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Research Article

Phytochemical And Antioxidant Studies Of Leaf Of *Tetrapleura Tetraptera* (Schum and Thon) Taubert (Mimosaceae)

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ABSTRACT

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Tetrapleura tetraptera (Schum and Thon) Taubert (Mimosaceae) has numerous ethnopharmacological relevance, the plant has played significant roles in the management of numerous health conditions including ulcer, inflammations, arthritis, malaria and even as molluscicidal agent. This research was aimed at evaluating the phytochemical and antioxidant properties of both fractions and volatile oil of leaf of *T. tetraptera*. Soxhlet apparatus was used in extraction of the leaves into fractions of n-hexane, dichloromethane, ethyl acetate, and methanol, respectively. Determination of total phenolic content, ferric reducing powers (FRAP) assay, and 2,2- diphenyl-1- picrylhydrazyl (DPPH) radical scavenging assay, were used to determine the antioxidant power. Isolation and purification of volatile oils of n-hexane fraction involved chromatographic techniques. Results of total phenolic content showed that methanol fraction had highest phenolic content of 16.66±0.0012 mg, and 4.165 mg GAE/g, and this is statistically significance at p<0.001. DPPH radical scavenging activity assay also showed an increase in percentage inhibition with a corresponding increase in concentration, sample NH1 (volatile oil) showed greater percentage inhibition of 80 %, and was statistically significant at p<0.001. The FRAP assay also showed an increase in percentage inhibition with a corresponding increase in concentration, this is also significant at p<0.001. The GC-MS of the volatile oil showed the presence of 25 compounds which are basically esters, terpenoids, alkenes, alkanes and fatty acids. From the results of this study, it can be inferred that various fractions and volatile oil of leaf of *T. tetraptera* may be used as antioxidant agents.

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INTRODUCTION:

T. tetraptera is an indispensable medicinal plant, it has proven efficacy in the management of numerous health conditions. It is valuable because it contains high amount of essential phytochemicals and nutrients that are significant for the normal functioning of the body, the plant is an excellent source of potassium, iron, calcium, zinc, phosphorus, flavonoids, tannins, alkaloids, saponins, steroids and phenolic compounds [1].

Studies have shown that T. tetraptera has potent molluscicidal properties [2] [3] [4] [5] [6]. The anticonvulsant and hypotensive properties of T. tetraptera have been reported by many researchers [7] [8] [9] [10] [11]. The extract of this plant is also known for its anti-inflammatory properties and its use in the management of leprosy has also been reported [12] [13] [14]. The potential activity of extracts of T. tetraptera fruit as an antioxidant, hypoglycaemic and hepatoprotective and antipathogenic agent have been reported [15] [16] [17] [18] [19] [20] [21] [22] [23]. The economic and medicinal importance of T. tetraptera are many; the fruits have been widely used in Nigeria for the manufacturing of seasoning spices, pomades and soaps due to its pleasant aroma characteristics and used in Ghana as additive to soups and as a source of vitamin and to prevent postpartum contractions [24] [25].

Oxygen as a major requirement for the continuous existence of life produces free radicals in the mitochondria in the process of oxidative phosphorylation and the production of Adenosine triphosphate (ATP). These free radicals cause oxidative stress which plays a significant role in the development of human diseases. Numerous diseases including cancer, diabetes, cardiovascular diseases, rheumatic arthritis etc can result from the over-production of free radicals in the body. Although reports has been made on the antioxidant properties of aqueous and ethanol fractions of the leaf of T. tetraptera there are no such reports the comparative antioxidant properties of gradiently extracted fractions of the leaf of T. tetraptera and the constituents of a column obtained volatile oil [27].

MATERIALS AND METHODS

Plant collection and Identification

The leaves of T. Tetraptera were collected from the front of Post Graduate Laboratory, Department of Pharmacognosy and natural medicine, University of Uyo. The plant was identified by a taxonomist in the Department of Botany and Ecological Studies with the herbarium number UUPHA32(f) and voucher specimen deposited in the herbarium of the Department of Pharmacognosy and natural medicine, University of Uyo, Uyo, Nigeria.

Preparation of Extract

T. tetraptera leaves were air dried and coarsely powdered. About 650 g of the powdered plant material was extracted continuously using soxhlet apparatus and successively with n-hexane, dichloromethane, ethyl acetate and methanol, respectively, and dried to obtain different fractions.

Phytochemical Screening

The various soxhlet extracts of T. tetraptera were screened for secondary metabolites using standard methods [28]

Open Column Chromatography

The column chromatography was carried out the n-hexane fraction (30 g) using Rotaflo glass column. Silica gel – G mesh 60 – 200 (Burgoyne, India) was used as adsorbent and a wad of glass wool was inserted into the constriction just above the stopcock prior to the packing of the column. Slurry of the silica gel was prepared in a beaker and transferred into the column to about $\frac{3}{4}$ of its length. The gel was allowed to settle uniformly by gentle tapping of the column to prevent channelling due to air bubbles. Filter paper was cut to fit the inner diameter of the column before loading the sample. A layer of sand was also introduced at the top of the sample to avoid disturbances when introducing the elution solvent systems. The flow rate of the column was kept constant at about 1ml/min and the fractions were collected in 20 mL using beakers. The eluates in beakers (3-11) were bulked and coded NH1 (volatile oil).

Gas chromatography – Mass Spectroscopy (GC-MS) Analysis of NH1 (volatile oil)

The analysis was done on Agilent 7890 A GC/ MS equipped with a Quadrupole Mass Spectra Detector and an Autosampler. GC-MS system settings were as follows; 200°C, interfaced temperature, 250°C,

solvent cut time; 2.50 min; relative detector mode, ACQ mode; Scan; start time – end time; 3.00 min – 56.00 min, event time, 0.50 sec; scan speed, 1428.

Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP activity of the plant extract and isolates were determined by the method described by [29]. Various concentrations (20, 40, 60, 80, 100 µg/mL) of volatile oil (NH1), and ascorbic acid (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.

Determination of 2,2 – Diphenyl – 1 – Picrylhydrazyl (DPPH) radical scavenging Activity

The DPPH free radical scavenging activity of volatile oil and ascorbic acid prepared in methanol at various concentrations (20, 40, 60, 80, 100 µg/mL) was evaluated according to the method of [30]. 2,2 – Diphenyl – 1 – Picrylhydrazyl (0.1 mg, 1 ml) was added to 3ml of the solutions prepared with compounds volatile oil, and ascorbic acid and stirred for 1 minute. Each mixture was incubated in the dark

for 30 minutes and absorbance (As) was measured at 517 nm. The assays were carried out in triplicates and the results expressed as mean values vs standard error of mean. The percentage DPPH scavenging effect was calculated using the following equation:

$$\text{DPPH Scavenging effect (\%)} \text{ or percentage exhibition} = [(A_0 - A_s)/A_0] \times 100$$

Where A₀ is the absorbance of control reaction and A_s is the absorbance of the test samples or standard sample (ascorbic acid).

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 [31].

Statistical Analysis.

Data was analyzed statistically using one way analysis of variance (ANOVA).

RESULTS

Table 1: Yield of Fractions

Fraction	Yield (g)
n-hexane	18.8
Dichloromethane	11.4
Ethyl acetate	7.2
Methanol	24.7

Table 2: Phytochemical Screening of Fractions of *T. tetraptera*

Test	n-hexane	Dichloromethane	Ethyl acetate	Methanol
	fraction	Fraction	Fraction	Fraction
Phenols	+	+	+	+
Cardiac glycosides	-	-	-	-
Alkaloids	-	+	-	-
Terpenoids	+	+	+	+
Tannins	+	+	-	+
Flavonoids	+	-	+	+
Saponins	-	+	-	+
Anthraquinone	-	-	-	-

+ present

- absent

Figure 1: Gallic acid calibration curve

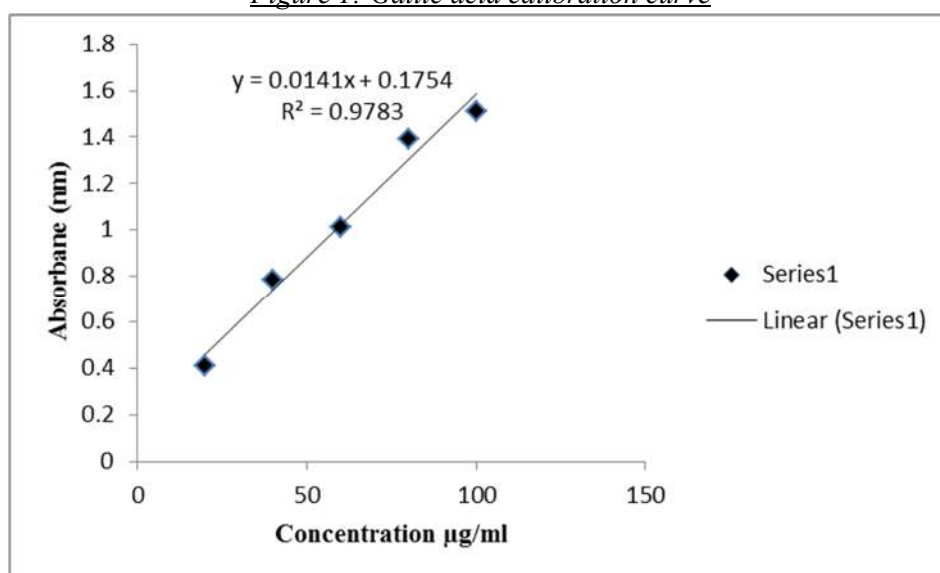


Table 3: Total phenolic content of fractions and gallic acid equivalent

Fractions	Total Phenolic (mg)±SEM	Gallic acid equivalent (mg GAE/g)
n-hexane	2.03±0.00152	0.507
Dichloromethane	5.72±0.00038	1.429
Ethyl acetate	13.16±0.0043	3.291
Methanol	16.66±0.0012	4.165

Table 4: 2,2 – Diphenyl – 1 – picrylhydrazyl (DPPH) radical scavenging activity

CONC ($\mu\text{g/mL}$)	Ascorbic acid	n-hexane fraction	Dichloromethane fraction	Ethyl acetate fraction	Methanol fraction	Volatile oil
20	0.0550 \pm 0.0010	0.2813 \pm 0.0008*	0.3200 \pm 0.0012*	0.3267 \pm 0.0008*	0.2843 \pm 0.0008*	0.1773 \pm 0.0007*
	+93%	+64%	+59%	+58%	+64%	+79%
40	0.0537 \pm 0.0008	0.2703 \pm 0.0007*	0.3173 \pm 0.0007*	0.2210 \pm 0.0006*	0.2570 \pm 0.0006*	0.1763 \pm 0.0008*
	+93%	+67%	+60%	+72%	+67%	+79%
60	0.0553 \pm 0.0003	0.2603 \pm 0.0007*	0.2830 \pm 0.0006*	0.0973 \pm 0.0008*	0.2230 \pm 0.0012*	0.1743 \pm 0.0009*
	+93%	+67%	+64%	+87%	+72%	+80%
80	0.0533 \pm 0.0007	0.2217 \pm 0.0003*	0.2243 \pm 0.0009*	0.0950 \pm 0.0006*	0.2000 \pm 0.0006*	0.1737 \pm 0.0009*
	+93%	+72%	+72%	+88%	+74%	+81%
100	0.0533 \pm 0.0003	0.1987 \pm 0.0003*	0.2223 \pm 0.0009*	0.0833 \pm 0.0009*	0.1977 \pm 0.0009*	0.1713 \pm 0.0007*
	+93%	+75%	+72%	+89%	+76%	+80%

Values represent Mean \pm S.E.M

Significance related to control: ^c $p < 0.01$; * $p < 0.001$

($n=3$); ns=not significant

Table 5: FRAP for ascorbic acid and fractions and isolates from *T. tetraptera* Taub

CONC $\mu\text{g/MI}$	Ascorbic acid	n-hexane fraction	Dichloromethane fraction	Ethyl acetate fraction	Methanol fraction	Volatile oil
20	0.3447 \pm 0.0003	0.2910 \pm 0.0006*	0.3010 \pm 0.0006*	0.3550 \pm 0.0006 ^c	0.3177 \pm 0.0007*	0.3270 \pm 0.0006 ^c
40	0.3617 \pm 0.0003	0.3233 \pm 0.0007*	0.3287 \pm 0.0003*	0.3673 \pm 0.0007 ^c	0.3403 \pm 0.0007*	0.3493 \pm 0.0003*
60	0.4103 \pm 0.0003	0.3587 \pm 0.0007*	0.3163 \pm 0.0009*	0.3797 \pm 0.0007*	0.4667 \pm 0.0007*	0.3943 \pm 0.0003 ^c
80	0.4523 \pm 0.0003	0.3913 \pm 0.0003*	0.3647 \pm 0.0013*	0.3837 \pm 0.0003*	0.4987 \pm 0.0003*	0.4097 \pm 0.0007*
100	0.4753 \pm 0.0003	0.4073 \pm 0.0003*	0.3723 \pm 0.0003*	0.4013 \pm 0.0003*	0.5657 \pm 0.0007*	0.4553 \pm 0.0003*

Values represent Mean \pm S.E.M

Significance related to control: ^c $p < 0.01$; * $p < 0.001$

($n=3$); ns=not significant

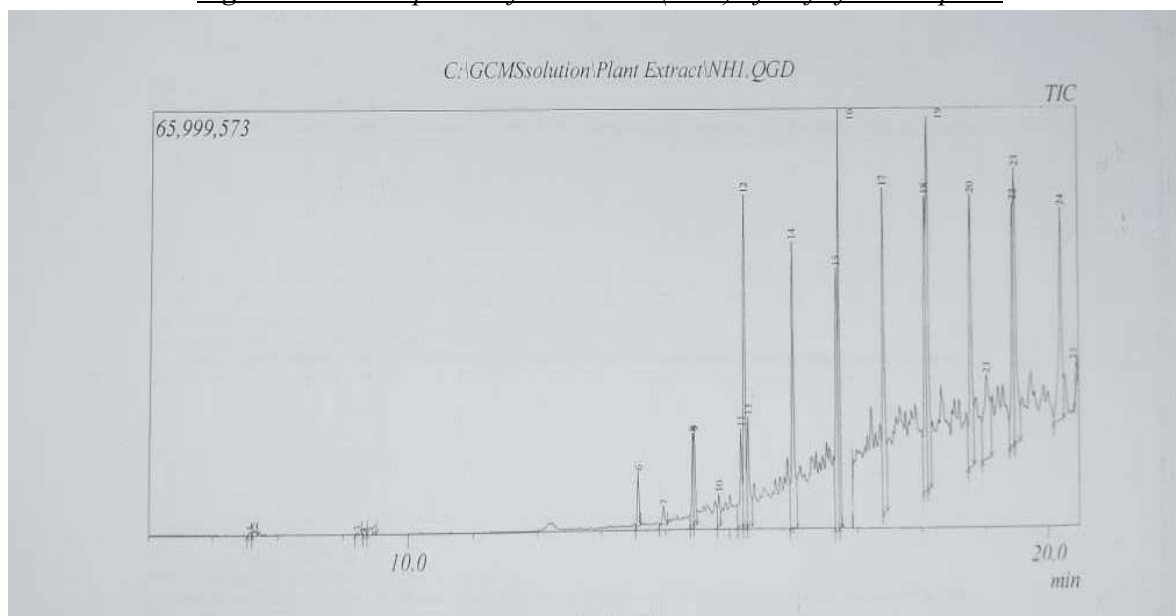
Figure 5: Gc-Ms spectra of volatile oil (NH1) of leaf of *T. tetraptera*

Table 6: Report peaks of gas chromatography-mass spectroscopy of volatile oil (NH1)

Peak	R.Time	Area	Area (%)	Height Molecular	Name(%)	Formula
1	7.57	1180683	0.08	0.08	C ₁₀ H ₁₄	Benzene, 1-methyl-3-
2	7.634	2633630	0.18	0.14	C ₁₀ H ₁₆	Limonene
3	9.217	711221	0.05	0.05	C ₁₀ H ₁₈	l-Menthone
4	9.34	188085	0.01	0.02	C ₁₀ H ₁₈	Cyclohexanone, 5-methyl-2-(1-methylethyl)
5	9.459	620051	0.04	0.05	C ₁₀ H ₂₀	Levomenthol
6	13.569	13115807	0.88	1.37	C ₁₆ H ₃₄	Hexadecane
7	13.967	7179999	0.48	0.47	C ₄₄ H ₉₀	Tetratetracontane
8	14.431	25305593	1.71	2.33	C ₁₉ H ₄₀	Nonadecane
9	14.453	24312582	1.64	2.36	C ₁₉ H ₄₀	Pentadecane, 2,6,10,14- tetramethyl
10	14.836	11487502	0.77	0.81	C ₂₅ H ₅₂	2-methyltetracosane
11	15.19	39366890	2.65	2.47	C ₁₈ H ₃₆	1-Octadecene
12	15.253	108596434	7.32	8.48	C ₂₀ H ₄₂	Eicosane
13	15.302	40416266	2.72	2.78	C ₂₀ H ₄₂	Eicosane
14	16.013	112838101	7.6	7.32	C ₂₀ H ₄₂	Eicosane
15	16.701	104307842	7.03	6.66	C ₂₃ H ₄₆	9-Tricosene, (Z)-
16	16.748	88935007	5.99	10.8	C ₂₀ H ₄₂	Eicosane
17	17.435	122639326	8.27	8.31	C ₂₈ H ₅₈	Octacosane
18	18.089	126107100	8.5	7.54	C ₂₁ H ₄₄	1-Heneicosanol
19	18.129	132138201	8.91	9.59	C ₂₄ H ₅₀	Tetracosane
20	18.797	112418152	7.58	6.99	C ₂₇ H ₅₆	2-methylhexacosane
21	19.035	84854245	5.72	2.15	C ₅₄ H ₁₀₈	Tetrapentacontane, 1,54-dibromo
22	19.466	101078834	6.81	6.24	C ₃₅ H ₇₀	17-Pentatriacontene
23	19.494	109489582	7.38	7.09	C ₂₄ H ₅₀	Tetracosane
24	20.219	108867266	7.34	5.45	C ₂₇ H ₅₆	2-methylhexacosane
25	20.464	5048092	0.34	0.43	C ₅₄ H ₁₀₈	Tetrapentacontane, 1,54-dibro

Table 7: NHI Gas Chromatography-Mass Spectroscopy (GC-MS) Spectral Pharmacological Information

S/N	Volatile oil	Biological activity	Reference
1	Benzene,1-methyl-3-(1-methylethyl)-	Nothing reported	
2	Limonene	Promotes weight loss, anticancer, antioxidant	[32]
3	l-Menthone	Antibacterial, antifungal	[33]
4	Cyclohexanone,5-methyl-2-(1-methylethyl)	Nothing reported	
5	Levomenthol	Analgesic	[34]
6	Hexadecane	Nothing reported	
7	Tetratetracontane	Antioxidant, antibacterial	[35]
8	Nonadecane	Antioxidant, antifungal, antibacterial	[36]
9	Pentadecane,2,6,10,14-tetramethyl-	Antibacterial, antioxidant	[37]
10	2-methyltetracosane	Antioxidant	[38]
11	1-Octadecene	Nothing reported	
12	Eicosane	Antibacterial	[39]
13	Eicosane	Antibacterial	
14	Eicosane	Antibacterial	
15	9-Tricosene, (Z)-	Antifungal, antioxidant	[40]
16	Eicosane	Antibacterial	
17	Octacosane	Antibacterial	[41]
18	1-Heneicosanol	Antibacterial, antifungal	[42]
19	Tetracosane	Antioxidant, allelopathic, antibacterial	[43]
20	2-methylhexacosane	Antioxidant, antibacterial	[44]
21	Tetrapentacontane, 1,54-dibromo-17-Pentatriacontene	Antioxidant, antitumour, antiviral, hypolipidemic	[45]
22	Tetracosane	Antioxidant, allelopathic, antibacterial	
23	2-methylhexacosane	Antioxidant, antibacterial	[46]
24	Tetrapentacontane, 1,54-dibromo-	Nothing reported	

DISCUSSION

T. tetraptera Taub has played significant roles in the management of numerous health conditions. Antidiabetic, antipyretic, anticancer, antioxidant, analgesic, anti-inflammatory, anticonvulsive and antitussive properties have been reported with different parts of the plant [47]. The antioxidant activity of the leaf was evaluated in this study.

In Table 1, the methanol fraction gave the highest yield of 24.7 g and this is followed by n-hexane fraction which yielded 18.8 g. The yield of the ethyl acetate fraction showed 7.2 g while dichloromethane fraction gave 11.4 g, respectively. The phytochemical screening of various fractions of T. tetraptera showed the presence of secondary metabolites such as terpenoids, tannins, flavonoids, saponins, phenols, and alkaloids,

respectively, others such as glycosides, cardiac glycosides and anthraquinones were reported absent.

The result of the total phenolic content differed from each fraction (Table 3). Data from table 3 revealed that n-hexane fraction had the least phenolic content of 2.03 mg while methanol fraction with 16.66 ± 0.0012 mg had the highest phenolic content among the fractions. The total phenolic content of ethyl acetate fraction and dichloromethane fraction were 13.16 ± 0.0043 mg and 5.72 ± 0.00038 mg, respectively. The gallic acid equivalent of n-hexane fraction was the least and was recorded as 0.507 mg GAE/g. The methanol extract, ethyl acetate fraction, and dichloromethane fraction showed gallic acid equivalent of 4.165 mg GAE/g, 3.30 mg GAE/g, and 1.429 mg GAE/g, respectively. These

figures are in harmony with the reported total phenolic contents.

Determination of the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity showed gradual increase with in a dose-related manner for the various fractions and volatile oil as shown in Table 4. Ascorbic acid recorded consistent percentage inhibition of 93% at all concentration different from both fractions and volatile oil which showed increasing percentage inhibition with increase in concentration. The average percentage inhibition recorded were; 69% for n-hexane fraction, dichloromethane fraction (65.4%), ethyl acetate fraction (78.8%), methanol fraction (70%) and volatile oil (NH1) (80%) with the ethyl acetate with 78.8% percentage inhibition as the highest and dichloromethane fraction with 65.4% as the lowest. The volatile oil showed 80% which is 13% less active when compared with the standard (Ascorbic acid 93%), this result was statistically significant at $p < 0.001$. This is consistent with the findings of an earlier report [48].

The ferric reducing power (FRAP) assay demonstrated a similar activity with the result of the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity with increasing percentage inhibition based on increasing concentration. The methanol fraction recorded the highest absorbance of 0.5657 ± 0.0007 nm at $100 \mu\text{g/mL}$ comparable to ascorbic acid which recorded 0.4753 ± 0.003 nm at $100 \mu\text{g/mL}$ and these were statistically significant at $p < 0.001$. This result supports an earlier report [49]. This evaluation supports the use of *T. tetraptera* Taub leaf as an antioxidant and is consistent with other findings in [50] [51].

The gas chromatography-mass spectroscopy spectra of sample NH1 (volatile oil) as shown in Figure 2 shows the presence of 25 peaks and twenty volatile oils which are mainly terpenoids, esters, fatty acids, alkanes, phenols, alcohols and benzene derivatives. The identified volatile oils include; Benzene, 1-methyl-3-(1-methylethyl)-, Limonene, 1-Methone, Cyclohexanone, 5-methyl-2-(1-methylethyl)-, Levomenthol, Hexadecane, Tetratetracontane, Nonadecane, Pentadecane, 2,6,10,14-tetramethyl, 2-Methyltetracosane, 1-Octadecene, Eicosane, 9-Tricosene, Octacosane, 1-Heineicosanol, Tetrapentacontane, 1,54-dibromo-, 17-Pentatriacontene, Tetracosane, 2-Methylhexacosane, and Tetrapentacontane, 1,54-dibromo.

Volatile oils have played significant roles as antioxidant agents. Over 50% of these volatile oils have been

reported as antioxidant agents as shown in Table 7. Phytochemical constituents of the volatile oil have shown valid applications as antibacterial, antioxidant, and anticancer, antifungal, antimicrobial and anti-inflammatory agents [57]. Tetracosane (C₂₄H₅₀) and 1-heneicosanol (C₂₁H₄₄) have the highest area (8.91 and 8.50%) respectively. 1-menthone and Cyclohexanone, 5-methyl-2-(1-methyl)- (2 have same molecular formula (C₁₀H₁₈) as does nonadecane and pentadecane, 2,6,10,14-tetramethyl-.

CONCLUSION

Total phenolic content assay of various fractions indicated ethyl acetate fraction had highest amount of phenolic components, determination of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity showed 69%, 65.4%, 78.8%, 90%, 93%, 80%, 77.4% and 71.2% percentage inhibition for n-hexane fraction, dichloromethane fraction, ethylacetate fraction, methanol fraction, ascorbic acid, and the volatile oil, respectively. The ferric reducing power assay (FRAP) also showed a minimum of 557% percentage inhibition, the nitric oxide assay showed an average of 57.85% percentage inhibition. The gas chromatography-mass spectral the volatile oil showed the presence of 31 and 25 volatile oils respectively. Over 40% of these volatile oil content have been reported to possess antioxidant properties. These results support the use of various fractions and volatile oil (NH1) of *T. tetraptera* Taub as an antioxidant agent.

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