Journal of Complementary and Alternative Medical Research

3(3): 1-10, 2017; Article no.JOCAMR.35156
ISSN: 2456-6276

Phytochemical and Antioxidant Assessments of Three Fractions from Methanol Extract of *Spathodea campanulata* Beauv. Leaves

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Authors' contributions

This work was carried out in collaboration between all authors. Author DOM designed the study and supervised the analyses. Authors CEU and EOO performed the analyses. Author JKA carried out the IR and GC-MS of the isolated compounds in the US. Authors CEU and DOM provided the analysis data interpretation for the isolates, managed the literature searches and wrote the first manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JOCAMR/2017/35156
Editor(s):
(1) Ramadoss Karthikeyan, Department of Pharmacognosy, Vignan Pharmacy College, Andhra Pradesh, India.
Reviewers:
(1) Roselena Silvestri Schuh, Federal University of Rio Grande do Sul, Brazil.
(2) Vanessa de Andrade Royo, Universidade Estadual de Montes Claros, Brazil.
Complete Peer review History: http://www.sciencedomain.org/review-history/20324

Original Research Article

Received 29th June 2017
Accepted 21st July 2017
Published 2nd August 2017

ABSTRACT

Aims: To screen hexane, ethyl acetate and methanol fractions of the methanol extract of *Spathodea campanulata* leaves for secondary metabolites, to isolate and to characterize constituents of the ethyl acetate fraction using GC-MS and IR and to determine the antioxidant activities of the three fractions.
Methodology: Methanol extract of *Spathodea campanulata* leaves was obtained by cold extraction, and partitioned into hexane, ethyl acetate and methanol fractions. Phytochemical screenings of the fractions were carried out using standard procedures to identify the class of constituents present in each of them. Ethyl acetate fraction was subjected to column chromatographic separations by gradient elution, and isolates were TMS (Trimethylsilyl)

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derivatised and characterized by GC-MS (Gas chromatography-mass spectrometry). Antioxidant content was also evaluated on the three fractions using 2, 2-diphenyl-picrylhydrazyl (DPPH) free-radical scavenging method. Percentage of inhibition and IC_{50} values were obtained for each fraction.

Results: Phytochemical screenings revealed presence of alkaloids, tannins, saponin, resins, phenol, cardiac glycosides, steroids, flavonoids, anthraquinones and terpenoids in the three fractions in varying concentrations. Alkaloids, resins, phenol and cardiac glycosides were found to be intense in the three fractions while phylobatannin was found to be absent in all the three fractions. Three compounds isolated from the ethyl acetate fraction were characterized based on MS and IR spectral interpretations as palmitic acid, ethylamine and caffeic acid. Percentage of inhibition of the three fractions indicates that they have substantial antioxidant activity with the standards at high concentration of 250 to 1000 µg/mL. The hexane fraction has the highest antioxidant activity with an IC_{50} of 178.46 µg/mL when compared to other fractions.

Conclusion: This paper reports phytochemical constituents and high antioxidant activity (at concentrations of 250 µg/ mL and above) of the African tulip tree (*Spathodea campanulata*) when compared to the standards. This has not been earlier reported in literature, our results supports its wide ethno-medicinal applications.

Keywords: Phytochemical screening; DPPH; antioxidant; GC-MS; Spathodea campanulata; bignoniaceae.

1. INTRODUCTION

Plants are good sources of drugs in many countries of the world; they are also utilized in ethno-medicine for treatment of diseases. The chemical compositions of these plants are responsible for the curative properties they display. These chemical contents include secondary metabolites [1,2] such as alkaloids, glycosides, corticosteroids, coumarins, flavonoids, essential oils and so on. Over 50% of modern clinical drugs are of natural origin [3] where plants play important roles in their development [4,5]. Nearly all culture and civilizations from ancient times to the present day have depended fully or partially on herbal medicines because of their effectiveness, low cost, low toxicity and acceptability [6].

Spathodea campanulata (Beauv.), a bignoniaceae, has many uses in folk medicine. The stem-bark is considered anti-hyperglycemic, anti-malaria, used in treating skin diseases, stomachaches and diarrhea. The leaves are utilized in curing kidney diseases, urethra inflammations and as an antidote against animal poisons [7]. Widespread use of *S. campanulata* in traditional medicine has stimulated more accurate pharmacological studies. Phytochemical compounds reported from different parts of *S. campanulata*, include spathodic acid, steroids, saponins, ursolic acid, tomentosolic acid and pectic substances from the stem bark [8-11]. Leaves contain spathodol, caffeic acid, other phenolic acids and flavonoids [12-14] while fruits have polyphenols, tannins, saponins and glucosides [11]. The flowers contain anthocyanins [15] while its floral nectar contains a complex mixture of triterpenoids and steroids [16].

Most plants owe their ethno-medicinal properties to presence of antioxidant compounds in them. Antioxidants play important roles as health protecting factor in organisms. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases such as cancer and heart problems. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables [17,18]. Plant sourced antioxidants contain vitamin C, vitamin E, carotenes, phenolic acids and so on, with potentials to reduce disease risk [19]. 2, 2-diphenyl-picrylhydrazyl (DPPH) is a stable free radical, which accept electron or hydrogen radical to become stable diamagnetic molecules [20,21]. The assay is widely used to evaluate antioxidant effect of plant extracts as well as pure compounds [22,23]. In recent times natural anti-oxidants are being studied since they prevent the formation of reactive species found to participate in a growing number of disorders, causing oxidative damage consequently altering the structure and function of cells of biological macro-molecules.

This research work is aimed at determining the phytochemical compositions and antioxidant evaluations of hexane, ethyl acetate and methanol fractions of methanol extract of *S. campanulata* leaves, which is scarce in literature.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

The solvents: n-hexane, ethyl acetate, methanol, chloroform were distilled before use. Other chemicals used include Fehling solution A and B, 5% ferric chloride, Dragendorff's reagent, 1% hydrochloric acid, sodium chloride, sodium hydroxide, silica gel, distilled water, DPPH (SIGMA-ALDRICH D9132-1G 2,2-Diphenyl-1-picrylhydrazyl LO STBD2362V Pcode 101341986 CAS: 1898-66-4), ice flakes, ginger, garlic, ascorbic acid, antibumping granules.

2.2 Equipment and Apparatus

The equipment and apparatus used include; water bath, round bottom flask, aspirator bottle, condenser, electric pump, Metler weighing balance, cotton wool, test tubes, test tube rack, oven, spatula, vials, reagent bottles, beakers, fume cupboard, UV spectrophotometer, distillation flask, oven, vortex mixer, measuring cylinder, clock, and tissue paper, thin layer glass plate for Thin Layer Chromatography (TLC), precoated TLC plates, capillary tube, sample vials, Infrared spectrometer (Spectrum Two PerkinElmer model L1600401, version 10.4.3), fractionating column, retort stand with clamp.

2.3 Plant Collection and Identification

Fresh leaves of *Spathodea campanulata* were collected from Ogun State, Nigeria during raining season in September. The identification and authentication were done at the herbarium, Botany department, University of Ibadan, Nigeria with voucher number UIH-22493.

2.4 Extract Partitioning

500 g of the leaves of *S. campanulata* was soaked in methanol (2.1 L) to obtain 14.5 g of methanol extract of the leaves, which was partitioned into hexane (1.1 g), ethyl acetate (4.4 g) and methanol (9 g) fractions. Each of the three solvents (500 mL) was added in batches, till all contents of each fraction were obtained.

2.5 Phytochemical Screening

The hexane, ethyl acetate, and methanol fractions of the leaf were analyzed for the presence of the following phytochemicals; alkaloid, anthraquinone, saponin, flavonoid, tannin, steroid, glycoside, and phenol using qualitative methods [24].

2.6 Column Chromatography

Ethyl acetate fraction (4.1 g) was adsorbed on silica gel (mesh size 70-230) and chromatographed on a glass-column (3 cm by 100 cm) using a gradient mixture of n-hexane, ethyl acetate and methanol as elution solvents. A total of 170 fractions were collected in volumes of 100-150 mL.

2.7 GC–MS of TMS Derivatives

Samples were weighed into GC vials to which CH_2Cl_2 containing anthracene as an internal standard was added. The samples were silylated with addition of N,O-bis(trimethylsilyl)-trifluoro-acetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) and pyridine and heated for 30 min at 70℃ until the solution became clear. The prepared TMS derivatives were analyzed by GC–MS EI (FOCUS-ISQ, Thermoscientific); temperature profile: 40℃ (1 min) ramped to 305℃ (10 min) at 5℃/min; GC capillary column (RTx-5 MS, 30 × 0.25 mm Φ, Restek). The eluted compounds were identified by spectral matching with the 2008 National Institute of Standard and Technology (NIST) spectral library and known standards.

2.8 Antioxidant Activities of the Fractions of *S. campanulata*

2.8.1 Scavenging effect on DPPH

A rapid, simple and inexpensive method to measure antioxidant capacity involves the use of the free radical DPPH which is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors and to evaluate antioxidant activity. A 3.94 mg of DPPH, a stable radical was dissolved in methanol (100 mL) to give a 100 µM solution. To 3.0 mL of the methanolic solutions of DPPH was added 0.5 mL of each of the fractions with doses ranging from 1000 µg/mL to 31.25 µg/mL. The mixture was shaken thoroughly using a vortex mixer and left to stand for 30 minutes after which the absorption was measured at 517 nm at each time using a UV spectrometer. Other concentrations were prepared from the stock solution through serial dilution. The same experiment was carried out on butylated hydroxylanisole (BHA), ginger and ascorbic acid, which are known antioxidants. All test and

analysis were carried out in triplicates and the results obtained were averaged. The radical scavenging activity (RSA) was calculated as the percentage of DPPH discolouration using the equation below:

$$\% \text{ Inhibition} = \frac{\text{Ac} - \text{As}}{\text{Ac}} \times 100$$

Where A_S is the absorbance of the solution and Ac is the absorbance of the DPPH solution [25].

The analysis was carried out on hexane, ethyl acetate and methanol fractions of the methanol extract of *S. campanulata* leaves. The same experiment was repeated using ascorbic acid, butylated hydroxyanisole (BHA) and ginger as standards.

3. RESULTS AND DISCUSSION

Phytochemical screenings revealed presence of alkaloids, tannins, saponin, resins, phenol, cardiac glycosides, steroids, flavonoids, anthraquinones and terpenoids in the three fractions in varying concentrations. Alkaloids, resins, phenol and cardiac glycosides were found to be intense in the three fractions while phylobatannin was found to be absent in all the three fractions (Table 1). These classes of compounds present are known to show curative activity against several pathogens and therefore could explain the medicinal use of the plant for treatment of wide array of illnesses [1,7].

3.1 Column Chromatography

A total of 170 fractions were collected in volumes of 100-150 mL. The fractions labelled 73-104 (2% ethyl acetate in hexane) yielded Mo1b, fractions labelled 127-151 (5% ethyl acetate in hexane) yielded Mo5 while fractions 159-161 (100% ethyl acetate) yielded Mo159b.

Table 1. Phytochemical screening of the fractions of the methanol extract of *S. campanulata* leaves

Secondary metabolites	HXF	EAF	MTF
Alkaloid	+++	+++	+++
Tannins	+	+++	+++
Phylobatannin	–	–	–
Saponin	+	+	+++
Resins	+++	+++	+++
Phenol	+++	+++	+++
Cardiac glycosides	+++	+++	+++
Steroids	+	++	+++
Flavonoids	+	+	+
Anthraquinone	+	+++	+
Terpenoid	+	+	+++

HXF=Hexane fraction, EAF= Ethyl acetate fraction, MTF=Methanol fraction,
+++ = intense, ++ = strong, + = weak, - =absent

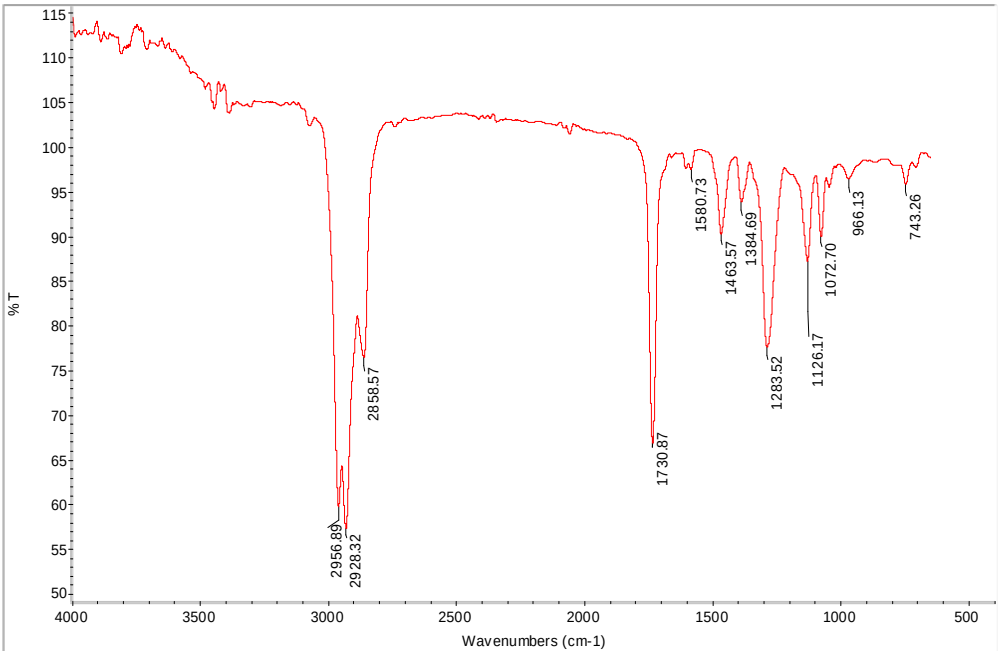

Fig. 1. Infrared spectrum of Mo1b

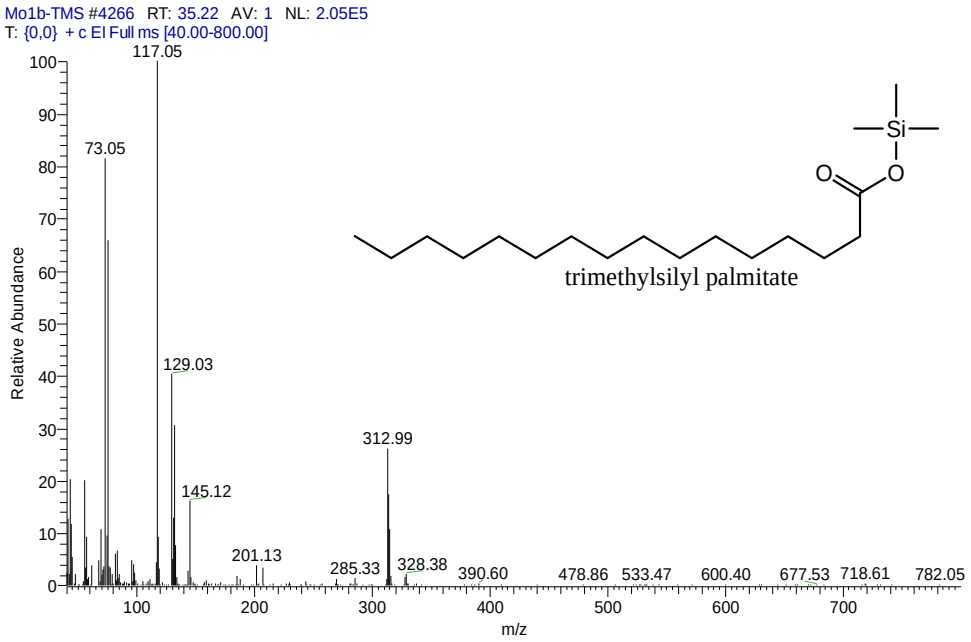

Fig. 2. GC-MS of TMS derivatised Mo1b

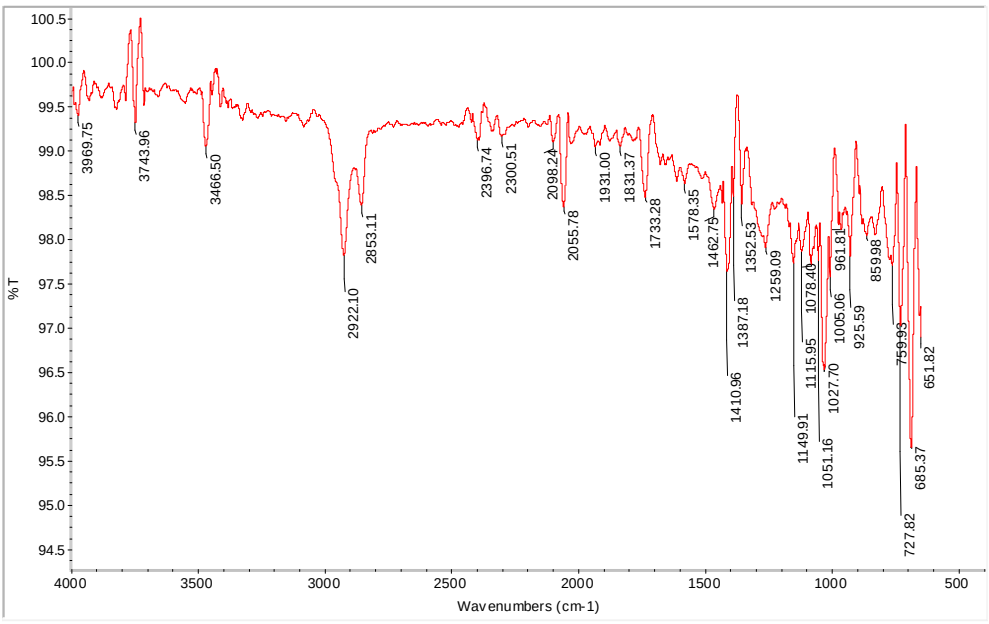

Fig. 3. Infrared spectrum of Mo5

The three isolated compounds were analysed thus: IR (Figs. 1, 3 and 5), GC–MS of the TMS derivative (Figs. 2, 4 and 6). The following are the characteristic absorptions:

Compound 1 (Mo1b): weight = 52 mg R_f = 0.4 IR λmax 2956 cm^{-1} (C-H) 1731 cm^{-1} (C=O) and 1283 cm^{-1} (C-O) and GC-MS m/z 328.38(3), 312.99(26), 145.12(16), 132.06(31), 129.03(40), 117.05(100), 83.10(7), 75.06(65.88), 73.05(81), 43.10(20).

Compound 2 (Mo5): weight = 30 mg R_f = 0.3 IR λmax 2922 cm^{-1} (C-H) 1027 cm^{-1} (C-N) and GC-MS m/z 189(3), 174.11(96), 130.10(7), 100.07(100), 73.05(62), 59.05(32).

Compound 3 (Mo159b): weight = 120 mg R_f = 0.4 IR λmax 3344 cm^{-1} (O-H), 1692 cm^{-1} (C=O), 1607(C=C), 1280(C-O) and GC-MS m/z 396.29(23), 380.91(9), 307.07(7), 249.13(6), 219.05(88), 191.03(17), 147.06(5), 75.07(11), 73.06(100), 45.05(16).

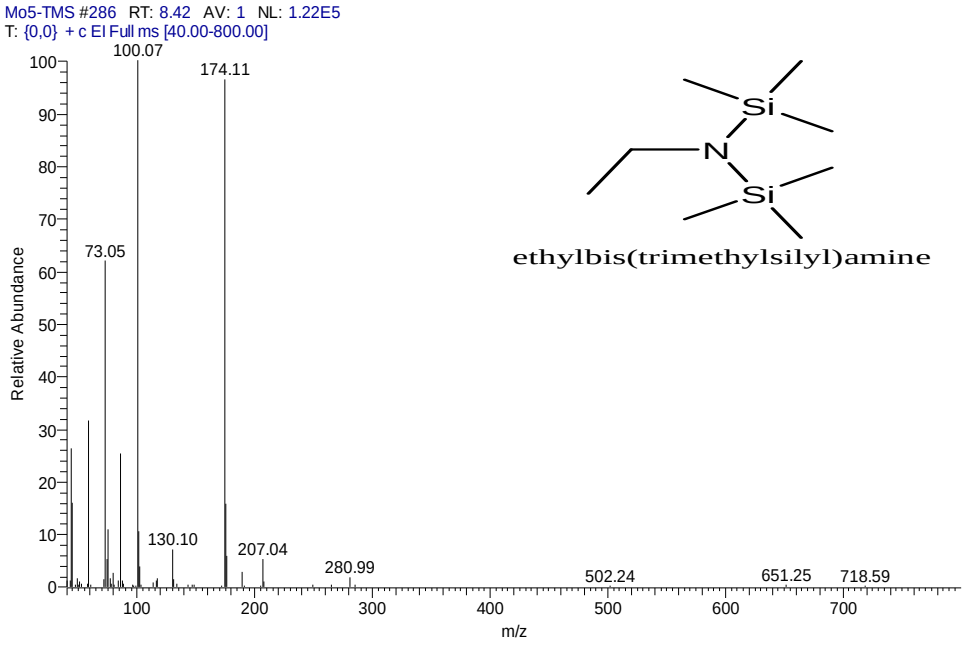

Fig. 4. GC-MS of TMS derivatised Mo5

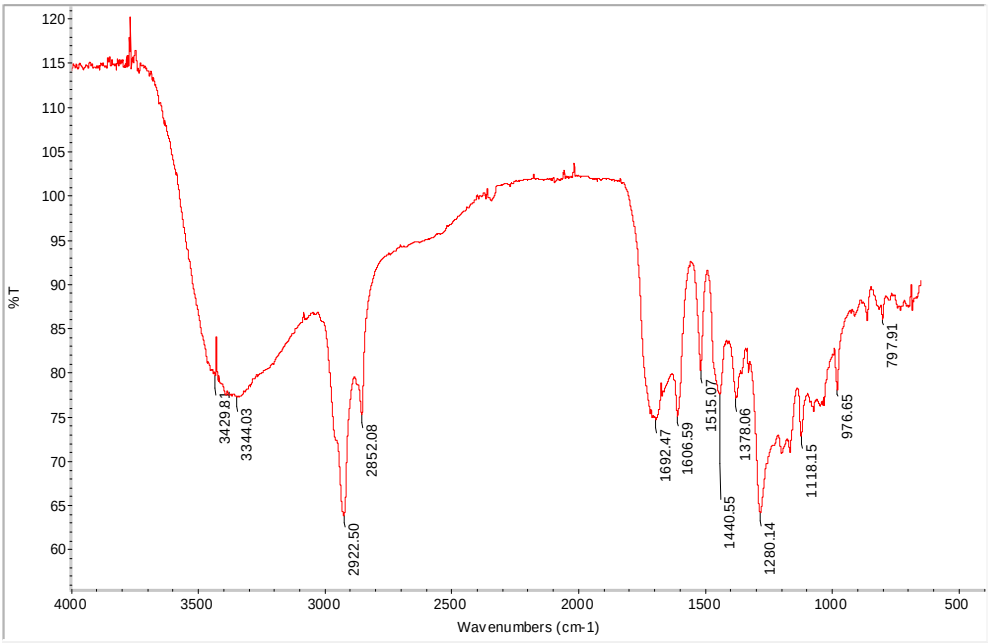

Fig. 5. Infrared spectrum of 159b

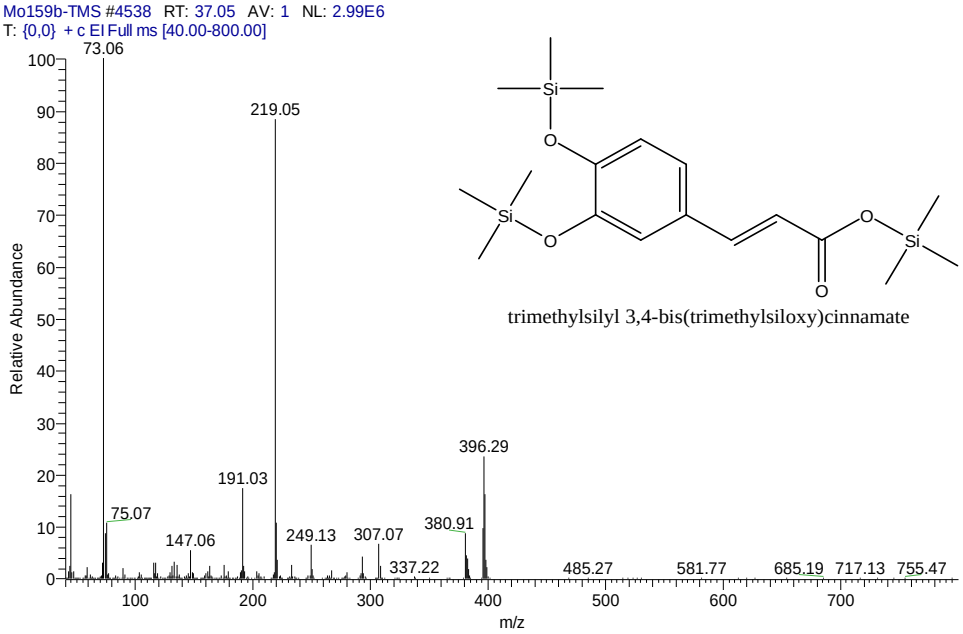

Fig. 6. GC-MS of TMS derivatised Mo159b

3.1.1 Compound 1

The identification of palmitic acid (Fig. 7) from ethyl acetate fraction was based on spectroscopic evidence and comparison of retention index and mass spectrum with the stored laboratory mass spectral library data. The IR spectrum of Mo1b showed absorption 2956 cm^{-1} indicative of sp^3 C-H stretch, 1731 cm^{-1} indicative of C=O stretch of an ester, 1283 cm^{-1} indicative of C-O stretch. GC–MS of the TMS derivative gave a molecular ion peak at m/z 328 in the spectrum corresponding to a molecular formula of $C_{19}H_{40}O_2Si$. The base peak of m/z 117 was as a result of $(CH_3)_3SiOCO^+$. Cleavage of the Si-O bond leads to a $(CH_3)_3Si^+$ peak for the fragment observed at m/z 73 while $(CH_3)_3SiOC(=OH^+)CH_2$. (m/z 132) is due to McLafferty rearrangement. The compound also displays prominent fragment ion at m/z 314, 145 and 43. Its MS fragmentation pattern is consistent with that of trimethylsilyl palmitate ($C_{19}H_{40}O_2Si$). The eluted compounds were identified by spectral matching with the 2008 National Institute of Standard and Technology (NIST) spectral library and known standards. Results of the IR, GC-MS and library search data suggest the identified compound (Mo1b) is palmitic acid.

3.1.2 Compound 2

The result of IR spectrum of Mo5 showed absorption at 2922 cm^{-1} indicative of sp^3 C-H stretch, 1027 cm^{-1} indicative of C-N stretch. GC–MS of the TMS derivative profile of the compound gave a molecular ion peak at m/z 189 and base peak at m/z 100. Loss of CH_3 from the molecular ion accounts for m/z at 174. The compound also displays prominent fragment ion at m/z 101, 73 and 59. Its fragmentation pattern is consistent with that of ethylbis (trimethylsilyl) amine ($C_8H_{23}NSi_2$) Results of the IR, GC-MS and library search data suggest the identified compound (Mo5) is ethylamine (Fig. 8).

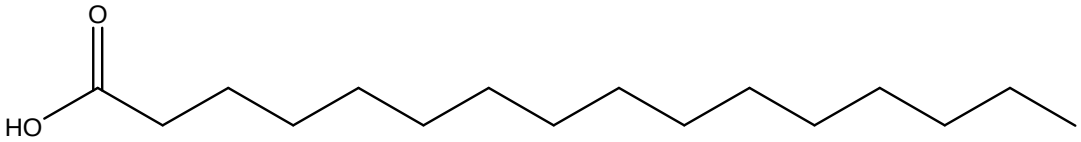

Fig. 7. Palmitic acid

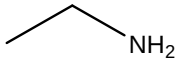

Fig. 8. Ethylamine

3.1.3 Compound 3

IR spectroscopic evidence and comparison of retention index and mass spectrum with the stored laboratory mass spectral library data revealed Mo159b to be caffeic acid (Fig. 9). The IR spectrum of Mo159b showed absorption 3344 cm^{-1} indicative of O-H stretch of carboxylic acid, 1692 cm^{-1} indicative of conjugated C=O stretch, 1607 cm^{-1} indicative of conjugated C=C stretch and 1280 cm^{-1} indicative of C-O stretch. GC–MS of the TMS derivative gave a molecular ion peak at m/z 396 in the spectrum corresponding to a molecular formula of $C_{18}H_{32}O_4Si_3$ and base peak of m/z 73. The compound also displays prominent fragment ion at m/z 381, 307, 219, 191 and 45. Its fragmentation pattern is in good agreement with that of Trimethylsilyl 3,4-bis(trimethylsiloxy) cinnamate ($C_{18}H_{32}O_4Si_3$). Results of the IR, GC-MS and library search data suggest the identified compound (Mo159b) is caffeic acid.

3.2 Antioxidant Activity

3.2.1 Scavenging effect on DPPH

DPPH is known to be a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule.

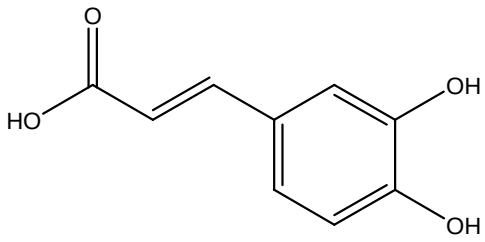

Fig. 9. Caffeic acid

The antioxidant activity of the hexane, ethyl acetate and methanol fraction of *S. campanulata* were measured and compared with the antioxidant activity of three standards (ascorbic acid, BHA, and ginger). The results were presented as absorbance (in nm) (Table 2) and % inhibition (Table 3).

Table 2. Absorbance (at 517 nm) of fractions of *S. campanulata* leaves and standard antioxidants

Conc. (µg/mL)	HXF	EAF	MTF	ASC*	BHA*	GIN*
1000	0.122±0.001	0.112±0.001	0.127±0.001	0.016±0.001	0.026±0.000	0.034±0.000
500	0.154±0.002	0.201±0.000	0.158±0.002	0.017±0.001	0.026±0.000	0.097±0.000
250	0.235±0.003	0.385±0.001	0.627±0.002	0.019±0.000	0.027±0.000	0.124±0.000
125	0.802±0.003	0.705±0.000	0.920±0.002	0.021±0.000	0.027±0.000	0.191±0.000
62.5	0.930±0.001	0.888±0.000	1.030±0.001	0.026±0.000	0.030±0.000	0.243±0.001
31.25	1.056±0.000	1.002±0.001	1.111±0.002	0.026±0.000	0.108±0.001	0.293±0.000

Key:
HXF= Hexane fraction of S. campanulata; BHA= Butylated hydroxyanisole; EAF= Ethyl acetate fraction of S.campanulata leaves; ASC*= Ascorbic acid; MTF= Methanol fraction of S.campanulata leaves; GIN*= Ginger; *= standards*

Table 3. Table showing the concentration (µg\mL) and percentage inhibition of the fractions and standards

Conc. (µg/mL)	HXF	EAF	MTF	ASC*	BHA*	GIN*
1000	91.66±0.09	91.66±0.09	91.31±0.09	98.03±0.10	96.99±0.00	95.71±0.06
500	89.53±0.14	85.10±0.03	89.19±0.12	97.95±0.12	96.95±0.05	87.83±0.06
250	83.96±0.20	71.42±0.06	57.18±0.14	97.67±0.00	96.91±0.05	84.46±0.06
125	45.33±0.20	47.64±0.03	37.18±0.14	97.38±0.06	96.88±0.00	76.08±0.06
62.5	36.58±0.09	34.10±0.03	29.72±0.09	96.81±0.00	96.49±0.05	69.67±0.16
31.25	27.99±0.03	25.61±0.06	24.16±0.11	96.76±0.06	87.50±0.09	63.38±0.00

Key:
Conc. = Concentration, HXF = Hexane Fraction, EAF = Ethyl acetate Fraction, MTF = Methanol Fraction, ASC = Ascorbic acid, BHA* = Butylated hydroxylanisole, GIN* = Ginger, *= standards*

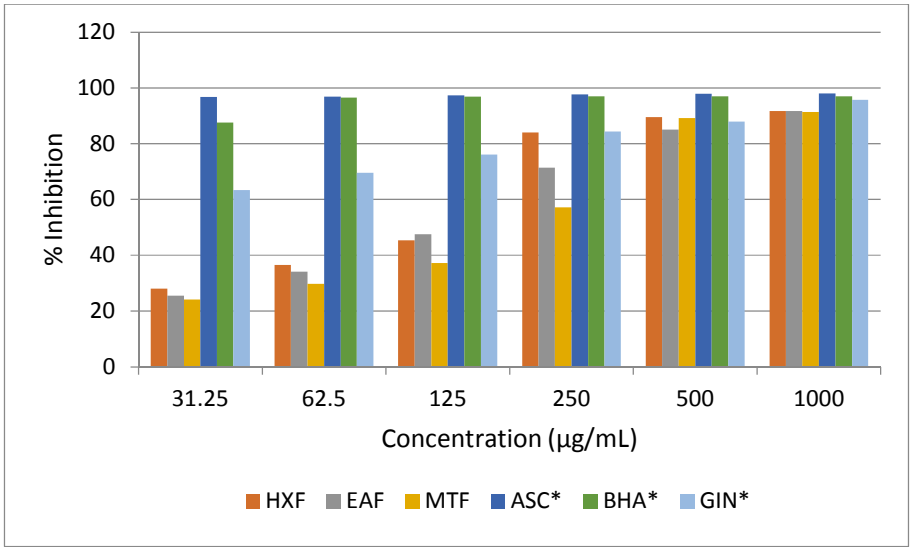

Fig. 10. DPPH free radical scavenging activity of fractions from the methanol extract of *Spathodea campanulata* leaves

The three fractions from the leaves gave % inhibition of 24.16 to 91.85% at 1000 - 31.25 µg/mL concentrations; all showed high antioxidant activities at 1000, 500 and 250 µg/mL concentrations, hence they are good antioxidants at higher concentrations. IC_{50} values of hexane, ethyl acetate and methanol fractions (178.46, 201.34, 227.68 µg/mL respectively) were found to be much higher than that of the standards used: ascorbic acid (21.87 µg/mL), BHA (23.91 µg/mL), and ginger (36.32 µg/mL). This shows that at 50% percentage inhibition, the fractions have low antioxidant properties when compared to the standards. As observed in the bar chart (Fig. 10) the methanol fraction was established to have the lowest antioxidant capacity with IC_{50} value of 227.68 µg/mL while the hexane fraction was found to be the richest of the three.

4. CONCLUSION

Phytochemical screening on the three fractions of methanol extract of *S. campanulata* leaves revealed presence of alkaloids, tannins, saponin, resins, phenol, cardiac glycosides, steroids, flavonoids, anthraquinones and terpenoids. Palmitic acid and ethylamine found in *S. campanulata* leaves were reported for the first time while caffeic acid has been earlier identified in the leaves [12-14]. The phytochemical constituents and high antioxidant activity (at concentrations of 250 µg/ mL and above) of the African tulip tree (*Spathodea campanulata*) when compared to the standards, supports its wide ethno-medicinal application.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENTS

We acknowledge plant collection and extractions done by DOM project students from Olabisi Onabanjo University (OOU), Ogun state Nigeria and use of IR, TMS GC-MS facilities in McDonald College of Natural Resources, University of Idaho, Moscow, USA.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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