Antimicrobial and spectroscopic analyses of the whole plant of Cassytha filiformis (Love vine) from Southeastern Nigeria

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Research Article

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Antimicrobial and spectroscopic analyses of the whole plant of *Cassytha filiformis* (Love vine) from Southeastern Nigeria

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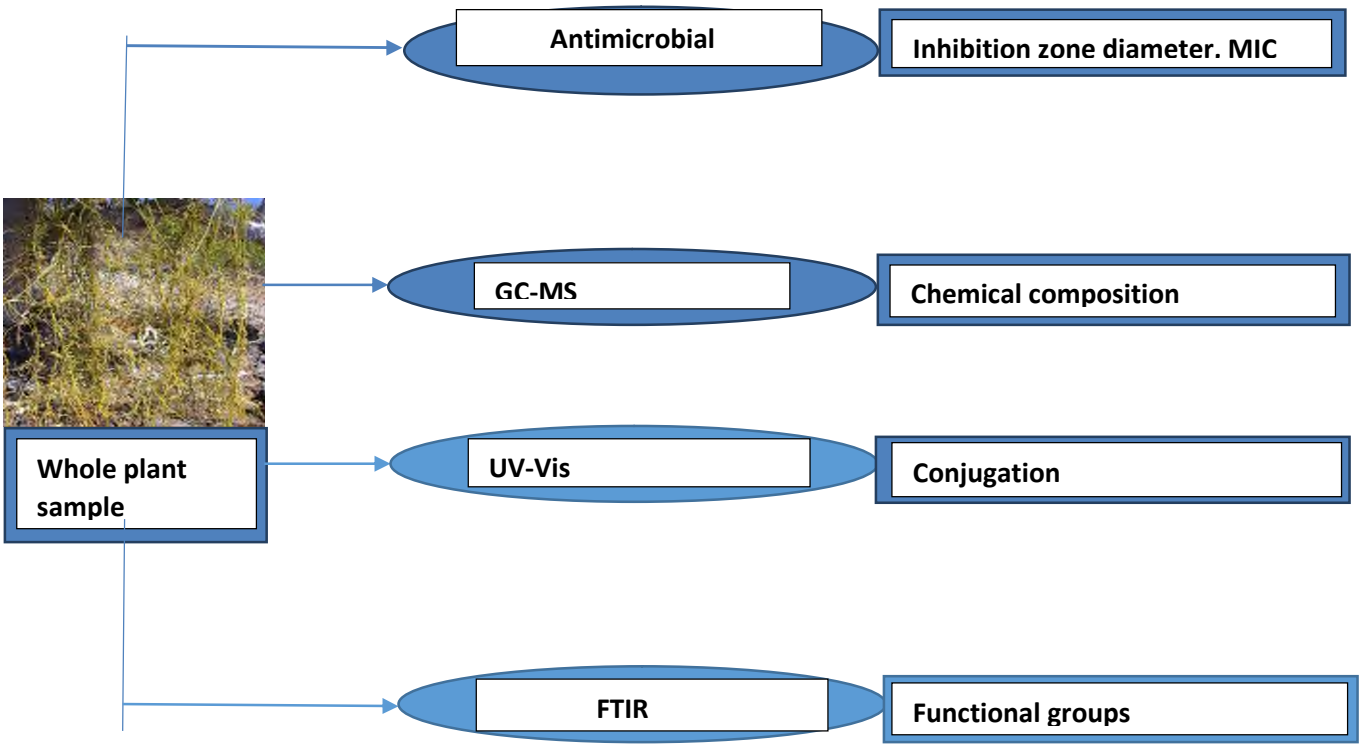
Abstract

This research investigated the efficacy of the solvent extracts of the whole plant of *Cassytha filiformis* in inhibiting microbial growth and employed spectroscopic techniques to analyze the chemical composition of the plant. Antimicrobial activity of the solvent plant extracts was evaluated in vitro by modified disc diffusion method against seven bacteria and seven fungi. Minimum Inhibitory Concentration (MIC) was determined by broth dilution method. Buck Scientific M530 USA FTIR was used for the FTIR analysis. UV-Vis spectrophotometer (Apel 3000UV) was used to conduct the UV-Vis spectrophotometric analysis. The quantification of phytochemicals by GC-MS was conducted using BUCK M910 with HP-5MS column. The UV-Visible results showed absorption in both the ultraviolet and visible regions indicating high conjugation. The antibacterial and antifungal activity screening showed strong inhibitory effect against some selected test bacteria and fungi organisms. Compounds having high concentrations were identified with the GC-MS analysis of the solvent extracts. The MIC results varied from turbidity to no turbidity at the different concentrations of the solvent extracts. The FTIR analysis revealed the presence of the predominant functional groups such as O-H, N-H, C=C and =C-H. The antimicrobial results revealed that the solvent extracts of the plant can be employed in the treatment of fungi and bacteria diseases. ^{1}H-NMR and ^{13}C-NMR studies of the plant solvent extracts should be conducted in order to elucidate the structures of the secondary metabolites responsible for the antimicrobial properties. Also, the antimicrobial activity of more plant solvent extracts against more bacteria and fungi should be further investigated and incorporated into orthodox drugs.

Keywords: *Cassytha filiformis***,** Antimicrobial activity, UV-Visible analysis, FTIR spectroscopy, GC-MS analysis

Graphical Abstract



1.0 Introduction

Biologically active compounds are constituents of the therapeutic plantnamed *Cassytha filiformis*. Previous research revealed that *C. filiformis* plant contains bioactive chemical compounds such as, tannins, proteins, carbohydrates, anthroquinones, alkaloids, terpenes, flavonoids, steroids, saponins, phenolics and glycosides. These bioactive compounds confer antimicrobial properties on the plant and are responsible for the utilization of the plant as traditional curative or preventive drugs for humans [1, 2, 3, 4] .The increasing resistance of bacteria and fungi to expensive antibiotics of synthetic medicine has led to the invention of antibiotics isolated from natural products which common people can afford. There has been an evolution of microorganisms with extraordinary ability to render conventional antibiotics relatively ineffective. Many studies have presented plants as alternative to conventional synthetic drugs for the treatment of infectious diseases. Many crude plant extracts have been reported to possess antibacterial and antifungal properties as a result of their phytochemical contents [5, 6, 7, 8]**.**

Cassytha filiformis is a leafless, vine-like, perennial, twining, climbing, parasitic and invasive plant commonly named ‘’Love-vine or Laurel dodder, from the Lauracaea family. Its flowers are small about 2mm and borne singly along the stem. It is widely distributed throughout India and is used medicinally in South Africa, China, India and Madagascar [9, 10]. The dispersion of *C. filiformis* seeds is by wind, water or animals. In traditional medicine, *C. filiformis* is extensively used to treat gonorrhea, cancer, hypertension, jellyfish stings, urinary tract infections, diarrhoea, erectile dysfunction, gastrointestinal infection, conjunctivitis, ulcer and post-partum haemorrhage. The water extract of the plant is used in the treatment of anuria. *C. filiformis* extracts possess diuretic, cytotoxic, antioxidant, antibacterial, antitripanosomal and antispasmodic effects [10, 9, 11, 8].

A few work has been carried out on the antimicrobial activity and spectroscopic analysis of the whole plant of *C. filiformis*. The objective of the study is to analyze the antifungal and antibacterial activities of the solvent extracts of *C. filiformis* against human pathogens and to characterize the plant using spectroscopic methods.

2.0 Materials and Methods

2.1 Sample collection, identification and preparation

The *Cassytha filiformis* (Love-vine) used in this research was identified and collected from an acacia plant in Ukpo, Dunukofia Local Government Area of Anambra State, which is located in the Eastern part of Nigeria. The fresh plant material was washed with clean water three times, air-dried for 15 days at room temperature and then pulverized to fine powder. The powder was stored inside an air-tight high density polyethylene bottle at room temperature for further use. The powdered sample was subjected to soxhlet extraction.

2.1.1 Soxhlet Extraction Procedure

500 mL of clean boiling flasks was oven-dried at 105-110^{0}C for about 30 mins and then transferred into a dessicator and was allowed to cool. 10g of the plant sample was weighed and transferred into a soxhlet thimble. The extraction thimble was lightly plugged with cotton wool to aid filter the extract. The boiling flask was filled with about 300 mL of the extraction solvent. The soxhlet apparatus was assembled and allowed to reflux for about 4 hrs at a temperature of 60^{0}C. Then the thimble was removed with care and the extract poured into a volumetric flask and allowed to cool. The content in volumetric flask was finally transferred into a rotary evaporator

in order to separate the solvent from the extract. The extraction solvents used were ethanol, diethyl ether, acetone and water [7].

2.1.2 Antimicrobial Assessment

McFarland Standard was used to carry out the adjustment of absorbance of the bacteria. The turbidities of the test organisms were adjusted to match the absorbance of the 0.5 McFarland Standards at the same wavelength, using nistatin. Modified disc diffusion method was employed in the determination of the antimicrobial properties. 50 µg/mL chloramphenicol and 50 µg/mL nistatin were used as reference standards/positive controls while dimethylsulfoxide (DMSO) served as the negative control.

About 25 µL of Muller-Hinton agar media for bacteria were poured into the sterilized petri dishes and allowed to solidify. The 24 hrs cultured 108 CFU/mL of microbial strains were swabbed on the solidified media and allowed to dry for 10 min. About 50 µL of the plant extract (1 mg/mL) were used to impregnate the 6 mm filter paper discs and placed on two portions of the agar plate and left for 1 hr at room temperature for compound diffusion. 50 µg/mL chloramphenicol and DMSO were used as positive control and negative control respectively. Incubation of the plates was carried out at 37^0C for 24 hrs. At the end of the incubation, the total microbe on the plate were counted and recorded in CFU/mL. The plate with the lowest count was recorded as MIC. The antimicrobial activity/microbial growth were evaluated by measuring the inhibition zone diameters in millimeter [3, 12].

The fungi was cultured on Sabourand dextrose agar media at 28^0C for 10days and the spores were collected using sterile double distilled water and homogenized. 50 µg/mL nistatin and DMSO were used as positive control and negative control respectively [3].

2.1.3 Determination of Minimum Inhibitory Concentration (MIC)

The Minimum inhibitory concentration (MIC) is defined as the lowest concentration required to arrest the growth of the microorganism at the end of 24 hr or 48 hr of incubation. The Minimum Inhibitory Concentration (MIC) was determined by the broth dilution method. Nutrient Broth and Sabouraud Dextrose Broth, which contained 20%, 40%, 60% and 80%, of the extracts were inoculated with known amounts of the bacterial and fungal inocula, with 0.5 McFarland adjusted cultures for bacteria and yeast; and dilution factor of 10-2 for fungi. Controls (negative and positive) were set up also. The tubes were incubated at room temperature for 24 hours for bacteria and 48 hours for fungi in a metabolic rotary shaker (220rev/min). Thereafter, the incubated test tubes were then subcultured onto sterile freshly prepared plates and incubated for 24 hours for bacteria and 48 hours for fungi. At the end of the incubation period, the plates were counted and the total microbial count recorded in CFU/ml. The plate with the lowest count was recorded as the MIC [12].

2.2 Procedure for FTIR spectroscopic analysis

Buck scientific M530 USA FTIR was used for the analysis. This instrument was equipped to obtain the spectra and to manipulate them. An approximate value of 1.0g of samples, 0.5ml of nujol were added, they were mixed properly and placed on a salt pellet. During measurement with a detector of deuterated triglycine sulphate and beam splitter of potassium bromide. The software of the Gram A1 was used. FTIR spectra was obtained at frequency regions of 4,000 – 600 cm^{-1} and co-added at 32 scans and at 4 cm^{-1} resolution. FTIR spectra were displayed as transmitter values [13,14].

2.3 Procedure for UV-Vis Spectroscopic analysis

UV-Visible spectrophotometric analysis was conducted on the plant extracts using a UV-Visible spectrophotometer (Apel 3000UV) with a slit width of 2nm, and a 10-mm cell at room temperature. The extract was examined under visible and UV light in the wavelength ranging from 200-1100nm [15, 16].

2.4 Quantification by GC-MS analysis

The dried powdered sample was subjected to soxhlet extraction using the solvents which include ethanol, diethyl ether, acetone and water respectively. Then the phytochemicals were extracted from the solvent plant extracts and identified by GC-MS using BUCK M910 Gas chromatography equipped with HP-5MS Column [17, 18].

2.5 Statistical methods

The antimicrobial assay was determined in three replicates and the results were calculated as the mean of the triplicates plus or minus standard deviation. Microsoft Excel 2013 was used to calculate the standard deviation and plot the bar charts representing the mean zones of inhibition diameter of the test microorganisms.

3.0 Results and discussions

3.1 Antimicrobial assay

The results obtained revealed that all the plant extracts exhibited very significant antimicrobial activity against the test organisms. The antibacterial activity was examined by modified disc diffusion method while the antifungal activity was assessed using Sabourand dextrose agar media. The zones of inhibition diameters in millimeter are presented in **Tables 1 and 3, Figures 2 and 3.** All the solvent extracts of the C. filiformis plant exhibited very strong antimicrobial

activity towards all the test micro-organisms. Phenolics and flavonoids contents of the plant extracts are responsible for the antimicrobial activity. In his present study, all the plant extracts exhibited resistance against the test bacteria such as *P. vulgaris, S. albus, S. typhi, E. aerogene, S. mutenus, K. pneumonia and S. pyogene*. The standard drug named chloramphenicol used as positive control strongly inhibited the growth of test bacteria making it a stronger and better antibiotic than the plant extracts.The negative control which is dimethyl sulfoxide (DMSO) exhibited no resistance against all the test bacteria [19, 12].

Table 1: Antibacterial Susceptibility Screening of plant extracts

Test Bacteria	**Mean Inhibitions Zone Diameters in Millimetres ± Standard deviation**				
	Diethylether extract	**Acetone extract**	**Ethanol extract**	**Water extract**	**Chloramp (Positive**
Proteus vulgaris	20.333 ± 0.577	19.333 ± 1.154	20.333 ± 1.528	20.667± 1.155	48.000
Staphylococcus albus	21.000 ± 1.000	18.667 ± 1.528	21.333 ± 0.577	21.000± 1.000	53.667
Salmonella typhi	22.000 ± 1.000	21.000 ± 1.000	21.333± 1.155	22.000 ± 1.000	44.333
Enterobacter aerogene	21.667 ± 2.082	20.667 ± 2.082	21.000± 1.000	22.333 ± 0.577	37.333
Streptoccucusmutenus	22.000 ± 1.000	19.000 ± 1.000	22.333 ± 1.528	20.667 ± 0.577	41.333
Klebsiella pneumonia	22.333 ± 1.155	21.333 ± 1.528	22.000 ± 1.000	22.333 ± 0.577	38.667
Streptococcus pyogene	21.333 ± 0.577	21.667 ± 2.082	21.333 ± 0.577	21.000 ± 1.000	41.667

Table 2: Minimum Inhibitory Concentration (MIC) of the extracts on the various test bacteria organisms

	Diethyl ether Extract				**Acetone Extract**				**Ethanol Extract**				**Water Extract**				**Chloramphenico**		
	MIC (Mg/ml)				**MIC (Mg/ml)**				**MIC(Mg/ml)**				**MIC (Mg/ml)**				**MIC (Mg/ml)**		
Test Bacteria	**500**	**250**	**125**	**62.5**	**500**	**250**	**125**	**62.5**	**500**	**250**	**125**	**62.5**	**500**	**250**	**125**	**62.5**	**500**	**250**	**12**
Proteus vulgaris	T	NT	NT	NT	T	NT	T	NT	T	NT	NT	NT	T	T	NT	NT	NT	NT	NT
Staphylococcus albus	NT	T	T	NT	NT	NT	T	NT	NT	T	T	NT	NT	T	T	T	NT	NT	NT
Salmonella typhi	NT	T	NT	T	NT	NT	NT	T	NT	T	NT	T	NT	NT	NT	T	NT	NT	NT
Enterobacter aerogene	T	NT	NT	NT	T	T	NT	NT	T	NT	NT	NT	T	T	T	NT	NT	NT	T
Streptoccucusmutenus	NT	NT	T	T	NT	NT	T	T	NT	NT	T	T	NT	T	T	NT	NT	NT	T
Klebsiella pneumonia	T	NT	NT	T	T	NT	NT	T	T	NT	NT	T	T	NT	T	T	NT	T	T
Streptococcus pyogene	NT	T	T	T	NT	T	T	T	NT	T	T	T	NT	NT	NT	T	NT	NT	T

Key:

T= Turbidity

NT = No turbidity

Table 3: Antifungal Susceptibility Screening of plant extracts

Test Fungi	Mean Inhibitions Zone Diameters in Millimetres ± Standard deviation				
	Diethylether extract	Acetone extract	Ethanol extract	Water extract	Nistatin (Positive C
Aspergillus niger	25.667 ± 3.215	25.667 ± 1.155	27.667 ± 1.528	20.333 ± 1.528	45.000 ± 4.3
Aspergillus flavus	24.000 ± 1.000	21.000 ± 1.000	28.333 ± 2.082	26.000 ± 2.000	47.333 ± 1.5
Candida albican	25.000 ± 1.000	26.000 ± 1.000	26.000 ± 1.000	26.667 ± 1.155	47.000± 1.0
Trichophyton mentagraphytes	25.667 ± 1.155	25.000 ± 1.000	28.333 ± 1.528	27.333 ± 0.577	44.667 ± 1.1
Alternaria alternate	25.000 ± 1.000	24.333 ± 1.528	27.667 ± 0.577	28.000 ± 1.000	48.000 ± 1.0
Fusarium graminearum	27.333 ± 0.577	23.333 ± 1.528	25.333 ± 0.577	35.333 ± 11.846	45.667 ± 0.5
Aspergillus nidulans	27.000 ± 1.000	26.333 ± 1.528	25.667 ± 0.577	27.000 ± 2.000	44.333 ± 1.5

Table 4: Minimum Inhibitory Concentration (MIC) of the extracts on the various test fungi organisms

	Diethyl ether Extract				Acetone Extract				Ethanol Extract				Water Extract				Nistatin		
	MIC (Mg/ml)				MIC (Mg/ml)				MIC(Mg/ml)				MIC (Mg/ml)				MIC (Mg/ml)		
Test Fungi	**500**	**250**	**125**	**62.5**	**500**	**250**	**125**	**62.5**	**500**	**250**	**125**	**62.5**	**500**	**250**	**125**	**62.5**	**500**	**250**	**125**
Aspergillus niger	NT	NT	T	NT	T	NT	T	T	T	T	NT	NT	NT	T	NT	NT	NT	NT	NT
Aspergillus	T	NT	NT	NT	T	NT	T	NT	NT	NT	NT	NT	T	T	T	T	T	NT	NT

flavus																			
Candida albican	NT	T	NT	T	NT	NT	NT	NT	NT	T	T	T	NT	T	NT	T	NT	NT	NT
Trichophyton mentagraphytes	T	T	NT	T	NT	NT	NT	NT	T	T	NT	NT	NT	T	NT	NT	NT	NT	NT
Alternaria alternate	T	T	NT	T	NT	NT	T	T	NT	NT	NT	NT	NT	NT	T	NT	NT	NT	T
Fusarium graminearum	T	NT	NT	T	NT	T	NT	T	T	NT	NT	NT	NT	NT	T	T	NT	T	T
Aspergillus nidulans	NT	T	T	NT	NT	T	T	T	NT	NT	T	T	T	NT	NT	T	NT	NT	T

Key:

T= Turbidity

NT = No turbidity

Proteus vulgaris was found to be most susceptible to water extract with a maximum inhibitory zone of 20.667mm followed by both the diethyl ether extract (20.333mm) and ethanol extract (20.333mm) and then acetone extract (19.333mm). *Staphylococcus albus* was found to be most sensitive to ethanol extract with a maximum inhibitory zone of 21.333mm followed by both diethyl ether extract (21.000mm) and water extract (21.000mm) and then acetone extract (18.667mm). *Salmonella typhi* was found to be most susceptible to both the diethyl ether extract (22.000mm) and water extract (22.000mm) succeeded by ethanol extract (21.333mm) and the acetone extract (21.000mm). *Enterobacter aerogene*was most sensitive to water extract with a maximum inhibitory zone of 22.333mm followed by diethyl ether extract (21.667mm), ethanol extract (21.000mm) and then acetone extract (20.667mm). *Streptococcus mutenus* was most susceptible to ethanol extract (22.333mm) succeeded by diethyl ether extract (22.000mm), water extract (20.667mm) and then acetone extract

(19.000mm). Klebsiella pneumonia was most sensitive to both water (22.333mm) and diethyl ether (22.333mm) extracts followed by ethanol extract (22.000mm) and then acetone extract (21.333mm). *Streptococcus pyogene* was most susceptible to the acetone extract (21.667mm) followed by both diethyl ether extract (21.333mm) and ethanol extract (21.333mm) and then water extract (21.000mm) [19, 5, 20, 21, 22, 23].

The diethyleher, water, acetone and ethanol plant extracts showed resistance against all the test fungi such as *A. niger, A. flavus, C. albicans, T. mentagraphytes, A.alternate, F. graminearum* and *A. nidulans.* The positive control named Nistatin showed very significant resistance against all the test fungi whereas the negative control which is dimethylsufoxide (DMSO) exhibited zero resistance against all the test fungi. DMSO failed to inhibit the growth of all the test fungi. The ethanol extract showed the most effective inhibition of 27.667mm against Aspergillus niger strain followed by both the diethyl ether (25.667mm) and acetone (25.667mm) extracts and then the aqueous extract (20.333mm) with the least effective inhibition. The highest antifungal activity of the plant extract against *Aspergillus flavus* was recorded with the ethanol extract (28.333mm) succeeded by water extract (26.000mm), diethyl ether extract (24.000mm) and acetone extract (21.000mm). The water extract exhibited the most effective inhibition of 26.667mm against *Candida albican* followed by both acetone (26.000mm) and ethanol (26.000mm) extracts, and then diethyl ether (25.000mm) with the least effective inhibition. The highest antifungal activity of the plant extract against *Trichophyton mentagraphytes* was recorded with the ethanol extract (28.333mm) followed by water extract (27.333mm), diethyl ether extract (25.667mm) and acetone extract (25.000mm). the water extract exhibited the most effective inhibition of 28.000mm against *Alternaria alternate* strain followed by ethanol extract (27.667mm), diethyl ether (25.000mm) and then acetone extract (24.333mm) with the least effective inhibition. The water extract (35.333mm) exhibited the highest inhibition against the growth of *Fusarium graminearum* followed by diethyl ether extract (27.333mm), ethanol extract (25.333mm) and then acetone extract (23.333mm) with the lowest

inhibition. The ethanol extract (27.667mm) showed the maximum inhibition against the growth of *Aspergillus nidulans* succeeded by both the diethyl ether extract (27.000mm) and water extract (27.000mm) and then the acetone extract (26.333mm) with the minimum inhibition [3, 6, 7, 5, 24].

The results of the Minimum Inhibitory Concentration (MIC) of the extracts represented in **Table**s **2 and 4** varied from turbidity to no turbidity. Turbidity means that the concentration was not able to prevent the growth of the bacteria or fungi while no turbidity implies that the concentration was able to prevent the growth of the bacteria or fungi [25, 26, 27]**.**

3.2 GC-MS Profiling

The GC Chromatogram of the phytochemical content of the ethanol extract of the plant gave a total of 86 compounds **(Figure 4).** The identified compounds having high concentrations, with their retention time and structures are shown in **Table 5.**The GC Chromatogram of the phytochemical content of the aqueous extract of the plant gave a total of 91 compounds as shown in **Figure 5**. The identified compounds having high concentrations with their structures and retention time are shown in **Table 6**. The GC Chromatogram of the phytochemical content of the diethyl ethyl extract of the plant yielded a total of 63 compounds (**Figure 6**). The identified compounds having high concentrations with the retention time and structures are presented in **Table 7.** The GC Chromatogram of the Phytochemical content of the acetone extract of the plant yielded a total of 43 compounds (**Figure7**). The identified compounds having high concentrations with their retention time and structures are presented in **Table 8** [17, 18].

Table 5: Compounds identified from the chromatogram of ethanol extract

Peak	RT	%Area	Compound name	Class of Secondary metabolite
1	6.496	0.23	Chloromethyl Oxirane	Not a secondary metabolite
2	6.848	0.28	1,4-dichlorobenzene	Not a secondary metabolite
3	7.202	0.26	p-Cymene	Terpenoid
4	8.162	1.07	gamma-Terpinene	Terpenoid
5	8.251	0.27	3,4,5,6-tetramethyl-octane	Not a secondary metabolite
6	8.380	0.82	2,6,7-trimethyl-decane	Not a secondary metabolite
7	8.534	0.55	Nonane	Not a secondary metabolite
8	8.591	0.19	Tetradecane	Not a secondary metabolite
9	8.644	2.23	2-methyl undecane	Not a secondary metabolite
10	8.957	2.14	2,6,10,14-tetramethyl heptadecane	Not a secondary

				metabolite
11	9.118	0.46	4-ethyl octane	Not a secondary metabolite
12	9.176	0.72	Tetradecane	Not a secondary metabolite
13	9.272	0.78	2,6,10,14-tetramethyl heptadecane	Not a secondary metabolite
14	9.339	0.89	Tridecane	Not a secondary metabolite
15	9.441	2.52	Linalool	Terpenoid
16	9.691	0.67	2,6,10,14-tetramethyl heptadecane	Not a secondary metabolite
17	9.735	0.42	2-ethylhexylester oxalic acid	Not a secondary metabolite
18	9.808	0.50	3-methyl nonane	Not a secondary metabolite
19	10.027	0.11	5-methylundecane	Not a secondary metabolite
20	10.101	0.09	2-methyl octane	Not a secondary metabolite

21	12.028	0.15	1-Dodecene	Not a secondary metabolite
22	12.262	0.44	Dodecane	Not a secondary metabolite
23	15.110	0.49	Tridecane	Not a secondary metabolite
24	16.471	0.13	alpha-Cubebene	Terpenoid
25	17.185	1.34	alfa-Copaene	Terpenoid
26	17.645	2.76	gamma-Elemene	Terpenoid
27	17.834	0.50	Tetradecane	Not a secondary metabolite
28	18.083	0.46	[1aR-(1a.alpha.,4.alpha.,4a .beta.,7b.alpha.)]- 1a,2,3,4,4 a,5,6,7b-octahydro-1,1,4,7-tetramethyl-, 1H-Cycloprop[e]azulene	Terpenoid
29	18.251	0.17	(Z,E)-3,7,11-trimethyl- 1,3,6,10-Dodecatetraene	Terpenoid
30	18.364	6.72	Aromandendrene	Terpenoid
31	18.737	0.63	Santolina triene	Terpenoid
32	18.980	0.28	(1R,3aS,8aS)-7-Isopropyl-1,4-dimethyl-1,2,3,3a,6,8a-hexahydroazulene	Terpenoid

33	19.251	1.90	Humulene	Terpenoid
34	19.364	4.34	(E)-beta-famesene	Terpenoid
35	19.478	0.68	[1R-(1R*,4 Z,9S*)- 4,11,11-trimethyl-8-methylene-Bicyclo[7.2.0]undec-4-ene]	Terpenoid
36	19.872	0.46	Gamma-Muurolene	Terpenoid
37	19.982	3.74	[3aS-(3a. alpha.,3b.beta.,4.beta.,7.alpha.,7 aS*)]octahydro-7-methyl-3-methy lene-4-(1-methylethyl) -1H-Cyclopenta[1,3]cyclopropa[1,2]benzene	Not a secondary metabolite
38	20.114	2.07	[2R-(2.alpha.,4a.alpha.,8 a.beta.)]- 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylet henyl)-Naphthalene	Not a secondary metabolite
39	20.349	2.97	(E,Z)-alpha-Farnesene	Terpenoid
40	20.481	0.37	alpha-Muurolene	Terpenoid
41	20.588	0.37	8-Isopropenyl-1,5-dimethyl-cyclodeca-1,5-diene	Terpenoid
42	20.718	10.87	(E)-beta-Famesene	Terpenoid
43	20.982	1.45	2,4-Di-tert-butylphenol	Phenolic compound
44	21.089	7.59	[S-(R*,S*)]-3-(1,5-dimethyl-4-hexenyl)-6-methylene-cyclohexene	Terpenoid

45	21.260	0.90	(E)-1-Methyl-4-(6-methylhept-5-en- 2-ylidene)cyclohex-1-ene	Terpenoid
46	21.531	0.53	4-[(1E)-1,5-dimethyl- 1,4-hexadien-1-yl]-1-methyl-cyclohexene	Terpenoid
47	21.748	0.37	4-ethenyl-.alpha.,.alpha.,4-trimethyl-3-(1-meth ylethenyl)-, [1R-(1.alpha.,3.alpha .,4.beta.)]-cyclohexanemethanol	Terpenoid
48	21.881	1.00	Alloaromadendrene	Terpenoid
49	22.078	2.59	3,7,11-trimethyl-1,6,10-Dodecatrien-3-ol	Terpenoid
50	22.450	0.19	[1ar-(1a.alpha.,4a.alpha.,7.beta., 7a.beta.,7b.alpha.)]-decahydro-1,1,7-trimethyl-4-methylene Cycloprop[e]azulen-7-ol	Terpenoid
51	22.533	0.79	1,3-Bis-(2-cyclopropyl,2-methylcyclopropyl)-but-2-en-1-one	Not a secondary metabolite
52	22.691	1.06	Cetene	Not a secondary metabolite
53	22.927	0.58	Guaiol	Terpenoid
54	23.181	0.11	3,4 -dimethyl -3-Cyclohexen-1-carboxaldehyde	Terpenoid
55	23.598	0.16	Apiol	Phenolic compound

56	23.682	0.29	Aromandendrene	Terpenoid
57	23.983	0.21	tau.-Muurolol	Terpenoid
58	24.299	0.80	[1R-(1.alpha.,7.beta.,8a.a lpha.)]- 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-Naphthalene	Not a secondary metabolite
59	24.607	0.21	Alloaromadendrene	Terpenoid
60	24.711	0.46	[1R-(1.alpha.,7.beta.,8a.alpha.)]-1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-Naphthalene	Terpenoid
61	25.073	0.79	[1aR-(1a.alp ha.,4.beta.,4a.beta.,7.beta.,7a.be ta.,7b.alpha.)]-decahydro- 1,1,4,7-tetramethyl-1H-Cycloprop[e]azulene	Terpenoid
62	25.259	0.42	[1R-(1.alpha.,7.beta.,8a.a lpha.)]-1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-Naphthalene	Not a secondary metabolite
63	27.255	0.86	1-Octadecene	Not a secondary metabolite
64	27.790	0.22	(Z)-14-methyl-8-hexadecenal	Fatty acid derivative
65	29.764	0.83	1-(4-methyl-1H-imidazol- 2-yl)-Ethanone	Alkaloid

66	30.016	0.36	butyl- 2-ethylhexyl ester- 1,2-Benzenedicarboxylic acid	Fatty acid derivative
67	30.256	1.20	3-Heptafluorobutyroxypentadecane	Not a secondary metabolite
68	30.432	0.12	6-(Trifluoromethoxy)-N-(trimethylsilyl)-1,3-benzothiazol-2-amine	Alkaloid
69	31.081	0.08	1-Octadecene	Not a secondary metabolite
70	31.306	0.11	Phytol	Terpenoid
71	31.628	0.26	Ethyl 9.cis.,11.trans.-octadecadienoate	Fatty acid derivative
72	31.664	0.26	9-Oxabicyclo[6.1.0]nonane	Not a secondary metabolite
73	31.813	0.36	1-Docosene	Not a secondary metabolite
74	32.966	0.23	1-Docosene	Not a secondary metabolite
75	33.264	1.59	(E)-5-(Benzo[d][1,3]dioxol-5-yl)-N -isobutylpent-2-enamide	Alkaloid
76	34.004	0.49	Bis(2-ethylhexyl) phthalate	Not a secondary

				metabolite
77	34.141	0.30	(1S,15S)-Bicyclo[13.1.0]hexadecan-2-one	Terpenoid
78	34.213	0.49	2-hydroxy- methyl ester Tetradecanoic acid	Fatty acid derivative
79	34.685	3.70	1-Piperonyl-3,5-diamino-1,2,4-triazole	Not a secondary metabolite
80	34.884	8.32	1-Piperonyl-3,5-diamino-1,2,4-triazole	Not a secondary metabolite
81	35.655	0.41	E-1,9-Hexadecadiene	Not a secondary metabolite
82	35.842	1.79	(1.alpha.,2.beta.,4.beta.,6a)-Tricyclo[4.2.0.0(2,4)]octan-5-one	Not a secondary metabolite
83	36.007	0.68	Piperine	Alkaloid
84	36.183	0.94	6-methyl-Furo[3,4-c]pyridine-3,4(1H,5H)-dione	Alkaloid
85	34.447	1.45	3,4-dimethoxy-N-(4-cyanomethylphenyl)- Benzamide	Not a secondary metabolite
86	37.409	-0.15	(E,E)-1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]- Pyrrolidine	Alkaloid

Table 6: Phytochemicals identified in the aqueous extract of the plant

Peak	RT	%Area	Compound name	Class of secondary metabolites
1	5.310	0.26	Methylene chloride	Not a secondary metabolite
2	5.547	0.33	Methylene chloride	Not a secondary metabolite
3	5.723	0.33	Methylene chloride	Not a secondary metabolite
4	6.298	0.18	Methylene chloride	Not a secondary metabolite
5	6.363	0.58	1,2,3-trimethyl-Benzene	Not a secondary metabolite
6	6.496	0.87	Decane	Not a secondary metabolite
7	6.774	0.34	1-methylbutyl ester chloro acetic acid	Not a secondary metabolite
8	6.846	1.27	1,4-dichlorobenzene	Not a secondary

				metabolite
9	6.951	0.81	dl-Threitol	Not a secondary metabolite
10	7.132	0.50	1,1-dimethyl ethyl ester chloro acetic acid	Not a secondary metabolite
11	7.199	0.79	p-Cymene	Terpenoid
12	7.944	0.58	5,6-dimethyl-undecane	Not a secondary metabolite
13	8.112	1.25	Isobutyl nonyl ester oxalic acid	Not a secondary metabolite
14	8.159	1.38	gamma-Terpinene	Terpenoid
15	8.256	0.92	3,6-dimethyl-decane	Not a secondary metabolite
16	8.380	2.29	3,4-dimethyl-decane	Not a secondary metabolite
17	8.537	1.72	Decane	Not a secondary metabolite
18	8.593	0.57	Decane	Not a secondary metabolite
19	8.643	0.74	Allyl nonyl ester oxalic acid	Not a secondary

				metabolite
20	8.700	1.11	2,3,4-trimethyl-hexane	Not a secondary metabolite
21	8.787	0.73	1-Iodo-2-methylnonane	Not a secondary metabolite
22	8.911	1.72	3-methyl-undecane	Not a secondary metabolite
23	8.959	3.26	2,6,11-trimethyl-dodecane	Not a secondary metabolite
24	9.039	1.19	Nonyl vinyl ester carbonic acid	Not a secondary metabolite
25	9.119	1.42	2,4-dimethyl-heptane	Not a secondary metabolite
26	9.176	2.23	Dodecane	Not a secondary metabolite
27	9.270	2.31	2,6,10,14-tetramethyl heptadecane	Not a secondary metabolite
28	9.339	3.82	2-methyl decane	Not a secondary metabolite

29	9.481	0.97	Nonyl vinyl ester carbonic acid	Not a secondary metabolite
30	9.541	1.06	Nonyl prop-1-en-2-yl ester carbonic acid	Not a secondary metabolite
31	9.636	1.01	Nonyl vinyl ester carbonic acid	Not a secondary metabolite
32	9.693	1.29	2,3,7-trimethyl-octane	Not a secondary metabolite
33	9.737	0.88	2,6-dimethyl-octane	Not a secondary metabolite
34	9.807	2.48	3-methyl-nonane	Not a secondary metabolite
35	9.916	0.53	Decyl octyl ether	Not a secondary metabolite
36	10.024	1.27	2,6-Dimethyldecane	Not a secondary metabolite
37	10.098	1.00	2,3,5-trimethyl-hexane	Not a secondary metabolite
38	10.156	1.48	4,7-dimethyl-undecane	Not a secondary

				metabolite
39	11.771	0.38	Naphthalene	Not a secondary metabolite
40	12.026	0.45	1-Tridecene	Not a secondary metabolite
41	12.261	1.19	Dodecane	Not a secondary metabolite
42	14.938	0.36	1-methyl naphthalene	Not a secondary metabolite
43	15.108	1.24	Tridecane	Not a secondary metabolite
44	17.182	0.41	1-(3,3-Dimethyl-but-1-ynyl)-1,2-dimethyl-3-methylene-cyclopropane	Not a secondary metabolite
45	17.628	1.61	1-Hexadecanol	Fatty acid derivative
46	17.828	1.14	Tetradecane	Not a secondary metabolite
47	18.345	1.59	[1R-(1R*,4 Z,9S*)]-4,11,11 -trimethyl-8-methylene Bicyclo[7.2.0]undec-4-ene	Terpenoid
48	19.243	0.27	Humulene	Terpenoid

49	19.348	0.60	3-methylene-7,11-dimethyl -1,6,10-Dodecatriene	Terpenoid
50	19.433	0.04	2,6,10,14-tetramethyl heptadecane	Not a secondary metabolite
51	19.964	0.75	[3aS-(3a. alpha.,3b.beta.,4.beta.,7.alpha.,7 aS*)]-octahydro-7-methyl-3-methylene-4-(1-methylethyl)-1H-Cyclopenta[1,3]cyclopropa[1,2]benzene	Not a secondary metabolite
52	20.102	0.50	[2R-(2.alpha.,4a.alpha.,8 a.beta.)]-1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-Naphthalene	Not a secondary metabolite
53	20.339	0.49	[1aR-(1a.alpha.,4.alpha.,4a .beta.,7b.alpha.)] 1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramethyl-1H-Cycloprop[e]azulene	Terpenoid
54	20.409	0.81	Pentadecane	Not a secondary metabolite
55	20.678	2.52	beta-Bisabolene	Terpenoid
56	20.975	4.04	2,4-Di-tert-butylphenol	Phenolic

				compound
57	21.057	2.47	[S-(R*,S*)]-3-(1,5-dimethyl-4-hexenyl)-6-methylene-cyclohexene	Terpenoid
58	22.073	0.55	3,7,11-trimethyl-1,6,10-Dodecatrien-3-ol	Terpenoid
59	22.688	1.93	1-Nonadecene	Not a secondary metabolite
60	22.858	0.28	Hexadecane	Not a secondary metabolite
61	24.729	0.22	Aromandendrene	Terpenoid
62	27.255	2.22	E-14-Hexadecenal	Fatty acid derivative
63	28.803	0.03	Piperine	Alkaloid
64	29.148	0.31	Piperine	Alkaloid
65	29.214	0.61	Piperine	Alkaloid
66	29.294	0.07	Piperine	Alkaloid
67	29.495	0.17	Piperine	Alkaloid
68	29.553	0.10	Piperine	Alkaloid
69	30.017	0.95	Di-sec-butyl phthalate	Not a secondary

				metabolite
70	30.283	5.54	Ethyl ester Hexadecanoic acid	Fatty acid derivative
71	30.357	0.21	Z-11-Hexadecenoic acid	Fatty acid
72	30.432	0.40	N1-(1a,2,7,7a-tetrahydronaphtho[2,3-b]ociren-3-yl) acetamide	Not a secondary metabolite
73	30561	0.14	ethyl ester -2-acetyl heptanoic acid	Fatty acid derivative
74	31.089	0.39	Palmitoleic acid	Fatty acid
75	31.150	0.67	1-bromo- tridecane	Not a secondary metabolite
76	31.190	0.23	Cis 9-Oxabicyclo[6.1.0]nonane	Not a secondary metabolite
77	31.629	4.05	Ethyl ester Linoleic acid	Fatty acid derivative
78	31.662	3.35	Ethyl ester- 9-Octadecenoic acid	Fatty acid derivative
79	31.816	1.84	4-trifluoroacetoxytetradecane	Not a secondary metabolite
80	32.432	0.20	Cyclopentane undecanoic acid	Fatty acid

				derivative
81	32.609	1.03	2-ethyl-5-methyl-tetrahydrofuran	Not a secondary metabolite
82	32.969	0.71	4-Trifluoroacetoxyhexadecane	Not a secondary metabolite
83	33.581	0.16	Hexacosanoic acid	Fatty acid
84	34.003	1.39	Bis(2-ethylhexyl) phthalate	Not a secondary metabolite
85	34.220	0.99	Nonyl pentadecyl ester fumaric acid	Fatty acid derivative
86	34.727	0.53	2-ethylhexyl ester-2-methoxybenzoic acid	Not a secondary metabolite
87	34.884	1.48	(E)-5-(Benzo[d][1,3]dioxol-5-yl)-1 -(piperidin-1-yl)pent-2-en-1-one	Alkaloid
88	34.992	0.18	Oleic Acid	Fatty acid
89	35.373	0.57	(Z)-2-Hexene	Not a secondary metabolite
90	35.608	1.79	Threo-2,3-dibromopentane	Not a secondary metabolite
91	35.880	0.48	2-Methyl-Z,Z-3,13-octadecadienol	Fatty acid

				derivative

Table 7: Phytochemicals present in diethyl ether extract of the plant

Peak	**RT**	**%Area**	**Compound name**	**Class of secondary metabolite**
1	6.659	0.90	Propyl ester chloroacetic acid	Not a secondary metabolite
2	8.104	0.64	Methylthio-2,3-dimethylbutane	Not a secondary metabolite
3	9.275	0.38	Isobutylamine	Alkaloid
4	12.374	0.34	Ethyl ester chloro-acetic acid	Not a secondary metabolite
5	15.371	0.24	Acetate chloro methanol	Not a secondary metabolite
6	15.398	0.13	S-(Propoxythiocarbonyl)thiohydroxylamine	Not a secondary metabolite
7	17.006	0.29	1-methylbutyl ester chloro acetic acid	Not a secondary metabolite

8	17.053	0.10	S-(Isopropoxythiocarbonyl)thiohydroxylamine	Not a secondary metabolite
9	19.383	0.00	Cis- 9-Oxabicyclo[6.1.0]nonane	Not a secondary metabolite
10	19.585	0.01	2-methyl- octane	Not a secondary metabolite
11	20.222	0.38	N-[4-bromo-n-butyl]-2-piperidinone	Alkaloid
12	21.997	0.04	2,6,10-trimethyl-dodecane	Not a secondary metabolite
13	23.023	0.06	Cyclotetradecane	Not a secondary metabolite
14	23.819	2.85	(Z)-3-Nonen-1-ol	Terpenoids
15	24.189	0.07	1-fluoro-Tetradecane	Not a secondary metabolite
16	26.460	0.07	methyl ester-10-methyl dodecanoic acid	Fatty acid derivative
17	26.620	0.01	1-Heptadec-1-ynyl-cyclopentanol	Not a secondary metabolite
18	27.023	2.43	Metolachlor	Not a secondary metabolite
19	27.454	0.13	Metolachlor	Not a secondary

				metabolite
20	27.909	3.47	N-[4-bromo-n-butyl]-2-Piperidinone	Alkaloid
21	28.760	0.02	(2-hexyloctyl)-cyclopentane	Not a secondary metabolite
22	28.825	0.01	(Z)-2-Octadecen-1-ol	Fatty acid derivative
23	29.151	0.20	(E)-8-Methyl-9-tetradecen-1-ol- acetate	Fatty acid derivative
24	29.243	0.23	(Z)-2-Octadecen-1-ol	Fatty acid derivative
25	29.351	0.05	Octadecanal	Fatty acid derivative
26	29.401	0.05	N-[4-bromo-n-butyl]- 2- Piperidinone	Alkaloid
27	29.472	0.09	Tetradecyl-Oxirane	Fatty acid derivative
28	29.571	0.23	N-[4-bromo-n-butyl]- 2-Piperidinone	Alkaloid
29	29.845	0.29	1-Docosene	Not a secondary metabolite
30	29.916	0.14	N-[4-bromo-n-butyl]- 2-Piperidinone	Alkaloid
31	30.609	9.45	Octadecanal	Fatty acid derivative
32	30.920	0.11	1,2-dibromo-dodecane	Not a secondary metabolite
33	31.018	0.43	1,2-dibromo-dodecane	Not a secondary metabolite

34	31.155	0.38	N-[4-bromo-n-butyl]- 2-Piperidinone	Alkaloid
35	31.213	0.38	1-Docosene	Not a secondary metabolite
36	31.318	0.36	N-[4-bromo-n-butyl]- 2-Piperidinone	Alkaloid
37	31.376	0.34	E,Z-2,15-Octadecadien-1-ol acetate	Fatty acid derivative
38	31.597	1.65	1-Docosene	Not a secondary metabolite
39	31.949	12.67	Methyl ester-13-Docosenoic acid	Fatty acid derivative
40	32.246	1.78	1-Docosene	Not a secondary metabolite
41	32.332	0.63	Methyl ester-13-Docosenoic acid	Fatty acid derivative
42	32.477	1.52	Methyl ester-13-Docosenoic acid	Fatty acid derivative
43	32.710	12.63	1-Docosene	Not a secondary metabolite
44	32.985	2.02	Methyl ester-13-Docosenoic acid	Fatty acid derivative
45	33.082	1.05	Methyl ester-13-Docosenoic acid	Fatty acid derivative
46	33.104	0.58	Methyl ester-13-Docosenoic acid	Fatty acid derivative
47	33.410	13.42	1,7,11-trimethyl -4-(1-methylethyl)-cyclotetradecane	Terpenoids

48	33.590	0.32	1-Docosene	Not a secondary metabolite
49	33.661	0.65	6-Nitroundec-5-ene	Not a secondary metabolite
50	33.710	0.40	Ethyl Cyclodocosane	Not a secondary metabolite
51	33.777	0.64	5.alpha.14-methyl-Cholest-8-en-3-one	Steroid
52	33.821	0.30	allyl hexadecyl ester oxalic acid	Not a secondary metabolite
53	33.883	0.44	Methyl ester-13-Docosenoic acid	Fatty acid derivative
54	34.321	12.21	Dimethyl ester-2-undecyl Octanedioic acid	Fatty acid derivative
55	34.554	0.32	Methyl ester-13-Docosenoic acid	Fatty acid derivative
56	34.682	0.62	5.alpha.14-methyl Cholest-8-en-3-one	Steroid
57	34.793	0.69	Dimethyl ester-2- undecyl Octanedioic acid	Fatty acid derivative
58	34.860	0.50	1-chloro-tetradecane	Not a secondary metabolite
59	35.038	1.25	allyl hexadecyl ester oxalic acid	Not a secondary metabolite
60	35.304	8.22	4-octyldodecyl)-cyclopentane	Not a secondary

				metabolite
61	35.965	0.18	Allyl octadecyl ester oxalic acid	Not a secondary metabolite
62	36.787	0.01	Allyl hexadecyl ester Oxalic acid	Not a secondary metabolite
63	36.858	0.00	tetradecyl-oxirane	Fatty acid derivative
64	36.914	0.00	Allyl hexadecyl ester oxalic acid	Not a secondary metabolite

Table 8: Phytochemicals present in acetone extract of the plant

Peak	**RT**	**%Area**	**Compound name**	**Class of secondary metabolite**
1	6.365	0.64	Methylene chloride	Not a secondary metabolite
2	6.496	1.19	chloromethyl-Oxirane	Not a secondary metabolite
3	6.848	2.36	1,4-dichloro-Benzene	Not a secondary metabolite
4	7.131	0.24	S-(Propoxythiocarbonyl)thiohydroxy lamine	Not a secondary metabolite

5	7.199	0.34	o-Cymene	Monoterpene/terpenoid
6	7.946	0.51	2,2,4-trimethyl-Hexane	Not a secondary metabolite
7	8.113	3.13	isobutyl nonyl ester oxalic acid	Not a secondary metabolite
8	8.256	1.11	3,6-dimethyl-Decane	Not a secondary metabolite
9	8.382	3.67	2,6,10-trimethyl-Dodecane	Not a secondary metabolite
10	8.534	3.43	Nonane	Not a secondary metabolite
11	8.643	1.21	Decane	Not a secondary metabolite
12	8.699	1.66	2-methyl-Octane	Not a secondary metabolite
13	8.958	10.12	2,6,10-trimethyl-Dodecane	Not a secondary metabolite
14	9.118	2.25	Nonyl vinyl ester Carbonic acid	Not a secondary metabolite

15	9.176	3.55	Decane	Not a secondary metabolite
16	9.272	3.90	2,6,10,14-tetramethyl Heptadecane	Not a secondary metabolite
17	9.339	5.62	2-methyl-Decane	Not a secondary metabolite
18	9.485	1.36	2,6,11-trimethyl- Dodecane	Not a secondary metabolite
19	9.542	1.49	nonyl vinyl ester carbonic acid	Not a secondary metabolite
20	9.692	5.95	7- methyl Heptadecane	Not a secondary metabolite
21	9.806	2.71	2,6,11-trimethyl- Dodecane	Not a secondary metabolite
22	10.026	1.68	2,6-Dimethyldecane	Not a secondary metabolite
23	10.101	2.90	2,6-Dimethyldecane	Not a secondary metabolite
24	12.027	0.56	(Z)-5-Dodecene	Not a secondary metabolite

25	12.262	2.04	Dodecane	Not a secondary metabolite
26	15.109	2.11	Tridecane	Not a secondary metabolite
27	17.625	1.56	1-Hexadecanol	Fatty alcohol/ lipid derivative
28	17.830	1.57	Hexadecane	Not a secondary metabolite
29	20.414	1.07	Pentadecane	Not a secondary metabolite
30	20.974	9.65	2,4-Di-tert-butylphenol	Phenolic compound (Antioxidant)
31	22.691	3.35	1-Pentadecene	Not a secondary metabolite
32	22.859	0.50	Hexadecane	Not a secondary metabolite
33	27.259	4.06	1-Octadecene	Not a secondary metabolite
34	30.022	1.19	butyl- 2-ethylhexyl ester-1, 2-Benzenedicarboxylic acid	Not a secondary metabolite

35	30.257	3.19	1,2-diethyl Cyclohexadecane	Not a secondary metabolite
36	30.433	0.68	1,5,6,7-tetrahydro-3,6,6-trimethyl-1-phthalazin-1-yl- Indazol-4-one	Not a secondary metabolite
37	31.324	0.23	1-bromoOctane	Not a secondary metabolite
38	31.665	0.65	Ethyl Oleate	Fatty acid ester/Lipid derivative
39	31.814	1.80	1-Docosene	Not a secondary metabolite
40	31.980	0.29	Octadecyl ester bromoacetic acid	Not a secondary metabolite
41	32.970	0.78	1-Docosene	Not a secondary metabolite
42	34.008	1.89	Bis(2-ethylhexyl) phthalate	Not a secondary metabolite
43	34.217	1.79	1-Hexacosene	Fatty acid derivative/lipid derived metabolite

3.3 FTIR Analysis

The results of the FTIR analysis are given in **Tables 8, 9, 10** and **11** and in **Figures 9, 10, 11** and **12.**

The=C-H out-of-plane bending vibrations of hydrogens attached to unsaturated carbons give rise to strong absorption bands in the 1000-800cm^{-1}region. The O-H bending of alcohols and phenols results in strong absorption band in the 1410-1260cm^{-1}region. The C=C stretching vibration of conjugated alkenes give rise to absorption band in the 1650-1600cm^{-1}region. All acid anhydrides show two strong absorption bands of C=O stretch near 1800cm^{-1} and 1750cm^{-1} region. –N=C=S stretch of isothiocyanates exhibit strong band within the 2140-1990 cm^{-1} range. The S-H stretch of organo-sulphur compounds show weak band within the range of 2600-2550cm^{-1}. C-H stretch of alkanes yield strong absorption band below 3000cm^{-1}. The O-H streching vibrations of carboxylic acids give rise to broad absorption band from 3300cm^{-1} to 2500cm^{-1}. Two absorptions band arise from the asymmetric and symmetric stretching vibrations of two N-H bonds of free primary amines, one near 3500cm^{-1} and the other near 3400cm^{-1}. C=O stretch of caboxylate ion (CO_2^-) give rise to strong absorption band in the 1420-1300cm^{-1} region. Allenes show moderately intense band, sometimes a double peak at 2000-1900cm^{-1} due to asymmeic C=C=C sretching vibration. N-H bending band of amines is very weak and appears at 1650-1550cm^{-1}. The C=C stretching vibration give rise to absorption band in the in the 1680-1620cm^{-1} region in simple alkenes. The C≡C stretch of alkynes with weak absorption band occurs within the range 2140-2100cm^{-1}. C-H stretch absorption results in two weak bands near 2820cm^{-1} and 2720cm^{-1}.A sharp band just above 3000cm^{-1} is as a result of the unsaturated =C-H stretching vibration in alkenes. The intramolecular hydrogen- bonded O-H stretch of alcohols and phenol give rise to sharp strong band within 3600-3200cm^{-1}. Free O-H sretching band which is sharp appear in the 3650-3200cm^{-1} region. The P-H stretch of phosphorus compounds with sharp strong bands falls within the the 2440-2350cm^{-1} region. Amino salts show broad absorption band at approximately 3000cm^{-1}. Out of plane C-H bending vibrations of meta-disubstituted benzene ring has strong band of 810-750cm^{-1} region. C-H stretch of –O-CO-CH_3group

results in strong absorption band at 1385-1365cm^{-1} region. N-H bending of amino group results in medium bands at 1650-1560cm^{-1} region. C=O stretch overlap of primary amide I and II in solid state absorbs at approximately 1640cm^{-1}. C=O stretch of extended quinones show absorption band in the 1655-1635cm^{-1} region. –C=C- stretch of conjugated alkenes give rise to very sharp absorption band at approximately 1625cm^{-1} [28, 29, 30, 31].

Table 9: FTIR Analysis of diethyl ether extract of *C. filiformis*

Absorption Frequency (cm^{-1})	**Band description**
878.7349	=C-H out of plane bending of alkenes
1391.126	-O-H Bending of alcohols and phenols
1615.566	C=C stretch of conjugated alkenes
1847.839	C=O stretch of acid anhydride
2073.922	Cyanide, thiocyanate and cyanate ions,-N=C=S stretch of isothiocyanates
2600.633	S-H stretch of organo-sulphur compounds: thiols
2717.155	C-H stretch of alkanes
3223.531	Strongly hydrogen-bonded O-H stretch of carboxylic acid
3345.79	N-H Symmetrical stretch of free primary amines

3492.406	N-H unsymmetrical stretch of free primary amines

Table 10: FTIR analysis of the acetone extract of *C. filiformis*

Absorption Frequency (cm^{-1})	**Band description**
841.0694	=C-H out of plane bending of alkenes
1420.436	C=O symmetrical stretch of carboxylate ions ($-CO_2^-$)
1618.805	C=C stretch of alkenes
1845.046	C-H vibration of adjacent H's in aromatic compounds
1920.277	C=C=C stretch of allenes
2121.931	C≡C stretch of alkynes
2509.521	O-H Stretch of carboxylic acid
2787.422	C-H stretch of aldehyde
2886.911	C-H stretch of alkanes
2998.896	C-H stretch of alkanes
3167.455	=C-H stretch of alkenes

3578.582	Intramolecular hydrogen bonded -OH of alcohols and phenols in chelate form
3792.477	Free –OH stretch of alcohols

Table 11: FTIR analysis of ethanol extract of *C. filiformis*

Absorption Frequency (cm^{-1})	**Band description**
872.3964	=C-H out of plane bending of alkenes
1373.27	-O-H bending of alcohols
1607.77	C=O anti-symmetrical stretch of carboxylate ions ($-CO_2^-$)
2031.799	Cyanide, thiocyanate and cyanate ions –N=C=S of isothiocyanates
2459.414	P-H stretch of Phosphorus compounds
2969.937	N-H stretch of amino salts
3185.705	Intramolecular hydrogen-bonded –O-H stretch of alcohol in chelate form
3304.363	N-H symmetrical stretch of aliphatic primary amine
3430.795	N-H asymmetrical stretch of aliphatic primary amine, intermolecular H-bonded O-H stretch of

	alcohols
3810.158	Free O-H stretch of alcohols

Table 12: FTIR analysis of water extract of *C. filiformis*

Absorption Frequency (cm^{-1})	**Band description**
780.3195	Out of plane C-H bending vibrations of meta-disubstituted benzene ring
1382.166	O-H bending of alcohol, C-H stretch $–O-CO-CH_3$ group
1639.613	C=O stretch of overlap of primary amide I and II, C=O stretch of extended quinones, -C=C- stretch of conjugated alkenes
2024.582	Cyanide, thiocyanate, cyanate ions, -N=C=S stretch of isothiocyanate
2623.294	Intramolecular hydrogen-bonded O-H of alcohols in chelate form
2899.572	N-H stretch of amino salts
3240.487	Intramolecular hydrogen-bonded O-H stretch of alcohols in chelate form

3801.152	Free O-H stretch of alcohols

3.4 UV-Vis profiling

The UV-Vis spectrum profiles of the ethanol and aqueous extracts of Cassytha filiformis were selected from wavelength 200 to 800nm at 50nm interval whereas those of the acetone and diethyl ether extracts were selected from wavelength 220 to 940nm at the range of 20-50nm interval. The diethyl ether extract exhibited highest absorbance at both 350nm and 450nm and lowest absorbance at 220nm (**Figure 12** and **Table 13**). The maximum absorbance of the acetone extract occurred within the wavelength range of 350-600nm while the minimum absorbance occurred at 220nm wavelength (**Figure 14** and **Table 13**). Also, the ethanol and aqueous extracts of the plant exhibited highest absorbance at the wavelength range of 600-800nm and lowest absorbance at wavelengths 250nm and 400nm respectively (**Figures 13 & 15 and Tables 13**). All the plant extracts exhibited significant absorption in all the wavelength range of the UV-Vis ray making the plant highly conjugated as well as a potential sunscreen agent [15, 16, 20]**.**

Table 13: Absorbance of the plant extracts at different wavelengths

Wavelenghth (nm)	**Acetone extract Abs.**	**Diethyl ether extract Abs.**	**Wavelength (nm)**	**Ethanol extract absorbance**	**water extract absorbance**
220	0.033	0.028	**200**	0.174	0.08
260	0.045	0.032	**250**	0.064	0.14

280	0.056	0.056
300	0.078	0.06
320	0.089	0.073
350	2.141	2.019
380	2.32	1.879
420	2.97	1.922
450	2.694	2.017
470	2.859	1.796
490	2.859	1.66
520	2.688	1.384
550	2.387	1.148
580	2.243	1.012
600	2.129	0.928
650	1.998	0.819
700	2.342	0.989
750	1.517	0.615
780	1.39	0.56

300	0.083	0.072
350	0.158	0.21
400	0.243	0.05
450	0.213	0.116
500	0.791	0.74
550	0.964	0.522
600	1.598	0.764
650	2.5	0.861
700	2.122	0.713
750	2.894	0.943
800	2.143	1.021

810	1.289	0.521
850	1.17	0.476
900	1.084	0.433
940	0.746	0.515
980	0.7	0.322

Conclusion

The In vitro antimicrobial analysis of the four solvent extracts of the plant indicated antimicrobial activities against the given seven test bacteria and seven test fungi. The results revealed that the *C. filiformis* whole plant extracts possess attractive antimicrobial activities and therefore can serve as a broad spectrum antibiotics for the treatment of fungi and bacteria diseases. The whole plant extracts could be applied in food and pharmaceutical fields. The assessment of the phytochemicals, proximate and mineral compositions of the whole plant of *C. filiformis* has been investigated and the bioactive agents responsible for its antimicrobial properties ascertained. It is recommended that the plant be used to develop new cost-effective antimicrobial drugs and pharmaceuticals. In addition, the in vivo antimicrobial assessment of the purified extracted compounds from *C. filiformis* should be investigated.

Declarations

Ethical Approval

No human or animal testing was involved in this study

Consent to Participate

Not Applicable

Consent to Publish

Not Applicable

Data availability

The complete data sets supporting the research findings are included in the supplementary files.

Author Contribution Statement

Ebele Joy Morah, Emeka Christian Ezeudu and Christian Chukwuemeka Oli were involved in the conception and design of the experiment, interpretation of results, writing and proofreading of the manuscript. Ozioma Juliana Anekwe-Nwekeaku provided resources and methodology. Project administration was done by Nkechi Chinwendu Nwakife. Sample collection and preparation was performed by Uzoamaka Anthonia Emezie, Theresa Uzoma Onuegbu supervised and validated the research. All authors read and approved the final manuscript.

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No financial sponsorship was in anyway received for the research.

Conflict of Interest Statement

Authors declare that there is no conflict of interest among them.

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APPENDICES

Figure 1. *Cassytha filiformis* vine

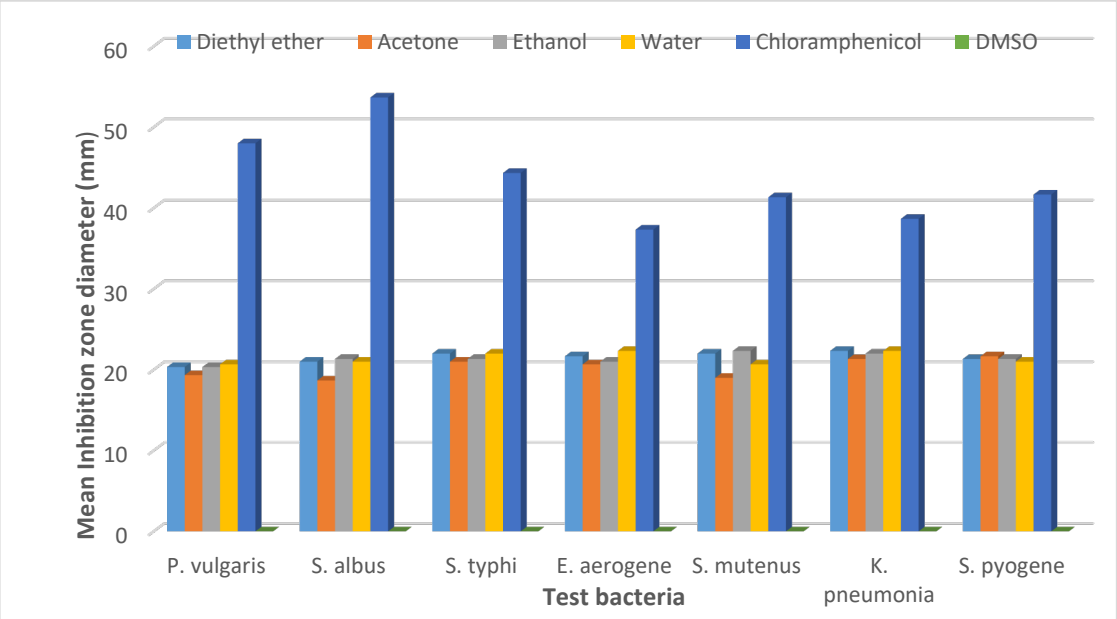


Figure 2. A plot of mean zones of inhibition of solvent extracts of *C. filiformis* against test bacteria

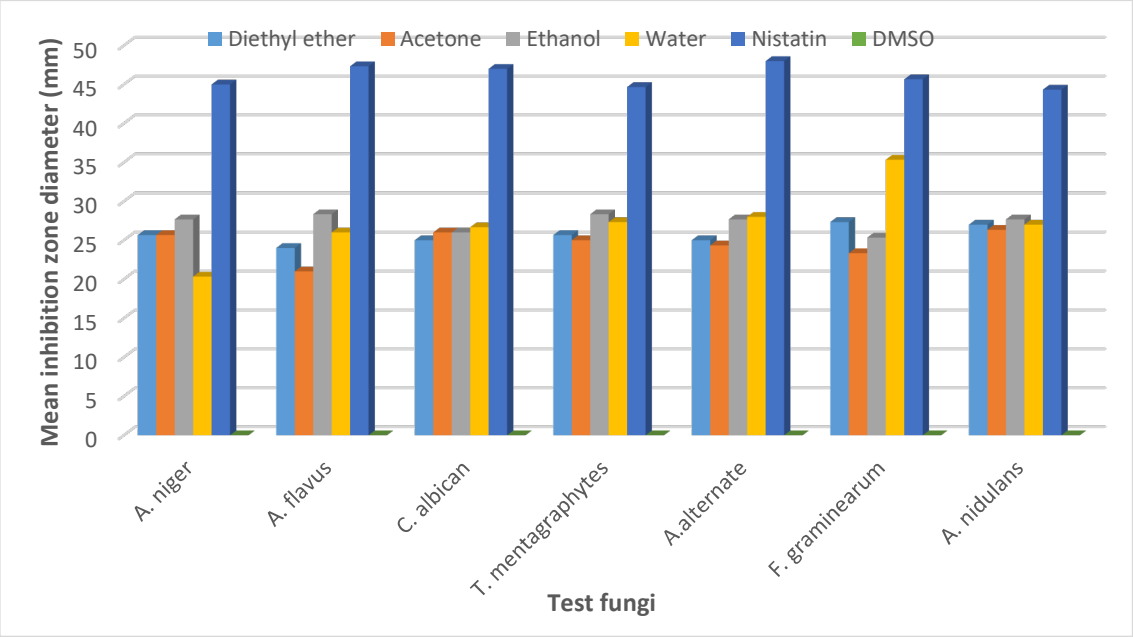


Figure 3. A plot of mean zones of inhibition of solvent extracts of *C. filiformis* against test fungi

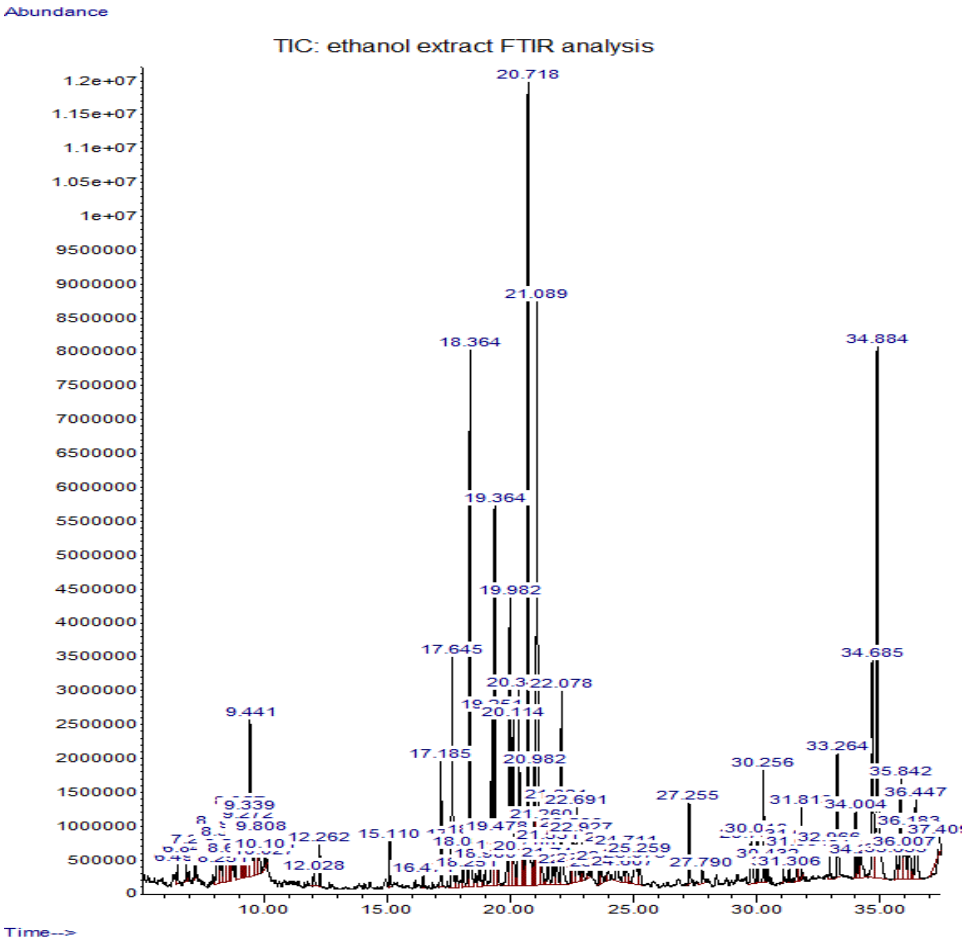


Figure 4. GC chromatogram of the phytochemical content of ethanol extract of the plant

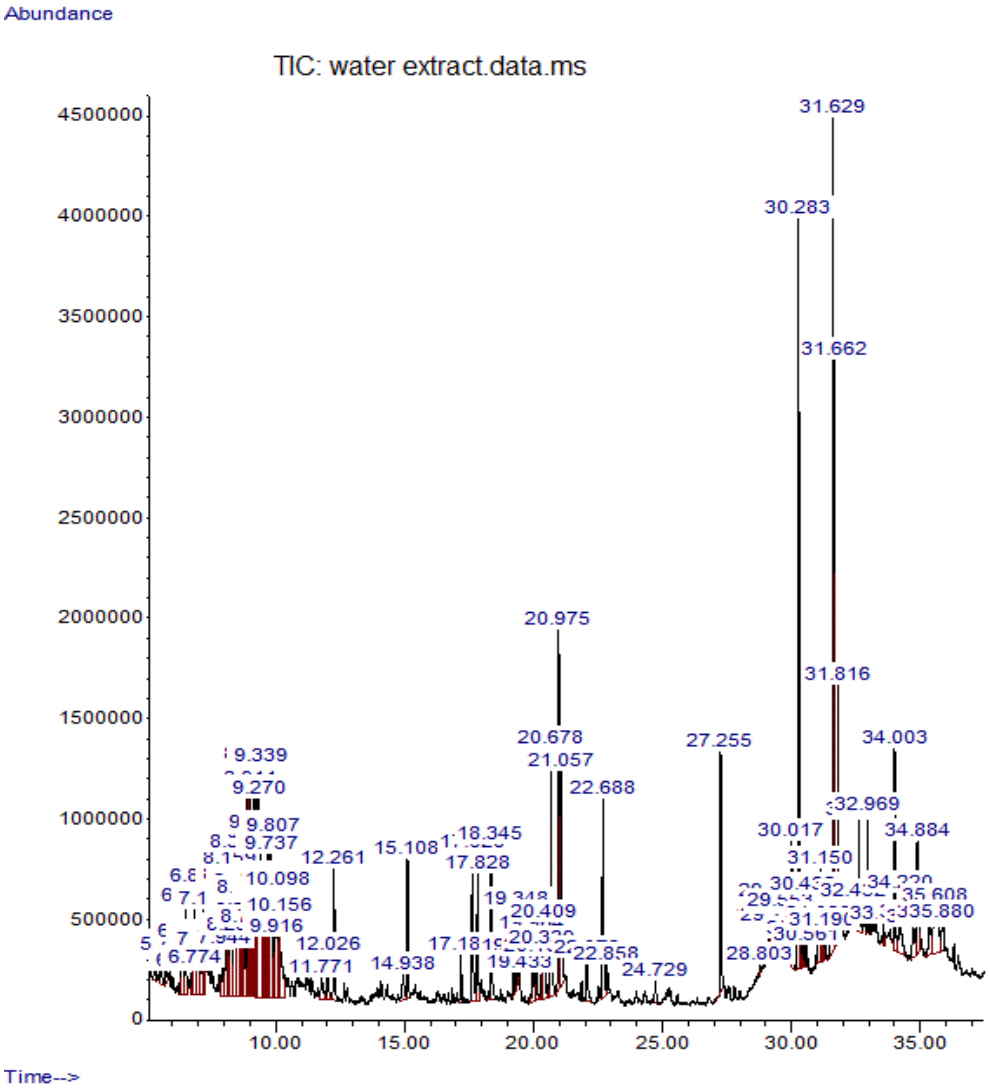


Figure 5. GC chromatogram of the phytochemicals in aqueous extract of the plant

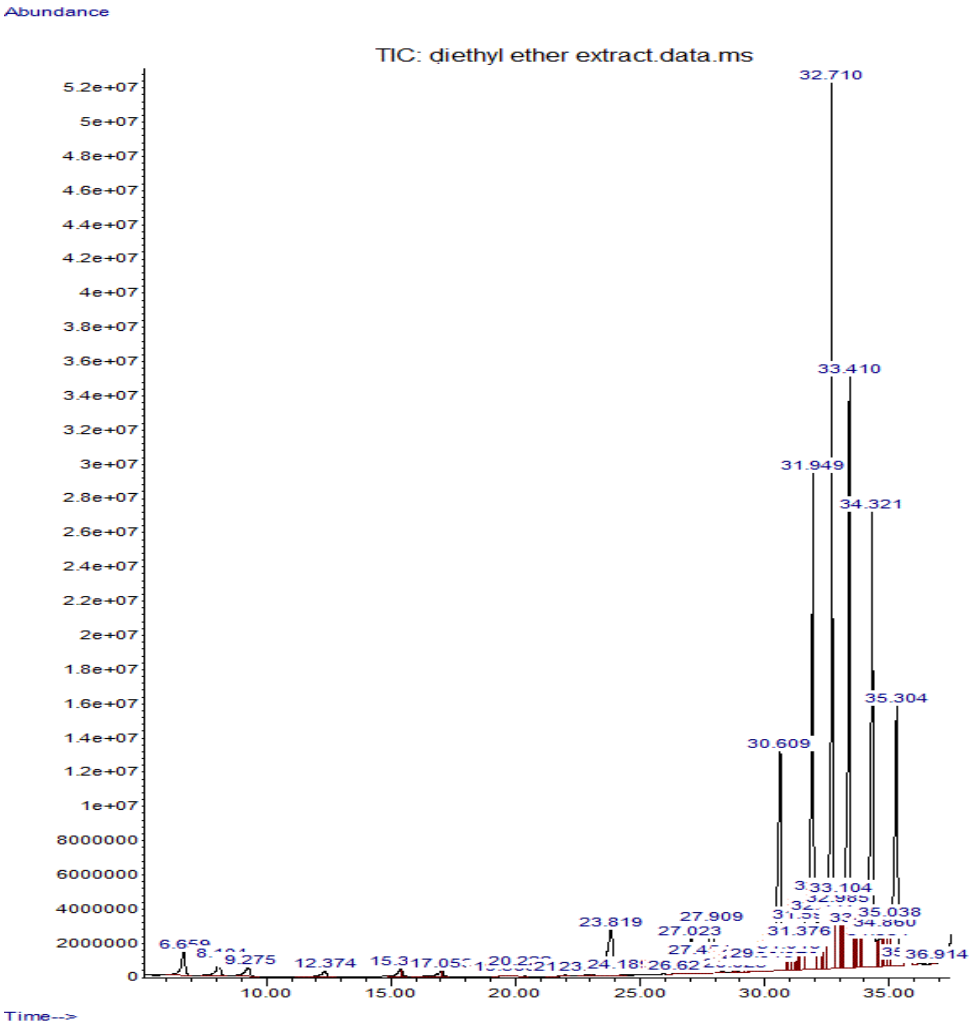


Figure 6. GC chromatogram of the phytochemicals present in diethyl ether extract of the plant

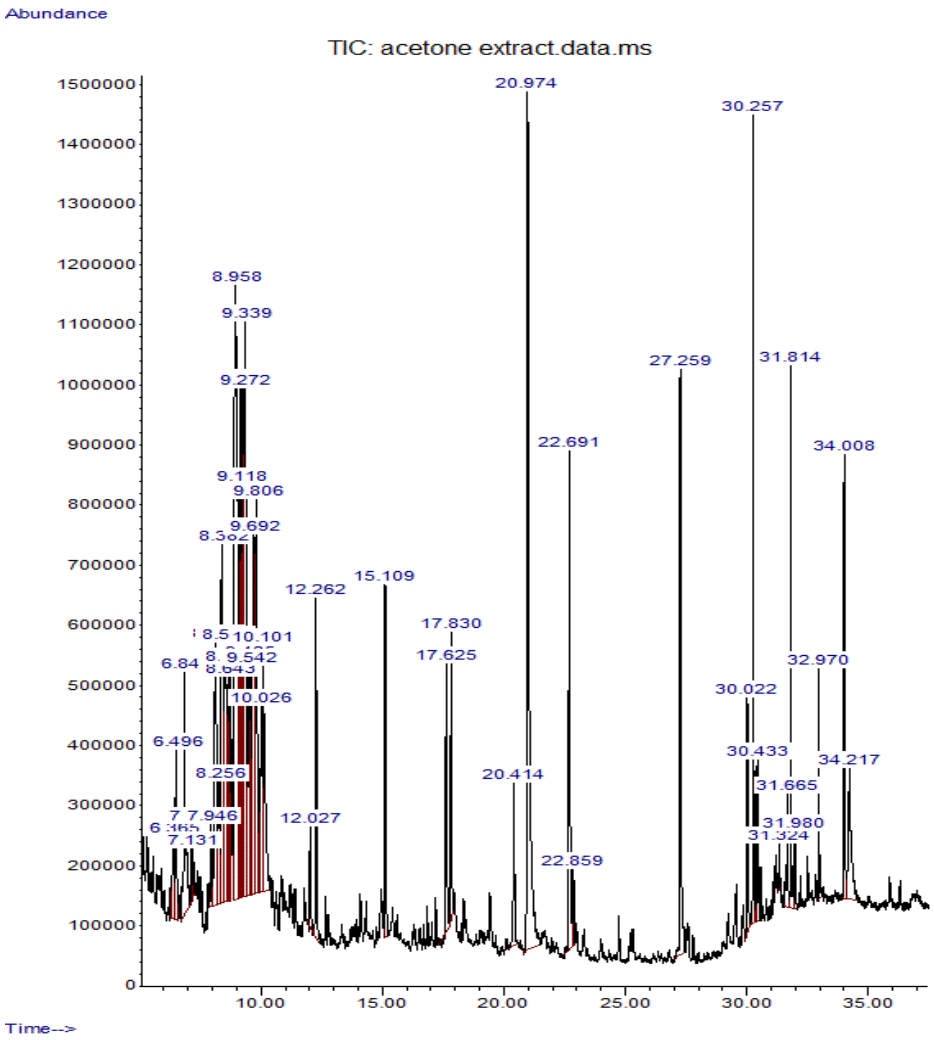


Figure 7. GC chromatogram of the phytochemicals present in acetone extract of the plant

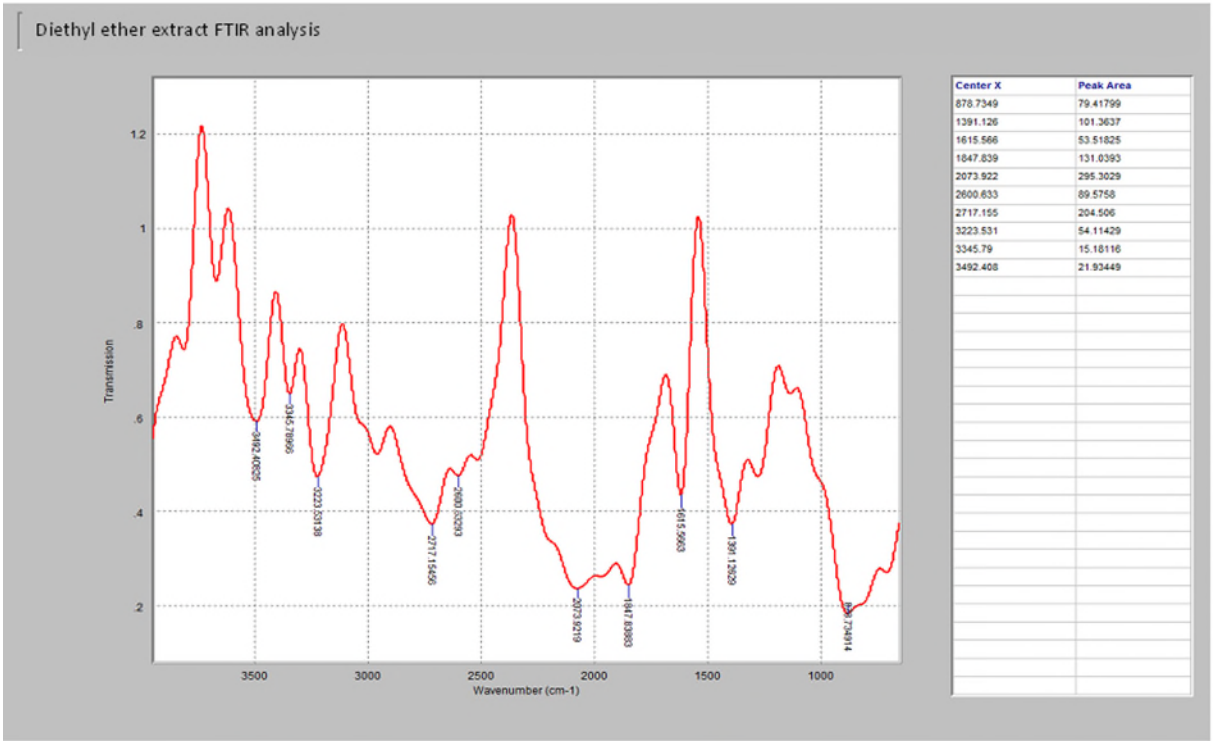
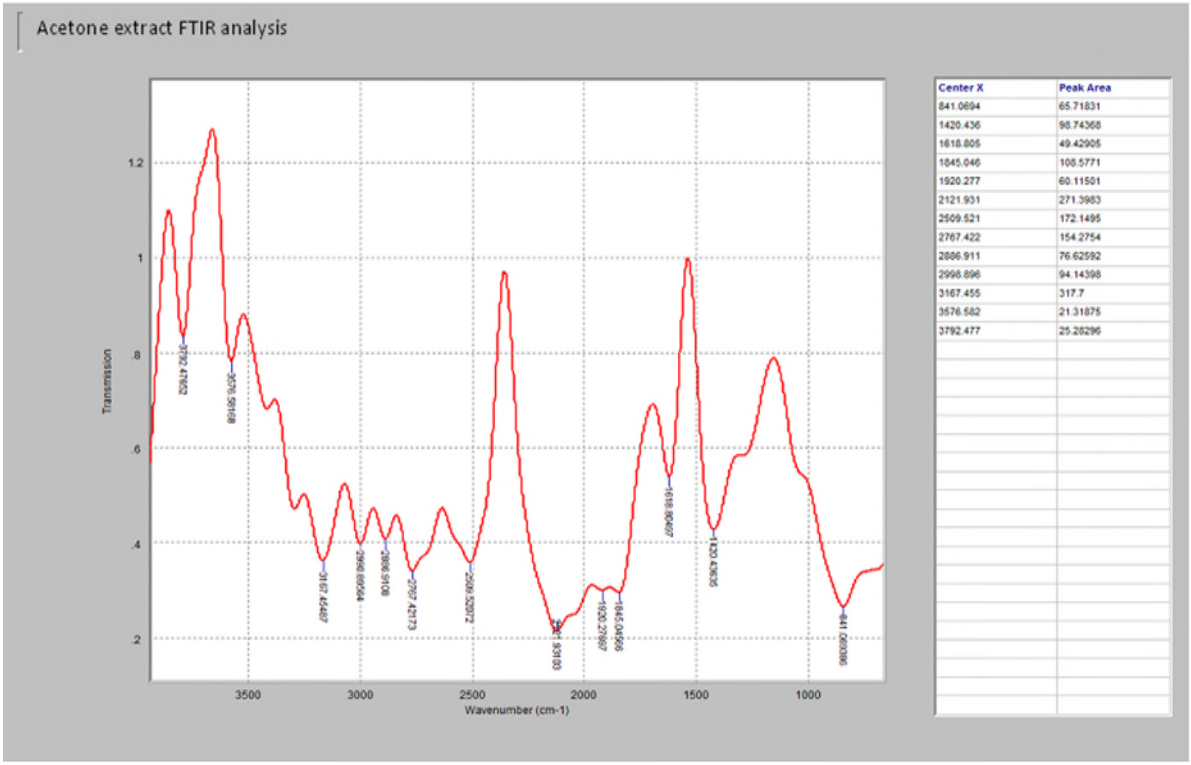


Figure 8. FTIR Spectra of diethyl ether extract



Center X	Peak Area
841.0694	65.71831
1420.436	98.74368
1618.805	49.42905
1645.046	106.5771
1920.277	60.11501
2121.931	271.3983
2509.521	172.1495
2767.422	154.2754
2886.911	76.62592
2998.896	94.14398
3167.455	317.7
3676.582	21.31875
3792.477	25.28296

Figure 9. FTIR Spectra of Acetone extract

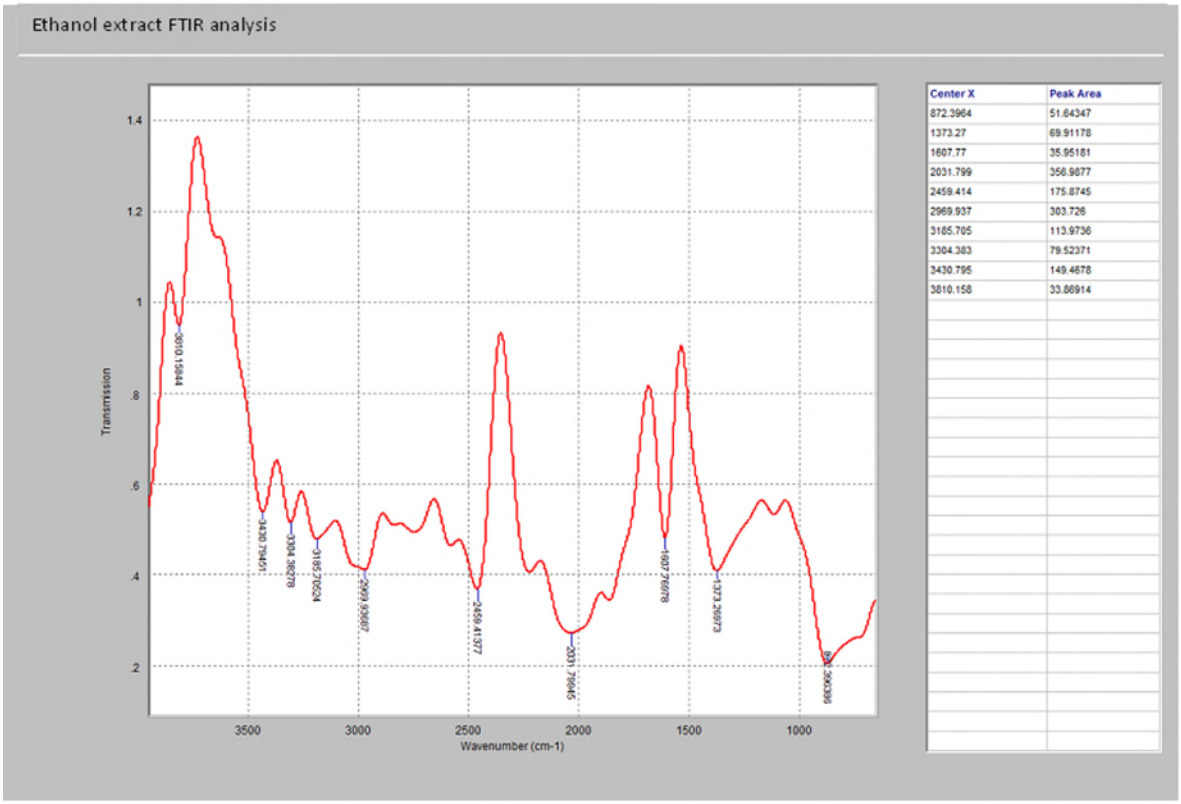


Figure 10. FTIR Spectra of ethanol extract

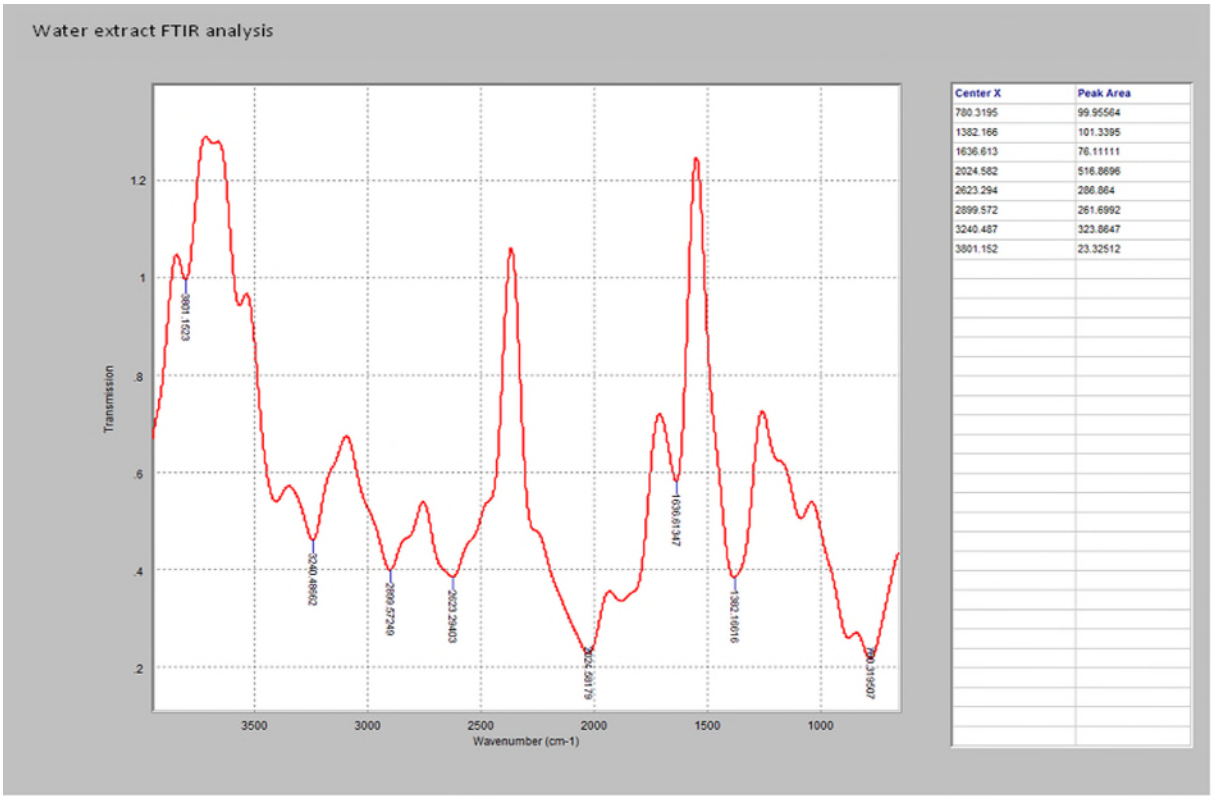


Figure 11. FTIR Spectra of water extract

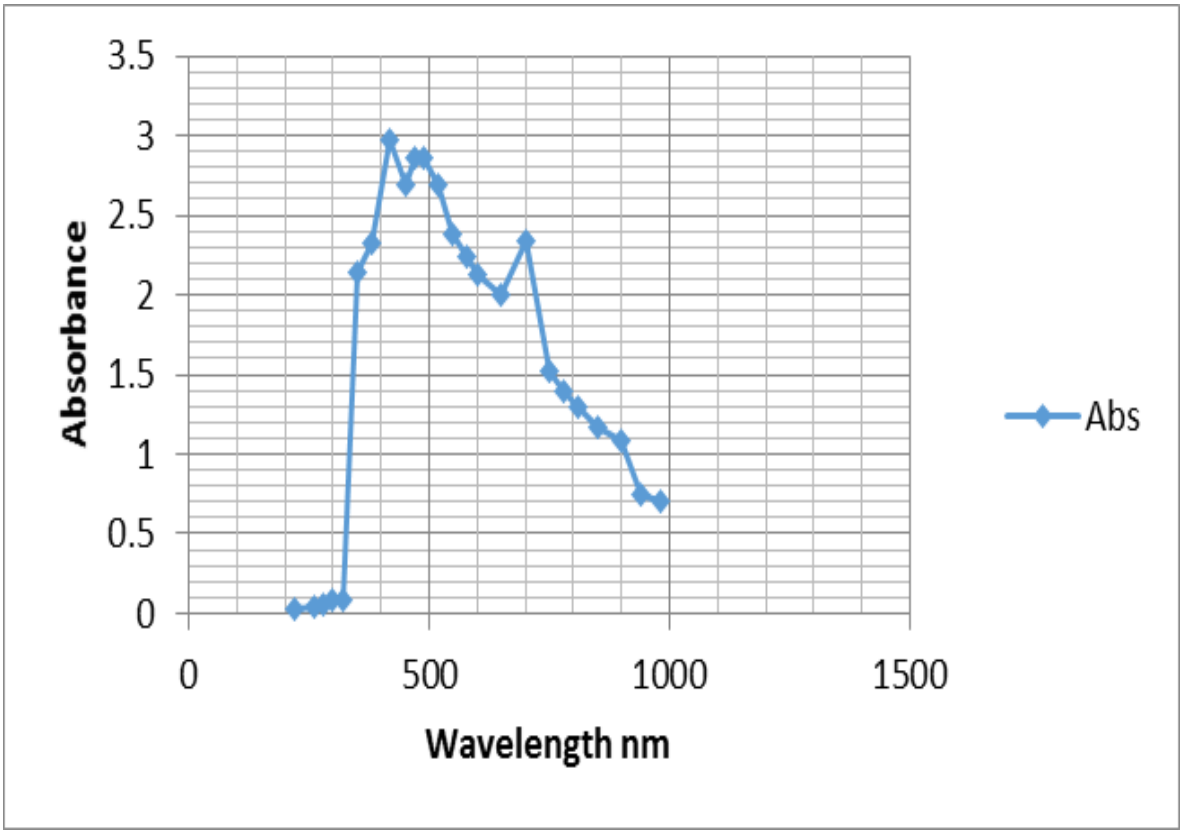


Figure 12. UV-Vis spectrum of diethyl ether extract

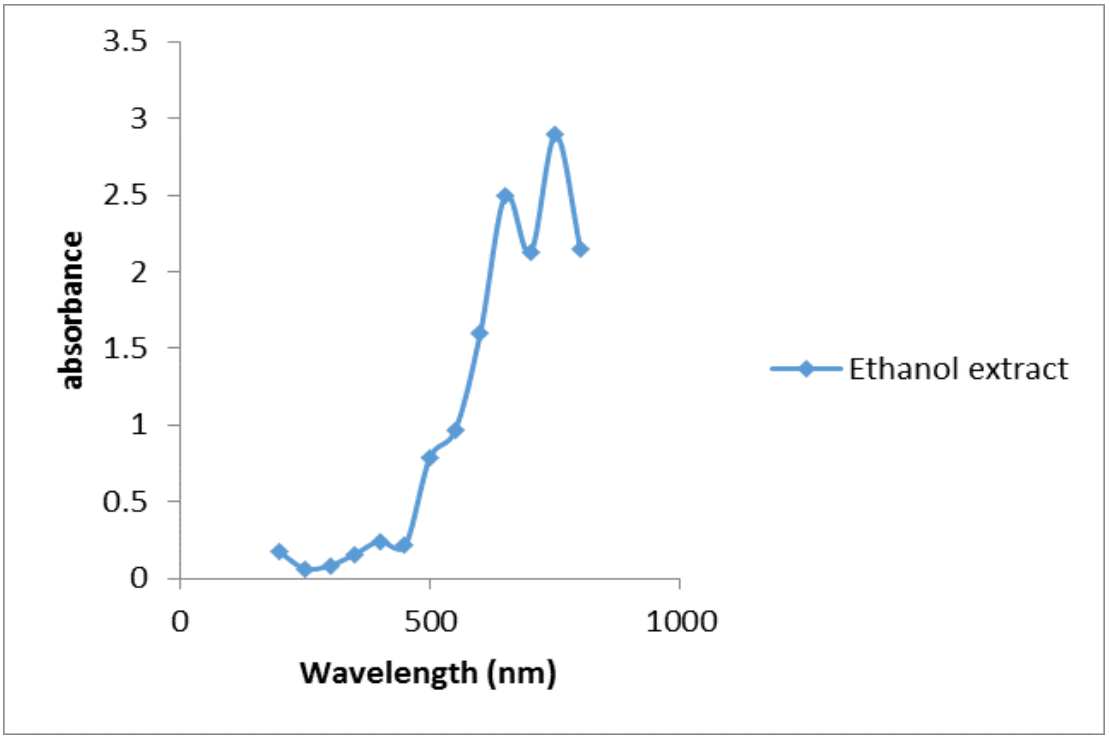


Figure 13. UV-Vis spectrum of ethanol extract

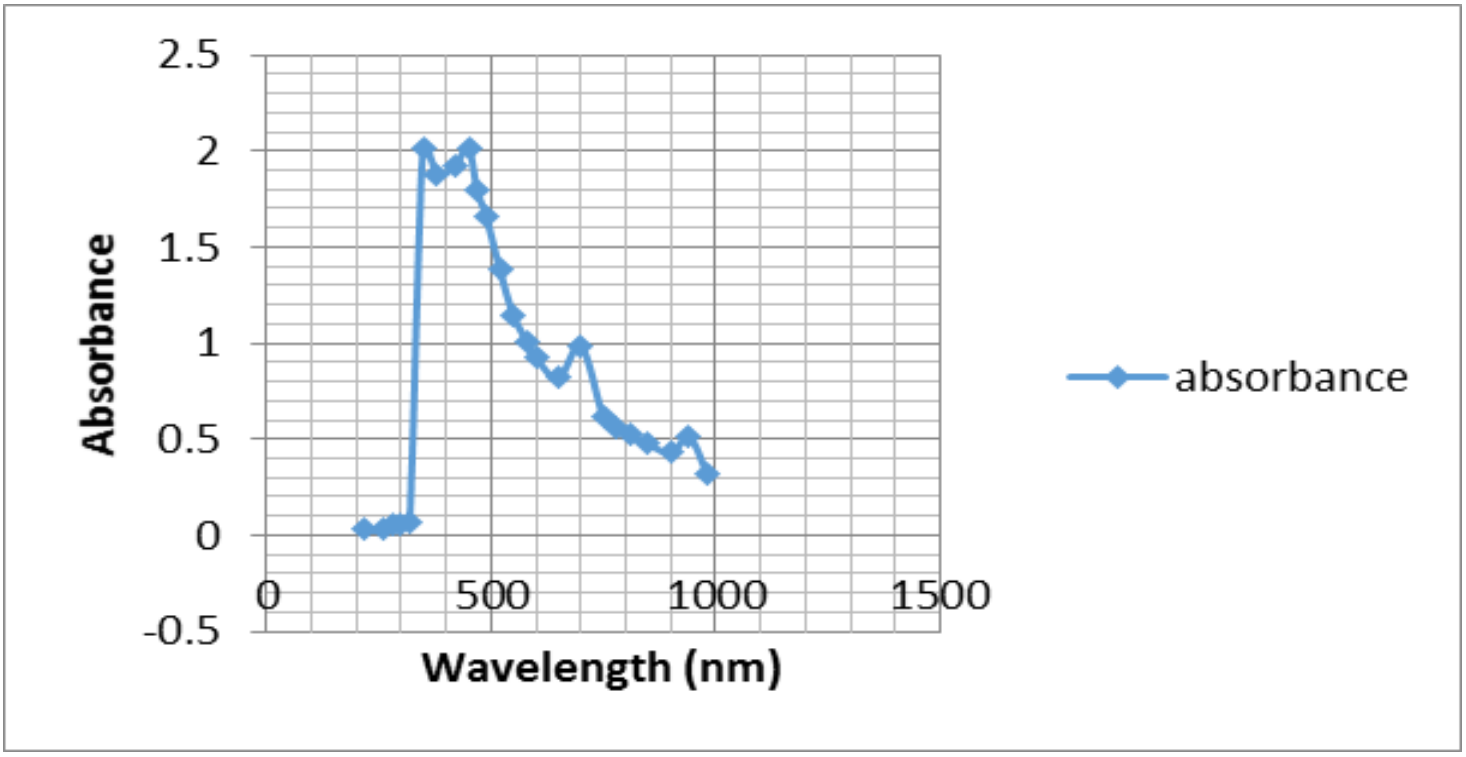


Figure 14. FTIR spectrum of acetone extract

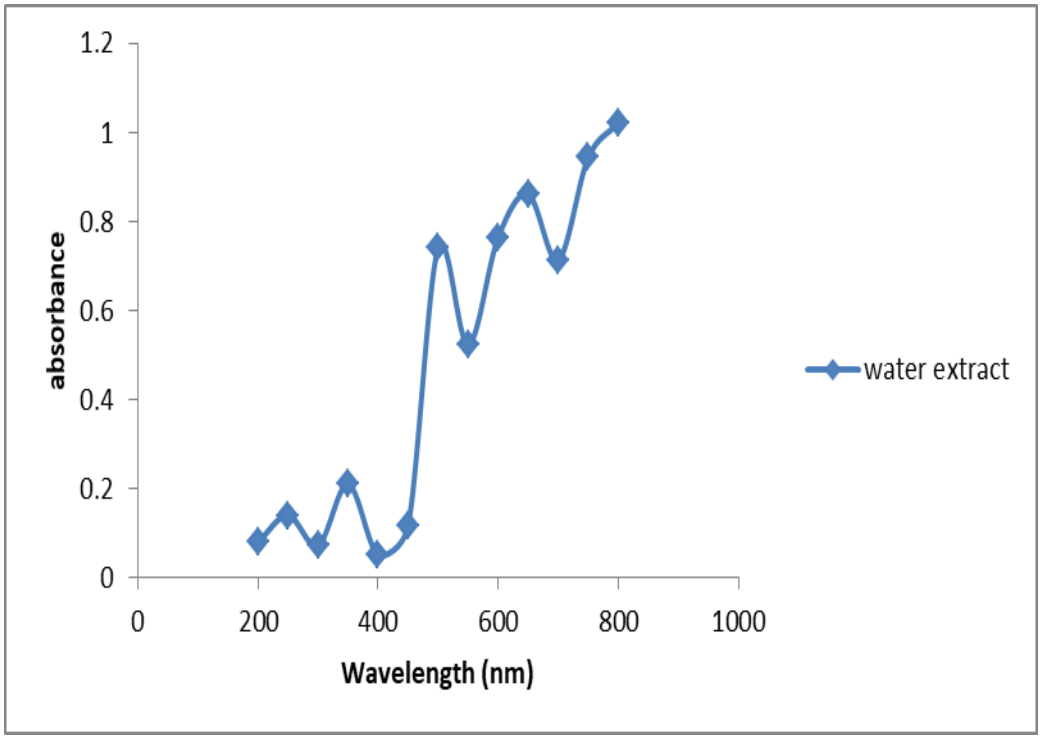


Figure15. UV-Vis spectrum of water extract