PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol x(x): xx–xx
https://doi.org/10.14719/pst.2349

HORIZON
e-Publishing Group
HePG

RESEARCH ARTICLE

Phytochemical screening and Gas chromatography-mass spectrometry analysis on *Ischaemum pilosum* (Kleinex Willd.)

Amanulla Khan[1*], K. C. More[2], M. H. Mali[3], Satish V. Deore[4] & M. B. Patil[4]

[1]Department of Botany, Islam Janjira Degree College of Science Murud-Janjira, 402401, India
[2]Department of Botany, Sant Gadge Baba Amravati University, Amravati, 444602, India
[3]Department of Botany, Centre for Post Graduate Studies and Research in Botany, C.H.C Arts, S.G.P Commerce and B.B.J.P Science CollegeTaloda-425413, India
[4]Department of Botany, J E S's, Arts, Science and Commerce College, Nandurbar-425412, India

***Email:** dramanullak@gmail.com

OPEN ACCESS

ARTICLE HISTORY
Received: 09 January 2023
Accepted: 16 April 2023
Available online
Version 1.0 : 10 September 2023

Check for updates

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS,UGC Care etc. See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

CITE THIS ARTICLE
Khan A, More K C, Mali M H, Satish V D, Patil M B. Phytochemical screening and Gas chromatography-mass spectrometry analysis on *Ischaemum pilosum* (Kleinex Willd.). Plant Science Today (Early Access). https://doi.org/10.14719/pst.2349

Abstract

Ischaemum pilosum (Kleinex Willd.) a weed among the grass is reported for ethno-medicinal practices for treatment of various treatments for human and domestic animals. The current work deals with phytochemical analysis in different parts of plants to find out bioactive compounds. The first-time reported results on *I. pilosum* reveal the significant phytochemicals by using preliminary phytochemical analysis, UV Visible spectral technique, FTIR analysis and GC-MS analysis. The preliminary phytochemical test confirms the presence of alkaloids, anthraquinone, cardiac glycosides, coumarins, flavonoids, glycosides, phenols, reducing sugars, saponins, steroids, tannin and triterpenes in *Ischaemum pilosum.* UV Visible spectra and FTIR gives the ranges of absorptions and functional group like Carboxylic acids (O-H) at 2956,92 cm^{-1}, Alkanes (O-H) at 2849,89 cm^{-1}, Aldehydes (C=O) at 1735,92 cm^{-1}, Aromatic Rings (C=C) at 1462,95 cm^{-1}, Alkanes (C-H) at 1377,97 cm^{-1}, Esters (C-O) 1166,95 $cm^{-1}$and Phenyl Ring (C-H) 758,97 cm^{-1}. The GC-MS analysis related twenty-one compounds like Phenol, 4-bis (1,1-dimethylethyl), Pentanoic acid, 5-hydroxy, 2,4-di-t-butylphenyl esters, E-15-Heptadecenal, 1-Hexadecanol, n-Hexadecanoic acid, l (+)-Ascorbic acid 2,6-dihexadecanoate, Palmitic anhydride, Cycloeicosane, Cis-13-Octadecenoic acid and Triacontane from *Ischaemum pilosum* leaves extract.

Keywords

Ischaemum pilosum, phytochemical, Fourier transform infrared spectrophotometer (FTIR) and gas chromatography: mass spectroscopy (GCMS)

Introduction

The medicinal value of plants lies in their specific chemical substances that produce a definite physiological action on the human and animal body (1–3). In many parts of the world plant-based medicine are used for human and animal illnesses from thousands of years due to its bioactive compounds (4–6). Plants used in ethno-medicinal practices are believed to have potential sources for effective and safe treatment due to their constituents (7). The most important bioactive constituents among these medicinal plants are alkaloids, flavonoids, tannins, and phenolic compounds. Due to great medicinal potential plants are used in various cultures and traditions and now-a-days in modern pharmacopoeial drugs (8–10). A large number of phytochemical investigations and therapeutics compound isolation are in practice (11–13). Plant-based different purified constituents and crude drugs

have been reported for their valuable therapeutic potential (14). To develop and promote the use of medicinal plants as potential sources for different modes of disease it is important to investigate phytochemicals and validate their bioactivities(14–16).

Ischaemum pilosum belongs to the family Poaceae, locally named as *Kunda, Kons*and *Khavon*. It is perennial creeping grass with stolons that covers shoots at the base with dry striate leaf sheaths (Fig. 1). *I. pilosum* is found commonly as a weed in many states of India (17,18). Plant is a source of good lignocellulosic material for the preparation of ethanol (19,20). A decoction of root powder is given against urine stone for urine disorder. A teaspoon of root powder is used to increase the flow of milk in cattle (19).

Fig.1. *Ischaemumpilosum*(Klein.exWilld.) Wt.

The determination of phytochemical compounds is largely practiced by developing spectroscopic and chromatographic techniques in medicinal plants (8,21,22). GC-MS analysis can act as a valuable tool for identifying pure compounds present in plant extracts (23,23–27). Similarly spectroscopic (UV-Vis, FTIR) methods together or separately can be used in this chemical analysis sense andalso act as supportive methods for phytoconstituents finding (28–31).This study evaluates the phytochemical in *Ischaemum pilosum* and help to serve as a basis for the development of novel plant based drugs from weed plants. The work is alsoafirst-time report to validate the medicinal importance of plants by chemical extractions with phytochemical techniques. (17–19).

Materials and Methods

Plant collection and identification

Ischaemum pilosum (Klein.exWilld.) Wt. plants were collected from forests of Toranmal of Shahda Tahsil (21.840213° N, 74.456583° E) of Nandurbar district. The collected plant are taxonomically identified and the herbarium (Voucher number: ANP-2204) is stored in the Department of Botany, Jijamata College Nandurbar MS. Ethnomedicinal importance has been validated from existing available literature about plant use. (32–34).

Preliminary phytochemical screening

Proximate analysis of leaves, stem and leaves powdered of *Ischaemum pilosum* are taken with the estimation of moisture content, dry matter content and ash content as determined using the AOAC method (35–38). Leaves, stem and root parts'soxhlet extract in six different solvents like aqueous (Aq), methanol (M), ethanol (E), pet-ether (Et), chloroform (C) and acetone (A) were used to perform preliminary phytochemicals test to identify phytochemical constituents (Table 01.) (39,40).

Spectral Analysis

UV Visible spectral analyses were carried out for leaves, stem and root soxhlet extract in 60% concentration ethanol and chloroform solvents with 200 nm to 11000 nm range (28,41–43). Fourier Transform Infrared Spectrophotometer (FTIR) is perhaps the most powerful tool for identifying the types of chemical bonds (functional groups) present in compounds. The wavelength of light absorbed is characteristic of the chemical bond as can be seen in the annotated spectrum. By interpreting the infrared absorption spectrum, acetone leaves samples of *Ischaemum pilosum* extracts were selected. 10 mg of the dried extract powder was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample discs. The powdered sample of the plant was loaded in an FTIR spectroscope (Shimadzu, IR Affinity 1, Japan), with a scan range from 400 to 4000 cm^{1} and a resolution of 4 cm^{1}. The intense bands of FTIR results were compared with standard values to recognize the functional groups of chemical compounds present in the leaves sample. (40,43–47).

Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS is one of the hyphenated analytical techniques used for *Ischaemum pilosum* acetone leaves extract by thesoxhlet extraction method. GC-MS study was conducted as per the Crop Processing Research Institute, Tanjore. The Clarus 500 GC used in the analysis used a fused silica column loaded with Elite-1 (100% dimethyl siloxane poly, 30 nm x 0.25 nm DI x 1 µmdf) and the components were separated using helium as a carrier gas at a constant rate of 1 mL/min. The Turbo Gold mass detectors (Perkin Elmer) were used to detect the 2 µl leaves acetone extract injected into the instrument using the Turbo mass 5.1 software. The injector temperature was set at 250 °C (mass analyzer). The parameters involved in the operation of the Clarus 500 MS have been standardized (input line temperature: 200 °C, source temperature: 200° C). Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments of 45 to 450 Da and MS detection was completed in 36 min (23,48).

Identification of compounds

GC-MS interpretation was performed using the NIST database (Multi format version of NIST (NIST20MF)) with more than 62,000 models. The spectrum of the unknown

Table 1. Preliminary phytochemical test

Sr. No.	*Name of the test*	*Procedure*	*Observation*	References
1	**Alkaloids**	0.5ml extract + treated with few drops of 1ml 2N HCl +Mayer's reagent / Dragandorf reagent Hager's reagent	Orange precipitate Orange color White ppt. Yellow ppt.	(42)
2	**Anthraquinone**	Few drops of extract was boiled with 10% HCl for few minutes and cool + CHCl3 (Chloroform) to filtrate and few drops of NH_3 added and heated	Rose pink color	(69)
3	**Cardiac glycosides**	0.5ml extract + 1ml water + aqueous solution NaoH some drops for color	Brown interface, violet ring below and greenish ring at lowest part	(42,70)
4	**Coumarins**	2 ml of extract, 3 ml of 10% NaOH	Appearance of yellow colour indicates presence of coumarins.	(71)
5	**Flavonoids**	0.5ml extract + 5-10 drops of dilute HCl + small amount / pieces + then boiled for few min. Shinodaw's Test	Red color Magenta color	(51,69,71)
6	**Glycosides**	Zn-HCl acid reduction Test Anthrone + H_2SO_4+ Heat	Purple or green	(69,71)
7	**Phenols**	$FeCl_3$ Sample + lead acetate + water	Intense color Formation of white ppt	(69–71)
8	**Reducing sugars**	0.5 ml extract was dissolved in 5ml of water and filter it + boiled with Fehling's solution A and B for few min.	Orange red precipitate positively detects reducing sugars.	(69)
9	**Saponins**	Sample + water + shaking	Formation of honey comb like froth Presence of froths/foams	(39,71)
10	**Steroids**	Salkowski's test and Liebermann burchard's test	Dark green color in the upper layer and red color in the lower layer indicating the presence of steroids	(40,42,69)
11	**Tannin**	0.5 ml of aqueous extract + 10% lead acetate few drops	Greenish-black colouration	(16,42,69)
12	**Triterpenes**	Liebermann Test Salkowski Test Noller's test	Bluish green Red and fluorescent Pink color	(9,70,70)

component was compared with the spectrum of known components stored in the NIST library. Prediction of the compound from the GCMS library was done with the help of Dr. Duke's phytochemical and ethnobotanical database by Dr. Jim Duke, Agricultural Research Service / USDA. The name, molecular weight and structure of the compounds were determined for acetone leaves extract (40,49–52).

Table 02. Proximate analysis test

Plant Parts Used	Moisture content	Dry matter Content	Ash Content
Leaves (w/w)	51.00 %	49.00 %	12.00%
Stem Parts (w/w)	54.00 %	46.50%	14.00%
Root (w/w)	51.50 %	46.50%	15.50%
Total	52.16%	47.33%	30.33%

Result

Preliminary phytochemicals

Proximate Analysis for moistures content, dry matter content and ash content is performed for *Ischaemum pilosum* leaves, stem and root parts as shows overall 52.16 % of moisture content, 47.33 % dry matter content and 30.33 % ash content in the plant (Table 02).Preliminary phytochemical test for the leaf, stem and root parts extract of *I. pilosum* for alkaloids, anthraquinone, cardiac glycosides, coumarins, flavonoids, glycosides, phenols, reducing sugars, saponins, steroids, tannin and triterpenes were found in different solvents (Table 02).

Spectral analysis outcome

UV-VIS spectrophotometer results show peaks at 450 nm and 675 nm in leaves, 450 nm and 670 nm in stem bark and 650 nm in root sample of ethanolic extracts (Fig. 2). Similarly chloroform extracts result gives a peak of absorptions at 430 nm and 670 nm in leaves, 650 nm in stem bark and 670 nm in root sample for same analysis (Fig. 3). FTIR results shows characteristic absorption bands for Carboxylic acids (O-H) at 2956,92 cm^{-1}, Alkanes (O-H) at 2849,89 cm^{-1}, Aldehydes (C=O) at 1735,92 cm^{-1}, Aromatic Rings (C=C) at 1462,95 cm^{-1}, Alkanes (C-H) at 1377,97 cm^{-1}, Esters (C-O) 1166,95 cm^{-1} and Phenyl Ring (C-H) 758,97 cm^{-1} peak value these compounds are found between 4000 to 650 cm^{-1} in spectral search (Table 04 and Fig. 4).

GC-MS profiling of *Ischaemum pilosum* leaves acetone extract

GC-MS test for leaves acetone extract were carried out for chemical compounds present in samples. Twenty-one compounds were listed from sample of *Ischaemum pilosum* leaves and identified by GC-MS analysis (Fig. 5). The listed, identified compounds are like Phenol, 4-bis (1,1 -dimethylethyl); Phenol, 2,6-bis (1,1-dimethylethyl);

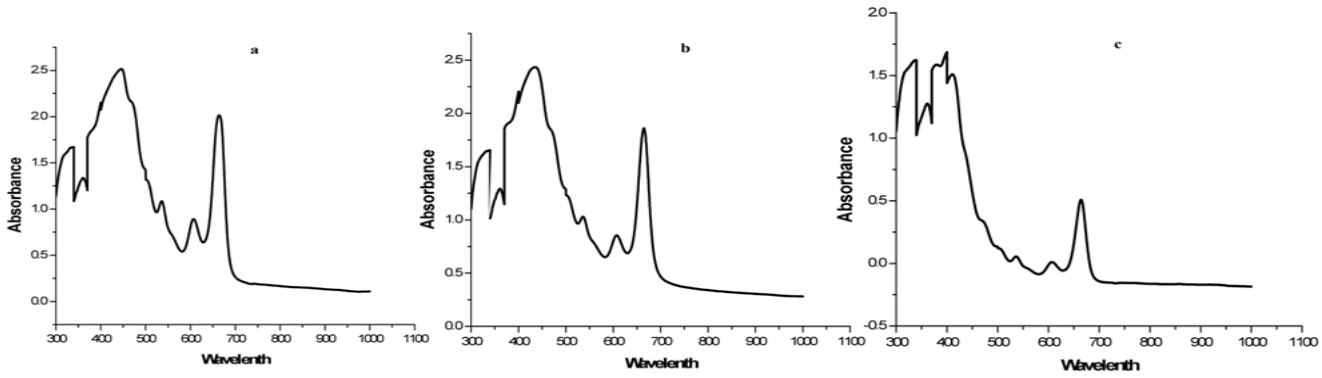


Fig. 2. UV-VIS spectral Analysis of in ethanol extract (a. leaves b. stem and c. root).

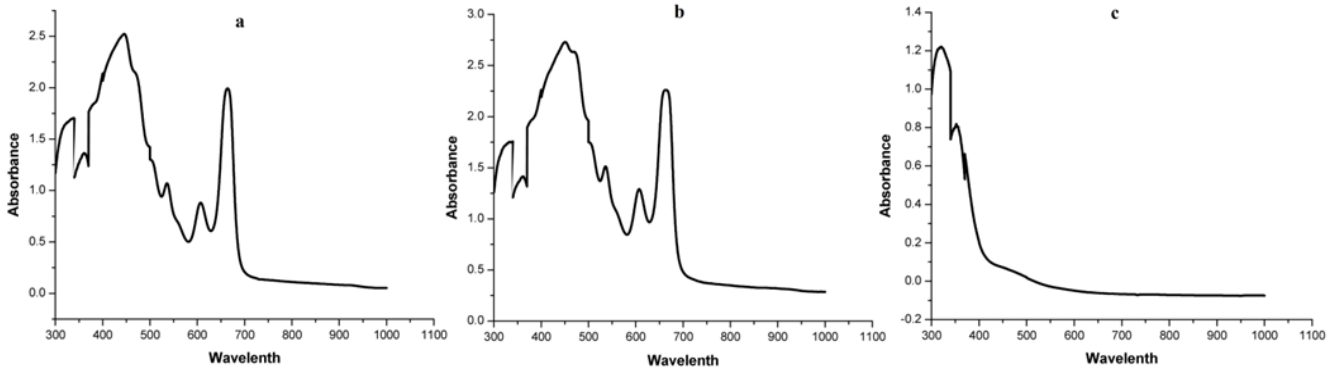


Fig. 3.UV-VIS spectral Analysis of in chloroform extract (a. leaves b. stem and c. root).

Table 03. Preliminary phytochemical test

Sr. No.	Phyto- constituents	*Ischaemum pilosum*																	
		Leaf						Stem						Root					
	Solvent System	Aq	M	E	Et	C	A	Aq	M	E	Et	C	A	Aq	M	E	Et	C	A
1	Alkaloids	-	+	+	-	-	+	-	+	+	-	-	+	+	+	+	-	-	-
2	Anthraquinone	-	+	-	-	-	-	-	+	-	-	-	-	+	+	+	-	-	-
3	Cardiac glycosides	+	+	+	-	+	-	+	+	+	-	-	-	+	+	+	-	-	-
4	Coumarins	-	-	+	-	-	+	-	-	+	-	-	+	-	-	-	+	-	-
5	Flavonoids	-	+	+	-	-	-	-	+	+	-	-	-	-	+	+	+	-	+
6	Glycosides	-	+	-	+	+	-	-	+	-	+	+	-	+	+	+	-	-	+
7	Phenols	-	+	+	-	-	+	-	+	+	-	-	+	-	+	+	+	-	-
8	Reducing sugars	+	+	-	+	-	-	+	+	-	+	-	-	-	+	+	-	-	-
9	Saponins	-	-	+	-	-	-	-	-	+	-	-	-	-	+	+	-	-	+
10	Steroids	-	+	+	-	-	+	-	+	+	-	-	+	-	-	-	-	+	-
11	Tannin	-	+	-	-	-	-	-	+	-	-	-	-	+	+	+	-	-	-
12	Triterpenes	-	+	-	-	-	-	-	+	-	-	+	-	-	+	-	+	+	-

Aq = Aqueous, M = Methanol, E = Ethanol, Et = Pet-ether, C = Chloroform, A = Acetone

Pentanoic acid, 5-hydroxy, 2,4-di-t-butylphenyl esters E-15; Heptadecenal; Hexadecen-1-ol,trans-9; 1-Hexadecanol; n-Hexadecanoic acid; l (+)-Ascorbic acid 2,6-dihexadecanoate; Palmitic anhydride; 1-Heneicosyl formate; 9-Nonadecene; Cycloeicosane; Cis-13-Octadecenoic acid; Cis-Vaccenic acid; Trans-13-Octadecenoic acid; Tetratriacontane; Squalane; Eicosane; Tetratriacontane; Squalane and Tetracosane (Table 5).

Table 04. FTIR spectral peak values and functional groups

Extracts prepared in	Peak values	IR Spectrum Frequency range (cm^{-1})	Functional groups	Compound Type
Acetone Extract	2956,92	2800-3200 cm^{-1}	O-H	Carboxylic acids
	2918,83	2800-3200 cm^{-1}	O-H	Carboxylic acids
	2849,89	At 2800 cm^{-1}	C-H	Alkanes
	1735,92	1600-1800 cm^{-1}	C=O	Aldehydes, Ketones,
	1462,95	1400-1600 cm^{-1}	C=C	Aromatic Rings
	1377,97	1200-1400 cm^{-1}	C-H	Alkanes
	1166,95	1000-1200 cm^{-1}	C-O	Carboxylic acids, Esters
	758,97	650-800 cm^{-1}	C-H	Phenyl Ring

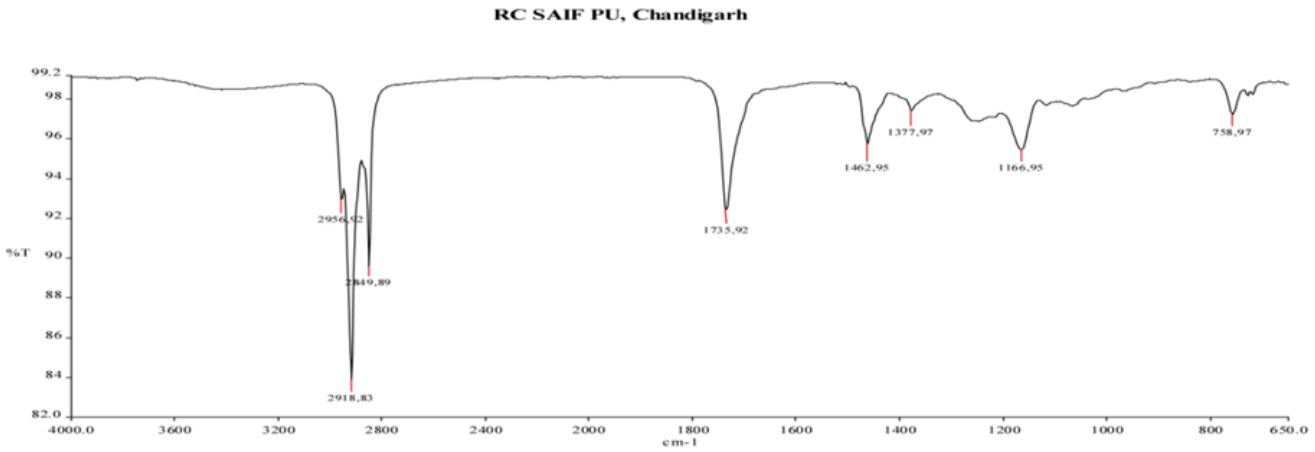


Fig. 4. FTIR results for *I. pilosum* leaves extract.

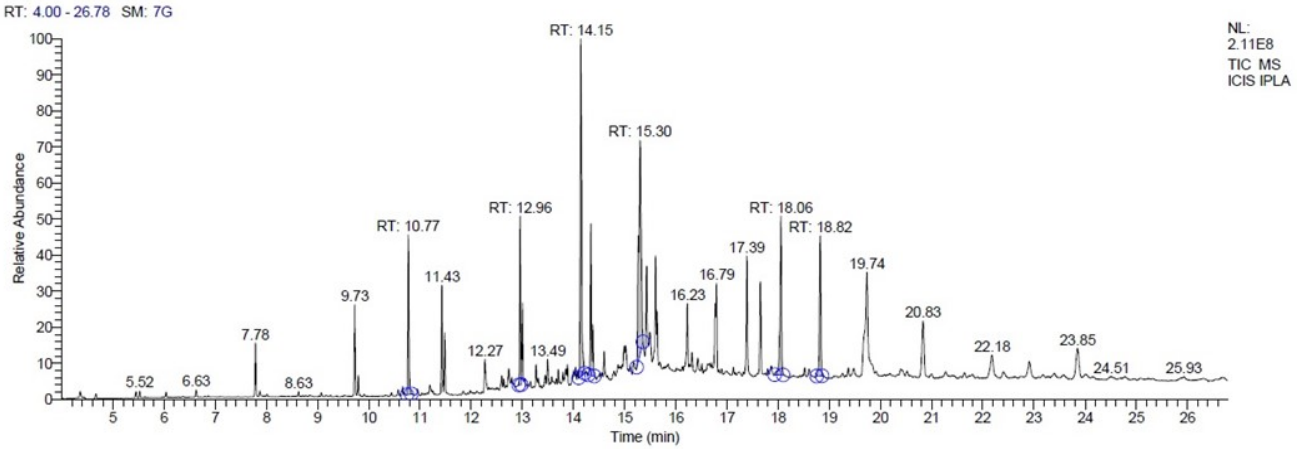
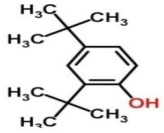
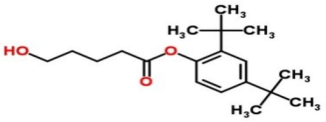
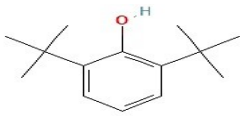
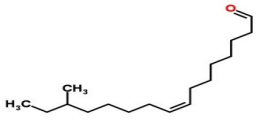
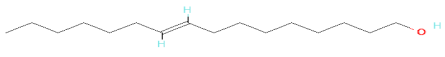
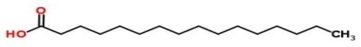
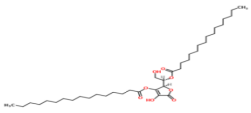
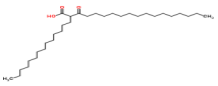
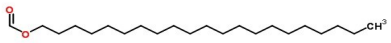
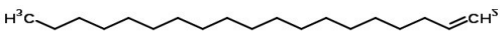
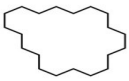
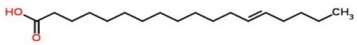
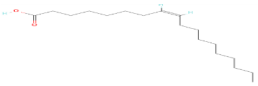
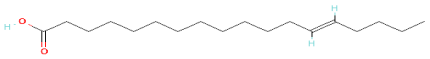
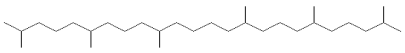
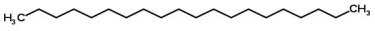
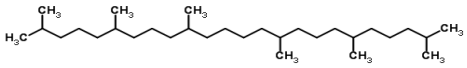
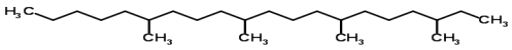


Fig. 5. GC MS analysis of *Ischaemum pilosum* (Klein.exWilld.) Wt(Leaves).

Table 05. Phytochemical Components found in acetone leaves extract of *Ischaemum pilosum by* GC-MS results

Sr No.	RT	Compound Name	Molecular Formula	MW (g/mol)	Structure of Compound
1	10.77	Phenol, ,4-bis(1,1-dimethylethyl)	$C_{14}H_{22}O$	206.33	
2	10.77	Pentanoic acid, 5-hydroxy, 2,4-di-t-butylphenyl esters E-15-	$C_{19}H_{30}O_3$	306.44	
3	10.77	Phenol, 2,6-bis(1,1-dimethylethyl)	$C_{14}H_{22}O$	206.33	
4	12.96	Heptadecenal	$C_{17}H_{32}O$	252.44	
5	12.96	Hexadecen-1-ol,trans-9	$C_{16}H_{32}O$	240.43	
6	12.96	1-Hexadecanol	$C_{16}H_{34}O$	242.44	
7	14.15	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	

Sr No.	RT	Compound Name	Molecular Formula	MW (g/mol)	Structure of Compound
8	14.15	l(+)-Ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	652.95	
9	14.15	Palmitic anhydride	$C_{32}H_{62}O_3$	494.84	
10	14.34	1-Heneicosyl formate	$C_{22}H_{44}O_2$	340.59	
11	14.34	9-Nonadecene	$C_{19}H_{38}$	266.51	
12	14.34	Cycloeicosane	$C_{20}H_{40}$	280.54	
13	15.30	Cis-13-Octadecenoic acid	$C_{18}H_{34}O_2$	282.46	
14	15.30	Cis -Vaccenic acid	$C_{18}H_{34}O_2$	282.46	
15	15.30	Trans-13-Octadecenoic acid	$C_{18}H_{34}O_2$	282.46	
16	18.06	Tetratriacontane	$C_{34}H_{70}$	478.93	
17	18.06	Squalane	$C_{30}H_{62}$	422.82	
18	18.06	Eicosane	$C_{20}H_{42}$	282.54	
19	18.82	Tetratriacontane	$C_{34}H_{70}$	478.93	
20	18.82	Squalane	$C_{30}H_{62}$	422.82	
21	18.82	Tetracosane	$C_{24}H_{50}$	338.66	

Discussion

Preliminary phytochemical screening of *Ischaemum pilosum* extracts reveals the presence of flavonoids that have been reported for antioxidant activities (10). The presence of flavonoids is a highly effective scavenger in various free radicals implicated in many diseases (53,54). The presence of alkaloid and terpenoids are reported for gastric ulcers (55). Terpenoidsare also important in cardiovascular muscle relaxation (56). Steroid's presence in leaves sample helps to relieve swelling and anti-inflammation properties (57,58). The presence of tannins have been reported as healing agents for inflammation and burn (7). Other phytochemicals present like anthraquinone, cardiac glycosides, coumarins, glycosides, phenols, reducing sugars and saponins are also reported in preliminary phytochemical reports are having various biological activities (7,59). Moreover, UV spectral and FTIR studies give absorbance peaks that reveals the functional group alkanes, aldehydes, aromatic rings and phenyl ring helps to confirm the structure (43,60).

The GC-MS analysis of the plant extract resulted in the identification of twenty-one phytochemicals. Among these compounds, several exhibited interesting biological activities. Phenol, 4-bis (1,1-dimethylethyl) compound was found to have antimicrobial, antifungal, and antitumor activity, as reported in previous studies (61–63). Eicosane, another compound identified, demonstrated antibacterial, antifungal, and cytotoxic activity (62,64). Furthermore, n-Hexadecanoic acid compound exhibited antibacterial inhibition activity, while 2,6-bis (1,1-dimethylethyl) phenol was reported to possess anti-inflammatory, antifungal, anti-malarial, and antioxidant activities (65,66). Additionally, heptadecane compound was found to be associated with the production of essential oil(66,67).

The identification and comparison of the GC-MS analyzed compounds were performed using established databases such as NIST and Dr. Duke's Phytochemical and Ethno-botanical databases (68). This allowed for accurate matching of the mass spectra and retention times of the compounds, aiding in their identification. The results of

this study highlight the potential bioactivity of the identified phytochemicals of *Ischaemum pilosum*. The antimicrobial, antifungal, antitumor, antibacterial, anti-inflammatory, antimalarial, antioxidant, and cytotoxic activities associated with these compounds indicate their potential as valuable therapeutic agents of plant. Further studies are warranted to explore their mechanisms of action and potential applications in the field of medicine.

Conclusion

This study first time reported Phytoconstituents from *Ischaemum pilosum* with GC-MS reports. The conformation tests of phytochemicals for alkaloids, phenolic etc helps to support the ethno-medical importance of the plant reported in the literature. Further detailed subsequent phytochemical analysis up to GC-MS analysis found 21 different phytoconstituents. The compounds like Phenol, 4 -bis (1, 1-dimethylethyl), Eicosane, n-Hexadecanoic acid, 2, 6-bis (1,1-dimethylethyl) phenol and heptadecane are having bioactive and therapeutic abilities as reported by various authors. In addition, many other compounds found in leaves' acetone extracts could have biological activities. We suggest the utilization of this weed for further pharmaceutical processing to make plant-based medicine from weed. This research contributes to the growing body of knowledge on plant-derived compounds and their potential applications in various fields, including medicine and drug discovery. Further investigations and clinical studies are needed to harness the full therapeutic potential of *Ischaemum pilosum.*

Acknowledgements

Authors would like to acknowledge their thanks for the support and guidance of Dr. S Gosavi Sir Taloda College, Mr. Javed, principal and staff of the Anjuman Islam Janjira Degree College of Science Murud.

Authors' contributions

AK, KC and MH designed concepts for the topic; where MH and SV provided the preliminary phytochemical analysis manuscript. AK, KC and MB did testing and analysis. AK and SV have supervised the work for its authentication. All authors carefully read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest : Authors do not have any conflict of interest to declare

Ethical issues : None

References

1. Garro, L.C., Intracultural variation in folk medical knowledge: A comparison between curers and noncurers. American Anthropologist. 1986;88(2):351–370. http://doi.wiley.com/10.1525/aa.1986.88.2.02a00040
2. Patil, M.B., Khan, P.A., Ethnobotanical, phytochemical and Fourier Transform Infrared Spectrophotometer (FTIR) studies of *Catunaregam spinosa* (Thunb.) Tirven. Journal of Chemical and Pharmaceutical Sciences. 2017;10(2)950–955. https://doi.org/10.5281/zenodo.7562415
3. Patil, M.B., Khan, P.A., Economical and ethical aspects in medicinal plant research. Research Review International Journal of Multidisciplinary. 2017;02(02):25–31.
4. Ani, NI, Okolo, K.O., Offiah, R.O., Evaluation of antibacterial, antioxidant, and anti-inflammatory properties of GC/MS characterized methanol leaf extract of *Terminalia superba* (Combretaceae, Engl. and Diels). Futur J. Pharm Sci. 2023;9 (1):3. https://doi.org/10.1186/s43094-022-00455-z
5. Dogara, A.M., A systematic review on the biological evaluation of *Calotropis procera* (Aiton) Dryand. Futur. J. Pharm Sci. 2023;9(1):16. https://doi.org/10.1186/s43094-023-00467-3
6. Zhang, S., Zhang, L., Zou, H., Qiu, L., Zheng, Y., Yang, D.,Effects of light on secondary metabolite biosynthesis in medicinal plants. Front Plant Sci. 2021;12:781236. https://doi.org/10.3389/fpls.2021.781236
7. Agidew, M.G., Phytochemical analysis of some selected traditional medicinal plants in Ethiopia. Bull Natl. Res. Cent. 2022; 46(1):87.https://doi.org/10.1186/s42269-022-00770-8
8. Eisenhauer, N., Klier, M., Partsch, S., Sabais, A.C.W., Scherber, C., Weisser, W.W., No interactive effects of pesticides and plant diversity on soil microbial biomass and respiration. Applied Soil Ecology. 2009;42(1):31–36.https://doi.org/10.1016/j.apsoil.2009.01.005
9. Harneti, D., Supratman, U., Phytochemistry and biological activities of *Aglaia* species. Phytochemistry. 2021;181:112540. https://doi.org/10.1016/j.phytochem.2020.112540
10. Olivia, N.U., Goodness, U.C., Obinna, O.M., Phytochemical profiling and GC-MS analysis of aqueous methanol fraction of *Hibiscus asper* leaves. Futur. J. Pharm Sci. 2021;7(1):59. https://doi.org/10.1186/s43094-021-00208-4
11. Khairnar, A.S., Gomase, P.V., Khan, T.A., Khan, P.A., Patil, M.B.,*Begonia picta* Sm.: A new record to Nandurbar and Dhule district flora of Maharashtra (India). Bionature. 2018; 38 333-336. https://doi.org/10.5281/zenodo.7562528
12. Khalaf N.A, Shakya A.K, Al-Othman A, El-Agbar Z, Farah H.,Antioxidant activity of some common plants. Turkish Journal of Biology. 2008; 32:18 51-55. Available at: https://journals.tubitak.gov.tr/biology/vol32/iss1/8
13. Prodhan, A.H.M.S.U, Mridu, F.S.,*Baccaurea motleyana* (Rambai): nutritional, phytochemical, and medicinal overview. Adv. Tradit. Med. (ADTM). 2023;23(1):11–35. https://doi.org/10.1007/s13596-021-00555-w
14. Chanda, S., Nair, R., Activity of some medicinal plants against certain pathogenic bacterial strains. Indian J. Pharmacol. 2006;38(2):142.https://doi.org/10.4103/0253-7613.24625
15. Ezeanyikaa, L.U., Anosikea, C.A., A.C.N.O, Chibuogwu, C.C., Phytochemistry, micronutrient composition, and antioxidant potentials of *Citrus maxima* (Shaddock) fruit juice. J. Pharmacogn Phytochem. 2022;11(5):20–3.https://doi.org/10.22271/phyto.2022.v11.i5a.14498
16. Momin, M.A.M., Bellah, S.F., Rahman, S.M.R., Rahman, A.A., Murshid, G.M.M., Emran, T.B., Phytopharmacological evaluation of ethanol extract of *Sida cordifolia* L. roots. Asian Pacific Journal of Tropical Biomedicine. 2014;4(1):18–24. https://doi.org/10.1016/S2221-1691(14)60202-1
17. Sharma, R. M., Check list of indian grass gall midges (diptera: cecidoymyiidae) with their host grass (poaceae) index. Zoo's print journal. 2000;15(7):297–299. https://doi.org/10.11609/JoTT.ZPJ.15.7.297-9
18. Sudip Ray, Jeetendra Sainkhediya. Threatened weeds of Bt cotton field in Nimar region of Madhya Pradesh. Asian Journal of Bio Science. 2014;9(1):84–87.
19. Rothe, S.P., Sapkal, S.S., Maheshwari, A.A., Traditional

ethnomedicinal investigation from Pohradevi forest of Washim district. IJARIIE. 2017;3(2) 4953–4956.

20. Than, S.S., Optimization of acid hydrolysis of grasses using response surface methodology for the preparation of bioethanol. Chemical Engineering Transactions. 2017;56:1615–1620. https://doi.org/10.3303/CET1756270

21. Patil, M.B., Khan, P.A., Review: techniques towards the plant phytochemical study. International Journal of Science Info. 2016;1(3):157–172. https://doi.org/10.5281/zenodo.7559052

22. Üzer, A., Erçağ, E., Apak, R., Selective spectrophotometric determination of TNT in soil and water with dicyclohexylamine extraction. Analytica Chimica Acta. 2005;534(2):307–317. https://doi.org/10.1016/j.aca.2004.11.045

23. Fiehn, O., Kopka, J., Dörmann, P., Altmann, T., Trethewey, R.N., Willmitzer, L., Metabolite profiling for plant functional genomics. Nat Biotechnol. 2000;18(11):1157–1161. https://doi.org/10.1038/81137

24. Górnaś, P., Baškirovs G., Siger, A., Free and esterified tocopherols, tocotrienols and other extractable and non-extractable tocochromanol-related molecules: compendium of knowledge, future perspectives and recommendations for chromatographic techniques, tools, and approaches used for tocochromanol determination. Molecules. 2022;27(19):6560. https://doi.org/10.3390/molecules27196560

25. Habib, H., Finno, C.J., Gennity, I., Favro, G., Hales, E., Puschner, B., Simultaneous quantification of vitamin E and vitamin E metabolites in equine plasma and serum using LC-MS/MS. J VET Diagn Invest. 2021;33(3):506–15. https://doi.org/10.1177/10406387211005433

26. Liebler, D.C., Burr, J.A., Philips, L., Ham, A.J.L., Gas chromatography–mass spectrometry analysis of vitamin e and its oxidation products. Analytical Biochemistry. 1996;236 (1):27–34. https://doi.org/10.1006/abio.1996.0127

27. Mottier, P., Gremaud, E., Guy, P.A., Turesky, R.J., Comparison of Gas chromatography–mass spectrometry and liquid chromatography–tandem mass spectrometry methods to quantify α-tocopherol and α-tocopherolquinone levels in human plasma. Analytical Biochemistry. 2002;301(1):128–135. https://doi.org/10.1006/abio.2001.5486

28. Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D., Lightfoot, D., Phytochemicals: extraction, isolation, and identification of bioactive compounds from plant extracts. Plants. 2017;6(4):42.https://doi.org/10.3390/plants6040042

29. Aysal, P., Ambrus, Á., Lehotay, S.J,, Cannavan, A., Validation of an efficient method for the determination of pesticide residues in fruits and vegetables using ethyl acetate for extraction. Journal of Environmental Science and Health. 2007;42(5):481–490. https://doi.org/10.1080/19312450701392490

30. Guemari, F., Laouini, S.E., Rebiai, A., Bouafia, A., Meneceur, S., Tliba, A., UV-visible spectroscopic technique-data mining tool as a reliable, fast, and cost-effective method for the prediction of total polyphenol contents: validation in a bunch of medicinal plant extracts. Applied Sciences. 2022;12 (19):9430. https://doi.org/10.3390/app12199430

31. Ibrahim, M., Aal, M.A.E., Spectroscopic study of the interaction of heavy metals with organic acids. IJEP. 2008;35 (1):99. https://doi.org/10.1504/IJEP.2008.021134

32. Cooke, Theodore., The Flora of the Presidency of Bombay. London: Taylor and Francis; 1908.

33. Patil, D. A. Flora of Dhule and Nandurbar District. M/S Bishen Singh Mahendra Pal Singh; 2003.

34. The Flora of the Presidency of Bombay. Nature. 1904;71 (1832):124–124. https://doi.org/10.1038/071124b0

35. AOAC (Official methods of analysis of the Association of Official Analytical Chemists). Washington, DC. 1990;2(15).

36. Hussain, J., Ullah, R., Khan, U., Hussain, S.T., Anwar, S., Endogenous transitional metal and proximate analysis of selected medicinal plants from Pakistan. Journal of Medicinal Plants Research. 2010;4(3):267–270.

37. Naqbi, K.M.A.A., Karthishwaran, K., Kurup, S.S., Abdul, Muhsen, Alyafei, M., Jaleel, A. Phytochemicals, proximate composition, mineral analysis and *in vitro* antioxidant activity of *Calligonum crinitum* boiss. Horticulturae. 2022;8 (2):156. https://www.mdpi.com/2311-7524/8/2/156

38. Shukla, A., Vats, S., Shukla, R. Phytochemical screening, proximate analysis and antioxidant activity of *Dracaena reflexa*Lam. leaves. Indian J Pharm Sci. 2015;77(5):640. https://doi.org/10.4103/0250-474X.169035

39. Ayoola, G., Coker, H., Adesegun, S., Adepoju-Bello, A., Obaweya, K., Ezennia, E., Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. Trop. J. Pharm Res. 2008;7(3):1019–1024. https://doi.org/10.4314/tjpr.v7i3.14686

40. Mohammed, J., Oba, O.A., Pasaoglulari̇, AydiNliK, N., Preliminary phytochemical screening, GC-MS, FTIR Analysis of ethanolic extracts of *Rosmarinus officinalis*, *Coriandrum sativum* L and *Mentha spicata*. Hacettepe J. Biol. and Chem., 2023, 51 (1), 93-102. https://doi.org/10.15671/hjbc.1073300

41. Moonmun,D. Majumder, R.,and Lopamudra, A., Quantitative phytochemical estimation and evaluation of antioxidant and antibacterial activity of methanol and ethanol extracts of *Heliconia rostrata*. Indian Journal of Pharmaceutical Sciences. 2017, 79(1): 79–90. https://doi.org/10.4172/pharmaceutical-sciences.1000204

42. Sandosh, T.A. Peter, M.P.J., Raj J.Y., Phytochemical analysis of *Stylosanthes fruticosa* using UV-VIS, FTIR and GC-MS. Research Journal of Chemical Sciences. 2013;3(11):14–23.

43. Umekar, M.J., Lohiya, R.T., Gupta, K.R., Kotagale, N.R., Raut, N.S., Studies on meropenem and cefixime metal ion complexes for antibacterial activity. Future Journal of Pharmaceutical Sciences. 2021;7(1):233. https://doi.org/10.1186/s43094-021-00379-0

44. Durães, N., Bobos, I., Ferreira, da Silva, E., Chemistry and FT-IR spectroscopic studies of plants from contaminated mining sites in the Iberian Pyrite Belt, Portugal. Mineral Mag. 2008;72 (1):405–409. https://doi.org/10.1180/minmag.2008.072.1.405

45. 45. Martin, J.A., Solla, A., Woodward, S., Gil, L., Fourier transform-infrared spectroscopy as a new method for evaluating host resistance in the Dutch elm disease complex. Tree Physiology. 2005 ;25(10):1331–1338. https://doi.org/10.1093/treephys/25.10.1331

46. Vejendla, A., Talari, S., Moturu, R., Boddapati, S.N.M., Kola, A.E., Method development and validation for Cabotegravir and Rilpivirine by using HPLC and its degradants are characterized by LCMS and FTIR. Futur J Pharm Sci. 2021;7 (1):226. https://doi.org/10.1186/s43094-021-00355-8

47. Yang, J., Yen, H.E., Early salt stress effects on the changes in chemical composition in leaves of ice plant and *Arabidopsis*. A Fourier Transform Infrared Spectroscopy study. Plant Physiology. 2002;130(2):1032–1042. https://doi.org/10.1104/pp.004325

48. Wu, D., Zhou, Y., Cai, P., Shen, S., Pan, Y., Specific cooperative effect for the enantiomeric separation of amino acids using aqueous two-phase systems with task-specific ionic liquids. Journal of Chromatography A. 2015;1395:65–72. https://doi.org/10.1016/j.chroma.2015.03.047

49. Chirumamilla, P., Dharavath, S.B., Taduri, S., GC–MS profiling and antibacterial activity of *Solanum khasianum* leaf and

root extracts. Bull Natl Res Cent. 2022;46(1):127. https://doi.org/10.1186/s42269-022-00818-9

50. Kalaichelvi, K., Dhivya, S. M., GC-MS analysis of ethanolic extract of *Micrococca mercurialis* (L.) Benth. (euphorbiaceae). WJPPS. 2017;6(9):1684–1696. https://doi.org/10.20959/wjpps20179-10118

51. Krishnaiah, D., Sarbatly, R., Bono, A., Phytochemical antioxidants for health and medicine -A move towards nature. Biotechnology and Molecular Biology Review. 2007;1 (4):097–104.

52. Mohan, S.C., Dinakar, S., Anand, T., Elayaraja, R., Sathiya, Priya, B., Phytochemical, GC-MS analysis and antibacterial activity of a medicinal plant *Acalypha indica*. Int. J. Pharm. Tech. Res. 2012;4(3):1050–1054.

53. Cao, H., Chai, T.T., Wang, X., Morais-Braga, M.F.B., Yang, J.H. Wong, F.C., Phytochemicals from fern species: potential for medicine applications. Phytochem Rev. 2017;16(3):379–440. https://doi.org/10.1007/s11101-016-9488-7

54. Saeed, N., Khan, M.R., Shabbir, M., Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. BMC Complement Altern Med. 2012;12 (1):221.https://doi.org/10.1186/1472-6882-12-221

55. Sreeja, P.S., Arunachalam, K., Saikumar, S., Kasipandi, M., Dhivya, S., Murugan, R., Gastroprotective effect and mode of action of methanol extract of *Sphenodesme involucrata var. paniculata* (C.B. Clarke) Munir (Lamiaceae) leaves on experimental gastric ulcer models. Biomedicine and Pharmacotherapy. 2018;97:1109–1118. https://doi.org/10.1016/j.biopha.2017.11.030

56. Alves-Silva, M., J., Zuzarte, M., Marques, C., Salgueiro, L., Girao, H., Protective effects of terpenes on the cardiovascular system: current advances and future perspectives. CMC. 2016;23(40):4559–600. https://doi.org/10.2174/0929867323666160907123559

57. Hill, J.A., Suker, J.R., Sachs, K., Brigham, C., The athletic polydrug abuse phenomenon: A case report. Am. J. Sports Med. 1983;11(4):269–271. https://doi.org/10.1177/036354658301100417

58. Phillipson, J.D., Phytochemistry and pharmacognosy. Phytochemistry. 2007;68(22–24):2960–2972. https://doi.org/10.1016/j.phytochem.2007.06.028

59. Rizwan, K., Majeed, I., Bilal, M., Rasheed, T., Shakeel, A., Iqbal, S., Phytochemistry and diverse pharmacology of genus *Mimosa*: A review. Biomolecules. 2022;12(1):83. https://doi.org/10.3390/biom12010083

60. Galdes, A., Hill, H.A., Baldwin, G.S., Waley, S.G., Abraham, E.P., The 1H nuclear-magnetic-resonance spectroscopy of cobalt(II)-β-lactamase II. Biochemical Journal. 1980;187 (3):789–795. https://doi.org/10.1042/bj1870789

61. Ajayi, G.O., Olagunju, J.A., Ademuyiwa, O., Martins, O.C., Gas chromatography-mass spectrometry analysis and phytochemical screening of ethanolic root extract of *Plumbago zeylanica*, Linn. J. Med. Plant. Res. 2011;5(9):1756–1761.

62. Belakhdar, G., Benjouad, A., Abdennebi, E.H., Determination of some bioactive chemical constituents from *Thesium humile* Vahl.J. Mater. Environ. Sci. 2015; 6 (10): 2778–2783.

63. Valentina Ramirez, Sandra Sulay Arango, Maria Elena Maldonado, Diego Uribe, Johanny Aguillon, Jenny Paola Quintero., Biological activity of *Passiflora edulis f. Flavicarpa* ethanolic leaves extract on human colonic adenocarcinoma cells. J. App. Pharm. Sci. 2019;9(2):64–71. https://doi.org/10.1186/s43094-021-00355-8

64. Sabeeh, S.A., Bioactive compounds of streptomyces QUS7 isolate ethyl acetate extract from sediment in aldiwanyia river. World Bulletin of Public Health. 2022;12: 29–34.

65. Goda, M.S., Eltamany, E.E., Habib, E.S., Hassanean, H.A., Ahmed, S.A., Abdelhameed, F.A. Gas chromatography-mass spectrometry analysis of marine seagrass*Thalassodendron ciliatum*collected from Red Sea. Records of Pharmaceutical and Biomedical Sciences. 2020;4(2):1–15.https://doi.org/10.21608/rpbs.2020.27958.1058

66. Khaled, J.M., Alharbi, N.S., Mothana, R.A., Kadaikunnan, S., Alobaidi, A.S., Biochemical profile by GC–MS of fungal biomass produced from the ascospores of *Tirmania nivea* as a natural renewable resource. J. Fungi. 2021;7(12):1083. https://doi.org/10.3390/jof7121083

67. Jiao, J., Ma, D.H., Gai, Q.Y., Wang, W., Luo, M., Fu, Y.J., Rapid analysis of *Fructus forsythiae* essential oil by ionic liquids-assisted microwave distillation coupled with headspace single-drop microextraction followed by gas chromatography–mass spectrometry. Analytica Chimica Acta. 2013;804:143–150. https://doi.org/10.1016/j.aca.2013.10.035

68. Jim Duke., Dr. Duke's phytochemical and ethnobotanical databases. 1994. https://phytochem.nal.usda.gov/phytochem/search

69. Yusuf, A.Z., Zakir, A., Shemau, Z., Abdullahi, M., Halima, S.A., Phytochemical analysis of the methanol leaves extract of *Paullinia pinnata*Linn. J. Pharmacognosy Phytother. 2014;6 (2):10–6. https://doi.org/10.5897/JPP2013.0299

70. Geoffrey, K.K., John, K.M., Naomi, M., Simon, K.M., Qualitative phytochemical screening of *Camellia sinensis* and *Psidium guajava*leave extracts from kericho and Baringo Counties. IJBR. 2014;5(3):506–512.

71. Dharmendra Singh, Poonam Singh, Abhishek Gupta, Shikha Solanki, Ekta Sharma, Raeev Nema., Qualitative estimation of the presence of bioactive compound in *Centella asiatica*: An important medicinal plant. International Journal of Life Science and Medical Science. 2012;2(1). https://doi.org/10.5963/LSMR0201002