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ANTIMICROBIAL ACTIVITY, PHYTOCHEMICAL SCREENING AND GC-MS ANALYSIS OF PETROLEUM ETHER EXTRACT OF THE MEDICINAL PLANT *CYATHEA NILGIRENSIS* HOLTTUM

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ABSTRACT

The present study was carried out to identify the bioactive compounds present in the Pet-ether extract of *Cyathea nilgirensis* Holttum and to study the antimicrobial activity of the petroleum ether extract of the plant. The zone of inhibition and percentage of inhibition of the extract against test microorganisms was determined using disc diffusion method. The test microorganisms included six standard bacterial species - *Staphylococcus aureus, Bacillus subtilis, Micrococcus luteus, Escherichia coli, Salmonella paratyphi and Klebsiella pneumoniae and two fungal species - Candida albicans* and *Aspergillus niger.* The primary and secondary metabolites were identified using spot tests. Various bioactive compounds were identified using GC-MS analysis. The petroleum ether fraction inhibited the chosen microbial species. GC-MS analaysis had shown the presense of compounds like Squalene, Pentacosane, Triacontane, 5,10 –dimethy- l6,8- bisdehydro pentapentadecafulvalene and 4-[10-(3,4-Dimethoxy) phenanthryl] morpholine in the petroleum ether extract.

KEYWORDS: *Cyathea nilgirensis* Holttum, Pet-Ether Extract, Preliminary screening, GC-MS Analysis & Antimicrobial Activity.

INTRODUCTION

Cyathea nilgirensis Holttum, a southern Indian endemic tree fern.[1] India has 11 species of *Cyathea* of which *C. nilgirensis* has been listed endangered.[2] *Cyathea nilgirensis* Holttum is used against snake bite.[3] The plant has central analgesic activity[4] and anti-diabetic activity.[5] Fresh rhizome of *Cyathea gigantea another species of cyathea is* mixed with powdered black pepper seeds and taken orally with milk twice a day for one week in empty stomach against white discharges.[6] Areal parts of *Cyathea gigantea has* anti-inflammatory properties.[7] Another species of *cyathea* named *Cyathea crinita* (Hook) Copel exhibit antibacterial properties.[3,8] This tree fern is found in the forests of Kerala, Karnataka, Tamil Nadu, and Andhra Pradesh. The presence of tannin, saponnin, flavonoids, steroids, terpenoids, triterpenoids, anthroquinone, polyphenol, glycoside, coumarin, carbohydrate and protein in the chloroform extract of *Cyathea nilgirensis* Holttum has been reported by G. Pradheesh et al. The antimicrobial efficiency of the chloroform extract of the plant has also been reported.[9] Janakiraman and Johnson[10, 11] studied the UV-Vis and FT-IR spectroscopic profile of *C. nilgirensis, C. gigantea* and *C. crinita*. So the present study is carried out to identify the phytoconstituents and antimicrobial efficiency of the petroleum ether extract of the plant *Cyathea nilgirensis* Holttum.

MATERIALS AND METHODS

Plant materials

The plant *Cyathea nilgirensis* Holttum was collected from Coonoor areas of Nilgiris District. The specimen was authenticated by Botanical Survey of India (BSI), located in Coimbatore, TamilNadu, India.

Extraction for preliminary screening

The leaves of *Cyathea nilgirensis* Holttum were cleaned and dried in shade. The shade dried leaves were cut in to small pieces and grinded in to powder using electric pulverizer. 5 g portions of powdered plant materials were dispersed in 50 ml of 100% petroleum ether. The solution was left to stand at room temperature for 24 hrs and was filtered with Whatman No. 1 filter paper. The filtrate obtained is used for the phytochemical screening of secondary metabolites as per the standard procedure from "Phytochemical Methods "by J.B Harborne.[12-15] The details of procedure are given in table1.

Extraction for GC-MS and Anti-microbial Activity

25 g of the leaf powder was weighed and subjected to extraction with 300 ml of petroleum ether solvent for 8 hr temperature ranging from 35-60˚C using Soxhlet apparatus. The leaf extract so obtained was concentrated by distillation. Then the crude extract was subjected to GC-MS analysis and Antimicrobial studies.

GC-MS Analysis

The trace GC Ultra and DSQ II model Mass Spectrometer was used for analysis. The instrument was set for the injector port temperature to 250 ^{0}C, Interface temperature to 250 ^{0}C, Source to 200^{0}C and 40 mtorr vaccum pressure. The oven temperature programmed as a variable, 70^{0}C for 2 minutes, 150^{0}C at 8 ^{0}C /min, up to 260 ^{0}C at 10 ^{0}C /min. The DB-35 MS Non –Polar column was used whose dimensions were 0.25mm OD x 0.25 µm ID. Helium was used as the carrier gas at 1ml / min. The mass spectrometer was set to scan the mass of the fragments ranging from from 50 to 650 Da. The ionization energy was -70 eV. The MS also had inbuilt pre-filter which reduced the neutral particles.[16]

Identification of compounds

GC-MS identification of compounds of the extract were based on the computer evaluation of the mass spectra of samples through NIST and WILEY each containing more than five million references[17] comparison of peaks and retention time with those of standard compounds and computer matching with the NIST as well as by following the characteristic fragmentation patterns of the mass spectra of particular class of compounds. The GC-MS Chromatogram is attached in the Fig. 2.

Growth and Maintenance of Test Microorganism for Antimicrobial Studies

A list of bacterial species (*Staphylococcus aureus, Bacillus subtilis, Micrococcus luteus, Escherichia coli, Salmonella paratyphi and Klebsiella pneumonia)* and fungal species (*Candida albicans* and *Aspergillus niger)* were obtained from the Culture Collection Centre, Department of Biotechnology, KMCH College of pharmacy, Coimbatore. They were used as antimicrobial test organisms. The bacteria were maintained on nutrient broth (NB) at 37°C and fungus was maintained on Potato dextrose agar (PDA) at 28°C.

The Disc Diffusion method

Disc diffusion method was employed to test the antibiotic sensitivity of microorganisms. Assay for antibacterial and antifungal testing had been carried as described here. Antibacterial activity of the petroleum ether extract was assayed separately using disc diffusion method. Nutrient agar (NA) and Potato Dextrose Agar (PDA) plates were swabbed (sterile cotton swabs) with 8 hour old - broth culture of respective bacteria and fungi. Petri plates containing 15 ml of Muller hinton agar and potato dextrose agar medium were inoculated with 10^8 CFU/ml of each test bacterial and fungal species. Sterile filter paper discs (6 mm in diameter) were impregnated with 10µl of the plant extract (100µg/disc) placed on the surface of the medium. Negative controls were prepared using the same solvent (petroleum ether). A standard disc containing ciprofloxacin antibiotic drug (30µg/disc) was used as a positive control for antibacterial activity, Clotrimazole (30µg/disc) was used as positive control for antifungal activity and then plates were incubated at 37°C for 18-24 h for bacterial pathogens, similarly 28°C for 48 hours for fungal pathogens. The assessment of antimicrobial activity was based on the measurement of the diameter of inhibition zone formed around the disc.

Calculation of % of Inhibition

The zone diameter values were averaged to get the mean diameter value. The zone of inhibition is measured and the diameter values were used to determine the % of inhibition.

% of Inhibition = $d_{av} - d_s / d_S \times 100$

Where, d_{av} - average zone diameter by the plant extract

$[d_{av} = (d_1 + d_2 + d_3) / 3]$

d_s -Zone diameter of the pure solvent (petroleum ether)

d_S -Zone diameter of the standard.

RESULTS AND DISCUSSION

***Qualitative analysis of Cyathea nilgirensis* Holttum**

Table 1 shows the presence of 9 metabolites and they are Tannin, Saponnin, Flavonoids, Steroids, Terpenoids, Triterpenoids, Protein, Anthroquinone and Glycosides.

GC-MS Analysis based on peak area

GC-MS analysis of pet-ether extracts of the plant *Cyathea nilgirensis* Holttum had shown the existence of 35 compounds (Table 2). From 35 compounds 10 compounds were to be at the peak area percent less than 1. Out of the remaining 25 compounds 8 had the peak area percentage of 1. From the remaining 17 compounds Tetratetracontane ($C_{44}H_{90}$) peak area percentage was 2.28 % and its probability of matching with NIST data is 9.84 %. One compound from the remaining 16 namely Squalene ($C_{30}H_{50}$) lies at the peak area 2.53 %, meanwhile the probability of matching with standard compounds from NIST for Squalene are 40.42%. Squalene is a structurally unique triterpene compound and its one of the main component of skin surface liquids. Squalene is not very susceptible to peroxidation and appears to function in the skin as a quencher of singlet oxygen, protecting human skin surfaces from lipid peroxidation due to exposure to UV light and other sources of oxidative damage. Structure of squalene[18] is shown here in fig.5a. Four compounds from the remaining 15 namely heneicosane ($C_{21}H_{44}$), docosane ($C_{22}H_{46}$), nonacosane ($C_{29}H_{60}$) and eicosane ($C_{20}H_{42}$) lies at the peak area of 2.12, 3.92, 4.22, and 4.93% respectively. Compounds Hexatriacontane ($C_{36}H_{74}$), Tetracosane ($C_{24}H_{50}$) and Heptacosane ($C_{27}H_{56}$) from the remaining 11 compounds lies at the peak area of approximately 6 % each. From the remaining eight compounds three compounds Di-(2-ethylhexyl) phthalate ($C_{24}H_{38}O_4$), Phthalic acid, di(2-propylpentyl) ester ($C_{24}H_{38}O_4$) and 1,2-Benzenedicarboxylic acid, bis (2-

ethylhexyl) ester ($C_{24}H_{38}O_4$) lies at the peak area of 8.16 % respectively. The percentage of matching with NIST data base for these three compounds is approximately 25% each. From the remaining 5 compounds three compounds namely Hexacosane ($C_{26}H_{54}$), 3-Methyl-6-isopropylcyclohex-3-en-1-one ($C_{10}H_{16}O$) and 3-Butyl-5-methyl-4-hxen-2-ol ($C_{11}H_{22}O$) lies at the peak area of 9.8% each and so these three compounds are found to be in higher proportion. Finally the remaining two compounds Pentacosane ($C_{25}H_{52}$) and Triacontane ($C_{30}H_{62}$) are found to be in higher proportion in the GC-MS report by both lying at peak area 20.62 %.

GC-MS Analysis based on probability percentage

`From 35 compounds in the GC-MS report 7 compounds match with NIST inbuilt data at the range of 1-10%. Of these 7 compounds 2 compounds namely 3-Methyl-6-isopropylcyclohex-3-en-1-one ($C_{10}H_{16}O$) and 3-Butyl-5-methyl-4-hxen-2-ol ($C_{11}H_{22}O$) lies at the peak area percentage is 9.82. From the remaining 28 compounds 16 compounds match the standard compound of the NIST data at the range of 10-20%. At the same time few compounds namely Heneicosane ($C_{21}H_{44}$), Eicosane($C_{20}H_{42}$), Hexatriacontane($C_{36}H_{74}$), Tetracosane ($C_{24}H_{50}$), Triacontane($C_{30}H_{62}$), Heptacosane ($C_{27}H_{56}$), Nonacosane ($C_{29}H_{60}$) of the above 16 lies at higher peak area percentage. From the remaining 12 compounds 6 compounds namely Octadecanoic acid, 2-propenyl ester ($C_{21}H_{40}O_2$), Docosane ($C_{22}H_{46}$), Heptadecane, 9-hexyl ($C_{23}H_{48}$), Di-(2-ethylhexyl) phthalate($C_{24}H_{38}O_4$), Phthalic acid, di(2-propylpentyl) ester ($C_{24}H_{38}O_4$) and 1,2-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester ($C_{24}H_{38}O_4$) match at the range of 20-30 percentage. Three compounds from remaining 6 namely Neophytadiene ($C_{20}H_{38}$), Pentacosane ($C_{25}H_{52}$) and Hexacosane ($C_{26}H_{54}$) match at the range of 30-40%.And the remaining 3 compounds 5,10 dimethy l6,8 bisdehydro pentapentadecafulvalene ($C_{22}H_{18}$), 4-[10-(3,4-Dimethoxy) phenanthryl] morpholine($C_{20}H_{21}NO_3$) and Squalene ($C_{30}H_{50}$) match higher in the result at the range of 40-50%.

Antibacterial Activity

Table 3 and fig 4 showed the antibacterial activity of Cyathea *nilgirensis* Holttum.The zone of inhibition of standard ciprofloxacin for the three gram positive bacteria are *S. aureus* (32 mm), *B.subtilis* (32 mm),and *M. luteus* (24 mm).The average zone zone of inhibition for the plant extract for above gram positive bacteria are 4.6, 5.3 and 4.6 mm. Negative control petroleum ether shows inhibition for *B.subtilis* (7mm) but other two gram positive bacteria does not show any zone of inhibition. The plant extract therefore possess good percentage of inhibition for *M. luteus and S. aureus.* The zone of inhibition of standard ciprofloxacin for the three gram negative bacteria are *E. coli* (20 mm), *S. paratyphi* (32 mm), and *K. pneumoniae* (34 mm).The average zone zone of inhibition for the plant extract for above gram negative bacteria are 0, 0 and 10 mm. Negative control petroleum ether does not show zone of inhibition for gram negative bacteria. The plant extract therefore possess good percentage of inhibition for *K. pneumoniae.*

Antibacterial Activity

Table 4 and fig 4 showed the antifungal activity of Cyathea *nilgirensis* Holttum. The zone of inhibition for standard Clotrimazole for two fungal species is *C. albicans* (29mm) and *A. niger* (34).The average zone of inhibition for these two above fungal species are 0 and 10.3mm respectively. The negative control petroleum ether inhibits *A. niger* (7mm) but it does not show any inhibition for *C. albicans.* Therefore petroleum ether extract inhibits *A. niger.*

Table 1: Result and procedure of phytochemical screening of the Ariel parts of the petroleum ether extract of *Cyathea nilgirensis* Holttum.

Sl.No	Phytoconstituent	Name of the test &Experimental Procedure	Inference	Response
1.	Tannin	**Ferric chloride test:** 1ml of extract is treated with few drops of neutral ferric chloride and observed for the formation brownish green or a blue-black colouration.	Appearance of brownish green colour.	+
2.	Phlobatannins	**Hydrochloric acid test:** 1ml of extract is boiled with 1% aqueous hydrochloric acid and observed for the formation of red precipitate.	No red precipitate.	---
3.	Saponnin	**Foam test:** 2 ml of extract was mixed with 6 ml of water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam.	Foam got generated.	+
4.	Flavonoids	**Ammonia test:** 1ml of extract was treated with 5 ml of dilute ammonia solution and Con.H_2SO_4.Formation of yellow colour indicates flavanoids and this yellow colour may disappear after few minutes.	Appearance of yellow colour.	+
5.	Steroids	**Liebermann-Burchard test** To 1 ml of the extract chloroform, acetic anhydride and drops of H2SO4 was added and observed for the formation of dark pink or red colour.	Solution turned dark pink colour.	+
6.	Terpenoids	**Salkowaski test:** 1 ml of extract was treated with chloroform and Con. H_2SO_4.Formation of reddish brown	Reddish brown colour formed	++

		colour at the interface indicates the presence of terpenoids.	at the interface.	
7.	Triterpenoids	**Chloroform Test:** 1 ml of extract was mixed with 1 ml of chloroform and 1ml of acetic anhydride. The mixture was treated with con. H_2SO_4. Formation of reddish violet colour indicates the presence of triterpenoids.	The solution turned light reddish violet colour.	++
8.	Alkaloids	**Mayer's test:** 1 ml of extract was treated with Mayer's reagent (potassiomercuric iodide solution) and observed for the formation of creamy or white colored precipitate.	No white precipitate got formed.	---
9.	Carbohydrate	**Benedict's test:** To 0.5 ml of the extract, 0.5 ml of Benedict's reagent is added. The mixture is heated on boiling water bath for 5 min. A characteristic red colored precipitate indicates the presence of sugar.	No red colour.	---
10.	Protein	**Sodium hydroxide test:** 1 ml of extract is mixed with 1ml of 40% NaOH and copper sulphate solution was added slowly in to the test tube. Appearance of violet or pink colour indicates that the presence of protein.	Appearance of violet colour at the bottom.	++
11.	Anthroquinone	**Sulphuric acid test:** 1 ml of the extract is hydrolysed with Conc. H_2SO_4 and 1 ml of dilute ammonia is added to it. Formation of Rose pink colour indicates the presence of anthraquinones.	Appearance of pink colour.	++
12.	Polyphenol	**Ferric cyanide test:** 0.5 ml of extract was mixed with 2ml of ethanol. The mixture was warmed in a water bath for 10 min then treated with freshly prepared ferric cyanide solution. Formation of blue green colour indicates the presence of polyphenols.	No blue green colour.	---
13.	Glycoside	**Killer – Killani test:** 1 ml of extract is treated with 1 ml of glacial acetic acid, ferric chloride and few drops of Conc. H_2SO_4. The above solution is observed for the below mentioned changes a) Brown ring at interface, b) Violet ring below the brown ring, c) Greenish ring in the acetic acid layer.	Formation of brown ring.	+
14.	Coumarin	**Sodium hydroxide test:** 1 ml of the extract is treated with drops of 10% NaCl. A yellow colour indicates the presence of coumarin.	No yellow colour.	---
15.	Anthocyanin	**Ammonia test:** 2 ml of extract is added to 2 ml of 2N HCl and NH_3.Pink red turns blue violet indicates presence of Anthocyanin	No blue violet colour formation.	---

(+) Presence; (-) Absence; (++) Highest concentrations.

Table 2: Phytocomponents in Petroleum ether extract of *Cyathea nilgirensis* Holttum in GC-MS Report.

S.NO	SI	RSI	Compound Name	Probability	Molecular Formula	Molecular Weight	Area %
1	990	999	3-Methyl-6-isopropylcyclohex-3-en-1-one	5.55	$C_{10}H_{16}O$	152	9.82
2	990	999	3-Butyl-5-methyl-4-hxen-2-ol	5.55	$C_{11}H_{22}O$	170	9.82
3	600	656	Tridecane, 7-methyl	15.58	$C_{14}H_{30}$	198	0.86
4	648	978	n-Cetyl thiocyanate	7.68	$C_{17}H_{33}NS$	283	0.58
5	471	799	1-[(1-Oxo-2-propenyl)oxy]-2,5 pyrrolidinedione	11.09	$C_7H_7NO_4$	169	0.79
6	469	630	1,5 Undecadiene,6,7,7,8,8,9,9,10,10,11, 11,11-dodecafluoro-3-methyl	10.23	$C_{12}H_{10}F_{12}$	382	0.79
7	432	855	5,10 dimethy l6,8 bisdehydro pentapentadecafulvalene	40.85	$C_{22}H_{18}$	282	1.08
8	404	814	9-(p-Toluoyl)-1-methyl-1,2,3,4-tetra hydropyrido [1,2-a] pyrimidin-6 (7H)-one	11.79	$C_{17}H_{18}N_2O_2$	282	1.08
9	889	919	Neophytadiene	36.14	$C_{20}H_{38}$	278	1.15
10	871	892	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-,[R-[R*,R*-(E)]]	18.64	$C_{20}H_{40}O$	296	1.15
11	650	672	Phytol, acetate	11.44	$C_{22}H_{42}O_2$	338	1.15

12	865	883	Iron, tricarbonyl [N-(phenyl-2-pyridinylmethylene) benzenamine-N,N']	15.57	$C_{21}H_{14}FeN_2O_3$	398	0.93
13	300	539	2-Phenyl-3-(p-tolyl)naphthalene	10.06	$C_{23}H_{18}$	294	1.02
14	290	598	5-Bromo-3,4-dimethoxy-2,2'-bipyridine	7.10	$C_{12}H_{11}BrN_2O_2$	294	1.02
15	891	903	Heneicosane	11.26	$C_{21}H_{44}$	296	2.12
16	622	633	Octadecanoic acid, 2-propenyl ester	24.91	$C_{21}H_{40}O_2$	324	0.73
17	891	909	Docosane	20.61	$C_{22}H_{46}$	310	3.92
18	580	605	Heptadecane, 9-hexyl	29.22	$C_{23}H_{48}$	324	0.87
19	555	627	Heneicosane, 11-decyl	10.74	$C_{31}H_{64}$	436	0.76
20	904	935	Eicosane	13.42	$C_{20}H_{42}$	282	4.93
21	347	366	17-(2-Hydroxy-1,5-dimethyl-hex-4-enyl)-4,4,10,13,14-pentamethyl-2, 3, 4, 5, 6, 7, 10, 11,12,13,14,15,16,17- tetradecahydro-1H-cyclopenta [a] phenanthrene	7.37	$C_{30}H_{50}O_2$	442	1.77
22	910	924	Hexatriacontane	11.13	$C_{36}H_{74}$	506	6.54
23	910	914	Tetracosane	11.13	$C_{24}H_{50}$	338	6.54
24	830	841	Pentacosane	30.30	$C_{25}H_{52}$	352	20.62
25	810	822	Triacontane	13.80	$C_{30}H_{62}$	422	20.62
26	619	880	4-[10-(3,4-Dimethoxy) phenanthryl] morpholine	40.07	$C_{20}H_{21}NO_3$	323	0.40
27	917	928	Hexacosane	39.15	$C_{26}H_{54}$	366	9.80
28	913	913	Heptacosane	16.09	$C_{27}H_{56}$	380	6.83
29	930	982	Di-(2-ethylhexyl) phthalate	26.68	$C_{24}H_{38}O_4$	390	8.16
30	929	938	Phthalic acid, di(2-propylpentyl) ester	25.65	$C_{24}H_{38}O_4$	390	8.16
31	903	919	1,2-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester	26.68	$C_{24}H_{38}O_4$	390	8.16
32	891	894	Nonacosane	13.75	$C_{29}H_{60}$	408	4.22
33	512	585	Hexacosane, 9-octyl	9.66	$C_{34}H_{70}$	478	0.80
34	857	874	Squalene	40.42	$C_{30}H_{50}$	410	2.53
35	805	840	Tetratetracontane	9.84	$C_{44}H_{90}$	618	2.28

Table 3: *Antibacterial activity of the plant Cyathea nilgirensis* Holttum.

S.NO.	ORGANISMS	Zone of Inhibition(mm)				d_{av} (d1+d2+d3) /3	Solvent Pet ether d_s	% of Inhibition
		Std ciprofloxacin (30µg/disc) (d_S)	Pet-Ether (100 µg/disc)					
			d1	d2	d3			
1.	*S. aureus*	32	7	0	7	4.6	-	14.3
2.	*B.subtilis*	32	8	8	0	5.3	7	-5.3(nil)
3.	*M. luteus*	24	6	0	8	4.6	-	19.1
4.	*E. coli*	20	-	-	-	-	-	-
5.	*S. paratyphi*	32	-	-	-	-	-	-
6.	*K. pneumoniae*	34	12	10	8	10	-	29.4

Table 4: *Antifungal activity of the plant Cyatheanilgirensis*Holttum.

S.NO.	ORGANISMS	Zone of Inhibition(mm)				d_{av} (d1+d2+d3) /3	Solvent Pet ether d_s	% of Inhibition
		Std Clotrimazole (30µg/disc) (d_S)	Pet-Ether (100µg/disc)					
			d1	d2	d3			
1.	*C. albicans*	29	-	-	-	-	-	-
2.	*A. niger*	34	10	10	11	10.3	7	9.7

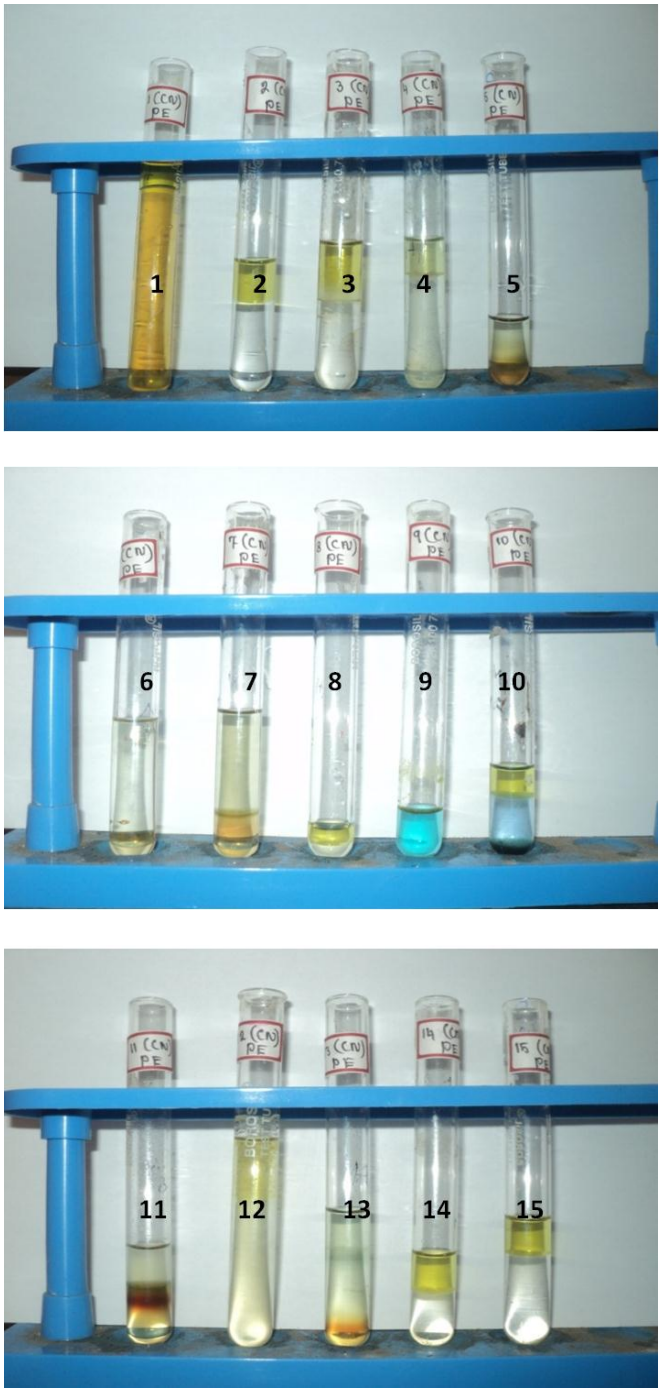


Fig 1. Qualitative Analysis Test of Petroleum ether of *Cyathea nilgirensis* Holttum.

1. Tannin, 2. Phlobatannins, 3. Saponnin, 4.Flavonoids, 5.Steroids, 6.Terpenoids, 7.Triterpenoids, 8.Alkaloids, 9. Carbohydrate, 10.Protein, 11.Anthroquinone, 12.Polyphenol, 13.Glycoside,14. Coumarine, 15. Anthocyanin.

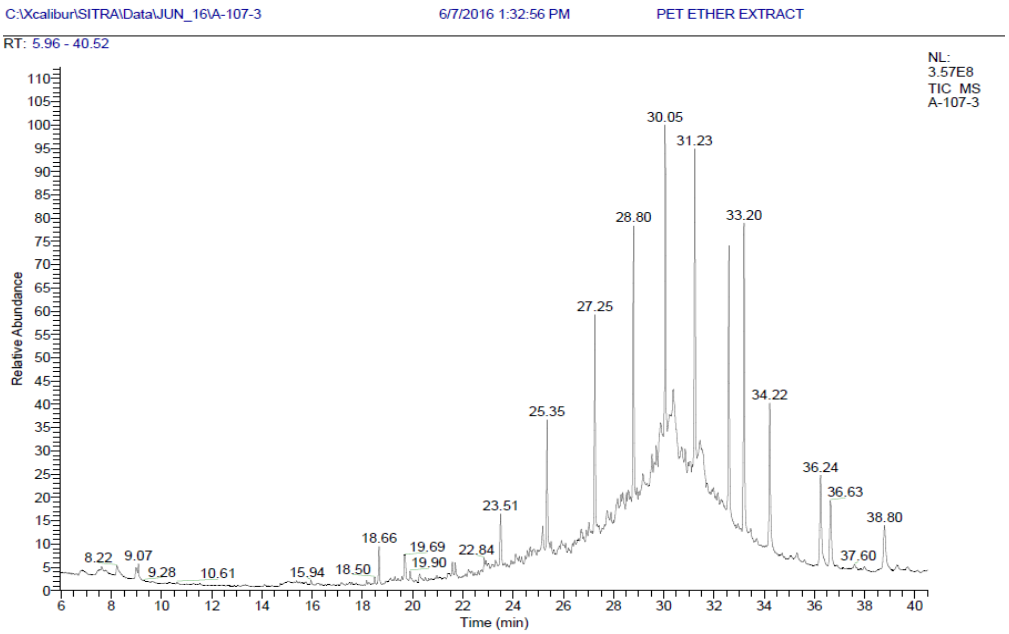
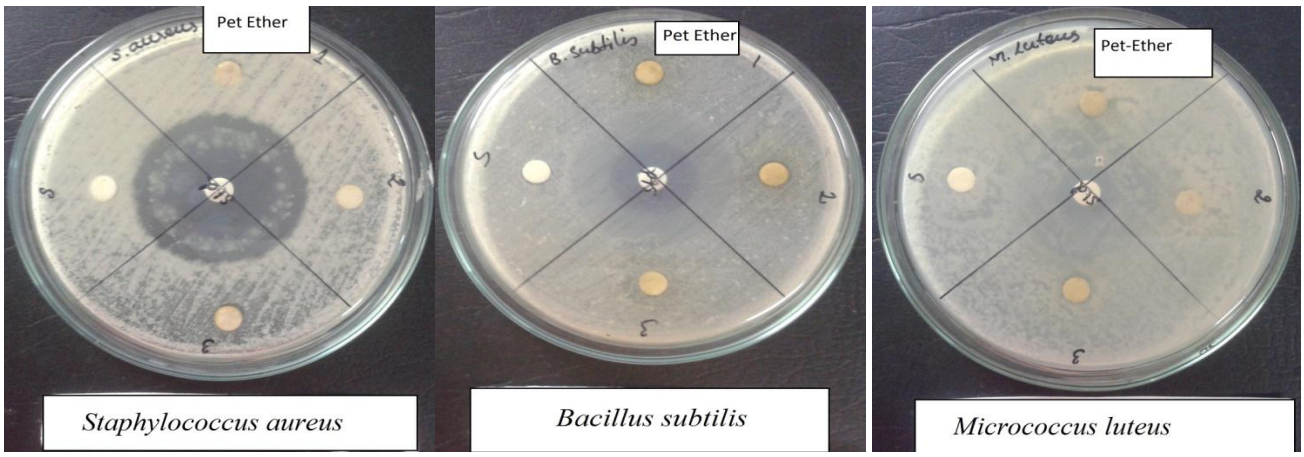
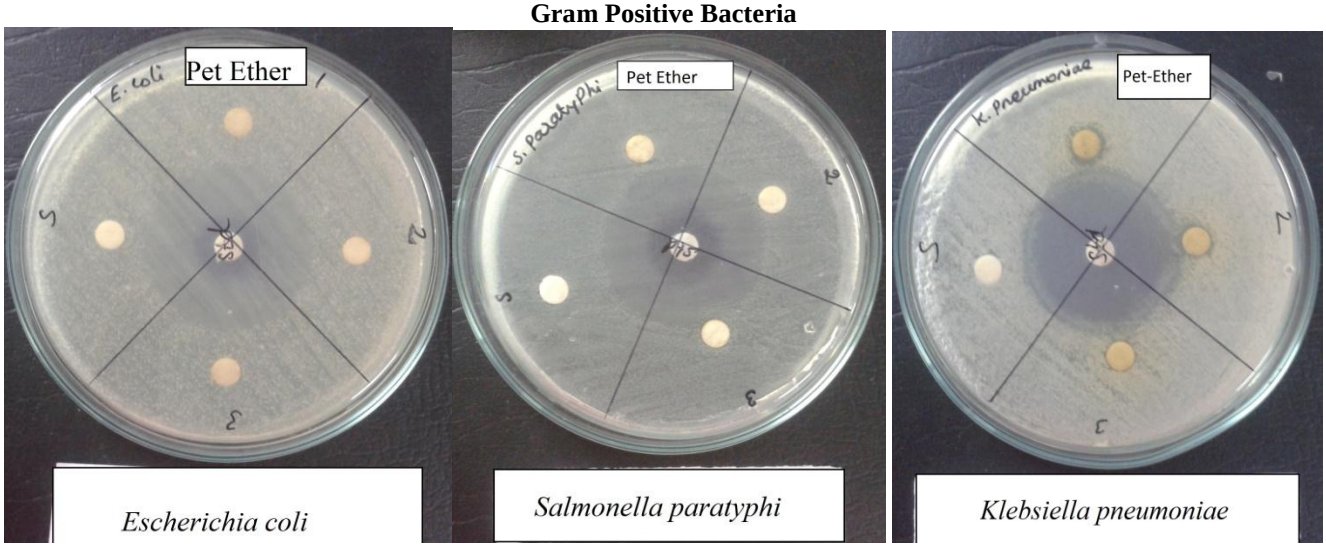


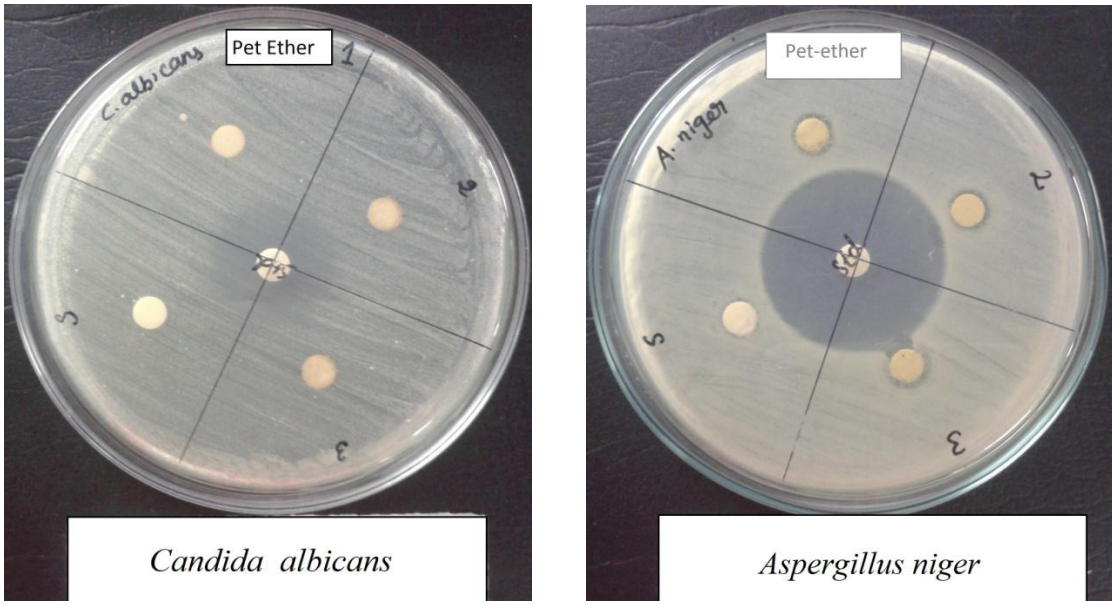
Fig 2.GC-MS spectrum of Petroleum ether Extract of *Cyathea nilgirensis* Holttum.



Gram Positive Bacteria



Gram Negative Bacteria



Fungal Species

Fig 3. Antibacterial and Antifungal Activity of Cyathea *nilgirensis Holttum* (Pet- ether Extract).

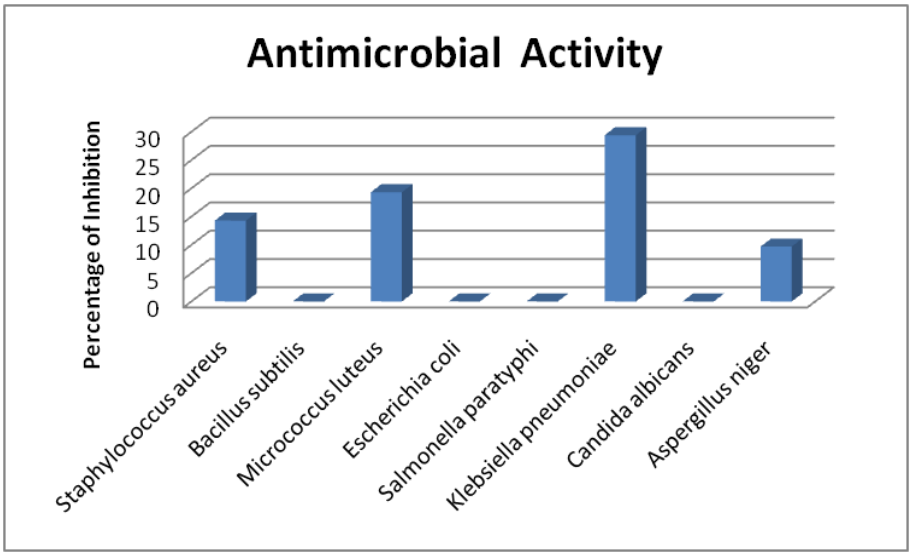
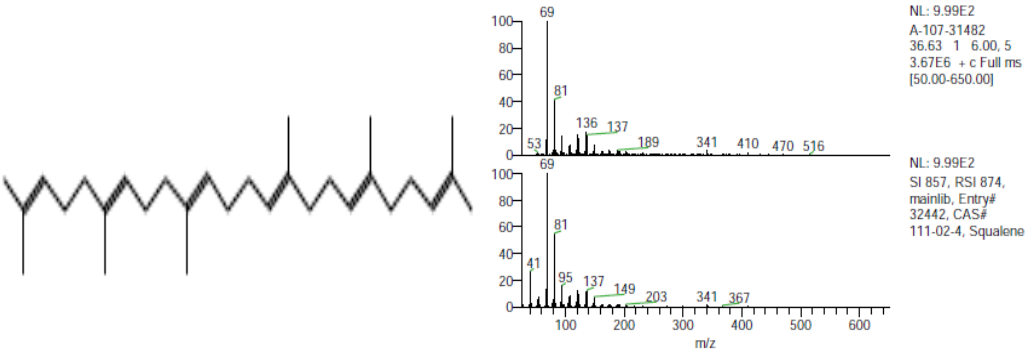
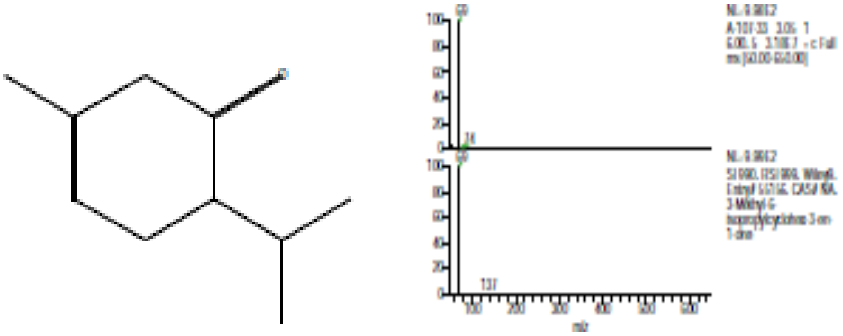


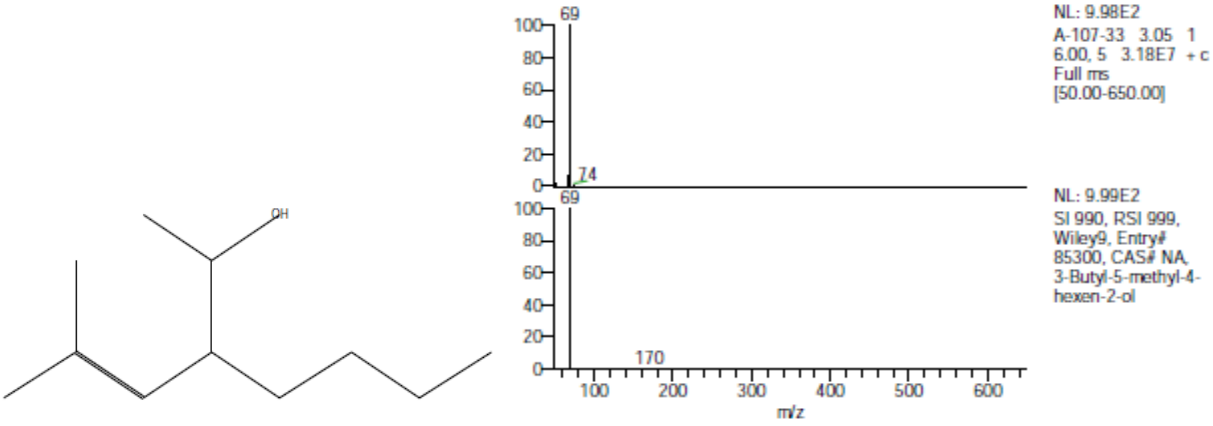
Fig 4: Percentage of inhibition of bacterial and fungal species.



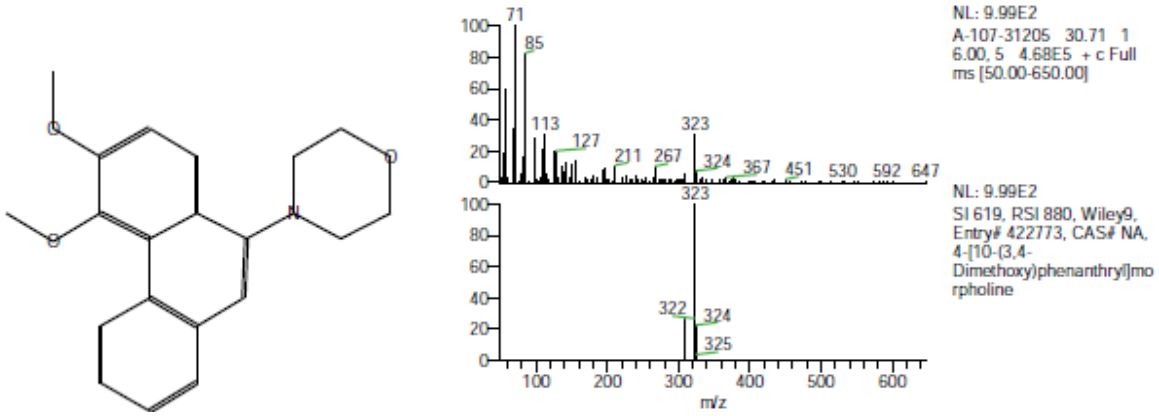
5a. Squalene ($C_{30}H_{50}$)



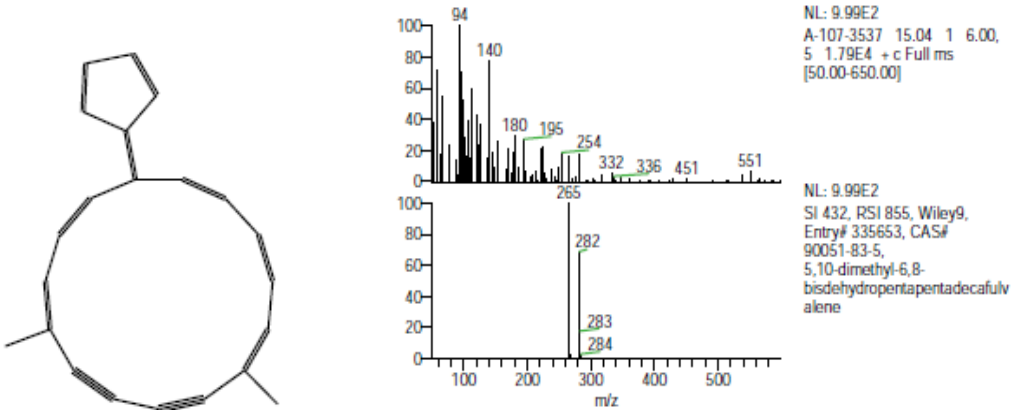
5b. 3-Methyl-6-isopropylcyclohex-3-en-1-one ($C_{10}H_{16}O$)



5c. 3-Butyl-5-methyl-4-hxen-2-ol ($C_{11}H_{22}O$)



5d. 4-[10-(3, 4-Dimethoxy) phenanthryl] morpholine ($C_{20}H_{21}NO_3$)



5e. 5,10 dimethy l6,8 bisdehydro pentapentadecafulvalene ($C_{22}H_{18}$)

Fig 5. Major Phytocomponents in Petroleum ether extract of *Cyathea nilgirensis* Holttum in GCMS Report.

CONCLUSION

The results of the present investigation complement the ethnobotanical usage of the studied plant which possesses several phytoconstituents with biological activity. Based on the present investigation, it is concluded that Cyathea *nilgirensis* Holttum have potential source of bioactive compounds with great pharmaceutical value. The study can be extended for isolation of bioactive compounds for novel drug discovery.

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REFERENCES

1. Fraser Jenkins CR. (Endemics and pseudo-endemics in relation to the distribution patterns of Indian pteridophytes). *Taiwania,* 2008; 53(3): 264–292.
2. Dixit RD. *A Census of Indian Pteridophytes: Flora of India - Series 4.* Calcutta; Botanical Survey of India, 1984; 177.
3. Singh HB. (Potential medicinal pteridophytes of India and their chemical constituents). Journal of Economic and Taxonomic Botany, 1999; 23: 63-77.
4. Dhawan BN. (Screening of Indian plants for biological activity). Indian Journal of Experimental Biology, 1977; 15: 208-219.
5. Kumar S. (Traditional medicinal plants curing diabetes: A promise for today and tomorrow). Asian Journal of Traditional Medicines, 2012; 7: 178-188.
6. Rout SD. (Ethnomedicinal studies on some Pteridophytes of Similipal Biosphere Reserve, Orissa, India). International Journal of Medicinal and Medicinal Sciences, 2009; 1: 192-197.
7. Asolkar LV. Glossary of Indian medicinal plants with active principles. New Delhi; CSIR, 1992.
8. Singh HB and Viswanathan MV. (Useful pteridophytes of India - A gift of nature for human beings) Journal of Economic and Taxonomic Botany, 1996; 12: 24-36.
9. Pradheesh G, Suresh J, Suresh S, Alex Ramani, Hong SI.(Antimicrobial activity and Identification of potential compounds from the chloroform extract of the medicinal plant *Cyathea nilgirensis* Holttum). World Journal of pharmacy and pharmaceutical sciences, 2017; 6(7): 1167-1184.
10. Janakiraman N, Johnson M. (UV-Vis Spectroscopic Profile as Taxonomic Criteria to distinguish the Tree Ferns (*Cyathea*)). International Journal of Research in Engineering and Bioscience, 2014; 2: 203- 212.
11. Janakiraman N, Johnson M.(Functional groups of tree ferns (*Cyathea*) using FT-IR: chemotaxonomic implications Romanian). J Biophys, 2015; 25: (In press).
12. Harborne JB. Phytochemical methods.London; Chapman and Hall, Ltd: 1973; 49-188.
13. Sofowara A. Medicinal plants and Traditional medicine in Africa. Nigeria; Spectrum Books Ltd, Ibadan, 1993; 289.
14. Trease GE, Evans WC. Pharmacognsy. 11th ed., London; Brailliar Tiridel and Macmillan Publishers, 1989.
15. Khandelwal K R. Practical Pharmacognosy. 16th ed., Pune; Nirali Prakashan Macmillian publishers, 2006; 98-106.
16. Ranganathan D. (Phytochemical Analysis of Caralluma Nilagiriana using GC-MS). J.of Pharmaco. And Phytochem, 2014; 3(1): 155-159.
17. Dool HVD, Kratz PD.(A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography). Journal of Chromatography, 1963; 11: 463-471.
18. Kohno Y, Egawa Y, Itoh S. (Kinetic study of quenching reaction of singlet oxygen and scavenging reaction of free radical by squalene in n-butanol). Biochim Biophys Acta, 1995; 1256: 52-56.