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ANTIMICROBIAL POTENTIAL OF CRUDE EXTRACTS AND FATTY ACID FRACTIONS OF FOUR PTERIDOPHYTE SPECIES FROM ASSAM, NORTHEAST INDIA, AND THEIR IDENTIFICATION BY GC-MS

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Keywords:

Pteridophytes, Fatty acids, Antimicrobials, GC-MS

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ABSTRACT: Fatty acids produced by plants protect themselves from pathogens, such as multidrug-resistant bacteria. In the present work, crude extracts, and fatty acid fractions of four pteridophyte species from Assam, Northeast India, were evaluated for their antimicrobial properties, and the bioactive compounds were identified using GC-MS analysis. The crude extract of *Pteris semipinnata* (Ps), *Lygodium microphyllum* (Lm), *Lycodium flexuosum* (Lf), and *Lycopodiella cernua* (Lc) was found to possess antibacterial and antifungal activities. The lowest MIC and MBC values were 0.25 mg/ml and 0.5 mg/ml for the fatty acid fractions against most of the microbial test strains. The GCMS analysis revealed 21 compounds in Ps, 35 in Lm, 33 in Lc, and 52 in Lf fractions. The present work supports the use of fern species in traditional medicine and therapies.

INTRODUCTION: About half of the deaths in tropical countries are caused by infectious diseases. Antibiotic resistance is a problem affecting the world and every nation. Antibiotic resistance occurs when bacteria become resistant and continue to proliferate in the presence of therapeutic amounts of an antibiotic, meaning the antibiotic can no longer effectively control or kill bacterial growth. Many factors contribute to antibiotic resistance. These include an inadequate grasp of how antibiotics function and how improper patient use promotes the development of resistance [1]. Drug-resistant bacteria have emerged because of the overuse of antibiotics in both medicine and food production.

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Utilizing plant remedies is a straightforward solution to this issue. Herbal medications are intricate biological structures that have progressively evolved and contain hundreds of active substances that interact harmoniously. Long known for their antimicrobial effects, fatty acids (FA) are produced by plants to protect themselves from pathogens, such as multidrug-resistant bacteria [2]. Recently, FAs have also come to light as a possible antibiotic substitute.

Numerous FAs have been found to selectively inhibit various microbial pathogens, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Burkholderia cenocepacia*, *Vibrio* spp., and *Candida albicans* suggesting their tremendous potential [3]. Pteridophytes have several medicinal uses in folk medicine. In the tribal communities of Assam, pteridophytes have been used to treat various ailments, including colds and coughs, boils, cuts and wounds, respiratory problems, diarrhea, inflammation, bodily pain, hair

loss and skin issues. From a single plant or a combination of plants, they create a paste, decoction, aqueous extract powder and juice [4]. The majority of the tribes also manufacture their own native beer made from rice grains. Each tribe raises its unique starting culture for fermentation, made of different plant parts.

In the current work, crude extracts and fatty acids of four pteridophyte species used in starter culture for the Mishing tribe of Assam's rice beer preparation are evaluated for their antimicrobial properties. The bioactive compounds were identified using GC-MS analysis.

MATERIALS AND METHOD:

Collection of Samples: The selected plant specimens were – *Pteris semipinnata*, *Lygodium microphyllum*, *Lygodium flexuosum*, and *Lycopodiella cernua*. Fresh plants were collected in the month of March from a Mishing village in Jorhat, Assam (26º 46' 33.012" N Latitude and 94º 15' 29.772" E Longitude). The leaves of *Pteris semipinnata* (Ps), *Lygodium microphyllum* (Lm), *Lygodium flexuosum* (Lf) and leaves along with stem of *Lycopodiella cernua* (Lc) were collected for the study. The herbarium specimens were prepared and identified in the Weed Herbarium of Assam Agricultural University. Plant material was collected, washed, shade dried, powdered with a blender, and stored in airtight bottles.

Preparation of Extracts: Five grams of each plant powder was extracted separately using different solvents based on polarity (ethyl acetate< acetone< ethanol< methanol) in the Soxhlet apparatus for 6 hours. The crude extracts were evaporated in a rotary vacuum evaporator at 37 ºC and stored at 4 ºC.

Extraction of Fatty Acids: 2 gm of aqueous extract was treated with 10 % lead acetate solution. The supernatant was collected, diluted with water and acidified with 1 % HCl before boiling for 2-3 hours. The precipitate was then extracted with ethanol and purified by fractional crystallization to obtain the fractions Ps-F, Lm-F, Lf-F and Lc-F. These fractions were then subjected to antimicrobial screening against clinical isolates and reference strains to establish their antimicrobial potential [5].

Antimicrobial Activity:

Microorganisms: The microorganisms selected for this study were isolated from clinical samples collected from Ayursundra Superspeciality Hospital, Guwahati, Assam. These included *Pseudomonas aeruginosa*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Acinetoba cterbaumannii*, *Proteus mirabilis*, *Candida albicans*, and *C. tropicalis*. The reference strains used in this study are *Staphylococcus epidermidis* ATCC 35984 and *K. pneumoniae* ATCC BAA-1705. Cultures were maintained on Luria Bertani Agar (for bacteria) and Potato Dextrose Agar (for yeast) at 4°C.

Determination of MIC and MBC: MIC and MBC were determined using resazurin microtiter assay. Two-fold serial dilutions of the crude extracts and fractionated extracts were made directly in a microtiter plate with Mueller Hinton broth (MHB) to produce varying concentrations. MHB was used to adjust microbial cultures to 0.5 McFarland turbidity standard (1.5×10^8 CFU/ml). Amoxicillin was used as a standard for positive control. The plate was sealed with a sterile sealer and kept at 37°C for 24 hours. After incubation, 0.02 % resazurin (Himedia-RM125-1G) was added to each microtiter plate well and incubated for 30 minutes at 37°C. The wells that had microbial growth became pink, whereas the wells that did not have microbial growth remained blue. The wells corresponding to the MIC and with higher concentration were streaked onto Mueller-Hinton Agar plates and incubated overnight at 37°C. The concentration corresponding to no bacterial growth was recorded as the minimum bactericidal concentration (MBC) for extract [6].

Identification of the Fatty Acid Fraction with Gas Chromatography and Mass Spectroscopy: The fatty acid fractions were re-dissolved in spectroscopy grade ethanol and filtered through 0.2 μm filter for GCMS analysis performed in a Perkin Elmer (USA) Clarus 680/600C unit fitted with Elite 5 MS column (length: 30 m, ID: 0.25 mm, film thickness: 0.25 μm). The software used in the system is TurboMassver 5.4.2. The oven program started at 60°C for 1 min and ramped at 7°C/min up to 200°C and held for 3 mins, again ramped at 10°C/min to 300°C and then held for 5 min. Next, 1.0 μl sample was injected at 280°C using He as

carrier gas with a solvent delay of 5 min. The split ratio was 10: 1. The mass spectrometer (Clarus 600C; single quad) was operated in the electron ionization (EI) mode at 70 eV with a source temperature of 150°C and a continuous scan from m/z 50 to 600. The peaks were identified by matching the mass spectra with the National Institute of Standards and Technology (NIST) library, USA.

RESULTS AND DISCUSSION: The development of new drugs also depends on the study of medicinal plants. Most underdeveloped nations presently use herbal treatment, and wealthy countries are also quickly adopting it. The pharmacological effects of plants are caused by metabolites, which are organic compounds divided into primary and secondary metabolites. Plants produce secondary metabolites such as alkaloids, flavonoids, saponins, terpenoids, steroids, glycosides, tannins, volatile oils, and other substances to protect themselves from microbial infections and insect invasions[7].

The experiment to ascertain the antimicrobial efficacy of the different crude extracts and fatty acid fraction utilized seven clinical and two control isolates. The antimicrobial activity of the different extracts was effective against all of the test microorganisms at dosages ranging from 0.29 to 17.75 mg/ml, as shown in **Table 1-3**. Fractionation sometimes leads to improved biological activity, as seen in this study. The lowest MIC and MBC values were 0.25 mg/ml and 0.5 mg/ml, respectively **Table 1-3**, recorded for the fatty acid fractions Ps-F, Lc-F, Lf-F, and Lm-F against most of the microbial test strains. The low MIC observed in this study further supports the potential of fatty acid as an antimicrobial resource. The results align with earlier research on the antimicrobial effectiveness of fatty acids against related species employed in this study [3, 8-9].

TABLE 1: MIC AND MBC OF THE DIFFERENT CRUDE EXTRACTS AND FRACTIONATED EXTRACTS AGAINST BACTERIAL CLINICAL ISOLATES

Sample		*P. aeruginosa*		*S. typhi*		*A. baumanii*		*K. pneumoniae*		*P. mirabilis*	
		MIC mg/ml	***MBC mg/ml***	***MIC mg/ml***	***MBC mg/ml***	***MIC mg/ml***	***MBC mg/ml***	***MIC mg/ml***	***MBC mg/ml***	***MIC mg/ml***	***MBC mg/ml***
Pteris semipinnata	**Methanol**	8.88	17.75	8.88	17.75	4.44	8.88	4.44	8.88	8.88	17.75
	Ethyl acetate	0.58	1.17	0.58	1.17	0.29	0.58	0.29	0.58	0.58	1.17
	Ethanol	9.33	18.67	9.33	18.67	4.67	9.33	4.67	9.33	9.33	18.67
	Acetone	2	4	2	4	1	2	1	2	2	4
	Ps-F	0.25	0.5	0.5	1	0.25	0.5	0.25	0.5	0.5	1
Lycopodiella cernua	**Methanol**	2.48	9.92	2.48	9.92	2.48	4.96	2.48	4.96	4.96	9.92
	Ethyl acetate	0.96	3.84	0.96	3.84	0.96	1.92	0.96	1.92	1.92	3.84
	Ethanol	2	8	2	8	2	4	2	4	4	8

Lygodium flexuosum	**Acetone**	0.88	3.5	0.88	3.5	0.88	1.75	0.88	1.75	1.75	3.5
	Lc-F	0.25	1	0.25	1	0.25	0.5	0.25	0.5	0.5	1
	Methanol	3.21	6.42	3.21	12.84	3.21	6.42	3.21	6.42	6.42	12.84
	Ethyl acetate	1.15	2.29	1.15	4.58	1.15	2.29	1.15	2.29	2.29	4.58
	Ethanol	1.79	3.58	1.79	7.17	1.79	3.58	1.79	3.58	3.58	7.17
	Acetone	1.63	3.25	1.63	6.5	1.63	3.25	1.63	3.25	3.25	6.5
	Lf-F	0.25	0.5	0.25	1	0.25	0.5	0.25	0.5	0.5	1
Lygodium microphyllum	**Methanol**	6.15	12.29	12.29	24.58	6.15	12.29	6.15	12.29	12.29	24.58
	Ethyl acetate	1.25	2.5	1.25	2.5	0.63	1.25	0.63	1.25	1.25	2.5
	Ethanol	9	18	9	18	4.5	9	4.5	9	9	18
	Acetone	3.67	7.34	3.67	7.34	1.83	3.67	1.83	3.67	3.67	7.34
	Lm-F	0.25	1	0.5	1	0.25	0.5	0.25	0.5	0.5	1

TABLE 2: MIC AND MBC OF THE DIFFERENT CRUDE EXTRACTS AND FRACTIONATED EXTRACTS AGAINST FUNGAL CLINICAL ISOLATES

Sample		***C. albicans***		***C. tropicalis***	
		MIC mg/ml	**MBC mg/ml**	**MIC mg/ml**	**MBC mg/ml**
Pteris semipinnata	Methanol	8.88	17.75	4.44	8.88
	Ethyl acetate	0.29	0.58	0.29	0.58
	Ethanol	4.67	18.67	2.33	4.67
	Acetone	1	4	0.5	1
	Ps-F	0.25	1	0.125	0.25
Lycopodiela cernua	Methanol	2.48	4.96	2.48	4.96
	Ethyl acetate	0.96	1.92	0.96	1.92
	Ethanol	2	4	2	4
	Acetone	0.88	1.75	0.88	1.75
	Lc-F	0.25	0.5	0.125	0.25
Lygodiu	Methanol	3.21	6.42	3.21	6.42
mflex	Ethyl acetate	1.15	2.29	0.57	1.15

	Ethanol	1.79	3.58	1.79	3.58
	Acetone	1.63	3,25	0.81	1.63
	Lf-F	0.25	0.5	0.125	0.25
Lygodium microphyllum	Methanol	6.15	12.29	6.15	12.29
	Ethyl acetate	0.63	1.25	0.63	1.25
	Ethanol	4.5	9	4.5	9
	Acetone	1.83	3.67	3.67	7.34
	Lm-F	0.25	0.5	0.25	0.5

TABLE 3: MIC AND MBC OF THE DIFFERENT CRUDE EXTRACTS AND FRACTIONATED EXTRACTSAGAINSTBACTERIAL CONTROL ISOLATES

Sample		*S. epidermidis*		*K. pneumoniae*	
		MIC mg/ml	**MBC mg/ml**	**MIC mg/ml**	**MBC mg/ml**
Pteris semipinnata	Methanol	8.88	17.75	8.88	17.75
	Ethyl acetate	0.58	1.17	0.29	0.58
	Ethanol	9.33	18.67	4.67	9.33
	Acetone	2	4	1	2
	Ps-F	0.25	0.5	0.25	0.5
Lycopodiela cernua	Methanol	4.96	9.92	4.96	9.92
	Ethyl acetate	1.92	3.84	1.92	3.84
	Ethanol	4	8	2	4
	Acetone	1.75	3.5	1.75	3.5
	Lc-F	0.25	0.5	0.25	0.5
Lygodium flexuosum	Methanol	6.42	12.84	3.21	1.60
	Ethyl acetate	2.29	4.58	1.15	0.57
	Ethanol	3.58	7.17	3.58	7.17
	Acetone	3.25	6.5	3.25	6.5
	Lf-F	0.5	1	0.5	1
Lygodium microphyllum	Methanol	12.29	24.58	12.29	24.58
	Ethyl acetate	1.25	2.5	0.63	1.25
	Ethanol	9	18	9	18
	Acetone	3.67	7.34	3.67	7.34
	Lm-F	0.5	1	0.25	0.5

Based on the promising result of the antimicrobial study, the fatty acid fractions were further subjected to GCMS analysis to identify the bioactive compounds. The four pteridophytes' fatty acid fraction included 184 peaks with retention times ranging from 6.044 to 38.157 **Fig. 1-4**. There were 21 compounds found in the *Pterissemipinnata* fraction, 35 compounds in the *Lygodium flexuosum* fraction, 33 compounds in the *Lycopodiella cernua* fraction, and 52 compounds in the *Lygodium microphyllum* fraction after a NIST library search was conducted for the significant peaks **Table 4-7.**

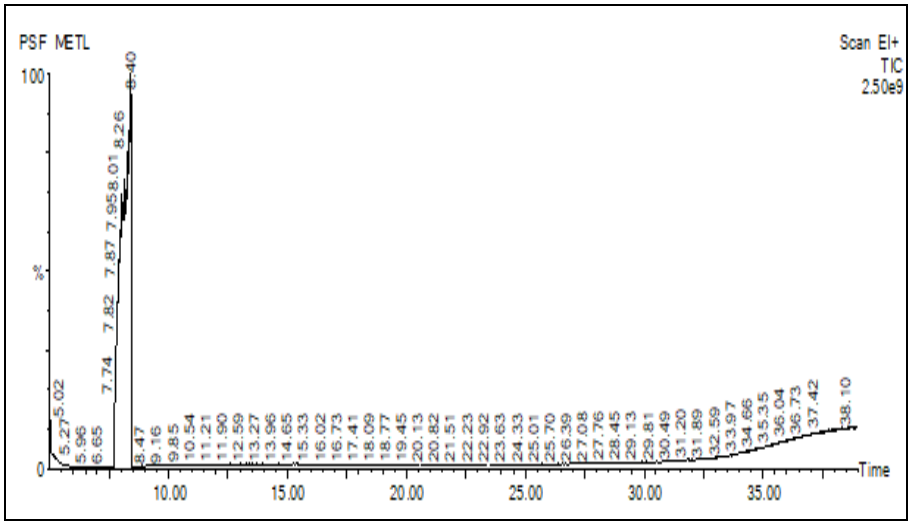
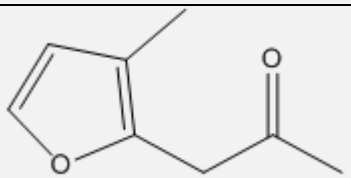
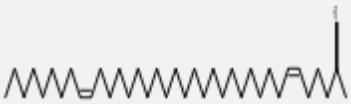
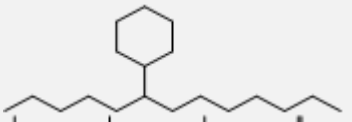
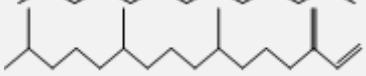
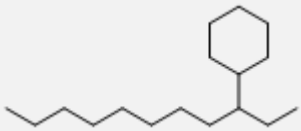
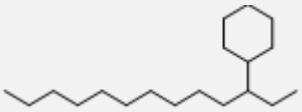
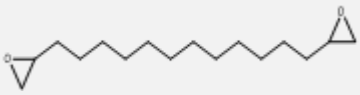
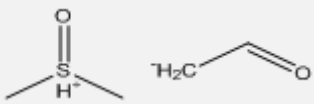
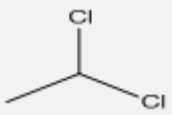
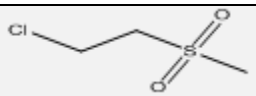
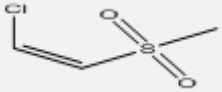
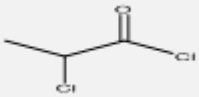
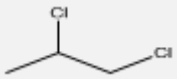
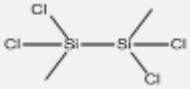


FIG. 1: GC-MS CHROMATOGRAM OF THE *PTERISSEMIPINATTA* FRACTION

TABLE 4: GC-MS ANALYSIS OF FRACTIONATED EXTRACT OF *PTERIS SEMIPINNATA*

Sl. no.	Compound name	Molecular structure	Mol formula	Mol.wt	Activity
1	3-methyl-2-(2-oxopropyl)furan		$C_8H_{10}O_2$	138	Antimicrobial activity[10-11], Antibiofilm, Anti-quorum sensing activity[12]; antioxidant, antipyretic, anti-inflammatory activity[13]
2	z,z-6,28-heptatriactontadien-2-one		$C_{37}H_{70}O$	530	Larvicidal activity[14]; Vasodialator, carcinogenic and antioxidant activity[15]
3	z,z-6,27-hexatriactontadien-2-one		$C_{36}H_{68}O$	516	Vasodialator[16]
4	11,14-eicosadienoic acid, methyl ester		$C_{21}H_{38}O_2$	322	Anti-infammatory, anti-oxidant, anti-arthritic, anticoronary[17]
5	Undec-10-ynoic acid, tetradecyl ester		$C_{25}H_{46}O_2$	378	Antimicrobial activity[18]
6	7-hexadecenal, (z)-		$C_{16}H_{30}O$	238	-
7	Undec-10-ynoic acid, octadecyl ester		$C_{29}H_{54}O_2$	434	Inhibitor of cytochrome P450 4A1[19]; Antioxidant, Antifungal, and Wound Healing Activity[20]
8	Tridecane, 6-cyclohexyl-		$C_{19}H_{38}$	266	-
9	Neophytadiene		$C_{20}H_{38}$	278	Anti-inflammatory and antiviral activity[21]; Antioxidant[22]
10	Undecane, 3-cyclohexyl-		$C_{17}H_{34}$	238	-
11	Tridecane, 3-cyclohexyl-		$C_{19}H_{38}$	266	-
12	1,2-15,16-diepoxyhexadecane		$C_{16}H_{30}O_2$	254	Cytotoxic activity[23]; Anti-tumour and anti-inflammatory activity[24]
13	Docosanal		$C_{22}H_{44}O$	324	Antiviral activity[25]
14	Dimethylsulfoxonium formylmethylide		$C_4H_8O_2S$	120	Antioxidant and Cytotoxic activity[26]; Antimicrobial activity[27]
15	Ethane, 1,1-dichloro-		$C_2H_4Cl_2$	98	-

16	2-chloroethyl methyl sulfone		$C_3H_7ClO_2S$	142	-
17	(z)-1-chloro-2-(methylsulfonyl)ethylene		$C_3H_5ClO_2S$	140	-
18	2-chloropropionyl chloride		$C_3H_4Cl_2O$	126	-
19	Propane, 1,2-dichloro-		$C_3H_6Cl_2$	112	-
20	Disilane, 1,1,2,2-tetrachloro-1,2-dimethyl-		$C_2H_6Cl_4Si_2$	226	-
21	Bis(methylsulfonyl)methane		$C_3H_8O_4S_2$	172	Anti-inflammatory activity [28]

*Molecular structures were generated using ChemDraw Ultra 12.0

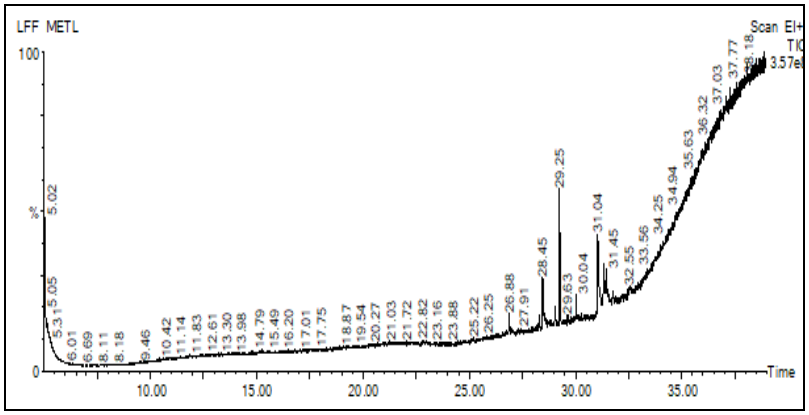
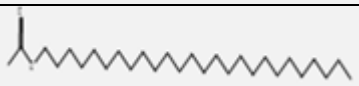
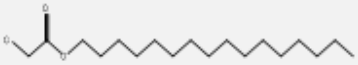
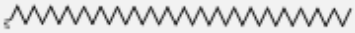
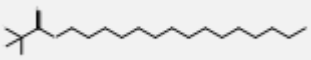
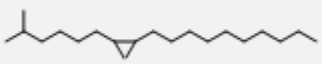
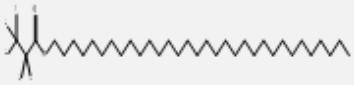
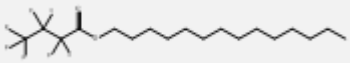
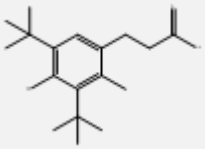
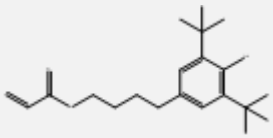
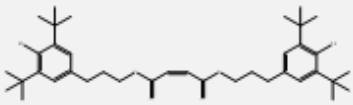
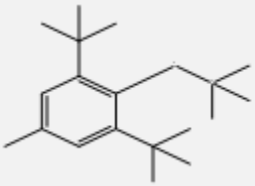
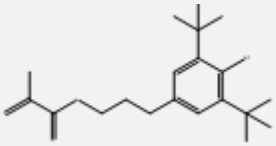
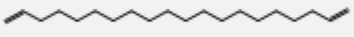
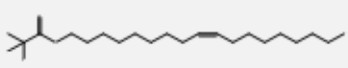


FIG. 2: GC-MS CHROMATOGRAM OF THE *LYGODIUM FLEXUOSUM* FRACTION

TABLE 5: GC-MS ANALYSIS OF FRACTIONATED EXTRACT OF *LYGODIUM FLEXUOSUM*

Sl. no.	Compound name	Molecular structure	Mol formula	Mol. wt	Activity
1	Hexacosyl acetate		$C_{28}H_{56}O_2$	424	Larvicidal activity[29]; Antifungal activity[30]
2	Chloroacetic acid, tetradecyl ester		$C_{16}H_{31}ClO_2$	290	Antioxidant, antimicrobial and bacteriocide, anti-infammatory activity[13]
3	1-hexadecanol		$C_{16}H_{34}O$	242	Antimicrobial activity[31]
4	Cis-1-chloro-9-octadecene		$C_{18}H_{35}Cl$	286	Antibacterial activity[32]
5	Acetic acid, chloro-, hexadecyl ester		$C_{18}H_{35}ClO_2$	318	antibacterial, anthelmintic, insecticidal activity[33]
6	N-nonadecanol-1		$C_{19}H_{40}O$	284	Antibacterial activity[34]
7	Behenic alcohol		$C_{22}H_{46}O$	326	Antiviral activity[35]
8	Octacosanol		$C_{28}H_{58}O$	410	Antinociceptive and Anti-Inflammatory activity[36]
9	1-heneicosanol		$C_{21}H_{44}O$	312	Antioxidant and Antimicrobial activity[37]
10	1-heneicosyl formate		$C_{22}H_{44}O_2$	340	Biocontrol activity[38]
11	1-		$C_{41}H_{84}O$	593	Antimicrobial activity[39]

12	hentetracontanol trifluoroacetic acid, pentadecyl ester		$C_{17}H_{31}F_3O_2$	324	-
13	Disparlure		$C_{19}H_{38}O$	282	-
14	Dotriacontyl pentafluoropropionate		$C_{35}H_{65}F_5O_2$	612	Cytotoxic activity[40]
15	Heptafluorobutyric acid, n-tetradecyl ester		$C_{18}H_{29}F_7O_2$	410	Antioxidant activity[41]
16	Benzene propanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl e		$C_{18}H_{28}O_3$	292	Antibacterial activity[42]
17	4-(3,5-di-tert-butyl-4-hydroxyphenyl)butyl acrylate		$C_{21}H_{32}O_3$	332	-
18	bis[3-(3,5-di-tert-butyl-4-hydroxyphenyl)propyl] maleate		$C_{38}H_{56}O_6$	608	Antioxidant activity[43]
19	butylated hydroxytoluene, tms derivative		$C1_8H_{32}OSi$	292	Antioxidant activity[44]
20	3-(3,5-di-tert-butyl-4-hydroxyphenyl)propyl methacrylate		$C_{21}H_{32}O_3$	332	-
21	Eicosen-1-ol, cis-9-		$C_{20}H_{40}O$	296	Antibacterial activity[45]
22	9-octadecen-1-ol, (z)-		$C_{18}H_{36}O$	268	-
23	13-docosen-1-ol, (z)-		$C_{22}H_{44}O$	324	-
24	z,z-6,28-heptatriactontadien-2-one		$C_{37}H_{70}O$	530	Larvicidal activity[46]
25	1,16-hexadecanediol		$C_{16}H_{34}O_2$	258	-
26	(z)-14-tricosenyl formate		$C_{24}H_{46}O_2$	366	Anticancer activity[47]
27	1,19-eicosadiene		$C_{20}H_{38}$	278	Antimicrobial activity[48]; Anti-quorum sensing and Anti-biofilm activity[49]
28	Oleyl alcohol, trifluoroacetate		$C_{20}H_{35}F_3O_2$	364	Antioxidant, Antidiabetic and Hypolipidemic[50]
29	Ethanol, 2-(9-octadecenyloxy)-, (z)-		$C_{20}H_{40}O_2$	312	Antimycotoxigenic activity[51]

30	Cyclododecanemethanol		$C_{13}H_{26}O$	198	Antioxidant and Metal Chelation Activity[52]
31	11-dodecen-1-ol trifluoroacetate		$C_{14}H_{23}F_3O_2$	280	Antiobesity and Antihyperlipidemic activity[53]
32	9-hexadecenoic acid, 9-octadecenyl ester, (z,z)-		$C_{34}H_{64}O_2$	504	Antibacterial activity[54]
33	17-octadecynoic acid		$C_{18}H_{32}O_2$	280	-
34	z,e-2-methyl-3,13-octadecadien-1-ol		$C_{19}H_{36}O$	280	-
35	1,21-docosadiene		$C_{22}H_{42}$	306	-

*Molecular structures were generated using ChemDraw Ultra 12.0

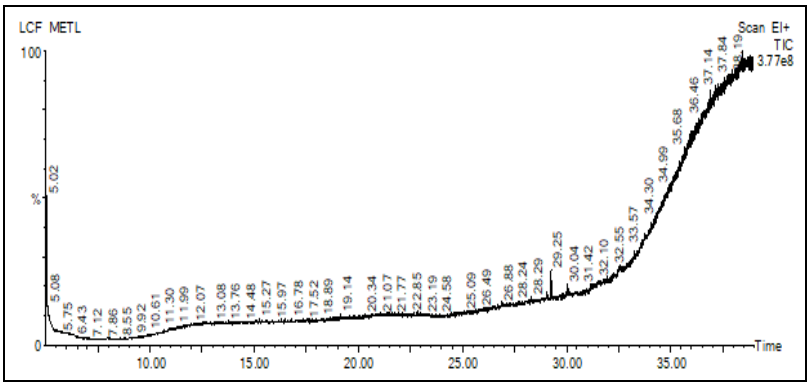
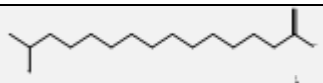
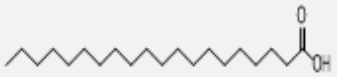
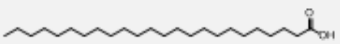
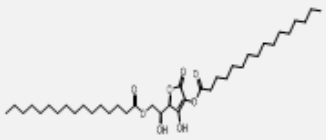
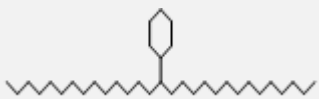
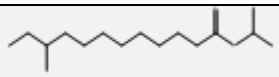
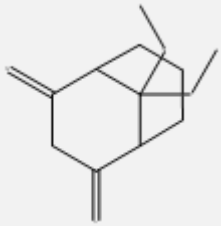
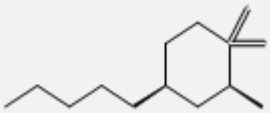
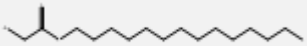
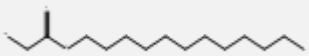
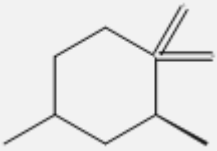
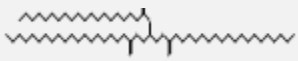
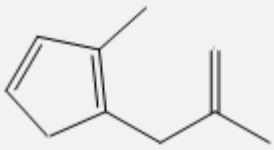


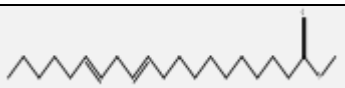
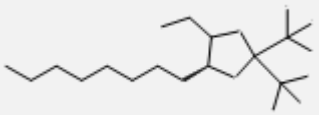
FIG. 3: GC-MS CHROMATOGRAM OF THE *LYCOPODIELLA CERNUA* FRACTION

TABLE 6: GC-MS ANALYSIS OF FRACTIONATED EXTRACT OF *LYCOPODIELLA CERNUA*

Sl. no.	Compound name	Molecular structure	Mol formula	Mol. wt	Activity
1	4-n-hexylthiane, s,s-dioxide		$C_{11}H_{22}O_2S$	218	-
2	oleic acid		$C_{18}H_{34}O_2$	282	Antimicrobial, Antifungal, anticonvulsive activity, Antiadhesive, Antiallergic, Antianalgesic, Antiatherosclerosis, Anesthetic, Antihelmenthic, Antianxiety, Antibacterial, Antiberiberi, Antibiotic, Anticancer, Anticonvulsant, Antidiabetic, Antidiarrheic, Antifertility, Antigastric, Anti-infammatory, Antiobesity, Antioxidant, Antiulcer, Antitubercellosic, Anticold, Antihepatotoxic and Antiviral activityanemiagenic, dermatitigenic[13]
3	2-nonadecanone 2,4-dinitrophenylhydrazine		$C_{25}H_{42}N_4O_4$	462	Antioxidant activity[55]

4	Pentadecanoic acid, 14-bromo-		$C_{15}H_{29}BrO_2$	320	-
5	Docosanoic acid		$C_{22}H_{44}O_2$	340	Antibacterial activity[56]
6	Tetracosanoic acid, isobutyl ester		$C_{28}H_{56}O_2$	424	-
7	Docosanoic acid, docosyl ester		$C_{44}H_{88}O_2$	648	Antidepressant and Cytotoxic activity[56]
8	Butyl 11-eicosenoate		$C_{24}H_{46}O_2$	366	-
9	Eicosanoic acid		$C_{20}H_{40}O_2$	312	Reduced heart diseases, kidney and liver function, blood Clotting[13]; Antibacterial and cytoprotective activity[57]
10	Propionic acid, 3-iodo-, octadecyl ester		$C_{21}H_{41}IO_2$	452	-
11	Tetracosanoic acid		$C_{24}H_{48}O_2$	368	-
12	l-(+)-ascorbic acid 2,6-dihexadecanoate		$C_{38}H_{68}O_8$	652	Antioxidant and reduces the triglycerides level—Protects LDL against peroxidation and inhibits the progression of atherosclerosis, Antiallergic, Antianemic, Antianxiety, Antibacterial, Antibronchitic, Anticancer, Anticarcinogenic, Anticataract, Anticoagulant, Anticonvulsant, Antidiabetic, Antidiarrheic, Antifatigue, Antifertility, Antigastric, Anti-infammatory, Antimalarial, Antioxidant, Antistress, Antiulcer, Antiatheroscelerotic, Anticold, Antiglaucomic, Antihepatic, Antihypertensive, Antiplague, Antiproliferant, Antiprotozoal, Antiseptic, Antistroke, Antituberculic, Antitumer, CNSStimulant, Chelator, Chemopreventive, CytochromeP450Inducer, Deodorant, Dermal, Detoxicant, Flavor, Hypolipidimic, Neuroprotective, Neurotransmitter, Termiticide and Antiviral activity[13]
13	Octadecanoic acid		$C_{18}H_{36}O_2$	284	Decreases cardiovascular and cancer risks, reduces LDL cholesterol levels, reduces blood pressure, improved heart function[58]
14	Cyclohexane, 1-(1-tetradecylpentadecyl)-		$C_{35}H_{70}$	490	-

15	i-propyl 10-methyl-dodecanoate		$C_{16}H_{32}O_2$	256	-
16	Butyl 15-methylhexadecanoate		$C_{21}H_{42}O_2$	326	-
17	Docosanoic acid, isobutyl ester		C26H52O2	396	-
18	9,9-dimethoxybicyclo[3.3.1]nona-2,4-dione		$C_{11}H_{16}O_4$	212	-
19	Cis-2-methyl-4-n-pentylthiane, s,s-dioxide		$C_{11}H_{22}O_2S$	218	Anti-proliferative activity[59]
20	Chloroacetic acid, tetradecyl ester		$C_{16}H_{31}ClO_2$	290	Antioxidant, antimicrobial and bacteriocide, anti- infammatory activity[13]
21	5-methyl-z-5-docosene		$C_{23}H_{46}$	322	Antibacterial, antidiabetic, antitumour activities[13]
22	Heptyl triacontyl ether		$C_{37}H_{76}O$	536	-
23	Chloroacetic acid, dodecyl ester		$C_{14}H_{27}ClO_2$	262	-
24	Eicosyl heptyl ether		$C_{27}H_{56}O$	396	-
25	Heptyl hexacosyl ether		$C_{33}H_{68}O$	480	-
26	Trans-2,4-dimethylthiane, s,s-dioxide		$C_7H_{14}O_2S$	162	Anti-infammatory activity[60]
27	Eicosyl nonyl ether		$C_{29}H_{60}O$	424	Antioxidant activity[61]
28	triarachine		$C_{63}H_{122}O_6$	975	Anti-hyperglycemic activity[62]
29	3-methyl-2-(2-oxopropyl) furan		$C_8H_{10}O_2$	138	Antioxidant, antimicrobial and bacteriocide, Antipyretic, antiinfammatory activity[13]
30	z,z-6,27-hexatriactontadien-2-one		$C_{36}H_{68}O$	516	Vasodilator[16]
31	Heptacosanoic acid, 25-methy—, methyl ester		C29H58O2	438	Antimicrobial activity[13]

32	11,14-eicosadienoic acid, methyl ester		C21H38O2	322	Anti-infammatory, anti-oxidant, anti-arthritic, anticoronary[13]
33	1,3-dioxolane, 4-ethyl-5-octyl-2,2-bis(trifluoromethyl)-, trans-		$C_{15}H_{24}F_6O_2$	350	Antioxidant activity[63]

*Molecular structures were generated using ChemDraw Ultra 12.0

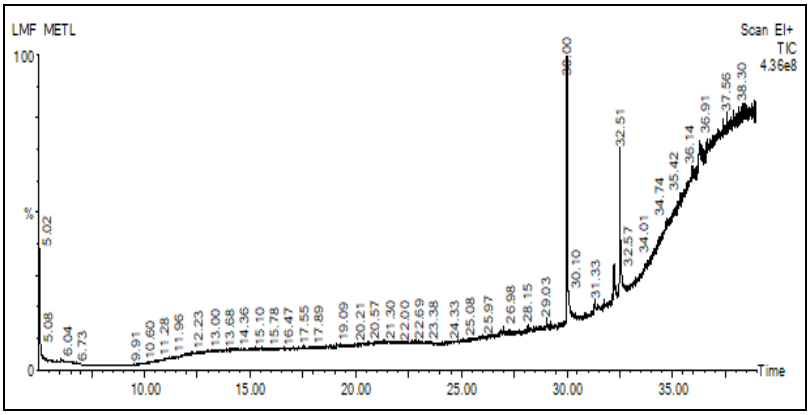
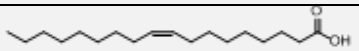
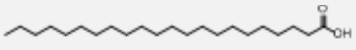
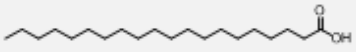
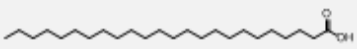
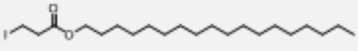
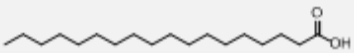
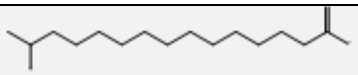
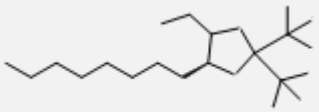
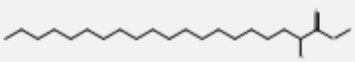
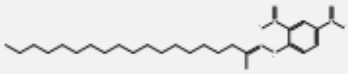
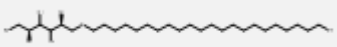
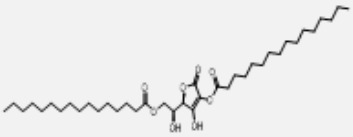
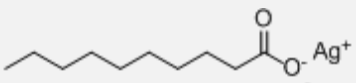
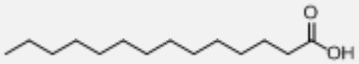
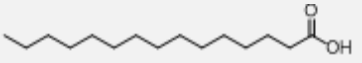
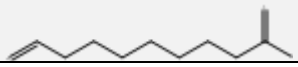
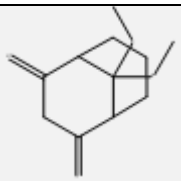
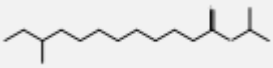
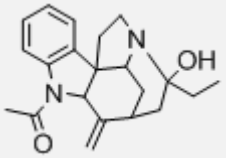
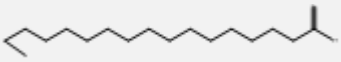
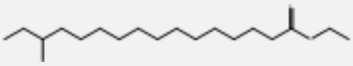
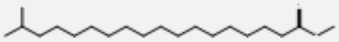
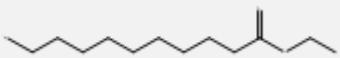
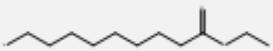
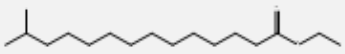
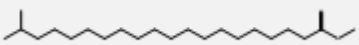
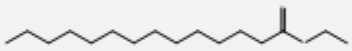
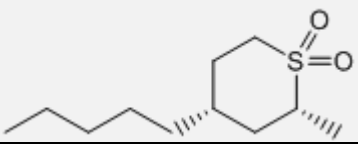


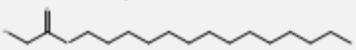
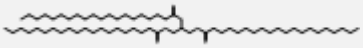
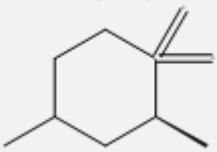
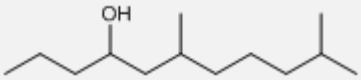
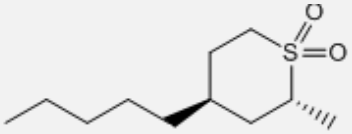
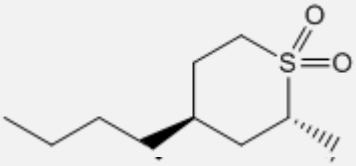
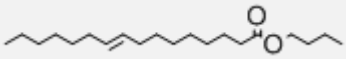
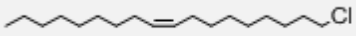
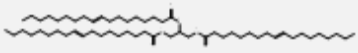
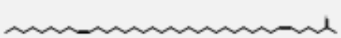
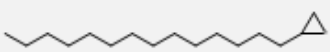
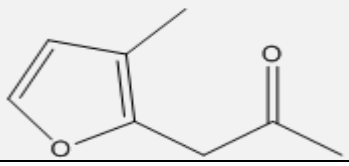
FIG. 4: GC-MS CHROMATOGRAM OF THE *LYGODIUM MICROPHYLLUM* FRACTION

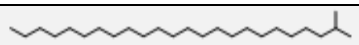
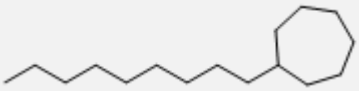
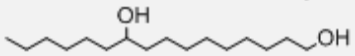
TABLE 7: GC-MS ANALYSIS OF FRACTIONATED EXTRACT OF *LYGODIUM MICROPHYLLUM*

Sl. no.	Compound name	Molecular structure	Mol formula	Mol.wt	Activity
1	Oleic acid		$C_{18}H_{34}O_2$	282	Antimicrobial, Antifungal, anticonvulsive activity, Antiadhesive, Antiallergic, Antianalgesic, Antiatherosclerosis, Anesthetic, Antihelmenthic, Antianxiety, Antibacterial, Antiberiberi, Antibiotic, Anticancer, Anticonvulsant, Antidiabetic, Antidiarrheic, Antifertility, Antigastric, Anti-infammatory, Antiobesity, Antioxidant, Antiulcer, Antitubercellosic, Anticold, Antihepatotoxic and Antiviral activityanemiagenic, dermatitigenic[13]
2	Docosanoic acid		$C_{22}H_{44}O_2$	340	Antibacterial and cytoprotective activity[57]
3	Eicosanoic acid		$C_{20}H_{40}O_2$	312	Reduced heart diseases, kidney and liver function, blood Clotting[13]; Antibacterial and cytoprotective activity[57]
4	Tetracosanoic acid		$C_{24}H_{48}O_2$	368	-
5	Propionic acid, 3-iodo-, octadecyl ester		$C_{21}H_{41}IO_2$	452	-
6	Octadecanoic acid		$C_{18}H_{36}O_2$	284	Decreases cardiovascular and cancer risks, reduces LDL cholesterol levels, reduces blood pressure, improved heart function[58]

7	Pentadecanoic acid, 14-bromo-		$C_{15}H_{29}BrO_2$	320	-
8	1,3-dioxolane, 4-ethyl-5-octyl-2,2-bis(trifluoromethyl)-, trans-		$C_{15}H_{24}F_6O_2$	350	Antioxidant activity[63]
9	methyl 2-hydroxy-eicosanoate		$C_{21}H_{42}O_3$	342	-
10	2-nonadecanone 2,4—dinitrophenylhydrazine		$C_{25}H_{42}N_4O_4$	462	Antimicrobial activity[15]
11	d-mannitol, 1-o-(22-hydroxydocosyl)-		$C_{28}H_{58}O_7$	506	-
12	Distearyl thiodipropionate		$C_{42}H_{82}O_4S$	682	Antioxidant activity[64]
13	l-(+)-ascorbic acid 2,6-dihexadecanoate		$C_{38}H_{68}O_8$	652	Antioxidant and reduces the triglycerides level—Protects LDL against peroxidation and inhibits the progression of atherosclerosis, Antiallergic, Antianemic, Antianxiety, Antibacterial, Antibronchitic, Anticancer, Anticarcinogenic, Anticataract, Anticoagulant, Anticonvulsant, Antidiabetic, Antidiarrheic, Antifatigue, Antifertility, Antigastric, Anti-infammatory, Antimalarial, Antioxidant, Antistress, Antiulcer, Antiatheroscelerotic, Anticold, Antiglaucomic, Antihepatic, Antihypertensive, Antiplague, Antiproliferant, Antiprotozoal, Antiseptic, Antistroke, Antituberculic, Antitumer, CNSStimulant, Chelator, Chemopreventive, CytochromeP450Inducer, Deodorant, Dermal, Detoxicant, Flavor, Hypolipidimic, Neuroprotective, Neurotransmitter, Termiticide and Antiviral activity[13]
14	Decanoic acid, silver(1+) salt		$C_{10}H_{19}AgO_2$	278	-
15	Tetradecanoic acid		$C_{14}H_{28}O_2$	228	Anti-virulence activity[65]
16	Pentadecanoic acid		$C_{15}H_{30}O_2$	242	Antibacterial Antifungal activity[66]
17	9-oxononanoic acid		$C9H_{16}O_3$	172	-

18	9,9-dimethoxybicyclo[3.3.1]nona-2,4-dione		$C_{11}H_{16}O_4$	212	antioxidants, anti-arthritic and antimicro-bial activity[67]
19	i-propyl 10-methyl-dodecanoate		$C_{16}H_{32}O_2$	256	-
20	Strychane, 1-acetyl-20.alpha.-hydroxy-16-methylene-		$C_{21}H_{26}N_2O_2$	338	Antimicrobial activity[68]
21	n-hexadecanoic acid		$C_{16}H_{32}O_2$	256	Anti-inflammatory activity[69]; Antioxidant, hypocholesterolemic, nematicide, pesticide, antiandrogenic, flavour, hemolytic, 5-alpha reductase inhibitor[70]
22	Ethyl 14-methyl-hexadecanoate		$C_{19}H_{38}O_2$	298	Insecticidal and Anti-helminthic activity[71]
23	octadecanoic acid, 17-methyl-, methyl ester		C20H40O2	312	-
24	10-bromodecanoic acid, ethyl ester		$C_{12}H_{23}BrO_2$	278	Antioxidant and Antibacterial activity[72]
25	nonanoic acid, 9-bromo-, ethyl ester		$C_{11}H_{21}BrO_2$	265	Antioxidant and Antimicrobial activity[73]
26	Octadecanoic acid, ethyl ester		$C_{20}H_{40}O_2$	312	Antimicrobial activity[74]
27	Hexadecanoic acid, ethyl ester			284	Antioxidant, hypocholesterolemic, nematicide, pesticide, antiandrogenic, flavor, hemolytic, 5-alpha reductase inhibitor[71]
28	Ethyl 13-methyl-tetradecanoate		$C_{17}H_{34}O_2$	270	Antimicrobial, Antioxidant and Anticancer activity[75]
29	methyl 19-methyl-eicosanoate		$C_{22}H_{44}O_2$	340	-
30	Tetradecanoic acid, ethyl ester		$C_{16}H_{32}O_2$	256	Cytotoxic activity[76]
31	Docosanoic acid, docosyl ester		$C_{44}H_{88}O_2$	648	Antidepressant and Cytotoxic activity[56]
32	Dotriacontyl isopropyl ether		$C_{35}H_{72}O$	508	-
33	cis-2-methyl-4-n-pentylthiane, s,s-dioxide		$C_{11}H_{22}O_2S$	218	Anti-proliferative activity[57]

34	5-methyl-z-5-docosene		$C_{23}H_{46}$	322	Antibacterial, antidiabetic, antitumour activities[13]
35	Chloroacetic acid, tetradecyl ester		$C_{16}H_{31}ClO_2$	290	Antioxidant, antimicrobial and bacteriocide, anti-infammatoryactivity[13]
36	triarachine		$C_{63}H_{122}O_6$	975	Anti-hyperglycemic activity[62]
37	Trans-2,4-dimethylthiane, s,s-dioxide		$C_7H_{14}O_2S$	162	Anti-infammatory activity[60]
38	Eicosyl nonyl ether		$C_{29}H_{60}O$	424	Antioxidant activity[61]
39	6,10-dimethyl-4-undecanol		$C_{13}H_{28}O$	200	Antioxidant And Anti-cancer activity[77]
40	Trans-2-methyl-4-n-pentylthiane, s,s-dioxide		$C_{11}H_{22}O_2S$	218	Antioxidant activity[63]
41	Trans-2-methyl-4-n-butylthiane, s,s-dioxide		$C_{10}H_{20}O_2S$	204	Antimicrobial, Antioxidant, cytotoxic activity[78]; Anti-proliferative and apoptosis-inducing activity[79]
42	Dodecane, 1-fluoro-		$C_{12}H_{25}F$	188	Antioxidant activity[80]
43	Butyl 9-hexadecenoate		$C_{20}H_{38}O_2$	310	-
44	cis-1-chloro-9-octadecene		$C_{18}H_{35}Cl$	286	Antiviral activity[81]
45	Undec-10-ynoic acid, tetradecyl ester		$C_{25}H_{46}O_2$	378	Antimicrobial activity[18]
46	9-octadecenoic acid, 1,2,3-propanetriyl ester, (e,e,e)-		$C_{57}H_{104}O_6$	884	Antispasmodic and immune modulators[82]
47	z,z-6,28-heptatriactontadien-2-one		C37H70O	530	Vasodilator[13]
48	oxirane, tetradecyl-		$C_{16}H_{32}O$	240	Antimicrobial activity[83]
49	3-methyl-2-(2-oxopropyl)furan		$C_8H_{10}O_2$	138	Antimicrobial activity[10-11], Antibiofilm, Anti-quorum sensing activity[12]; antioxidant, antipyretic, anti-inflammatory activity[13]

50	2-methyltetracosane		$C_{25}H_{52}$	352	Antioxidant Activity[84]
51	1-nonylcycloheptane		$C_{16}H_{32}$	224	Antimicrobial activity[85]
52	1,10-hexadecanediol		$C_{16}H_{34}O_2$	258	Antioxidant and Antibacterial activity[86]

*Molecular structures were generated using ChemDraw Ultra 12.0

The various medicinal uses of pteridophytes [87-88] may be due to the bioactive chemicals found in the fractions, which have been documented to exhibit a variety of medicinal properties **Table 4-7.** With the existence of these bioactive chemicals, the MIC and MBC values of the pteridophyte extracts in the current investigation may also be confirmed.

To have a better understanding of the therapeutic activities of the examined pteridophytes, it will be interesting to document the biological roles of some of the compounds present in greater quantities, such as Tridecane, 6-Cyclohexyl-, Undecane, 3-Cyclohexyl-, Ethane, 1,1-Dichloro-, 2-Chloroethyl Methyl Sulfone, (Z)-1-Chloro-2-(Methylsulfonyl) Ethylene, 2-Chloropropionyl Chloride, Propane, 1,2-Dichloro-, Disilane, 1,1,2,2-Tetrachloro-1,2-Dimethyl-, Disparlure, 4-(3,5-Di-Tert-Butyl-4-Hydroxyphenyl)Butyl Acrylate, 3-(3, 5-Di-Tert-Butyl – 4 - Hydroxyphenyl) Propyl Methacrylate, 9-Octadecen-1-Ol, (Z)-, 1,16-Hexadecanediol, 17-Octadecynoic Acid, Z,E-2-Methyl - 3, 13 – Octadecadien – 1 - Ol, 1, 21-Docosadiene, Pentadecanoic Acid, 14-Bromo-, Tetracosanoic Acid, D-Mannitol, 1-O-(22-Hydroxydocosyl)- and Decanoic Acid, Silver(1+) Salt, which has not yet been published.

CONCLUSION: The present work found that the four pteridophyte species possess antibacterial and antifungal activities. The study revealed encouraging outcomes for fatty acids as antimicrobial agents. The GCMS analysis of the fatty acid fractions revealed bioactive compounds with reported biological activity, which supports the study's results. The present work supports the use of fern species in traditional medicine and therapies. These results will also be useful for undertaking *in-silico* and cell-line studies for potential pharmacological lead compounds for drug discovery in future research.

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REFERENCES:

1. Tillotson G: The Fight against Bacterial Resistance – New Initiatives But Much Still Needed.Journal of Infectious Diseases and Therapy 2016. doi:10.4172/2332-0877.1000e109.
2. Giancarlo CV, Carlimar OM, Solymar M, Christian MG, Valle RGD, Carballeira N and Sanabria-Ríos DJ: Antibacterial fatty acids: An update of possible mechanisms of action and implications in the development of the next-generation of antibacterial agents, Progress in Lipid Research 2021; 82: 101093, https://doi.org/10.1016/j.plipres.2021.101093.
3. Kumar P, Lee JH and Lee HBJ: Fatty Acids as Antibiofilm and Antivirulence Agents, Trends in Microbiology, 2020; 28(9): 753-768.
4. Sen A and Ghosh PD: A note on the ethnobotanical studies of some pteridophytes in Assam, IJTK 2011; 10(2): 292-295.
5. Twitchell E: The Precipitation of Solid Fatty Acids with Lead Acetate in Alcoholic Solution. Ind Eng Chem 1921; 13(9): 806–807 https://doi.org/10.1021/ie50141a024
6. Khanal L, Sharma K, Pokharel Y and Kalauni S: Assessment of Phytochemical, Antioxidant and Antimicrobial Activities of Some Medicinal Plants from Kaski District of Nepal. American Journal of Plant Sciences 2020; 11: 1383-1397.
7. Antonisamy MA, Johnson & Johnson, Antonisamy, Madona C, Ray A, Cruz-Martins N and Coutinho H: Antibiotics *in-vitro* toxicity, antioxidant, anti-inflammatory, and antidiabetic potential of *Sphaerostephanos unitus* (L.) Holttum. Antibiotics 2020; 9: 333. 10.doi: 10.3390/antibiotics9060333
8. Desbois A: Potential applications of antimicrobial fatty acids in medicine, agriculture and other industries. Recent Pat Antiinfect Drug Discov 2012; 7(2): 111–22.

9. Yoon B, Jackman J, Valle-Gonz´ alez E and Cho N: Antibacterial free fatty acids and monoglycerides: biological activities, experimental testing, and therapeutic applications. Int J Mol Sci 2018; 19(4): 1114.
10. Singh N, Sudandiradoss C and Abraham J: Screening of furanone in cucurbitamelo and evaluation of its bioactive potential using *in-silico* studies. Interdiscip Sci Comput Life Sci 2016; 8: 395–402. https://doi.org/10.1007/s12539-016-0161-z
11. Karthik R, Saravanan R and Ebenezar KK: Isolation, Purification, and Characterization of Avian Antimicrobial Glycopeptide from the Posterior Salivary Gland of Sepia pharaonis. Appl Biochem Biotechnol 2015; 175: 1507–1518. https://doi.org/10.1007/s12010-014-1370-8
12. Gupta K, Singh SP and Manhar AK: Inhibition of *Staphylococcus aureus* and *Pseudomonas aeruginosa* Biofilm and Virulence by Active Fraction of *Syzygium cumini* (L.) Skeels Leaf Extract: In-Vitro and In-Silico Studies. Indian J Microbiol 2019; 59: 13–21. https://doi.org/10.1007/s12088-018-0770-9
13. Ralte L, Khiangte L and Thangjam NM: GC–MS and molecular docking analyses of phytochemicals from the underutilized plant, Parkiatimoriana revealed candidate anti-cancerous and anti-inflammatory agents. Sci Rep 2022; 12: 3395. https://doi.org/10.1038/s41598-022-07320-2
14. Manjari MS, Karthi S, Ramkumar G, Muthusamy R, Natarajan D and Shivakumar MS: Chemical composition and larvicidal activity of plant extracts from *Clausena dentata* (Willd) (Rutaceae) against dengue, malaria, and filariasis vectors. Parasitol Res 2014; 113(7): 2475-81. doi: 10.1007/s00436-014-3896-7. Epub 2014 May 7. PMID: 24802866.
15. Ramkumar G, Karthi S, Muthusamy R, Suganya P, Natarajan D, Kweka J and Eliningaya: Chemical composition of chloroform leaf extract from *Glycosmis pentaphylla*. PLOS ONE. Dataset. 2016https://doi.org/10.1371/journal.pone.0158088.t004
16. Mallikadevi T, Paulsamy S, Jamuna S and Karthika K: Asian J Pharm Clinical Res., 2012, 5(4), 163-168.
17. Nithyadevi J and Sivakumar R: Phytochemical screening and GC-MS, FT-IR analysis of methanolic extract leaves of Solanumtorvum Sw. Int J Res Stud Biosci 2015; 3: 61–66.
18. Hasan MD, Basher M, Shyama M, Fatema J, Islam MD and Sarmina Y: Antimicrobial activities and GC-MS chemical profiling of methanol extracts of different parts of Morus alba (Tut) in Bangladesh. IJB 2021; 18: 156-166. 10.12692/ijb/18.5.156-166.
19. Khiralla A, Spina R, Varbanov M, Philippot S, Lemiere P, Slezack-Deschaumes S, André P, Mohamed I, Yagi SM and Laurain-Mattar D: Evaluation of Antiviral, Antibacterial and Antiproliferative Activities of the Endophytic Fungus *Curvularia papendorfii*, and Isolation of a New Polyhydroxyacid. Microorganisms 2020; 8(9): 1353. https://doi.org/10.3390/microorganisms8091353
20. Abdel-Motaal FF, Maher ZM, Ibrahim SF, El-Mleeh A, Behery M and Metwally AA: Comparative Studies on the Antioxidant, Antifungal, and Wound Healing Activities of Solenostemmaarghel Ethyl Acetate and Methanolic Extracts. Applied Sciences 2022; 12(9): 4121. https://doi.org/10.3390/app12094121
21. Ratheesh M, Sunil S and Sheethal S: Anti-inflammatory and anti-COVID-19 effect of a novel polyherbal formulation (Imusil) via modulating oxidative stress, inflammatory mediators and cytokine storm. Inflammopharmacol 2022; 30: 173–184. https://doi.org/10.1007/s10787-021-00911-x
22. Bhardwaj M, Sali VK and Mani S: Neophytadiene from Turbinariaornata Suppresses LPS-Induced Inflammatory Response in RAW 264.7 Macrophages and Sprague Dawley Rats. Inflammation 2020; 43: 937–950. https://doi.org/10.1007/s10753-020-01179-z
23. Ganesh M and Mohankumar M: Extraction and identification of bioactive components in Sidacordata (Burm.f.) using gas chromatography-mass spectrometry. J Food Sci Technol 2017; 54(10): 3082-3091. doi: 10.1007/s13197-017-2744-z. Epub 2017 Jul 17. PMID: 28974793; PMCID: PMC5602971.
24. Shareef HK, Muhammed HJ, Hussein HM and Hameed IH: Antibacterial effect of ginger (*Zingiber officinale*) roscoe and bioactive chemical analysis using gas chromatography mass spectrum. Oriental Journal of Chemistry 2016; 32(2): 817-837. Doi: 10.13005/ojc/320207
25. Shankar VK, Wang M, Ajjarapu S, Kolimi P, Avula B, Murthy R, Khan I and Murthy SM: Analysis of docosanol using GC/MS: Method development, validation and application to ex vivo human skin permeation studies. Journal of Pharmaceutical Analysis 2022; 12(2): 287-292. https://doi.org/10.1016/j.jpha.2021.08.004.
26. Paudel MR, Chand MB, Pant B and Pant B: Antioxidant and cytotoxic activities of *Dendrobium moniliforme* extracts and the detection of related compounds by GC-MS. BMC Complement Altern Med 2018; 18(1): 134. doi: 10.1186/s12906-018-2197-6. PMID: 29685150; PMCID: PMC5913799.
27. Paudel MR, Chand MB, Pant B and Pant B: Assessment of Antioxidant and Cytotoxic Activities of Extracts of *Dendrobium crepidatum*. Biomolecules 2019; 9(9): 478. https://doi.org/10.3390/biom9090478
28. Butawan M, Benjamin RL and Bloomer RJ: Methylsulfonylmethane: Applications and Safety of a Novel Dietary Supplement. Nutrients 2017; 9(3): 290. doi: 10.3390/nu9030290. PMID: 28300758; PMCID: PMC5372953.
29. Hari I and Mathew N: Larvicidal activity of selected plant extracts and their combination against the mosquito vectors *Culexquinque fasciatus* and Aedesaegypti. Environ Sci Pollut Res 2018; 25: 9176–9185. https://doi.org/10.1007/s11356-018-1515-3
30. Prakash J and Arora NK: Novel metabolites from *Bacillus safensis* and their antifungal property against *Alternaria alternata*. Antonie van Leeuwenhoek 2021; 114: 1245–1258. https://doi.org/10.1007/s10482-021-01598-4
31. Mani G: Extraction and identification of bioactive components in *Sida cordata* (Burm.f.) using gas chromatography–mass spectrometry. Journal of Food Science and Technology 2017; 54. Doi: 10.1007/s13197-017-2744-z.
32. Xiong H, Qi S, Xu Y, Miao L and Qian PY: Antibiotic and antifouling compound production by the marine-derived fungus Cladosporium sp. F14. J Hydro Environ Res 2009; 2: 264–271. https://doi.org/10.1016/j.jher.2008.12.002
33. Zeb A, Ullah F, Ayaz M, Ahmad S and Sadiq A: Demonstration of biological activities of extracts from Isodonrugosus Wall. Ex Benth: Separation and identification of bioactive phytoconstituents by GC-MS analysis in the ethyl acetate extract. BMC Complement Altern Med 2017; 17(1): 284. doi: 10.1186/s12906-017-1798-9. PMID: 28558679; PMCID: PMC5450350.
34. Chatterjee S, Karmakar A and Azmi SA: Antibacterial Activity of Long-Chain Primary Alcohols from *Solena*

amplexicaulis Leaves. Proc Zool Soc 2018, 71, 313–319. https://doi.org/10.1007/s12595-017-0208-0

35. Katz DH, Marcelletti JF, Khalil MH, Pope LE and Katz LR: Antiviral activity of 1-docosanol, an inhibitor of lipid-enveloped viruses including Herpes simplex. Proc Natl Acad Sci USA 1991; 88(23): 10825-9. doi: 10.1073/pnas.88.23.10825. PMID: 1660151; PMCID: PMC53024.
36. Oliveira AMD, Conserva LM, De Souza Ferro JN, BritoFd A, Lemos RPL and Barreto E: Antinociceptive and Anti-Inflammatory Effects of Octacosanol from the Leaves of *Sabiceagrisea var*. grisea in Mice. International Journal of Molecular Sciences 2012; 13(2): 1598-1611. https://doi.org/10.3390/ijms13021598
37. Rhetso T, Shubharani R and Roopa MS: Chemical constituents, antioxidant, and antimicrobial activity of *Allium chinense* G. Don.Futur J Pharm Sci 2022; 6: 102. https://doi.org/10.1186/s43094-020-00100-7
38. Hirpara DG, Gajera HP and Bhimani RD: The SRAP based molecular diversity related to antifungal and antioxidant bioactive constituents for biocontrol potentials of Trichoderma against *Sclerotium rolfsii* Scc..Curr Genet 2016; 62: 619–641. https://doi.org/10.1007/s00294-016-0567-5
39. Moin S, Mahalakshmipriya A and Kuppusamy S: Chemical Composition and *In-vitro* Antimicrobial Activity of Barlerialupulina Essential Oil. Journal of Herbs, Spices & Medicinal Plants 2012; 18: 101-109. 10.1080/10496475.2011.653711.
40. Aina DA, Oloke JK, Awoyinka OA, Adebayo EA, Akoni OI and Agbolade JO: Comparative cytotoxic effect of metabolites from wild and mutant strains of Schizophylum commune grown in submerged liquid medium. Am J Res Comm 2013; 1: 219-40.
41. Shah MD, Yong YS and Iqbal M: Phytochemical investigation and free radical scavenging activities of essential oil, methanol extract and methanol fractions of *Nephrolepis biserrata*. International Journal of Pharmacy and Pharmaceutical Sciences 2014; 6: 269-77, https://innovareacademics.in/journals/index.php/ijpps/article/view/1512.
42. Alqahtani SS, Makeen HA, Menachery SJ and Moni SS: Documentation of bioactive principles of the flower from *Carallum aretrospiciens* (Ehrenb) and in vitro antibacterial activity – Part B, Arabian Journal of Chemistry 2020; 13(10): 7370-7377.
43. Suluvoy JK and Berlin Grace VM: Phytochemical profile and free radical nitric oxide (NO) scavenging activity of *Averrhoa bilimbi* L. fruit extract 3 Biotech 2017; 7(1): 85..
44. Fries E and Puttmann W: Analysis of the antioxidant butylated hydroxytoluene (BHT) in water by means of solid phase extraction combined with GC/MS. Water Research 2002; 36: 2319-27. 10.1016/S0043-1354(01)00453-5.
45. Roselin I, Srinivasan S, Mahalingam G and Sara C: Gas Chromatography and mass spectroscopy analysis of bioactive compounds of *Adiantum latifolium* Lam. International Research Journal of Pharmacy 2018; 9: 125-130. 10.7897/2230-8407.0910239.
46. Manjari MS, Karthi S, Ramkumar G, Muthusamy R, Natarajan D and Shivakumar MS: Chemical composition and larvicidal activity of plant extracts from *Claus enadentata* (Willd) (Rutaceae) against dengue, malaria, and filariasis vectors. Parasitol Res 2014; 113(7): 2475-81. doi: 10.1007/s00436-014-3896-7. Epub 2014 May 7. PMID: 24802866.
47. Padmini R, Uma Maheshwari V and Saravanan P: Identification of novel bioactive molecules from garlic bulbs: A special effort to determine the anticancer potential against lung cancer with targeted drugs. Saudi Journal of Biological Sciences 2020; 27(12): 3274-3289. DOI: 10.1016/j.sjbs.2020.09.041. PMID: 33304133; PMCID: PMC7715046.
48. Oluwasina OO, Ezenwosu IV and Ogidi CO: Antimicrobial potential of toothpaste formulated from extracts of *Syzygium aromaticum*, *Dennettia tripetala* and Jatrophacurcas latex against some oral pathogenic microorganisms. AMB Expr 2019; 9: 20. https://doi.org/10.1186/s13568-019-0744-2
49. Nagar N, Aswathanarayan JB and Vittal RR: Anti-quorum sensing and biofilm inhibitory activity of Apium graveolens L. oleoresin. J Food Sci Technol 2020; 57: 2414–2422. https://doi.org/10.1007/s13197-020-04275-y
50. Moodley K, Joseph K and Naidoo Y: Antioxidant, antidiabetic and hypolipidemic effects of *Tulbaghia violacea* Harv. (wild garlic) rhizome methanolic extract in a diabetic rat model. BMC Complement Altern Med 2015, 15, 408. https://doi.org/10.1186/s12906-015-0932-9
51. Youssef NH, Qari SH, Behiry SI, Dessoky ES, El-Hallous EI, Elshaer MM, Kordy A, Maresca V, Abdelkhalek A and Heflish AA: Antimycotoxigenic Activity of Beetroot Extracts against AlternariaalternataMycotoxins on Potato Crop. Applied Sciences 2021; 11(9): 4239. https://doi.org/10.3390/app11094239
52. Baky MH, Shawky EM, Elgindi MR and Ibrahim HA: Comparative Volatile Profiling of Ludwigiastolonifera Aerial Parts and Roots Using VSE-GC-MS/MS and Screening of Antioxidant and Metal Chelation Activities. ACS Omega 2021; 6(38): 24788-24794. DOI: 10.1021/acsomega.1c03627
53. Anyanwu GO, Anzaku D, Donwell CC, Usunobun U, Adegbegi AJ, Ofoha PC and Rauf K: Chemical composition and *in-vitro* antiobesity and *in-vivo* anti-hyperlipidemic effects of *Ceratothecase samoides*, *Jatropha tanjorensis*, *Mucuna flagellipes*, *Pterocarpus mildbraedii* and *Piper guineense*. Phytomedicine Plus 2021; 1(3): 100042, ISSN 2667-0313, https://doi.org/10.1016/j.phyplu.2021.100042.
54. Rizwana H: Chemical Composition, FTIR Studies and Antibacterial Activity of *Passiflora edulis* f. edulis (Fruit). Journal of Pure and Applied Microbiology 2019; 13(4): 2489. Gale Academic OneFile, link.gale.com/apps/doc/A674565479/AONE?u=anon~3a7a86d&sid=googleScholar&xid=ed1846d8. Accessed 6 Aug. 2022.
55. Al-Huqail AA, Elgaaly GA and Ibrahim MM: Identification of bioactive phytochemical from two Punica species using GC-MS and estimation of antioxidant activity of seed extracts. Saudi J Biol Sci 2018; 25(7): 1420-1428. doi: 10.1016/j.sjbs.2015.11.009. Epub 2015 Nov 26. PMID: 30505191; PMCID: PMC6252002.
56. Bravo-Santano N, Ellis JK, Calle Y, Keun HC, Behrends V and Letek M: Intracellular *Staphylococcus aureus* Elicits the Production of Host Very Long-Chain Saturated Fatty Acids with Antimicrobial Activity. Metabolites 2019; 9(7): 148. https://doi.org/10.3390/metabo9070148
57. Senyilmaz-Tiebe D, Pfaff DH, Virtue S, Schwarz KV, Fleming T, Altamura S, Muckenthaler MU, Okun JG, Vidal-Puig A, Nawroth P and Teleman AA: Dietary stearic acid regulates mitochondria *in-vivo* in humans. Nat Commun 2018; 9(1): 3129. doi: 10.1038/s41467-018-05614-6. PMID: 30087348; PMCID: PMC6081440.

58. Rahman J, Tareq A, Hossain MM, Sakib S, Islam M, Hazra M, Uddin ABMN, Hoque M, Nasrin S, Emran T, Capasso R, Reza ASMA and Simal-Gandara J: Biological Evaluation, DFT Calculations and Molecular Docking Studies on the Antidepressant and Cytotoxicity Activities of Cycaspectinata Buch.-Ham. Compounds. Pharmaceuticals 2020; 13: 232. 10.3390/ph13090232.
59. Hakkim FL, Al-Buloshi M and Achankunju J: Chemical composition and anti-proliferative effect of Oman's *Ganoderma applanatum* on breast cancer and cervical cancer cells, Journal of Taibah University Medical Sciences 2016; 11(2): 145-151, ISSN 1658-3612, https://doi.org/10.1016/j.jtumed.2016.01.007.
60. Agarwal H and Shanmugam VK: Anti-inflammatory activity screening of *Kalanchoe pinnata* methanol extract and its validation using a computational simulation approach, Informatics in Medicine Unlocked 2019; 14: 6-14., https://doi.org/10.1016/j.imu.2019.01.002.
61. Erwin E, Pusparohmana W, Sari I, Hairani R and Usman U: GC-MS profiling and DPPH radical scavenging activity of the bark of Tampoi (*Baccaureama crocarpa*). F1000Research 2019; 7: 1977.
62. Bhaskar A, Nithya V and Gopalakrishnan V: Phytochemical evaluation by GC-MS and antihyperglycemic activity of *Mucuna pruriens* on streptozotocin induced diabetes in rats. Journal of Chemical and Pharmaceutical Research 2011; 3: 689-696.
63. Khan M, Khan Y, Samina, Kaun L, Shah M and Idris R: Chemical composition and antioxidant activity of essential oil of leaves and flowers of *Alternanthera sessilis* red from Sabah. Journal of Applied Pharmaceutical Science 2016; 6: 157-161. 10.7324/JAPS.2016.601222.
64. Chao M: Antioxidant synergism between synthesised alkylated diphenylamine and dilaurylthiodipropionate in polyolefin base fluid. Journal of Thermal Analysis and Calorimetry 2014; 117(2): 925. Gale Academic OneFile, link.gale.com/apps/doc/A384543099/AONE?u=anon~7158b1e3&sid=googleScholar&xid=ce4d26b2. Accessed 17 Aug. 2022.
65. Juárez-Rodríguez MM, Cortes-López H, García-Contreras R, González-Pedrajo B, Díaz-Guerrero M, Martínez-Vázquez M, Rivera-Chávez JA, Soto-Hernández RM Ceratothecasesamoides, Castillo-Juárez I: Tetradecanoic acids with anti-virulence properties increase the pathogenicity of *Pseudomonas aeruginosa* in a murine cutaneous infection model. Front Cell Infect Microbiol 2021; 10: 597517. doi: 10.3389/fcimb.2020.597517. PMID: 33585272; PMCID: PMC7876447.
66. Ghazala B, Shameel M, Choudhary MI, Shahzad S and Leghari SM: Phycochemistry and bioactivity of Tetraspora (Volvocophyta) from Sindh. Pakistan Journal of Botany 2004; 36(3): 531-548.
67. Ololade Z: Pharmacological Potential of the Stem Extract of *Melissa officinalis*: Compositional Profile, Polyphenol, Ascorbic Acid Contents, Antioxidant, Anti-Inflammatory and Antimicrobial Activities 2018; 1: 43-52.
68. Kadhim MJ, Mohammed GJ and Hameed IH: *In-vitro* antibacterial, antifungal and phytochemical analysis of methanolic extract of fruit *Cassia fistula*. Orient J Chem 2016; 32(3).
69. Aparna V, Dileep K, Mandal P, Karthe P, Sadasivan C and Haridas M: Anti-Inflammatory Property of n-Hexadecanoic Acid: Structural Evidence and Kinetic Assessment. Chemical Biology & Drug Design 2012; 80(3): 434-439.
70. Zayed MZ, Ahmad FB, Ho WS and Pang SL: GC-MS analysis of phytochemical constituents in leaf extracts of *Neolamarckia cadamba* (Rubiaceae) from Malaysia. Int J Pharm Pharm Sci 2014; 6(9): 123-127.
71. Innovare I: Phytochemical screening, gc-ms analysis and biological activitiys of *Ipomoea eriocarpa* leaf extracts. Int J Pharm Pharm Sci 2014; 6: 4.
72. Akwu NA, Naidoo Y, Singh M, Nundkumar N Ceratothecasesamoides, Lin J: Phytochemical screening, *in-vitro* evaluation of the antimicrobial, antioxidant and cytotoxicity potentials of Grewialasiocarpa E. Mey. ex Harv., South African Journal of Botany 2019; 123: 180-192
73. Jodallah N and Ali-Shtayeh MS: Antioxidant and antimicrobial activity of *Mandragoraau tumnalis* Bertol Extracts 2013.
74. Fernandes CP, Corrêa AL, Lobo JF, Caramel OP, De Almeida FB, Castro ES and Rocha L: Triterpene esters and biological activities from edible fruits of *Manilkara subsericea* (Mart.) Dubard, Sapotaceae. BioMed Research International 2013.
75. Rahman S, Ismail M, Shah MR, Iriti M and Shahid M: GC/MS analysis, free radical scavenging, anticancer and β-glucuronidase inhibitory activities of *Trillium govanianum* rhizome. Bangladesh Journal of Pharmacology 2015; 10(3): 577-583.
76. Letaief T, Garzoli S, LaghezzaMasci V, Mejri J, Abderrabba M, Tiezzi A and Ovidi E: Chemical Composition and Biological Activities of Tunisian Ziziphus lotus Extracts: Evaluation of Drying Effect, Solvent Extraction, and Extracted Plant Parts. Plants 2021; 10(12): 2651. https://doi.org/10.3390/plants10122651
77. Paudel MR, Joshi PR, Chand K, Sah AK, Acharya S, Pant B and Pant B: Antioxidant, anticancer and antimicrobial effects of *In-vitro* developed protocorms of *Dendrobium longicornu*. Biotechnol Rep (Amst) 2020; 28: 00527. doi: 10.1016/j.btre.2020.e00527. PMID: 32983924; PMCID: PMC7494665.
78. Naine J, Devi CS, Mohanasrinivasan V and Vaishnavi B: Antimicrobial, Antioxidant and Cytotoxic Activity of Marine *Streptomyces parvulus* VITJS11 Crude Extract. Brazilian Archives of Biology and Technology 2015; 58: 198-207. 10.1590/S1516-8913201400173.
79. Swargiary A, Roy M, Boro H and Verma A: Phytochemical analysis, antiproliferative and apoptosis-inducing properties of *Persicaria strigosa* Nakai. 2022 https://doi.org/10.21203/rs.3.rs-1383078/v1
80. Nonglang FP, Khale A and Bhan S: Phytochemical characterization of the ethanolic extract of *Kaempferia galanga* rhizome for anti-oxidant activities by HPTLC and GCMS. Futur J Pharm Sci 2022; 8(9). https://doi.org/10.1186/s43094-021-00394-1
81. Tassakka ACMAR, Sumule O, Massi MN, Sulfahri, Manggau M, Iskandar IW, Alam JF, Permana AD and Liao LM: Potential bioactive compounds as SARS-CoV-2 inhibitors from extracts of the marine red alga *Halymenia durvillei* (Rhodophyta) - A computational study. Arab J Chem 2021; 14(11): 103393. doi: 10.1016/j.arabjc.2021.103393. Epub 2021 Aug 23. PMID: 34909061; PMCID: PMC8381616.
82. Al-Marzoqi AH, Hadi MY and Hameed IH: Determination of metabolites products by *Cassia angustifolia* and evaluate antimicobial activity. Journal of Pharmacognosy and Phytotherapy 2016; 8(2): 25-48.
83. Musa AM, Ibrahim MA, Aliyu AB, Abdullahi MS, Tajuddeen N, Ibrahim H and Oyewale AO: Chemical composition and antimicrobial activity of hexane leaf extract of *Anisopus mannii* (Asclepiadaceae). J Intercult Ethnopharmacol 2015; 4(2): 129-33. doi:

10.5455/jice.20150106124652. Epub 2015 Jan 11. PMID: 26401399; PMCID: PMC4566775.

84. Thekkumalai M, Sivanandham V and Parthasarathy R: GC-MS analysis of bioactive compounds in *Bryonopsis laciniosa* Fruit Extract 2015; 10.13140/RG.2.2.20114.30401.
85. Nas F, Aissaoui N and Mahjoubi M: A comparative GC–MS analysis of bioactive secondary metabolites produced by halotolerant Bacillus spp. isolated from the Great Sebkha of Oran. Int Microbiol 2021; 24: 455–470. https://doi.org/10.1007/s10123-021-00185-x
86. Abbassy MMS, Salem MZM and Rashad NM: Nutritive and biocidal properties of agroforestry trees of *Moringa oleifera* Lam., Cassia fistula L., and *Ceratonia siliqua* L. as non-conventional edible vegetable oils. Agroforest Syst 2020; 94: 1567–1579. https://doi.org/10.1007/s10457-018-0325-4
87. Baskaran XR, Geo Vigila AV, Zhang SZ, Feng SX and Liao WB: A review of the use of pteridophytes for treating human ailments. Journal of Zhejiang University-Science B 2018; 19(2): 85-119.
88. Sureshkumar J, Silambarasan R, Bharati KA, Krupa J, Amalraj S & Ayyanar M: A review on ethnomedicinally important pteridophytes of India. Journal of Ethnopharmacology 2018; 219: 269-287.

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