GC-MS Analysis, Quantitative Phytochemical Profile and *in vitro*Aantioxidant Sstudies of the Sstem Bbark of *Dennettia tripetala*~~BAK. F. (Annonaceae).~~

Abstract

Plants have played a significant role in preventing degenerative diseases and maintaining good health.This study aims to evaluate the chemical profile and antioxidant properties of the methanol extract of the stem bark of Dennettia tripetala. The stem bark was collected, air-dried, and extracted with 70% methanol. Quantitative phytochemical screening was performed to determine the concentrations of tannins, phenolics, alkaloids, flavonoids, saponins, and terpenoids using in vitro techniques. Antioxidant activity was evaluated using ferric-reducing antioxidant power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, and nitric oxide (NO) scavenging assays and determination of total phenolic content. ~~Gas Chromatography-Mass Spectrometry (GC-MS) analysis identified the chemical constituents of the extract.~~ The GC-MS analysis revealed the presence of bioactive compounds including phenol, eugenol, caryophyllene, naphthalene, trimethyl arsenate, and other phenolic and flavonoid compounds. The methanol extract and its fractions demonstrated significant antioxidant activity, with high concentrations of phenolicsand flavonoids contributing to their potency. The FRAP, DPPH, and NO scavenging assays confirmed the extract's ability to neutralize free radicals effectively. The study concludes that the stem bark of D. tripetala possesses substantial antioxidant properties, which supports its traditional use in managing oxidative stress-related ailments.

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

Introduction

Plants have played a significant role in the treatment and prevention of degenerative diseases and maintenance of good health [1]. The rich biological activities of plants and their various parts are often due to their chemical constituents. Different parts of plants may possess different biochemical agents; this may also be affected by season and time of collection [2].

Traditional and Complementary Medicine (T&CM) is an important and often underestimated health resource with many applications in meeting the health needs of aging populations. *D. tripetala*also known as pepper fruit, widely consumed in West Africa, has been used in traditional medicine for the management of inflammations, diabetes, cough, fever, nausea, and even as an antioxidant agent. The plant possesses phytochemicals that have been shown to elicit antimicrobial, insecticidal, analgesic, and anti-inflammatory properties [3].

Cancer is another main cause of death globally, in Nigeria; over 100000 new cases are recorded annually. Nigeria represented 15% of the estimated 681000 cancer cases in Africa in 2008; this is due to increasing life expectancy, reduced risk of death from infectious diseases, increasing prevalence of smoking, physical inactivity, obesity and poor dietary habits [4].

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Comment [U2]: Why the choice of your plant (*Dennettia tripetala)*? You should write out few of its local uses. This will justify the work and link with your conclusion.
The knowledge gap, which was stated in the introduction (no research has been reported on the quantitative phytochemical study, GCMS profiling, and antioxidant properties of the stem bark) should be also be incorporated here.

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Comment [U5]: You reported 0.0 for phenol in the quantitative phytochemical in Table 1. This contradicts that of phenolics in the GC-MS result. How will you resolve this?

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Reactive oxygen species (ROS) such as hydrogen peroxide, hypochlorus acid, D. tripsuperoxide anion, single oxygen, lipid peroxides, hypochlorite and hydroxyl radicals play significant roles in growth, differentiation, progression and subsequently death of the cells. Free radicals have special affinity for lipids, proteins, carbohydrates, and nucleic acids [5].

The fruit and leaf of *D. tripetala* have been shown to have medicinal properties ranging from anti-inflammatory, antioxidant, and antibacterial effects [6][7].López-Martín*et al.* [8] reported three alkaloids from the crude methanol extract of the root (uvaropsine, argentinine, stephenanthine) and a chromene, called Dennetine. However, no research has been reported on the quantitative phytochemical study, GCMS profilling, and antioxidant properties of the stem bark hence, the novelty of this research. This study aimed to evaluate the chemical profile of the methanol extract of the stem bark of *D. tripetala* and the antioxidant properties of the methanol extract of the stem bark and various partitioned fractions of *D. tripetala*.

Materials and Methods

Plant collection and identification

The stembark of *D. tripetala* was collected from Itak Ikot Akap village in Ikono Local Government, Akwa Ibom State, Nigeria. The plant was identified by Dr. Imeh Imoh Johnny of the Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Uyo and authenticated by Prof Mrs. M.E Bassey ofat the Department of Botany and Ecological Studies, Faculty of Science, University of Uyo, Uyo.

Preparation of Extract

*Dennettia. tripetala*stem barkwas air-dried and coarsely powdered with hammer mill. About 1 kg of the powdered plant material was extracted in70% methanol. The extract was dried and weighed.

Quantitative Phytochemical Screening of Extracts

Determination of tannin Content

The tannin constituents of *D. tripetala*stem bark extract was determined by Folin-Ciocalteu method described by [9]. 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 % Na_2CO_3 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-Ciocalteureagent and 2mL of Na_2CO_3 (7%). The resulting mixture was vortexed for 15 seconds and incubated at 40°c 40°Cfor 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 [10].

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Determination of aAlkaloid

The stem bark extract (1mg) was dissolved in dimethyl sulphoxide (DMSO), added 1mL of 2N 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 vigorouslyshaken 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 [11].

Determination of tTotal flavonoid content

The total flavonoid content was determined by the method described by Lee and Intan [12]. 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 sSodium 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 sSaponins

The total saponins of the stem bark extract was determined by the method described by Ameen*et al.* [13]. 1g of the dried sample of the stem bark 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 $MgCO_3$. About 1 mL of the colourless filtrate was pipette into a 50 mL volumetric flask and 2 mL of 5% $FeCl_3$ 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:

% Saponin = AS x AG x DF / Wt of sample x 10,000

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

Total tTerpenoid determination

The total terpenoid was determined by the method described by[14]. 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 µg/ mL, which was added to all of the calibration and sample solutions.

Calibration curves:

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

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Sample solution preparation:

The stem bark 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 µg/mL of the IS in ethyl acetate) by sonication for 15 min. The mixture was centrifuged for 5 min at 1252 × g and the supernatants (without filtration) were used for the GC/MS analysis.

Antioxidant evaluation of extracts and fractions

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

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Ferric reducing antioxidant power (FRAP) assay

The FRAP activity of the methanol stem bark extract and fractions were determined by the method described by[15]. Various concentrations (20, 40, 60, 80, 100 ug/mL) of the methanol extract and fractions of stem bark 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 pPotassium Ffericyanide ($K_2Fe(CN)_6$) (1% w/v). The resulting mixture was incubated at 50^{o}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.

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Determination of 2,2-—Diphenly-—1-—picrylhydrazyl (DPPH) radical scavenging aActivity)

The DPPH free radical scavenging activity of methanol extract and fractions of stem bark of *D. tripetala* and ascorbic acid prepared in methanol at various concentrations (20, 40, 60, 80, 100 µg/mL) was evaluated according to the method of[16]. 2,2—-Diphenyl—-1-—pPicrylhydrazyl (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 versusstandard error of mean. The percentage DPPH scavenging effect was calculated using the following equation:

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DPPH sScavenging effect (%) or percentage exhibition = $[(A_0 - A_s)/A_0]$ x 100, Where A_o is the absorbance of control reaction and A_s is the absorbance of the test samples or standard sample (ascorbic acid).

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Nitric oxide (NO) scavenging assay

Nitric oOxide generated from sodium nitroprusside (SNP) was measured according to the modified method of[17]. 3.0 mL of 10 um of SNP in phosphate buffered saline (PH 7.4) was added to 2mL of different concentrations of methanol extract and fractions of stem bark of *D. tripetala* and ascorbic acid (20, 40, 60, 80, 100 µg/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 sulphamilamide 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 ± standard deviation.

Determination of total phenolic content

The total phenolic content of the fractions was determined spectrophotometrically with Ffolin-Ciocalteu reagent 0.5 mL (1 mg/mL) of the fractions was mixed with 2.5 mL of 10% Folin-Ciocalteu ~~folin-ciocalteu~~ reagent and 2mL of Na_2CO_3 (7%). The resulting mixture was vortexed for 15 seconds and incubated at 40°~~c~~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 [10].

Gas Chromatography Mass Spectrum (GC-MS) aA~~A~~nalysis

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 µm and a thickness of 250µm, 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, 1µl 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 4^0C/min, the temperature was raised to 2500C at the 162 rate of 20^0C/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 bark of *D. tripetala*.

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Results and Discussion

Quantitative phytochemical screening

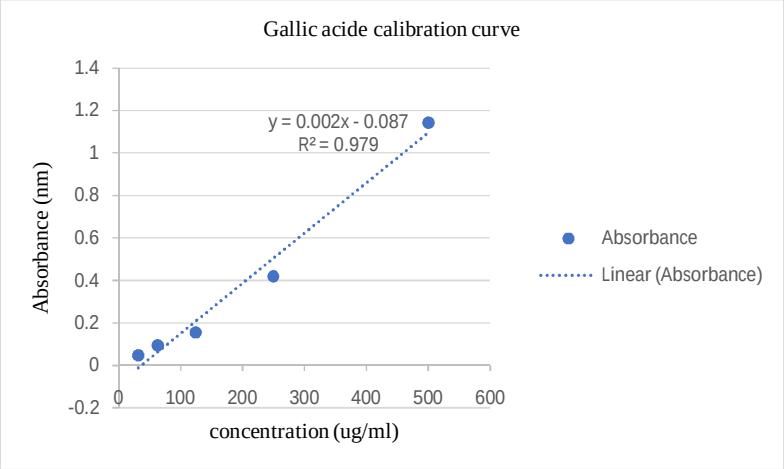

Figure 1: Gallic acid calibration curve for total phenols determination

~~Source: Experimental data (2024)~~

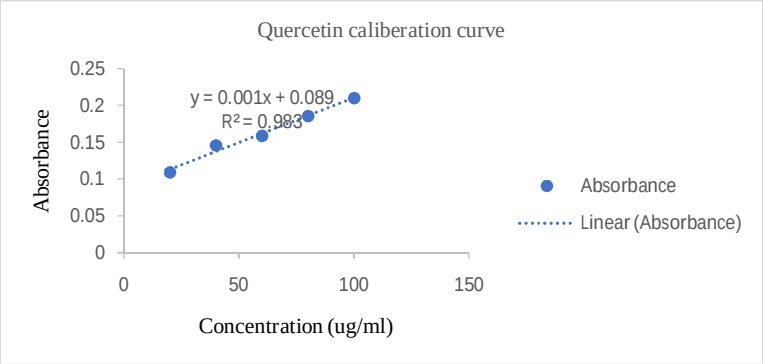

Figure 2: Quercetin calibration curve for total flavonoid determination

~~Source: Experimental data (2024)~~

Table 1: ~~Summary of results of~~ Qquantitative phytochemical screening~~(%w/w)~~

Sample	Alkaloids	Flavonoids	Saponins	Tannin	Terpenoids	Phenol
EPFS~~(%w/w)~~	19.5	241.4	1.75	53.7	0.9	0.0

~~Source: Experimental data (2024)~~

EPFS = Methanol extract of stem bark of *D. tripetala*

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Quantitative pPhytochemical screening of the stem bark 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's parts in the treatment of ailments traditionally. 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 µg/mL) to 2.075 ± 0.001 (at 1000 µg/mL), with the calibration curve described by the equation y = 0.0024x - 0.0878 (R^2 = 0.9795). The Total phenolic content in the stem bark extract was calculated from the calibration curve as 180 mg GAE/g. The linear relationship of the gallic acid calibration curve, with a correlation coefficient (R^2) of 0.9795 also suggests a highly reliable measurement.

Table 1 shows the result of the total flavonoid in the stem bark extract of *D. tripetala*. The total flavonoid content showed a progressive increase with higher concentrations. The concentration-dependent increase, especially noted at 1000 µg/mL with totals reaching 313 µg QE/g for the stem bark extract, suggesting strong anti-inflammatory and antioxidative potential. The total saponin component was also quantified, with the stem extract presenting a yield of (1.75 % 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 in the stem bark extract (EPFS) showed a maximum of 60.2 µg QE/g. Alkaloids, which have diverse pharmacological activities, were found to be concentrated in the stem bark (19.5 % w/w).Phenol was as absent in the stem bark extract. This is similar to the quantitative determination of the leaves of *D. tripetala*, reported by[18].

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Antioxidant analysis

Table 2: DPPH absorbance (nm) and percentage inhibition (%) of EPFS and fractions of EPFS

	Absorbance (nm) and percentage inhibition (%)						
Concentration (µg/mL)	**AS**	**EPFS**	**NH**	**DCM**	**ETH**	**nBT**	**AQ**
20	0.119 ± 0.001* (85)	0.737 ± 0.000* (10)	0.604 ± 0.000* (26)	0.475 ± 0.000* (42)	0.65 ± 0.000* (21)	0.672 ± 0.000* (18)	0.649 ± 0.000* (21)
40	0.116 ± 0.000* (86)	0.576 ± 0.000* (30)	0.461 ± 0.000* (44)	0.452 ± 0.000* (45)	0.545 ± 0.000* (34)	0.568 ± 0.000* (31)	0.619 ± 0.000* (25)
60	0.114 ± 000* (86)	0.573 ± 0.000* (30)	0.398 ± 0.000* (52)	0.344 ± 0.333* (58)	0.542 ± 0.000* (34)	0.478 ± 0.000* (42)	0.605 ± 0.000* (26)
80	0.110 ± 001* (87)	0.509 ± 0.000* (38)	0.362 ± 0.000* (56)	0.314 ± 0.333* (61)	0.509 ± 0.000* (38)	0.453 ± 0.001* (45)	0.571 ± 0.000* (30)
100	0.09 ± 0.001* (89)	0.444 ± 0.000* (46)	0.251 ± 0.000* (69)	0.197 ± 0.000* (76)	0.418 ± 0.000* (49)	0.396 ± 0.000* (52)	0.264 ± 0.000* (68)

~~Source: Experimental data (2024)~~ ($p<0.05$)

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

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You don't need to combine absorbance and the percentage inhibition. The latter is better and should be presented as bar chats, like in figure 3.

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DPPH assay is considered a valid, accurate, easy and economical method to evaluate antioxidants' ~~radical scavenging~~ activity, since the radical compound is stable and need not be generated [19]. The technique of DPPH testing is associated with eliminating DPPH, which would be a stabilized free radical [20]. The result of the DPPH assay of the stem bark extract of *D. tripetala* showed a significant increase with an increase in concentration of the sample when compared to the standard compound (ascorbic acid), this is shown in Table 2. The methanol stem bark extract had an average percentage inhibition of 30.8% when compared to ascorbic acid which had an average percentage inhibition of 87%. The statistical analysis of the absorbance (nm) of the samples was conducted using two-way ANOVA. The result showed that the measurements were statistically significant at ($~~P~~p<0.05$).

Among the partitioned fractions, the dichloromethane fraction also recorded the highest 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of 56.4%. This was followed by the n-butanol fraction which recorded a percentage inhibition of 37.6% and ethyl acetate fraction which recorded 35.2% as average percentage inhibition. The result was dose dependent and statistically significant ($~~P~~p<0.05$)when compared to ascorbic acid, with n-hexane fraction showing the least percentage inhibition of 30.8% (Table 2).

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Table 3: NO absorbance (nm) and percentage inhibition (%) of EPFS and fractions of EPFS

	Absorbance (nm) and percentage inhibition (%)						
Concentration (µg/mL)	**AS**	**EPFS**	**NH**	**DCM**	**ETH**	**nBT**	**AQ**
	0.086 ± 0.000*	0.169 ± 0.000*	0.262 ± 0.000*	0.132 ± 0.000*	0.443 ± 0.000*	0.386 ± 0.000*	0.295 ± 0.000*
20	(85)	(71)	(55)	(77)	(25)	(34)	(50)
	0.072 ± 0.000*	0.130 ± 0.000*	0.240 ± 0.333*	0.125 ± 0.000*	0.358 ± 0.000*	0.267 ± 0.000*	0.258 ± 0.000*
40	(87)	(77)	(59)	(78)	(39)	(55)	(56)
	0.07 ± 0.000*	0.128 ± 0.001*	0.179 ± 0.333*	0.125 ± 0.000*	0.231 ± 0.001*	0.128 ± 0.000*	0.127 ± 0.000*
60	(88)	(78)	(69)	(78)	(61)	(78)	(78)
	0.062 ± 0.000*	0.091 ± 0.001*	0.145 ± 0.000*	0.097 ± 0.000*	0.198 ± 0.000*	0.116 ± 0.000*	0.127 ± 0.000*
80	(89)	(84)	(75)	(83)	(66)	(80)	(78)
	0.054 ± 0.000*	0.084 ± 0.000*	0.110 ± 0.333*	0.071 ± 0.000*	0.120 ± 0.000*	0.108 ± 0.000*	0.114 ± 0.000*
100	(90)	(86)	(81)	(88)	(79)	(82)	(80)

~~Source: Experimental data (2024)~~ ($p<0.05$)

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

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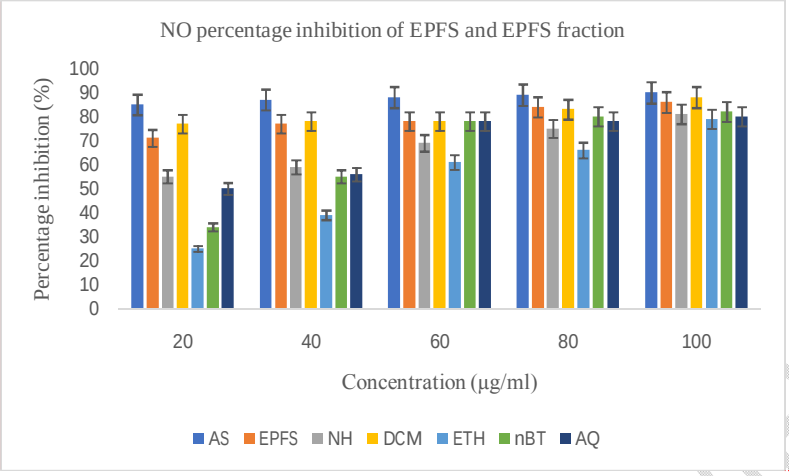

Figure 3: ~~Comparisons of~~ NO percentage inhibition of EPFS and EPFS fraction

~~Source: Experimental data (2024)~~

The result of the nitric oxide assay illustrated in F~~f~~igure 3also showed an increase in percentage inhibition of NO with increase in concentration of the sample. Like the DPPH scavenging activity assay, the results are statistically significant at all concentrations at (~~P~~p<0.05). Among the fractions, the dichloromethane fraction of the stem showed the highest nitric oxide scavenging potential of 80.8.At 20 µg/mL, the ethyl acetate fraction showed the least percentage inhibition when compared to other fractions of the stem bark extract. 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 and the dichloromethane fraction of the stem indicates the presence of effective nitric oxide scavengers in these fractions. ~~This is illustrated in table 3.~~

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Table 4: Absorbance (nm) of ferric reducing antioxidant power assay of EPFS and fractions of EPFS

	Absorbance (nm)						
Concentration (µg/mL)	**AS**	**EPFS**	**NH**	**DCM**	**ETH**	**nBT**	**AQ**
20	0.421 ± 0.001*	0.471 ± 0.001*	0.421 ± 0.001ns	0.457 ± 0.001*	0.403 ± 0.001*	0.394 ± 0.000*	0.382 ± 0.000*
40	0.394 ± 0.001*	0.421 ± 0.001*	0.386 ± 3.333*	0.325 ± 3.333*	0.398 ± 0.000ns	0.367 ± 0.001ns	0.324 ± 0.000*
60	0.298 ± 0.001*	0.398 ± 0.001*	0.321 ± 0.001*	0.304 ± 0.001*	0.387 ± 0.000*	0.299 ± 0.001ns	0.321 ± 0.000*
80	0.286 ± 0.001*	0.396 ± 0.001*	0.271 ± 0.001*	0.282 ± 0.001ns	0.362 ± 0.000*	0.329 ± 0.001*	0.292 ± 0.000*
100	0.187 ± 0.001*	0.291 ± 0.001*	0.242 ± 3.333*	0.243 ± 0.001*	0.298 ± 0.000*	0.322 ± 0.001*	0.242 ± 0.000*

~~Source: Experimental data (2024)~~ ($p<0.05$)

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

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Table 4 shows the ferric reducing antioxidant power assay of the stem bark extract and fractions of *D. tripetala*. The ferric reducing antioxidant power measurement of the stem bark was also statistically significant at (p~~P~~<0.05). Unlike the DPPH and nitric oxide assays where the measurements were significant across all concentrations, the ferric reducing power assay of the stem showed that at 20 µg/mL, the absorbance of the n-hexane fraction was not significant (~~P~~p<0.05). At 80 µg/mL, the dichloromethane fraction was also reported as not significant (~~P~~p<0.05). This was also noted in the aqueous fraction at 40 µg/mL (~~P~~p<0.05).

IC_{50} determination

Table 5: IC_{50} and L~~l~~ogIC_{50} of EPFS and fractions of EPFS

	$IC_{50\ (\mu M)}$						
	AS	EPFS	NH	DCM	ETH	nBT	AQ
IC_{50}	0.006218	0.03187	0.02364	0.02021	0.02971	0.02888	0.03039
L~~l~~ogIC_{50}	-2.206	-1.497	-1.626	-1.695	-1.527	-1.539	-1.517

IC_{50} = ~~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 IC_{50} of the stem bark extract (EPFS) is presented in Table 5. The IC_{50} values for the fractions n-hexane (NH), dichloromethane (DCM), ethyl acetate (ETH), n-butanol (nBT), and aqueous (AQ) are 0.02364, 0.02021, 0.02971, 0.02888, 0.03039 µM, respectively. In this case, the dichloromethane fraction also shows the lowest IC_{50} value, indicating the highest inhibitory potency among these fractions against the target. The ethyl acetate fraction, while still potent, shows a higher IC_{50} value than in the root, suggesting a different level of activity in the stem extract. The LogIC_{50} values are -1.626, -1.695, -1.527, -1.539, and -1.517, respectively for the various fractions of n-hexane, dichloromethane, ethyl acetate, n-butanol and aqueous.

From these observations, it is clear that the dichloromethane fraction exhibits a particularly high potency as an antioxidant agent in both fractions of the stem and root, with relatively low IC_{50} and LogIC_{50} 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 IC_{50} and LogIC_{50} 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.

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). The ethyl acetate fraction of the stem had 139.1 mg GAE/g phenolic content and was noted as the second most active fraction when compared to all others. These effects were statistically significant (p<0.05).

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GC-MS analysis

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Instrument : GCMSD
Sample Name: PQXY.
Misc Info :
Vial Number: 3

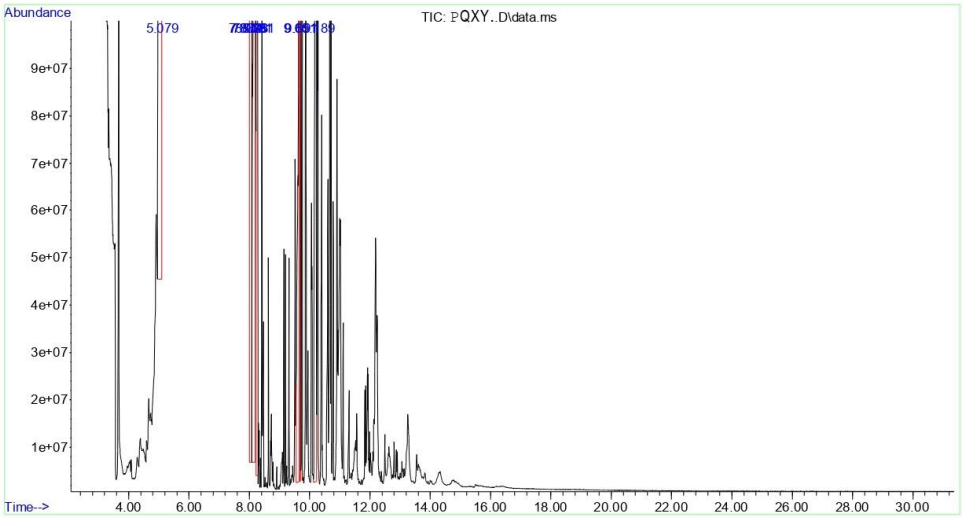

Figure 4: GC-MS spectra of methanol extract of the stem bark of *Dennettia~~.~~ tripetala*

Table 6: Identified compounds from GC-MS analysis of methanol extract of the stem bark of *Dennettia~~D.~~ tripetala*.

S/N	Retention time	Compound name	Molecular formula	Molecular weight	Area %
1	5.079	Phenol, 2-methoxy-3-(2-propenyl)-	$C_{10}H_{12}O_2$	164	17.232
2	7.832	Eugenol	$C_{10}H_{12}O_2$	164	7.666
3	7.872	Caryophellene	$C_{15}H_{24}$	204	8.894
4	7.958	Naphthalene	$C_{10}H_8$	128	10.853
5	8.038	Ethyl α-d-glucopyranoside	$C_8H_{16}O_6$	208	15.426
6	8.181	Clionasterol acetate	$C_{31}H_{52}O_2$	456	14.835
7	8.261	α-Farnesene	$C_{15}H_{24}$	204	8.264
8	9.651	Cycloundecene, 1-methyl	$C_{12}H_{22}$	166	3.602
9	9.691	1,2-Benzenedicarboxylic acid	$C_8H_6O_4$	166	5.423
10	10.189	Trimethyl arsenate	$C_3H_9AsO_4$	184	7.806

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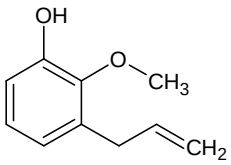
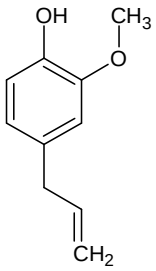
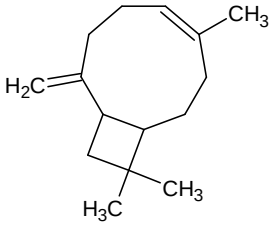


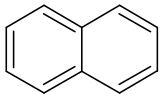
Phenol, 2-methoxy-3-(2-propenyl)-



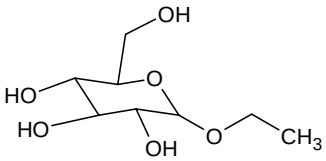
Eugenol



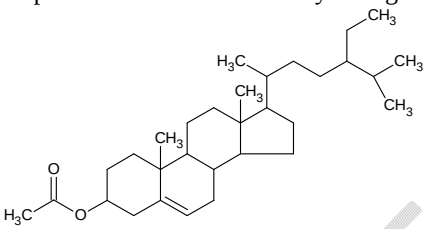
Caryophyllene



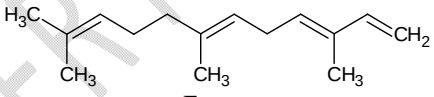
Naphthalene



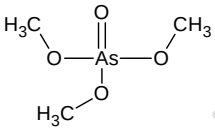
Ethyl α-d-glucopyranoside



Clionasterol acetate



α-Farnesene



Trimethyl arsenate

Figure 5: Chemical structures of compounds from the stem bark extract of *Dennettia~~D.~~ tripetala*

The GC-MS analysis of the methanol extract of the stem bark of *D. tripetala* revealed the presence of 10 compounds. Phenol, 2-methoxy-3-(2-propenyl)- (also known as 3-Allylguaiacol or 3-Allyl-2-methoxyphenol) has been studied for its various applications. Researchers have identified it as a potential flavouring agent, food additive, and component in food contact materials. It has also been reported to possess analgesic, antibacterial and anticancer properties. Additionally, phenol finds use in dental products (such as disinfectants and impression pastes), pet cleaners, and as a fungicide, insecticide, and pesticide. While these findings are promising, further research and clinical trials are necessary to fully validate its efficacy and safety across various contexts[21].

Eugenol~~,~~ has been reported to exhibit a wide range of pharmacological properties, including antibacterial, anticancer, antiviral, antifungal, anti-inflammatory, and antioxidant effects. It is also used as a flavouring agent, food additive, and in dental products.

Caryophellene, which is found in various plants, has anti-inflammatory, antioxidant, analgesic, and immunomodulatory properties. It has also been reported as a potential candidate for treating various conditions, including pain and inflammation [22].

Naphthalene is historically known for its use in mothballs, naphthalene repels moths and insects. It has been used widely in dermatology, gastroenterology, ophthalmology, and veterinary medicine. Ethyl α-d-glucopyranoside has antioxidant, anti-inflammatory, and skin-whitening effects. It is used in cosmeceuticals. Clionasterol acetate, a plant-originated sterol, exhibits antiviral, antioxidant, and antitumor activities. It holds promise for various therapeutic applications [23].

1,2-Benzenedicarboxylic acid has been isolated from *Salvadora persica* and shows antidiabetic activity [24]. Trimethyl arsenate has been studied for its clinical applications, particularly in treating diseases such as leukemia and tumors. It has a long history of use in traditional Chinese medicine [25]. Researchers have explored its pharmacological effects, including anti-tumor properties, while being mindful of its dose-dependent toxicity.

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Conclusion

The chemical profile analysis of *D. tripetala* stem bark extract through GC-MS and *in vitro* antioxidant assays revealed its rich phytochemical profile and significant antioxidant properties. The high concentrations of flavonoids and alkaloids identified in the stem bark indicate its potent bioactivity. The methanol extract and its fractions, especially dichloromethane, ethyl acetate and butanol, exhibited strong radical scavenging activities in both DPPH and nitric oxide assays. These findings support the traditional use of *D. tripetala* in herbal medicine and suggest its potential for development as a natural antioxidant agent. Further studies are warranted to isolate specific bioactive compounds and explore their mechanisms of action, which could lead to the development of novel therapeutic agents for oxidative stress-related diseases. The study highlights the need for continued research into the pharmacological applications of *D. tripetala*, emphasizing its promise in the fields of natural product chemistry and medicinal plant research.

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Comment [U71]: Correct also these ones like in 23 above. Moreover, I will suggest that you cross check all the references for necessary adjustment. Thanks.