the standard mass spectra from National Institute of Standard and Technology (NIST) database. The identity of the spectra above 95% was used to ascertain the name, molecular weight and structure of the components in the stem and roots of *D. tripetala*.

III. Results and Discussion

Quantitative Phytochemical Screening of methanol extract of root of *D. tripetala*

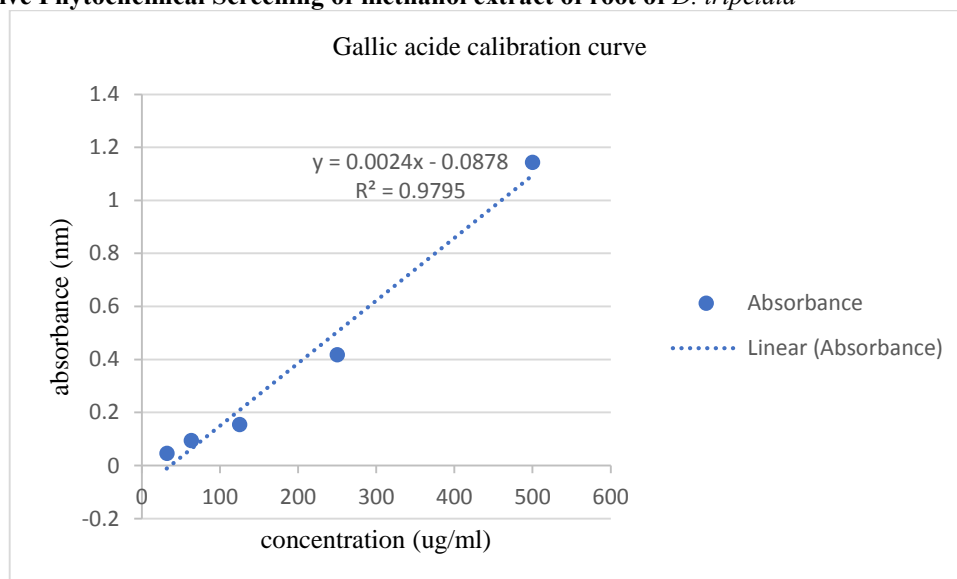


Figure 1: Gallic acid calibration curve

Source: Experimental data (2024)

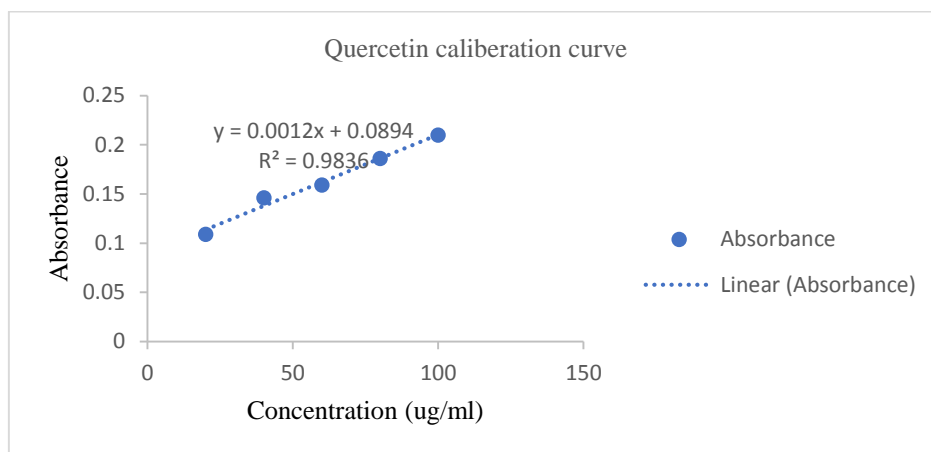


Figure 2: Quercetin calibration curve
Source: Experimental data (2024)

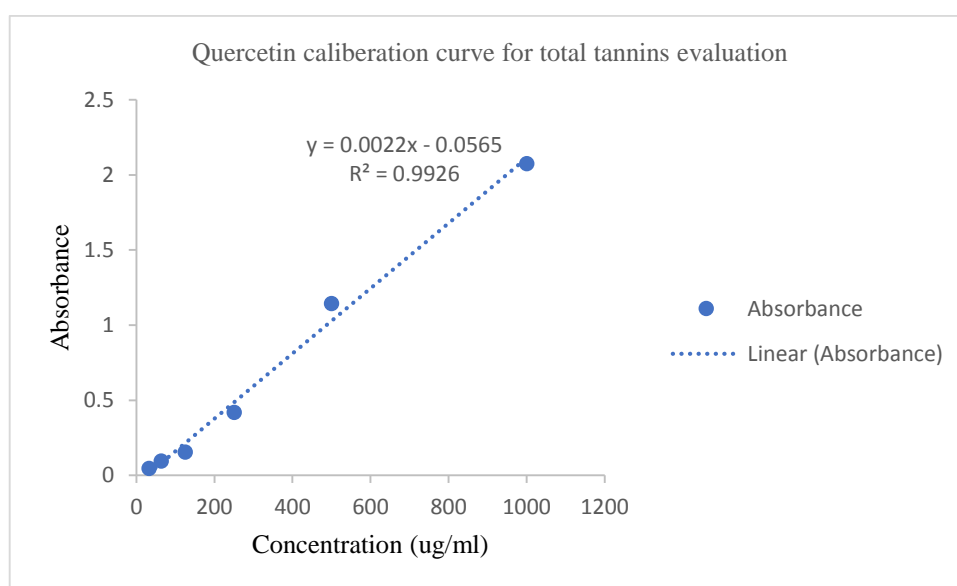


Figure 3: Calibration curve of quercetin for total tannin determination
Source: Experimental data (2024)

Table 1: Total tannins of EPFR

Concentration (µg/ml)	Absorbance (nm)	Total tannins (µg QE/g)
	EPFR	EPFR
32	0.027 ± 0.000	38
63	0.029 ± 0.000	38.9
125	0.069 ± 0.000	57
250	0.077 ± 0.000	60.7
500	0.092 ± 0.000	67.5
1000	0.12 ± 0.000	80.22

Source: Experimental data (2024)

EPFR = Methanol root extract of *D. tripetala*

Table 2: Summary of results of quantitative phytochemical screening (%w/w)

sample	Alkaloids	Flavonoids	Saponins	Tannin	Terpenoids	Phenol
EPFR	11.75	245.1	0.95	68.5	0.65	0.79

Source: Experimental data (2024)

EPFR = Methanol root extract of *D. tripetala*

Quantitative Phytochemical

Quantitative Phytochemical Screening of the root and stem of *D. tripetala* was carried out to determine the concentration of various bioactive compounds such as phenolics, flavonoids, saponins, tannins, terpenoids, and alkaloids which are responsible for the various medicinal applications of the plant parts in the treatment of ailments traditionally [16]. The total phenolic content was evaluated using a calibration curve based on gallic acid (Figure 1). The absorbance values for gallic acid ranged from 0.046 ± 0.000 (at 32 $\mu\text{g/ml}$) to 2.075 ± 0.001 (at 1000 $\mu\text{g/ml}$), with the calibration curve described by the equation $y = 0.0024x - 0.0878$ ($R^2 = 0.9795$). The Total phenolic content in the root extract was calculated from the calibration curve as 231 mg GAE/g. This indicates that the root extract has higher antioxidative properties, given the role of phenolics in scavenging free radicals. The linear relationship of the gallic acid calibration curve, with a correlation coefficient (R^2) of 0.9795 also suggests a highly reliable measurement. The total flavonoid was estimated from the calibration curve of quercetin with the correlation equation $y = 0.0012x + 0.0894$ and correlation coefficient of $R^2 = 0.9836$. The total flavonoid content in the root extracts showed a progressive increase with higher concentrations. The concentration-dependent increase, especially noted at 1000 $\mu\text{g/ml}$ with totals reaching 313.8 $\mu\text{g QE/g}$ for the root extract, suggesting strong anti-inflammatory and antioxidative potential.

Saponins, recognized for their cholesterol-lowering and immune-boosting properties, were also quantified, with the root extract presenting a yield of 0.95 % w/w. Tannin compounds which are known for their astringent properties and potential health benefits ranging from antioxidative to anticancer effects were also quantified through a quercetin calibration curve that demonstrated an exceptionally high correlation coefficient ($R^2 = 0.9926$), signifying the accuracy of the measurement. The total tannins present in the root (EPFR) peaked at the value of 80.22 $\mu\text{g QE/g}$ at a 1000 $\mu\text{g/ml}$ concentration. It is also important to note that the concentration of tannins increased with an increase in the concentration of the samples across both root and stem extracts, respectively. Alkaloids were reported to be 11.75 % w/w and 0.9 % w/w for terpenoids, respectively.

Antioxidant analysis

Table 3: DPPH absorbance (nm) and percentage inhibition (%) of EPFR and EPFR fractions

Concentration ($\mu\text{g/ml}$)	Absorbance (nm) and percentage inhibition (%)						
	AS	EPFR	NH	DCM	ETH	nBT	AQ
20	$0.119 \pm 0.001^*$ (85)	$0.678 \pm 0.005^*$ (17)	$0.698 \pm 0.001^*$ (14)	$0.497 \pm 0.000^*$ (39)	$0.674 \pm 0.003^*$ (18)	$0.577 \pm 0.001^*$ (29)	$0.679 \pm 0.001^*$ (17)
	$0.116 \pm 0.000^*$ (86)	$0.5156 \pm 0.001^*$ (37)	$0.684 \pm 0.000^*$ (17)	$0.303 \pm 0.000^*$ (63)	$0.575 \pm 0.003^*$ (30)	$0.533 \pm 0.000^*$ (35)	$0.668 \pm 3.333^*$ (19)
60	$0.114 \pm 0.000^*$ (86)	$0.514 \pm 0.000^*$ (38)	$0.597 \pm 0.001^*$ (27)	$0.300 \pm 0.000^*$ (63)	$0.418 \pm 0.001^*$ (49)	$0.462 \pm 0.000^*$ (44)	$0.591 \pm 3.333^*$ (28)
	$0.110 \pm 0.001^*$ (87)	$0.408 \pm 3.333^*$ (50)	$0.589 \pm 0.000^*$ (28)	$0.206 \pm 3.333^*$ (75)	$0.316 \pm 0.000^*$ (61)	$0.342 \pm 0.001^*$ (58)	$0.547 \pm 3.333^*$ (33)
80	$0.09 \pm 0.001^*$ (87)	$0.376 \pm 0.001^*$ (50)	$0.379 \pm 0.001^*$ (28)	$0.203 \pm 0.001^*$ (75)	$0.276 \pm 0.000^*$ (61)	$0.492 \pm 0.000^*$ (58)	$0.466 \pm 3.333^*$ (33)
	$0.09 \pm 0.001^*$ (89)	$0.376 \pm 0.001^*$ (54)	$0.379 \pm 0.001^*$ (53)	$0.203 \pm 0.001^*$ (75)	$0.276 \pm 0.000^*$ (66)	$0.492 \pm 0.000^*$ (40)	$0.466 \pm 3.333^*$ (43)

Source: Experimental data (2024)

AS = Ascorbic acid

EPFS = Methanol stem bark extract

NH = n-hexane fraction

DCM = Dichloromethane fraction

ETH = Ethyl acetate fraction

nBT = n-butanol fraction

AQ = Aqueous fraction

DPPH scavenging activity

The result of the DPPH assay of the root extract of *D. tripetala* (Table 3) showed a significant increase with an increase in concentration of the sample when compared to the standard compound (ascorbic acid). Ascorbic acid which is the standard compound recorded the highest antioxidant activity of 89% as percentage inhibition at 100 µg/ml while the root recorded a percentage inhibition of 54%, respectively. The percentage inhibition also increased with an increase in concentration of the sample. The root extract had an average percentage inhibition of 39.2% when compared to ascorbic acid which had an average percentage inhibition of 87%.

When compared to ascorbic acid, the result of the DPPH radical scavenging activity was statistically significant at all concentrations at ($P < 0.05$). The fractions were also evaluated statistically and are statistically significant at ($P < 0.05$) at all concentrations. The dichloromethane fraction of the root of *D. tripetala* recorded the highest average percentage inhibition of free radicals (63%) when compared to other fractions of the root, this was followed by the ethyl acetate fraction of the root with the average percentage inhibition of 44.8%. It is also important to note that at 40 µg/ml and 60 µg/ml, the percentage inhibition of the dichloromethane fraction of the root did not change (63%). This was also noted at 80 µg/ml and 100 µg/ml where the percentage inhibition of the dichloromethane fraction was recorded as 75%, respectively. Among the fractions of the root, the n-hexane fraction had the lowest percentage inhibition (27.8%) as illustrated in Figure 4.

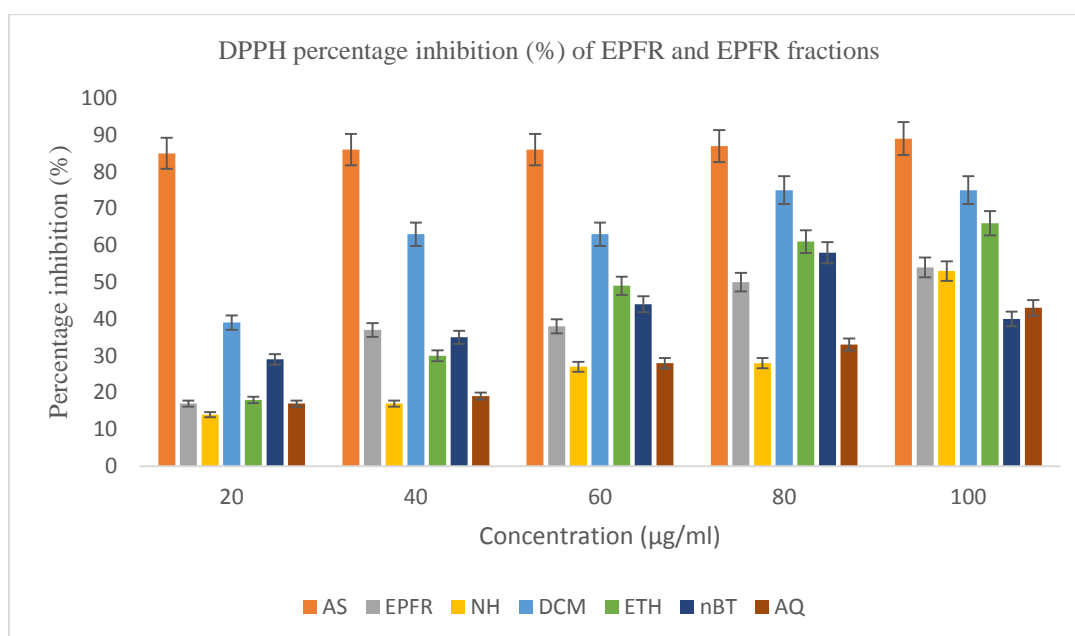


Figure 4: Comparisons of DPPH percentage inhibition (%) of EPFR and EPFR fractions
Source: Experimental data (2024)

Total phenolics content

The total phenolic content of the fractions of the fractions was estimated from the gallic acid calibration curve equation $y = 0.0024x - 0.00878$. The n-hexane fraction of the stem had the lowest phenolic content recorded (41.2 mg GAE/g) which was slightly lower than that of the root (42 mg GAE/g). The dichloromethane fraction of the root had the highest phenolic content when compared to other fractions with the dichloromethane fraction of the root recording the highest value of 160.8 mg GAE/g. Among the fractions of the root, the ethyl acetate fraction was reported as the second most active fraction with a total phenolic content of 126.2 mg GAE/g. These are statistically significant at ($p < 0.05$) using two-way ANOVA.

Table 4: NO absorbance (nm) and percentage inhibition (%) of EPFR and fractions of EPFR

Absorbance (nm) and percentage inhibition (%)							
Concentration (µg/ml)	AS	EPFR	NH	DCM	ETH	nBT	AQ
20	0.086 ± 0.000*	0.213 ± 0.000*	0.335 ± 0.000*	0.273 ± 0.000*	0.239 ± 0.000*	0.199 ± 0.000*	0.385 ± 0.000*
	(85)	(63)	(43)	(53)	(59)	(66)	(35)
40	0.072 ± 0.000*	0.180 ± 0.000*	0.259 ± 0.000*	0.183 ± 0.000*	0.191 ± 0.000*	0.177 ± 0.333*	0.262 ± 0.000*
	(87)	(69)	(56)	(68)	(67)	(69)	(55)
60	0.07 ± 0.000*	0.145 ± 0.000*	0.195 ± 0.023*	0.122 ± 0.000*	0.135 ± 0.000*	0.135 ± 0.000*	0.139 ± 0.000*
	(88)	(75)	(67)	(69)	(77)	(77)	(76)
80	0.062 ± 0.000*	0.118 ± 0.000*	0.155 ± 0.001*	0.120 ± 0.000*	0.141 ± 0.001*	0.160 ± 0.333*	0.114 ± 0.000*
	(89)	(80)	(75)	(69)	(76)	(73)	(80)
100	0.054 ± 0.000*	0.084 ± 0.000*	0.099 ± 0.010*	0.173 ± 0.000*	0.169 ± 0.000*	0.104 ± 0.000*	0.084 ± 0.000*
	(90)	(85)	(83)	(70)	(71)	(82)	(85)

Source: Experimental data (2024)

AS = Ascorbic acid
 EPFS = Methanol stem bark extract
 NH = n-hexane fraction
 DCM = Dichloromethane fraction
 ETH = Ethyl acetate fraction
 nBT = n-butanol fraction
 AQ = Aqueous fraction

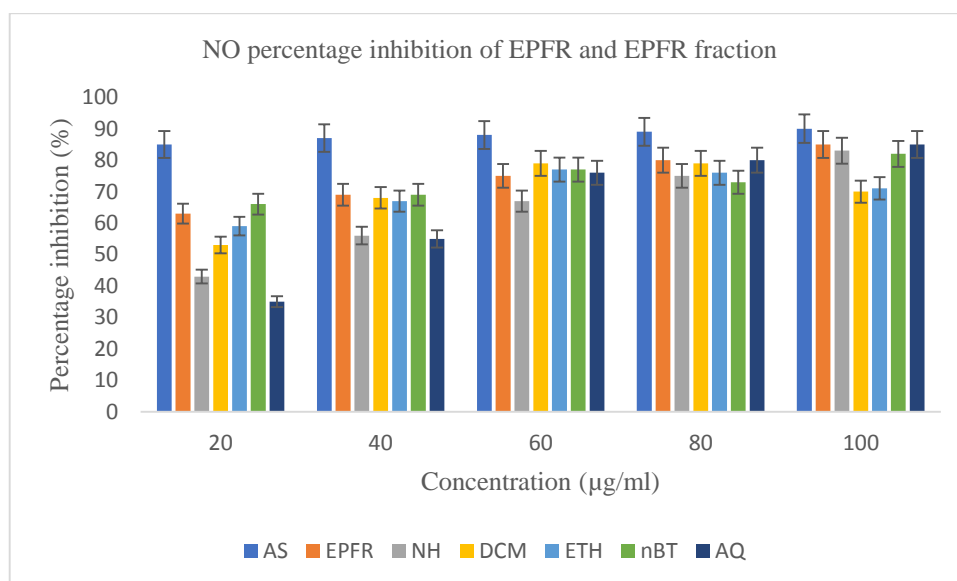


Figure 5: Comparisons of NO percentage inhibition of EPFR and EPFR fraction

Source: Experimental data (2024)

The result of the nitric oxide (NO) assay showed that the root extract of *D. tripetala* (Table 4) recorded 74.4% NO scavenging potential. The result also showed an increase in percentage inhibition of NO with increase in concentration of the sample (Figure 5). This is shown in Figure 17. The results are also statistically significant at ($P < 0.05$). Like the DPPH scavenging activity assay, the results are statistically significant at all concentrations at ($P < 0.05$). The n-hexane fraction of the root had the lowest nitric oxide scavenging potential when compared to other fractions of the root. This assay measures the ability to scavenge nitric oxide radicals, which may involve different mechanisms or compounds than those effective in the DPPH assay. The high activity in the n-butanol fraction of the root indicates the presence of effective nitric oxide scavengers in these fractions.

Table 5: Absorbance (nm) of ferric reducing antioxidant power assay of EPFR and fractions of EPFR

Concentration (µg/ml)	Absorbance (nm)						
	AS	EPFR	NH	DCM	ETH	nBT	AQ
20	0.421 ±	0.401 ±	0.602 ±	0.401 ±	0.571 ±	0.400 ±	0.401 ±
	0.001*	0.000*	0.000*	0.001*	0.001*	0.001*	0.001*
40	0.394 ±	0.397 ±	0.407 ±	0.318 ±	0.543 ±	0.326 ±	0.415 ±
	0.001*	0.001 ^{ns}	0.001*	0.000*	0.001*	0.001*	0.001 ^{ns}
60	0.298 ±	0.284 ±	0.265 ±	0.232 ±	0.392 ±	0.311 ±	0.341 ±
	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*
80	0.286 ±	0.200 ±	0.250 ±	0.194 ±	0.224 ±	0.206 ±	0.241 ±
	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*
100	0.187 ±	0.184 ±	0.187 ±	0.097 ±	0.202 ±	0.198 ±	0.218 ±
	0.003*	0.001 ^{ns}	0.001 ^{ns}	0.001*	0.001*	0.001*	0.001*

Source: Experimental data (2024)

AS = Ascorbic acid
 EPFS = Methanol stem bark extract
 NH = n-hexane fraction
 DCM = Dichloromethane fraction
 ETH = Ethyl acetate fraction
 nBT = n-butanol fraction
 AQ = Aqueous fraction

The result of the ferric reducing antioxidant power (FRAP) assay (Table 5) showed a very close relationship between the root extract and its fractions of *D. tripetala* at different concentrations. The result also showed an increase in percentage reducing power of the extracts with increase in concentration of the sample. The results are statistically significant at (P<0.05). The absorbance of the root extract at 40 µg/ml and 100 µg/ml was reported as not significant. The absorbance of the n-hexane fraction of the root at 100 µg/ml was also reported as not significant (P<0.05). At 40 µg/ml, the absorbance of the aqueous fractions of the root was also reported as not significant.

Table 6: IC₅₀ and logIC₅₀ of EPFR and fractions of EPFR

	IC ₅₀ (µM)						
	AS	EPFR	NH	DCM	ETH	nBT	AQ
IC ₅₀	0.006218	0.02813	0.03298	0.0263	0.02594	0.02678	0.03287
logIC ₅₀	-2.206	-1.551	-1.482	-1.58	-1.586	-1.572	-1.483

Source: Experimental data (2024)

AS = Ascorbic acid
 EPFS = Methanol stem bark extract
 NH = n-hexane fraction
 DCM = Dichloromethane fraction
 ETH = Ethyl acetate fraction
 nBT = n-butanol fraction
 AQ = Aqueous fraction

Determination of IC₅₀

Table 6 shows the IC₅₀ of the root extract (EPFR) and the fractions of the root of *D. tripetala*. The IC₅₀ values listed in the tables represent the concentration at which 50% inhibition of the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical is observed, a common measure of antioxidant activity. Lower IC₅₀ values indicate higher antioxidant activity since less substance is required to inhibit 50% of the free radical activity. The LogIC₅₀ values are the logarithmic transformations of the IC₅₀ values, providing a different scale to interpret the data that can sometimes make patterns more evident or statistical analysis more straightforward. The IC₅₀ value of the standard compound (ascorbic acid, AS) is remarkably low at 0.006218 µM, indicating a high potency of the drug as an antioxidant compound. Among the fractions of the root, the NH (n-hexane) fraction shows the highest IC₅₀ value (0.03298 µM), suggesting it is the least potent among the fractions. The dichloromethane fraction (DCM) has an IC₅₀ value of 0.0263µM suggesting it is the most potent antioxidant agent among the fractions. LogIC₅₀ values for these fractions are -1.482, -1.58, -1.586, -1.572 and -1.483 µM, for n-hexane, dichloromethane, ethyl

acetate, n-butanol and aqueous fractions, respectively. The LogIC₅₀ values, which are the logarithm of the IC₅₀ values, further support the potency ranking, with lower values indicating higher potency in a logarithmic scale.

From these observations, it is clear that the dichloromethane fraction exhibits a particularly high potency as an antioxidant agent in both fractions of the root, with relatively low IC₅₀ and LogIC₅₀ values, indicating that it might be the most effective inhibitory fraction among the tested fractions, excluding the standard ascorbic acid (AS) which is the standard compound. The differences in IC₅₀ and LogIC₅₀ values among the fractions indicate varying levels of inhibitory activity, which could be due to differences in their chemical composition and mechanism of action of the bioactive compounds.

GC-MS Analysis

File :E:\Austin\PZX20.D
 Operator : AUSTIN
 Acquired : 28 Ma 2024 8:49 using AcqMethod SCAN.M
 Instrument : GCMS
 Sample Name: PZX20
 Misc Info :
 Vial Number: 1

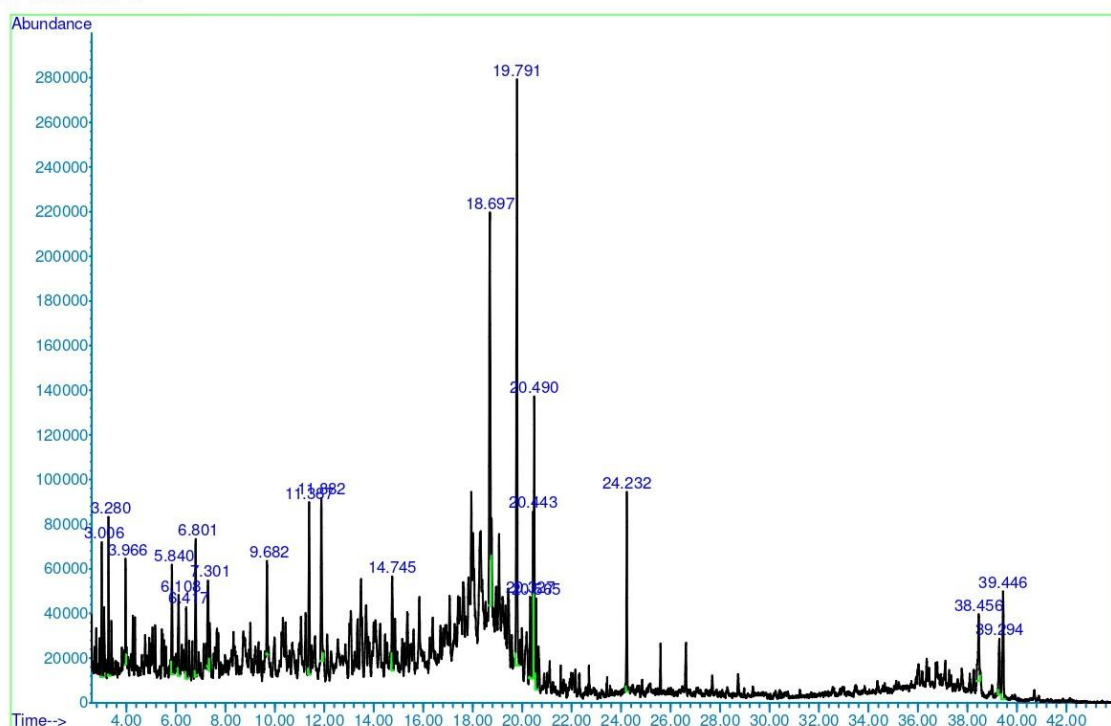


Figure 6: GC-MS spectra of methanol extract of the root of *D. tripetala*

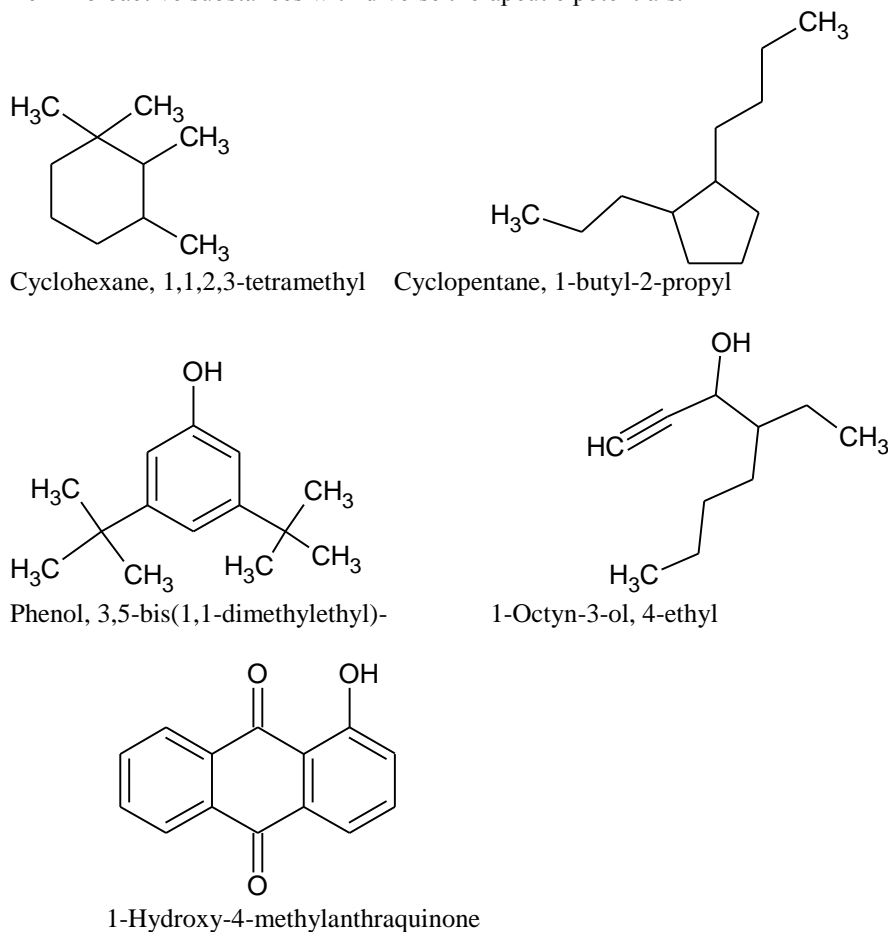
Table 7: Identified compounds from GC-MS analysis of methanol extract of the root of *D. tripetala*.

S/N	Retention time	Compound name	Molecular formula	Molecular weight	Area %
1	3.006	1,2-Cyclooctanedione	C ₈ H ₁₂ O ₂	140	2.894
2	3.280	2-Methylenecyclohexanol	C ₇ H ₁₂ O	112	3.301
3	3.966	5-Hydroxymethylfurfural	C ₆ H ₆ O ₃	126	2.064
4	5.840	5-Acetoxyethyl-2-furaldehyde	C ₈ H ₈ O ₄	168	3.226
5	6.108	2-Methoxy-4-vinylphenol	C ₉ H ₁₀ O ₂	150	2.490
6	6.417	Ethanone, 1-(2-hydroxy-5-methylphenyl)-	C ₉ H ₁₀ O ₂	150	2.115
7	6.801	N-Chloroacetylglucylglycine	C ₆ H ₉ ClN ₂ O ₄	208	3.791
8	7.301	Carbamic chloride, dimethyl	C ₃ H ₆ ClNO	107	3.216
9	9.682	2,4-Di-tert-butylphenol	C ₁₄ H ₂₂ O	206	2.500
10	11.387	Phenol, 3,5-bis(1,1-dimethylethyl)-	C ₁₄ H ₂₂ O	206	5.180
11	11.882	1-Octyn-3-ol, 4-ethyl	C ₁₀ H ₁₈ O	154	5.270
12	14.745	4-Trifluoroacetoxytridecane	C ₁₅ H ₂₇ F ₃ O ₂	296	3.127

13	18.697	Cyclohexane, 1,1,2,3-tetramethyl	C ₁₀ H ₂₀	140	15.298
14	19.791	Cyclopentane, 1-butyl-2-propyl	C ₁₂ H ₂₄	168	17.206
15	20.327	Eseroline, 1-desmethyl-1-ethoxycarbonyl-, methyl(ether)	C ₁₆ H ₂₂ N ₂ O ₃	290	2.464
16	20.443	Physostigmine	C ₁₅ H ₂₁ N ₃ O ₂	275	3.978
17	20.490	1-Hydroxy-4-methylanthraquinone	C ₁₅ H ₁₀ O ₃	238	6.230
18	20.565	1-Hydroxy-2-methylanthraquinone	C ₁₅ H ₁₀ O ₃	238	2.179
19	24.232	cis-Decalin, 2-syn-methyl	C ₁₁ H ₂₀	152	4.120
20	38.456	Naphthalene, decahydro-2-methyl	C ₁₁ H ₂₀	152	4.288
21	39.294	E,Z-1,3,12-Nonadecatriene	C ₁₉ H ₃₄	262	2.309
22	39.446	1,3,12-Nonadecatriene	C ₁₉ H ₃₄	262	4.154

The GC-MS analysis of the root methanol extract of *D. tripetala* revealed the presence of 22 chemical compounds. Among these, the notable compounds include 1,2-Cyclooctanedione, 2-Methylenecyclohexanol, 5-Hydroxymethylfurfural, and 5-Acetoxyethyl-2-furaldehyde. Additionally, 2-Methoxy-4-vinylphenol, Ethanone (1-(2-hydroxy-5-methylphenyl)-), and N-Chloroacetylglycylglycine were identified. Other significant compounds include Carbamic chloride (dimethyl), 2,4-Di-tert-butylphenol, and Phenol (3,5-bis(1,1-dimethylethyl)-). Noteworthy is the presence of Physostigmine and its derivative, Eseroline, along with 1-Hydroxy-4-methylanthraquinone and 1-Hydroxy-2-methylanthraquinone. Compounds such as Cyclohexane (1,1,2,3-tetramethyl), Cyclopentane (1-butyl-2-propyl), and various forms of Nonadecatriene were also detected.

These compounds possess a range of pharmacological applications. Physostigmine is a well-known acetylcholinesterase inhibitor used in the treatment of glaucoma and Alzheimer's disease [17]. 5-Hydroxymethylfurfural has been investigated for its potential anti-inflammatory and antioxidant properties [18]. 2-Methoxy-4-vinylphenol, often found in natural products, has antimicrobial activities [19]. Anthraquinone derivatives, such as 1-Hydroxy-4-methylanthraquinone, are known for their laxative effects and potential anticancer properties. Compounds like 2,4-Di-tert-butylphenol and its derivatives have antioxidant properties, making them useful in preserving food and cosmetic products [20]. Eseroline, being a derivative of Physostigmine, also holds potential in neurological applications. The variety of compounds detected suggests the extract could be rich in bioactive substances with diverse therapeutic potentials.



IV. Conclusion

This study elucidated the rich biochemical profile and potent antioxidant capabilities of the root of *D. tripetala*. The GC-MS analysis identified numerous bioactive compounds, with significant levels of phenolics and flavonoids, known for their strong antioxidant properties. Quantitative phytochemical screening confirmed the presence of these compounds in high concentrations, which contribute to the plant's overall antioxidant activity. Notably, the dichloromethane fraction demonstrated superior radical scavenging activity, underscoring its potential as a natural antioxidant agent.

These results validate the traditional use of *Dennetia tripetala* in Nigeria traditional medicine and highlight its promise for inclusion in antioxidant formulations. The observed antioxidant activity suggests that the plant could be beneficial in managing oxidative stress-related conditions such as cardiovascular diseases, neurodegenerative disorders, and certain cancers. The high content of phenolics and flavonoids indicates that the root extract may also possess anti-inflammatory and antimicrobial properties, offering a broad spectrum of therapeutic applications.

Future research should focus on isolating specific compounds responsible for the observed bioactivity and exploring their mechanisms of action in greater detail. Studies should also investigate the bioavailability and toxicity profiles of these compounds to ensure their safety and efficacy for clinical use. Additionally, *in vivo* studies and clinical trials are necessary to confirm the therapeutic potential of *Dennetia tripetala* root extracts. This study lays the groundwork for further pharmacological investigations and the potential development of novel therapeutic agents derived from *Dennetia tripetala*, contributing to the advancement of natural product chemistry and medicinal plant research.

Conflict of Interest Disclosure

The authors declare no conflict of interest.

References

- [1]. Mahunakpon, N.G., Assogba G. A., Habib G., Engelbert J. A., Fernand A. G., and Virgile A. *Annona muricata* L. and *Annona squamosa* L. (Annonaceae): a review of their traditional uses and anticancer activities. *Journal of Pharmacognosy and Phytochemistry*. 2021, 10(1): 40-51.
- [2]. Oyemitan, I. A., Iwalewa, E. O., Akanmu, M. A., Asa, S. O., and Olugbade, T. A. The abusive potential of habitual consumption of the fruits of *Dennetia tripetala* G.baker (Annonaceae) among the people in Ondo township (Nigeria). *Nigerian Journal of Natural Products Medicine*, 2006, 10: 55-62.
- [3]. Ugochukwu S. C., Arukwe, U. I., and Ifeanyi, O. Preliminary phytochemical screening of different solvent extracts of stem bark and roots of *Dennetia tripetala* G. Baker. *Pelagia Research Library*. 2013, 3(3):10-13.
- [4]. Enema, O. J., Umoh, U. F., Thomas, P. S., Adesina, S. K., and Eseyin, O. A. Phytochemical and antioxidant studies of leaf of *Tetrapleura Tetraptera* (Schum and Thon) Taubert (Mimosaceae). *British Journal of Pharmaceutical and Medicinal Research*. 2019, 4(3): 1865 - 1875.
- [5]. WHO. (2021). World Health Organisation. Definition and Diagnosis of Diabetes. Geneva, Switzerland. https://www.who.int/diabetes/publications/Definition%20and%20diagnosis%20of%20diabetes_new.pdf (Retrieved on 20th November 2021).
- [6]. Elekwa, I., Okereke, S.C., and Chukwudoruo, C. S. Phytochemical screening and GC-MS analysis of the essential oil of *Dennetia tripetala* (pepper fruit) Seeds. *ABSU Journal of Environment, Science and Technology*. 2011, 1: 93-98.
- [7]. Rajeev, S., Pawan K. V., Gagandeep, S. Total phenolic, flavonoids and tannin contents in different extracts of *Artemisia absinthium*. *J Intercult Ethnopharmacol*. 2012, 1 (2): 101-104.
- [8]. Kaur, C., Kapoor, H. C. Antioxidant Activity and total phenolic content of some Asian vegetables. *International Journal of Food Science and Technology*. 2002, 37(2): 9-26.
- [9]. Fazel, S., Hamidreza M., Rouhollah G., Mohammadreza, V. Spectrophotometric determination of total alkaloids in some Iranian medicinal plants. *Thai Journal of Pharmaceutical Sciences (TJPS)*. 2008, 32: 17-20.
- [10]. Lee W. H. and Intan S. I. Antioxidant activity, total phenolics and total flavonoids of *Syzygium polyanthum* (Wight) Walp leaves. *Int J Med Arom Plants*. 2012, 2(2): 219-228.
- [11]. Ameen, O. A., Hamid, A. A., Yusuf, Q., Njoku, O. G., Oseni, T. O., Jamiu, W. Quantitative and Qualitative Assessment of Phytochemicals in Methanolic Extracts of Hurricane Weed (*Phyllanthus amarus* Schumach. &Thonn) Plant. *Journal of Applied Sciences and Environmental Management*. 2021, 25 (2): 159 - 165.
- [12]. Elsayed A. I., Mei W., Mohamed M. R., Amira S. W., Chandrani G. M., Baharathi A., Yan-Hong W., Ikhlās A. K., Suman C., Hemant L., Ghada M. H., Randa A. A., Amany K. I., Safwat A. A., Mahmoud A. E.

- Analysis of Terpenes in Cannabis sativa L. Using GC/MS: Method Development, Validation, and Application. *Planta Medica*. 2019, 85: 431–438.
- [13]. Yen, G. C., and Chen, H. Y. Comparison of antimutagenic effect of various tea extract (green, oolong, pouching and black tea). *Journal of Clinical Investigation*. 1995, 120(11): 3742-3744.
- [14]. Shekhar, T. C., and Anju, G. Antioxidant activity by DPPH radical scavenging method of *Ageratum conyzoides* Linn leaves. *American Journal of Ethnomedicine*. 2014, 1(4): 244-249.
- [15]. Marcocci, L. L., Packer, M. T., Droy-Lefaix, A. S., and Albert, M. Antioxidant action of Ginkgo biloba extract EGb 761. *Methods and Enzymology*. 1994, 234(23): 462-475.
- [16]. Mehta M, Adem A, Sabbagh M. New acetylcholinesterase inhibitors for Alzheimer's disease. *Int J Alzheimers Dis*. 2012, 2012: 728983.
- [17]. Kong F, Lee BH, Wei K. 5-Hydroxymethylfurfural Mitigates Lipopolysaccharide-Stimulated Inflammation via Suppression of MAPK, NF- κ B and mTOR Activation in RAW 264.7 Cells. *Molecules*. 2019, 24(2): 275.
- [18]. Rubab M, Chelliah R, Saravanakumar K, Barathikannan K, Wei S, Kim JR, Yoo D, Wang MH, Oh DH. Bioactive Potential of 2-Methoxy-4-vinylphenol and Benzofuran from *Brassica oleracea* L. var. capitata f. rubra (Red Cabbage) on Oxidative and Microbiological Stability of Beef Meat. *Foods*. 2020 May 4;9(5):568.
- [19]. Shakira RM, Abd Wahab MK, Nordin N, Ariffin A. Antioxidant properties of butylated phenol with oxadiazole and hydrazone moiety at ortho position supported by DFT study. *RSC Adv*. 2022, 12(27): 17085-17095.
- [20]. Sharma K. Cholinesterase inhibitors as Alzheimer's therapeutics (Review). *Mol Med Rep*. 2019, 20(2): 1479-1487.