

Phytochemical Screening, GC-MS Characterisation and Antioxidant Activity of N-Hexane Fraction of *Microtrichia perotitii* DC

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Abstract

Microtrichia perotitii DC belongs to the family Asteraceae (Compositae) and it is an herb found in the West African countries. The herb has been used ethnomedicinally to treat children's rashes, burns, wounds, jaundice, rheumatism, diarrhea, and skin conditions. The n-hexane fraction of the ethanolic extract of the plant was investigated for the presence of secondary metabolites, characterisation as well as its antioxidant activity with a view to exploring other significant information that would be of benefit to scientific study. The n-hexane extract was subjected to partitioning, phytochemical screening using standard methods as well as spectroscopic characterizations using UV, FTIR and GC-MS analysis including antioxidant activity. The result of the phytochemical screening revealed the presence of alkaloids, tannins, saponins, phenols, glycosides, steroids, terpenoids and flavonoids, but anthraquinones and phlobatannins were absent. The spectroscopy analysis showed a high degree of conjugation and it also reveals the presence of Hydroxyl (O-H), carbonyl (C=O) and methyl (C-H) groups, with the compound, Pentadecanoic acid, 14-methyl-, methyl ester, having the highest abundance while the antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Nitric oxide (NO), radical scavenging assays exhibited antioxidant activity with an IC₅₀ value of 4.61mg/mL but less than ascorbic acid which has an IC₅₀ value of 0.84mg/mL. The phytochemical compounds present in the plant could be responsible for its antioxidant property which suggests the use of the plant in the treatment of diseases induced by free radicals. In the light of the importance of this herb in local applications, the research was aimed at exploring the scientific evidences to support the local claims as well as improving information of the efficacy of the plant.

Keywords: *Microtrichia*; *perotitii*; phytochemical; GC-MS; DPPH.

INTRODUCTION

There is a long history of using herbal therapy to treat many diseases. Man has used them for the treatment of disease for a very long time, and they are still commonly used today. Before the advent of conventional medicine, humans had long amassed a body of empirical data pertaining to the therapeutic benefits of regional flora. These herbalists and their apprentices have amassed a substantial body of knowledge on medicinal plants over periods of trial, error, and success. The first generation of plant medications, according to Iwu *et al.* (1999), was often simple botanicals used in a more or less raw form. Several potent medications that are utilized in their natural form have been chosen as therapeutic agents (Eluoba *et al.*, 2005; Abdullahi *et al.*, 2011; Abdullahi *et al.*, 2013).

Microtrichia perotitii DC belongs to the family Asteraceae (Compositae) and it is a herb found in West African countries (Hutchinson & Dalzie, 1963). In Nigeria it is found in the northern part of the country where it is known as *Maijankai* or *Sawun keke* in Hausa, *Osete* in Igbara and *Shaware pepe* in Yoruba.

Traditionally, *M. perotitii* is used for treating pain related diseases such as toothaches, cuts and burns and rashes in children. Others include, skin disease, rheumatism, diarrhea and jaundice (Magaji, 2003; Abdullahi *et al.*, 2011).



Figure1. Picture of *Microtrichia perotitti* DC.

MATERIALS AND METHODOLOGY

Sample collection

The herb *Microtrichia perotitii* was collected from Zaria Kaduna state, northern Nigeria in July 2023. The fresh herb has already been identified and authenticated by Mal. Shehu Gallah Umar, of herbarium unit of the Department of Biological sciences, Kaduna State University KASU and was given specimen number KASU/BSH/9002 for future references. The fresh herb was allowed to dry under the shade for three weeks and later reduced to coarse powder with a traditional pestle and mortar.

Extraction of sample

150g of the powdered herb (*Microtrichia perotitii*) was macerated in 1,500 ml of ethanol for 72 Hrs at room temperature with intermittent shaking. The residue was extracted twice with the same fresh solvent and later combined and filtered using whatman filter paper (5 mm) under suction. The extract was concentrated under reduced pressure (Rotary evaporator) at temperature of 45 °C. The extract was stored in desiccators until needed for the work (Abdullahi & Mainul, 2020).

Phytochemical screening

The crude ethanol extract of *Microtrichia perotitii* was evaluated for the presence of its phytoconstituents by using standard methods Trease and Evans, 2009, Sofowora, 1993 to test for, carbohydrates(Molisch's test), Tannins (Ferric chloride Test), Phlobatannins, Flavonoids; (Shinoda's test), Saponins (Frothing test), Alkaloids (Dragendoff's reagent), Cardiac Glycosides(Keller-Kiliani test), Steroids (Salkowski's test), Terpenoids (Liebermann-Burchard test) and Anthraquinones (Borntrager's Test).

Solvent partitioning

Fractionation of the crude ethanol extract of *Microtrichia perotitii* was done according to the scheme proposed by (Qing-Wen, et al. 2018) but modified. Thus 25 g of the crude was suspending crude in 300 ml of water separately and then partitioned with hexane, n-Butanol, and ethyl acetate that is in an order of increasing polarity by using separating funnel. All the three fractions obtained were dried by evaporation using rotary evaporator and then later weighed.

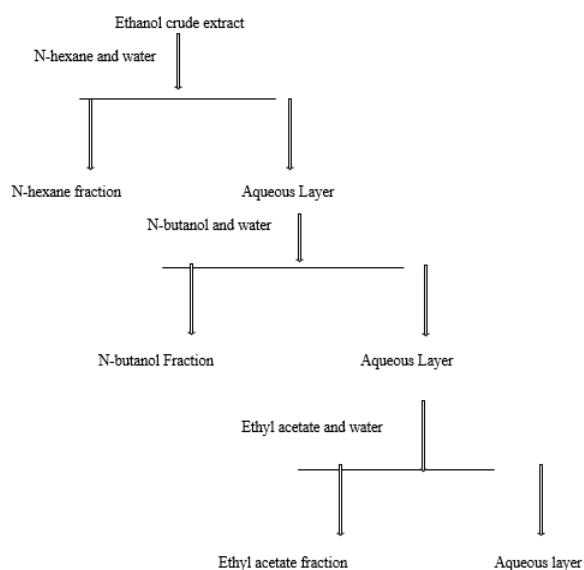


Figure 2. Scheme for Solvent Partitioning (Qing-wen, et al. 2018).

Antioxidant Activities

DPPH Radical Scavenging Activity Assay

The free RSA of the N-hexane fraction of *Microtrichia perotitii* was tested using a 2,2-diphenyl-1-picryl hydrazyl (DPPH) technique. A total of 24 milligrams of DPPH were dissolved in 100 mL of methanol to make the stock solution. Filtration of DPPH stock solution using methanol yielded a usable mixture with an absorbance of around 0.973 at 517 nm. In a test tube, 3 mL DPPH workable solutions were combined with 100 µL. Three milliliters of solution containing DPPH in 100 µL of methanol is often given as a standard

(Goldschmidt, et al 1922). After that, the tubes were kept in complete darkness for 30 min. The absorbance was therefore determined at 517 nm. The following formula was used to compute the percentage of antioxidants or RSA:

$$\% \text{ of antioxidant activity} = [(Ac - As) \div Ac] \times 100$$

where:

Ac : Control reaction absorbance;

As : Testing specimen absorbance.

Nitric oxide radical scavenging assay

The procedure was adopted with some modifications to determine the scavenging activity of the plant extracts against nitric oxide radical. Nitric oxide was generated from sodium nitroprusside and measured by the Greiss reaction. Ascorbic acid was used as a standard. Ascorbic acid inhibits induction of nitric oxide synthase and is a naturally occurring direct scavenger of nitric oxide. It reduces the amount of nitrite formed between oxygen and nitric oxide generated from sodium nitroprusside. The absorbance was measured at 596 nm and the percentage of antioxidant activity was calculated using the formula in the equation (Elizabeth & Rao, 1990; Ebrahimzadeh *et al.*, 2008).

$$\% \text{ of antioxidant activity} = [(Ac - As) \div Ac] \times 100$$

Where:

Ac : Control reaction absorbance;

As : Testing specimen absorbance.

Chromatographic Procedure

Gas Chromatography-Mass Spectrometry Analysis

GC-MS analysis was carried out on a GC system comprising a Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) instrument; Shimadzu GCMS-QP2010, employing the following conditions: Column Elite-1 fused silica capillary column (30×0.25 mm ID×1EM df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70 eV; helium (99.999%) as carrier gas at a constant flow of 1ml/ minute and a sample injection volume of 1 µl which was employed (split ratio of 10:1) injector temperature 250°C; ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 minutes), with an increase of 10°C/minute, to 200°C, then 5°C/minute to 280°C, ending with a 9 minutes isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 40 to 550 Da. Total run time was 30 min. The compounds were then identified from the GC-MS peaks, using

library data of the corresponding compounds. GC-MS was analyzed using electron impact ionization at 70 eV and data was evaluated using total ion count (TIC) for compound identification and quantification. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS library using NISP Search. The relative % amount of each component was calculated by comparing its average peak area to the total areas. Measurement of peak areas and data processing were carried out by Turbo-Mass-OCPTVS-Demo SPL software (Duraisamy *et al.*, 2015).

UV Analysis

Two ml of the sample was placed in beaker of 50 ml then 5 ml of n-hexane was added and shaken thoroughly to reach solution consistency. The cuvette was filled with sample solution and inserted in the device and the results were recorded. The cell was emptied and was read in the instrument at wave length 200 - 800 nm). The absorptions results were recorded (Jain *et al.*, 2016).

FTIR Analysis

Fourier-transform infrared spectroscopy (FT-IR) analysis was performed using a Perkin Elmer Spectrophotometer system (PerkinElmer, Waltham, MA, USA) over a frequency range of 400–4000 cm, which was used to detect the characteristic peaks (Dhivya & Kalaichelvi, 2016; Jain, *et al.*, 2016).

RESULTS AND DISCUSSION

Results

The results obtained from the Research have shown promising outcomes and thereby suggested the importance of being used locally. Similarly, it has provided an insight in to further Research in to certain areas beyond the scope of this study. The results below are a testimony to the outcome.

Table 1. Phytochemical analysis of n-hexane fraction of ethanolic extract of *Microtrichia perotitii*

Constituent	Test	Observation	Inference
Carbohydrates	Molisch test	Purple colour at interphase	+
Tannins	Ferric Chloride test	Greenish black coloration	+
Phlobatannins	Hydrochloric acid	No change	-
Flavonoids	Shinoda test	A red coloration	+
Saponins	Frothing test	Frothing which persist	+
Alkaloids	Dragendorff test	Orange yellow precipitate	+
Cardiac glycosides	Keller-Kiliani test	Purple ring at interphase	+
Steroids	Salkowski test	Pale green colour in the acetic layer	+
Terpenoids	Lieberman-Burchard test	Red colour ring	+
Anthraquinones	Bontragers test	Bright pink colouration	-

Key: + = Present and - = Absent

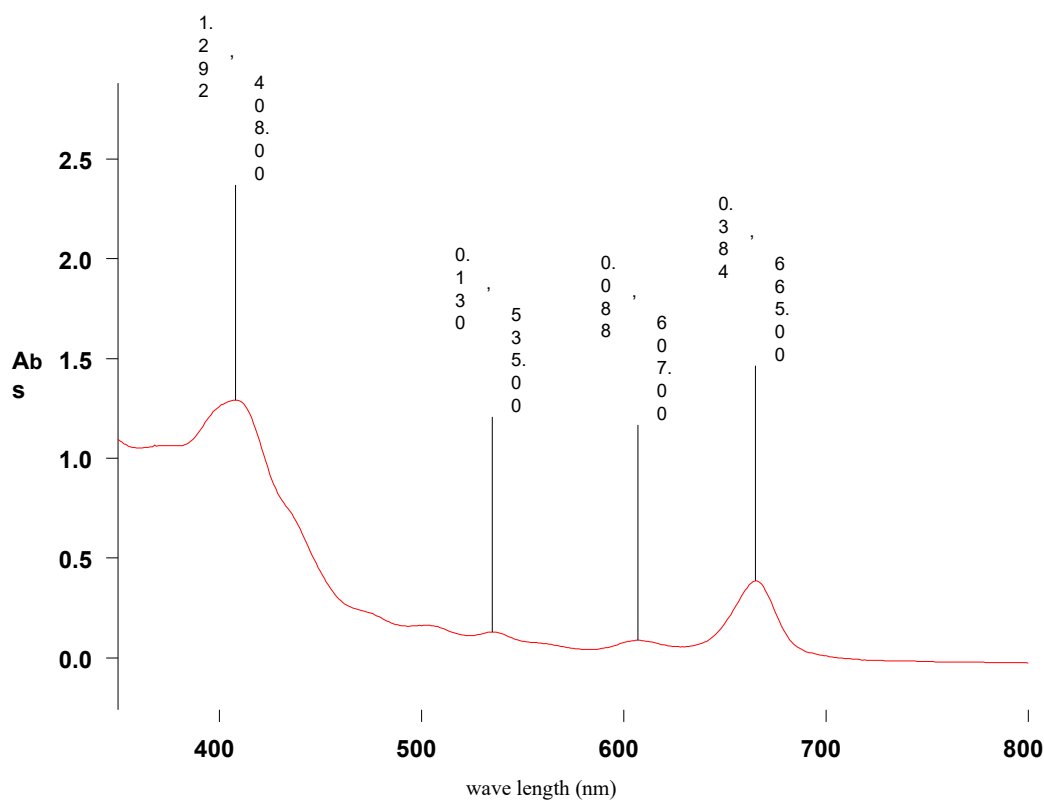


Figure 3. UV spectroscopy of n-hexane fraction of *M. perotiti*.

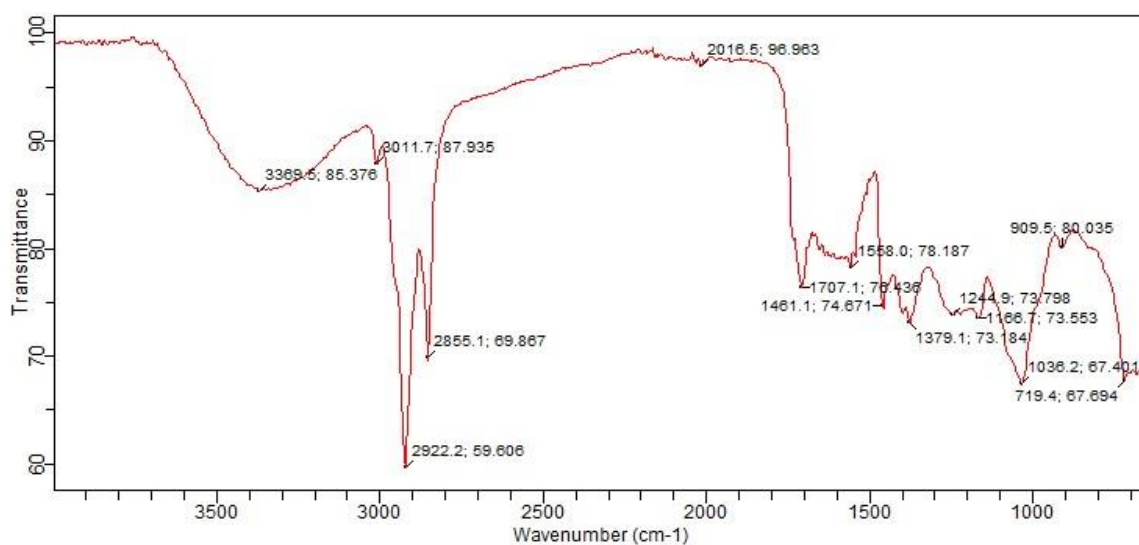


Figure 4. FT-IR spectroscopy of n-hexane fraction of *Microtrichia perotiti*.

Table 2. GC-MS Analysis.

No	Rt	Area %	Name	Mol. wt	Structure
1	11.2367	0.3862	Dodecanoic acid, methyl ester	214.1	<chem>CCCCCCCCCCCC(=O)OC</chem>
2	15.7246	0.3286	Methyl tetradecanoate	242.4	<chem>CCCCCCCCCCCCCCCC(=O)OC</chem>
3	18.2053	0.2057	2-Pentadecanone, 6,10,14-trimethyl-	268.4	<chem>CC(C)CCCC(C)CCCC(C)CCCC=O</chem>

Table 2. Cont.

No	Rt	Area %	Name	Mol. wt	Structure
4	19.3304	0.9035	(Z)-Methyl hexadec-11-enoate	268.4	
5	19.8907	29.1986	Pentadecanoic acid, 14-methyl-, methyl ester	270.0	
6	20.6915	0.7743	cis-Vaccenic acid	282.4	
7	20.94	3.589	n-Hexadecanoic acid	256.4	
8	21.2237	3.6954	Hexadecanoic acid, ethyl ester	284.5	
9	22.909	4.2005	9,12-Octadecadienoic acid, methyl ester	280.4	
10	23.1473	25.6492	9-Octadecenoic acid (Z)-, methyl ester	296.5	
11	23.4392	1.0329	Phytol	296.5	
12	23.7034	3.5635	Methyl stearate	298.5	
13	24.1454	2.9496	Linoleic acid ethyl ester	310.5	
14	24.3212	0.7167	9-Tetradecen-1-ol, (E)-	212.3	
15	26.635	0.3222	cis-Methyl 11-eicosenoate	324.5	
16	27.1937	0.8927	Eicosanoic acid, methyl ester	340.5	
17	30.4322	1.5348	Docosanoic acid, methyl ester	354.6	
18	32.589	1.8744	Oleic Acid	282.4	
19	33.4393	5.0067	beta-Sitosterol	414.7	
20	33.974	0.1669	Cyclopentane undecanoic acid	254.4	
21	34.6516	0.4221	2-Dodecen-1-yl(-)succinic anhydride	266.3	
22	36.3376	4.7332	cis-Vaccenic acid	282.4	
23	36.4854	1.2812	Erucic acid	338.5	
24	37.386	6.5723	1,4-Dimethyl-7-oxo-4,7-dihydro-triazolo(3,4-c)triazine		

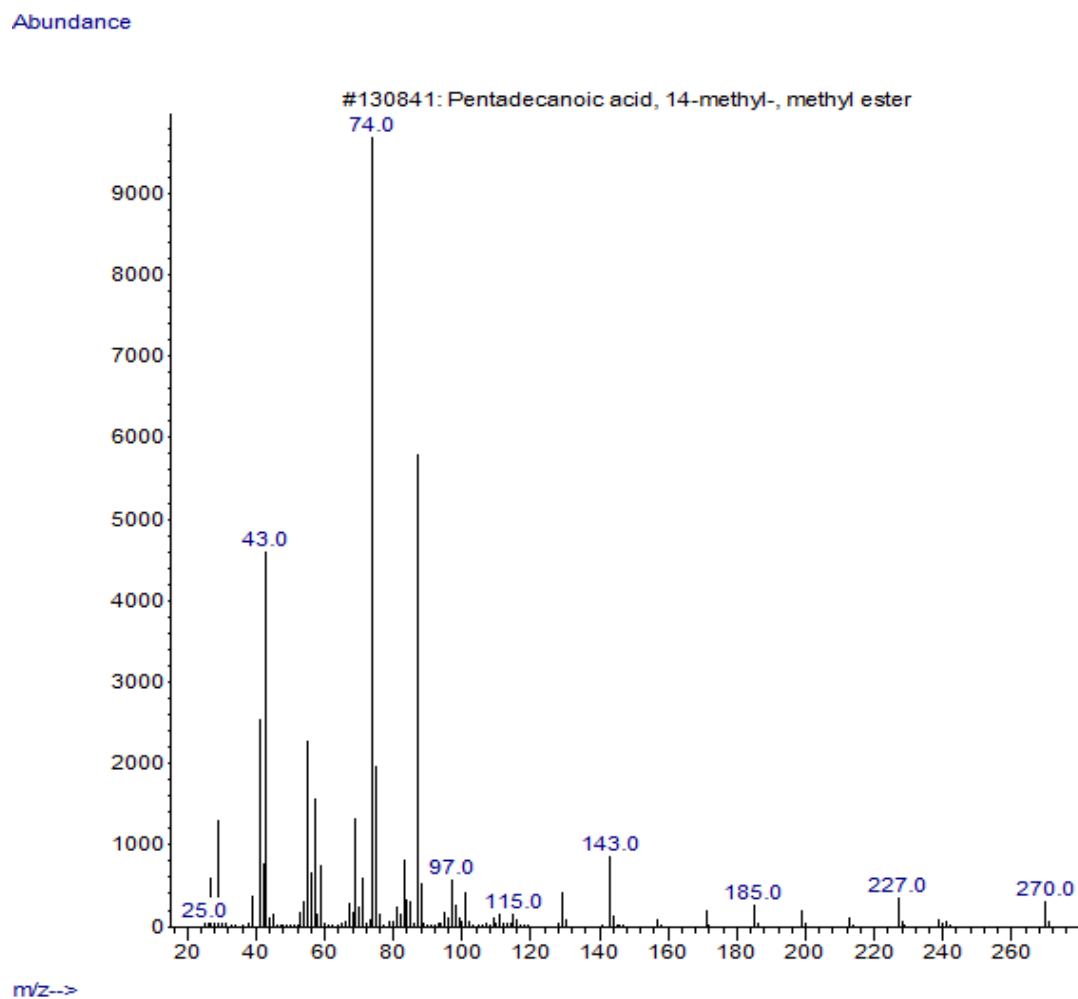


Figure 5. Fragmentation of the highest occurring compound (pentadecanoic acid, 14-methyl-, methyl ester).

Table 3. DPPH Scavenging activities.

	Concentration Mg/ml	Absorbance at 517NM	% Antioxidant
Blank		2.9015	-
Control	40	0.381	86.87
Sample	40	1.2931	55.43

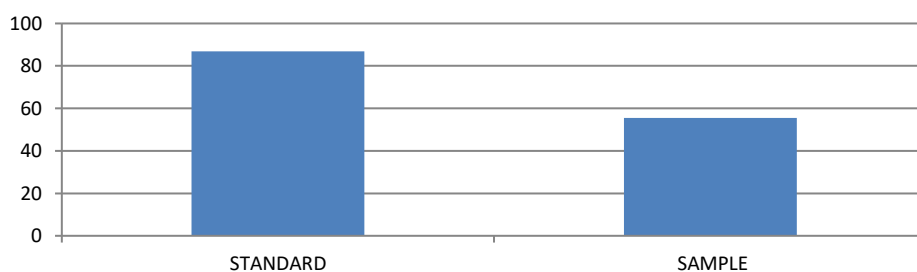


Figure 6. Percentage inhibition of the DPPH scavenging activity of sample and ascorbic acid.

	Absorbance at 542nm				% Antioxidant (RSA)			
	10ml/ml	20mg/ml	30mg/ml	40mg/ml	10ml/ml	20mg/ml	30mg/ml	40mg/ml
Sample	1.7340	1.4680	1.4680	3.0982	53.64	60.75	60.75	17.16
Control	0.4142	0.3557	0.3913	0.4057	89.89	90.51	89.53	89.15
Blank	3.74							

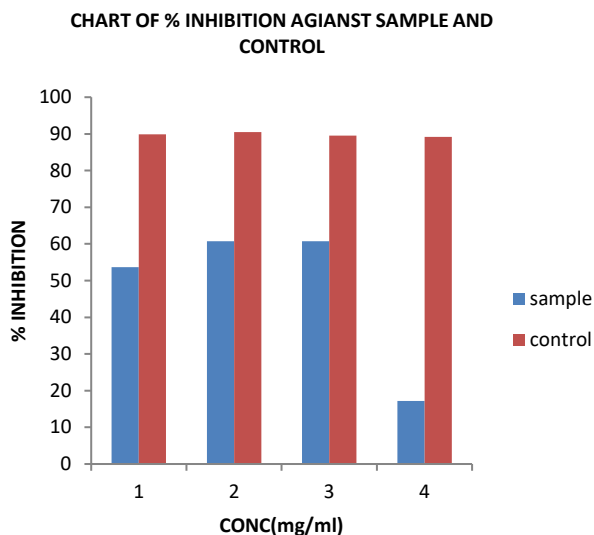
Table 4. Nitric oxide (NO) scavenging activity.

Sample			Ascorbic Acid		
CONC (mg/ml)	% RSA	IC ₅₀	CONC (mg/ml)	% RSA	IC ₅₀
10	53.64	5.98	10	88.93	0.34
20	60.75	5.07	20	90.48	0.67
30	60.75	4.15	30	89.55	1.01
40	17.17	3.24	40	89.14	1.34

Table 5. Mean IC₅₀ value of Sample and Standard.

	Mean IC ₅₀ Value (mg/mL)
Sample	4.61
Standard	0.84

The IC₅₀ (Inhibitory concentration) values, representing the amount of extract required to decrease the absorbance of DPPH by 50% was calculated from the percentage radical scavenging activity.

**Figure 7.** Percentage inhibition of nitric oxide of sample and ascorbic acid.

Discussion

The discussions below were driven from the outcomes of the results with specific comparisons as the case may be.

Preliminary Phytochemical Screening

The preliminary phytochemical screening of the crude ethanol extract of *Microtrichia perotitii* revealed the presence of secondary metabolites of therapeutically importance. They included alkaloids, saponins tannins, flavonoids, terpenoids, cardiac glycosides while Anthraquinones and phlobatannins were not detected. (Idris *et al.*, 2009; Junaid *et al.*, 2020). Therefore, the N-hexane fraction of the *Microtrichia perotitii* DC contains phytochemicals that contribute significantly to its antioxidant activity. In other studies important phytochemicals identified were phenols, flavonoids, and triterpenes, that is alpha-amyrin acetate, which has been

isolated from the plant (Abdullahi *et al.*, 2018; Abdullahi *et al.*, 2011).

Spectroscopy Analysis

UV spectroscopy

The n-hexane fraction of the sample's UV spectroscopy yielded the highest wavelength of 665 nm. Organic molecules absorb light in the UV region of the electromagnetic spectrum, especially those with a high degree of conjugation (Dyah *et al.*, 2016; Tarun *et al.*, 2020).

Table 6 UV Spectroscopy of n-hexane fraction of n-ethanolic extract of *Microtrichia perotitii*.

S/no	Wave length (nm)	Absorbance
1	665.00	0.384
2	607.00	0.088
3	535.00	0.130
4	408.00	1.292

FT-IR Spectroscopy

The FT-IR analysis of the n-hexane fraction of *Microtrichia perotitii* shows various bands, indicating the presence of different functional groups. Peaks 3369.5cm⁻¹ indicates strong stretching of a hydroxyl group (OH), band 2922.2cm⁻¹ indicates the presence of a methyl group (C-H), and band 1707.1cm⁻¹ indicates the presence of a carbonyl functional group (C=O) (Perveen & Zaib, 2013; Dyah, *et al.*, 2016).

Table 7. FTIR Spectroscopy of n-hexane fraction of ethanolic extract of *Microtrichia perotitii*.

No	Peaks /Bands (cm-1)	Functional Groups
1	3369.5	Alcohol (O-H)
2	3011.7	Alkenes (C=H)
3	2922.2	Methylene (C-H)
4	2855.1	Aliphatic (C-H)
5	2016.5	Alkynes (C≡C)
6	1707.1	Ketone (C=O)
7	1558.0	Alkene (C=C)
8	1461.1	Alkene (C=C)
9	1379.1	Ester (C-O)
10	1244.9	Ester (C-O)
11	1166.7	Ether (C-O)
12	1036.2	Trans (C-H)
13	909.5	Vinyl (C-H)
14	719.4	Alkene (C-H) cis

GC-MS Spectrometry

The GC-MS analysis of the n-hexane fraction of *Microtrichia perotitii* shows the presence of 27 compounds of which Pentadecanoic acid, 14-methyl-, methyl ester, has the highest abundance with about 29.1986% occurrence, followed by 9-Octadecenoic acid (Z)-, methyl ester with about 25.6492% abundance, 1,4-Dimethyl-7-oxo-4,7-dihydro-triazolo(3,4-c)triazine,(6.5723%),beta.-Sitosterol (5.0067%), cis-Vaccenic acid (4.7332%), 9,12-Octadecadienoic acid, methyl ester(4.2005), Hexadecanoic acid, ethyl ester (3.6954%), n-Hexadecanoic acid (3.589%), Methyl stearate (3.5635%), Linoleic acid ethyl ester (2.9496%). Other compounds with below 2% abundance are Docosanoic acid, methyl ester, Erucic acid, oleic acid, phytol, 2-Dodecen-1-yl(-)succinic anhydride, Cyclopentane undecanoic acid, Dodecanoic acid, methyl ester, Methyl tetradecanoate, 2-Pentadecanone, 6,10,14-trimethyl-Z)-Methyl hexadec-11-enoate, cis-Vaccenic acid, 9-Tetradecen-1-ol, (E)-, cis-Methyl 11-eicosenoate, and Eicosanoic acid, methyl ester. 14-methylpentadecanoic acid is a methyl-branched fatty acid that is pentadecanoic acid substituted by a methyl group at position 14. It is a biomarker for rheumatoid arthritis. It has a role as a biomarker and a mammalian metabolite. It is a branched-chain saturated fatty acid, a methyl-branched fatty acid, a long-chain fatty acid and a fatty acid 16:0. It is functionally related to pentadecanoic acid. It is a conjugate acid of a 14-methyl pentadecanoate (Njoku *et al.*, 2021).

Antioxidant Activity

DPPH activity

When free-radical DPPH interacts with an odd electron, the greatest absorption occurs at 517 nm (purple color). A free-radical scavenger antioxidant reacts to DPPH to form DPPHH, which has a lower absorbance than DPPH because of the lower amount of hydrogen. Compared to the DPPH-H state, this radical version causes decolourisation (a yellow hue) as the number of electrons collected increases.

It can be observed from Table 3 that the antioxidant activity of the n-hexane fraction of *Microtrichia perotitii* 40mg/ml shows 1.293 absorbance at 517nm with 55.43 percentage of antioxidant while 100mg/ml shows 1.5018 absorbance at 517 nm with 48.28 percentage of antioxidant. (Siddhartha *et al.*, 2022) The phytochemicals in *M. perotitii* exhibit strong radical scavenging properties, which are crucial for combating oxidative stress as well as reducing cellular damage. The antioxidant activity is often measured using assays such as DPPH and nitric oxide scavenging, where extracts show significant inhibition rates (Bisso *et al.*, 2022; Hamsalakshmi *et al.*, 2020).

Nitric oxide activity

Nitric oxide is a potent pleiotropic mediator of physiological processes such as smooth muscle radical relaxation, inhibition of platelet aggregation, neuronal signaling, and regulation of cell mediated toxicity. It is a diisable free radical which plays many important roles as an e ector molecule in diverse biological systems (vasodilation, neuronal, messenger, and antimicrobial and antitumor activities). Ascorbic acid used as the standard for NO radical scavenging in this analysis showed maximum activity with a percentage RSA (%RSA) of 90.51% and a maximum IC50 value of 1.34 while the 20mg/ml and 30mg/ml concentration of the n-hexane fraction of *Microtrichia perotitii* shows a maximum RSA value of 60.75% and a maximum IC50 value of 5.98. The mean IC50 value of the sample is 4.61mg/mL while the IC50 value of the standard is 0.84mg/ML (Avani, 2010). Specifically, the n-hexane extract demonstrated an IC50 value comparable to other plant extracts, suggesting its efficacy in scavenging free radicals (Putu *et al.*, 2023).

CONCLUSION

The phytochemical screening of the crude ethanol extract of *Microtrichia perotitii* revealed the presence of carbohydrate, saponins, flavonoids, terpenoids, alkaloids, and cardiac glycosides. The antioxidant activity reveals that the N-hexane fraction of *m perotitii* inhibits free radicals. The presence of different tannins, phenolics, carbohydrates, flavonoids etc, may be responsible for the antioxidant property of the sample. The antioxidant properties were highest at a concentration of 30mg/ml and lowest at 40mg/ml. This study shows that the N-hexane fraction of *Microtrichia perotitii* has antioxidant properties and might be used in the treatment of diseases induced by free radicals.

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Authors' Contributions: Dr Abdullahi Maikudi Nuhu designed the research project as well the interpretation of the spectra. Istifanus and Idris participated fully in the laboratory activities including travelling to collect the plant sample. All of us the Authours have read and approved the final manuscript and there were no ambiguities.

Competing Interests: The Authours wish to declare without any uncertainty that there was no competing interest with regards to this work.

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