Research Article

Assessment of Phytochemical, FT-IR and GC-MS Fingerprint Profiling of Marine Angiosperms *Enhalus acoroides* (L.f) Royle and *Syringodium isoetifolium* (Asch) Dandy, Gulf of Mannar Biosphere Reserve, Tamil Nadu

Murugeswaran Santhiya Selvam[1], Pious Soris Tresina[2], Jeben Benjamin[3], Veerabahu Ramasamy Mohan[2,*]

[1]Department of Botany, V.O.Chidambaram College, Thoothukudi, (Affiliated to Manonmaniam Sundaranar University), Tirunelveli, Tamil Nadu, INDIA.
[2]Ethnopharmacology Unit, Research Department of Botany, V.O. Chidambaram College, Thoothukudi, Tamil Nadu, INDIA.
[3]Department of Zoology, Scott Christian College, Nagercoil, Tamil Nadu, INDIA.

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ABSTRACT

Objectives: Seagrasses, the marine angiosperm plants, grow in saline environments. Fully submerged in the sea, they are widely distributed along the Gulf of Mannar biosphere reserve regions of Tamil Nadu. The current study was carried out to evaluate the possible bioactive components, present in seagrasses *Enhalus acoroides* and *Syringodium isoetifolium*. **Methodology :** Petroleum ether, benzene, ethyl acetate, methanol, ethanol and aqueous extracts were subjected to qualitative test for the identification of phytoconstituents as per standard procedure. The functional groups of plant powder were identified using FTIR analysis. The ethanol extraction were analyzed via GC-MS techniques. **Results:** The presence of catechin, flavonoid, phenol, saponin, tannin and glycoside is revealed through the phytochemical screening of the diverse solvent extracts of *E. acoroides* and *S. isoetifolium*. The Fourier Transform-Infra Red (FT-IR) spectroscopy analysis confirmed the presence of hydroxyl, phenolics/alcohol, alkane, ester, ether, amine and others. The Gas Chromatography-Mass Spectrum (GC-MS) analysis showed the presence of each ten phytocompounds including n-Hexadecanoic acid, phytol, stigmasterol, hexadecanoic acid and methyl ester. **Conclusion:** The analyses proved the presence of important bioactive compounds with medicinal properties in the marine angiosperms found in the Gulf of Mannar biosphere reserve regions of Tamil Nadu. The therapeutic effects of seagrasses were the results of the presence of bioactive components in them.

Keywords: Marine angiosperm, Phytochemical, Flavonoids, FT-IR, GC-MS, Stigmasterol and Phytol.

Correspondence:
Dr. Veerabahu Ramasamy Mohan
Ethnopharmacology Unit, Research Department of Botany, V.O. Chidambaram College, Thoothukudi, Tamil Nadu, INDIA.

Email: vrmohanvoc@gmail.com

INTRODUCTION

The marine environment (>70% of the planet's surface) possesses exceptional biological and chemical characters that show a vital role in the discovery of many drug leads. In response to physical, chemical and biological modifies in the environment, seagrass generates bioactive compounds. Under stress conditions, seagrass acts as a defence mechanism due to the production of secondary metabolites.[1] Biomass of seagrass has been used often as food and medicine by coastal indigenous society.[2] It has been recognized as marine forms and it can be used against microbial attack.[3] In addition, it has been used as outdated medicine for the treatment of fever, muscle pain, skin diseases, stomach problems, wounds, etc., and also used as an,

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anti-oxidant, anti-inflammatory, anti-viral, anti-diabetic, anti-cancer medicine as well as a tranquilizer.[4] Some of the previous studies reported the isolation and identification of different phytochemicals.[5-6] To the best of our knowledge, these studies were concerned with the chemical investigation of *Enhalus acoroides* and *Syringodium isoetifoilum*. However, in order to discover the other natural compounds present in seagrasses, further studies are needed. In the current study, therefore, the different solvent extracts of *E. acoroides* and *S. isoetifolium* were made to qualitative and quantitative phytochemical analysis, while the ethanol extracts were subjected to the GC-MS analysis to explicate the phytochemical present behind its bioactivity.

METHODOLOGY

Collection of Seagrass

Seagrass samples (*Enhalus acoroides* (l.f) Royle and *Syringodium isoetifolium* (Asch) Dandy) were gathered from Mandapam, the Gulf of Mannar biosphere reserve of Tamil Nadu. The collected seagrasses were found, by referring to the taxonomical keys of Karo and Hartog.[7] A voucher specimen number was assigned (*Enhalus acoroides* EPH 375; *Syringodium isoetifolium* EPH 236). They were cleaned, dried, powdered separately and stored for further studies.

Preparation of Extracts

The coarse powder (100g) of *E. acoroides* and *S. isoetifolium* was extracted sequentially and separately with petroleum ether, methanol, benzene, ethyl acetate, ethanol and water, each 250mL in a soxhlet apparatus for 24 hr. Through whatman No. 41 filter paper, all the extracts were filtered. All the extracts were subjected to qualitative tests for the identification of various phyto-chemical constituents as per standard procedures.[8-10] The ethanol extracts were used for the estimation of total phenolics and flavonoids. In a rotary evaporator, the ethanol extracts were concentrated. The ethanol extracts thus attained were employed for GC-MS analysis.

Estimation of Total Phenolics

Using Folin-Ciocalteu reagent-based assay the total phenolic content was estimated as previously described[11] with little modification. To 5mL of Folin-Ciocalteu reagent (diluted ten-fold) and 4mL (75g/L) of Na_2CO_3 1mL of each extract (100µg/ml) were added. The mixture was allowed to stand at 20°C for 30min. The absorbance of the developed colour was recorded at 765nm using UV-VIS spectrophotometer. As standard for calibration curve, 1mL aliquots of 20, 40, 60, 80, 100 µg/mL methanolic gallic acid solutions were used. The absorbance of solution was contrasted with gallic acid calibration curve. The whole phenolic content was assumed as gallic acid equivalents (GAE g/100g dry weight of extract).

Estimation of Flavonoids

The total flavonoids content was estimated as per Eom *et al*.[12] with 0.1mL of 10% aluminium chloride and 0.1mL of potassium acetate (1M) an aliquot of 0.5mL of sample (1mg/mL) was mixed. In this mixture, to make 5mL volume 4.3mL of 80% methanol was added. The mixture was vortexed. The absorbance was measured spectrophotometrically at 415nm. The value of optical density was used to calculate the total flavonoids content present in the sample.

Fourier Transform Infrared Spectroscopy Analysis

Using a mortar and pestle, little powdered of plant samples were separately mixed with KBr salt and compressed in to a thin pellet. On Thermoscientific transmission, between 4000-400 cm^{-1}.[13] infrared spectra were recorded as KBr pellets.

GC-MS Analysis

The GC-MS analysis of ethanol extracts were accomplished by employing a Perkin-Elmer GC Clarus 500 system and Gas chromatograph interfaced to a Mass spectrometer (GC-MS) equipped with Elite-I, fused silica capillary column (30 X 0.25 mm 1D X 1µMdf, composed of 100% Dimethyl polysiloxane). An electron ionization system with ionizing energy of 70 eV was employed for GC-MS detection. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1mL/min. An injection volume of 2µl was employed (split ratio of 10:1) with Injector temperature 250°C and Ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2min.), with an increase of 10°C/min, to 200°C, then 5°C/min to 280°C, closing with a 9 min isothermal at 280°C. Mass spectra were involved at 70 eV; a scan interval of 0.5 sec and portions from 45 to 450 Da. The whole GC running time was 36 min. By comparing its regular peak area to the total areas, the relative % amount of each component was found. The software assumed to handle mass spectra and chromatograms was a Turbo mass.

Identification of Compounds

By employing the database of National Institute of Standard and Technology (NIST) having more than 62,000 patterns, the interpretation of mass spectrum of GC-MS was conducted. With the spectrum of the recognized components stored in the NIST library, the

Table 1: Phytochemical Screening of *E. acoroides* and *S. isoetifolium.*

Bioactive Compounds	Petroleum ether		Benzene		Ethyl acetate		Methanol		Ethanol		Aqueous	
	EA	SI	EA	SI	EA	SI	EA	SI	EA	SI	EA	SI
Alkaloid	-	-	-	-	+	+	-	-	-	-	-	-
Anthraquinone	-	-	-	-	-	-	-	-	-	-	-	-
Catechin	-	-	+	+	-	+	+	+	+	-	-	-
Coumarin	-	-	+	+	-	-	-	+	-	-	+	+
Flavonoid	-	-	+	+	+	+	+	+	+	+	+	+
Phenol	+	+	+	+	+	+	+	+	+	+	+	+
Quinone	+	+	+	+	+	+	-	-	+	+	+	+
Saponin	+	+	+	+	+	+	+	+	+	+	-	-
Steroid	-	-	-	-	-	-	+	+	+	+	+	+
Tannin	+	+	+	+	+	+	+	+	+	+	-	-
Terpenoid	+	+	-	-	-	-	-	+	-	+	-	-
Glycoside	+	+	+	+	+	+	+	+	+	+	+	+
Carbohydrate	-	-	+	+	-	-	-	-	+	-	-	-
Xanthoprotein	+	+	-	-	-	-	+	+	-	+	+	+
Fixed oil	+	+	+	+	-	+	+	-	+	-	+	+

EA - *E. acoroides*, SI - *S. isoetifolium.*
+ Present –Absent.

spectrum of the unknown components was evaluated. The molecular weight, name and structure of the elements of the test materials were determined.

RESULTS

The phytochemical screening of ethyl acetate, methanol benzene and ethanol extracts of *E. acoroides* illustrated the presence of catechin, saponin, flavonoid, phenol, tannin and glycoside. Similarly, *S. isoetifolium* exhibited the presence of catechin, flavonoids, phenol, quinone, saponins, tannin, glycoside and carbohydrate. Aqueous extracts of *E. acoroides* and *S. isoetifolium* demonstrated the presence of coumarins, steroid, flavonoid, phenol, quinone, glycoside and xanthoprotein (Table 1).

Total phenolic and flavonoid content present in the ethanol extract of *E. acoroides* and *S. isoetifolium*, respectively, were 0.33g100g^{-1}, 0.17g100g^{-1}, 0.23 g100g^{-1} and 0.21g100g^{-1} (Table 2).

The plant powder of *E. acoroides* and *S. isoetifolium* analysed by FT-IR identifies the functional groups based on its peak ratio and elution transition of compounds (Figure 1 and 2).

The *E. acoroides* exposed the characteristic bonds via 3741, 3429, 1649, 1485, 1332, 1255, 1135, 1105, 1028, 808, 661 and 575 cm^{-1}. In the same way, *S. isoetifolium* showed the bonds via 3735, 3446, 2923, 1643, 1419, 1108, 874, 595 and 462 cm^{-1} (Table 3).

Table 2: Total Phenolic and Flavonoids content of *E. acoroides* and *S. isoetifolium.*

Seagrass	Total Phenolics g 100g^{-1}	Total Flavonoid g 100g^{-1}
E. acoroides	0.33±0.02	0.23±0.01
S. isoetifolium	0.17±0.01	0.21±0.01

[a] All values are names of triplicate determination expressed on dry weight basis.
± Standard error.

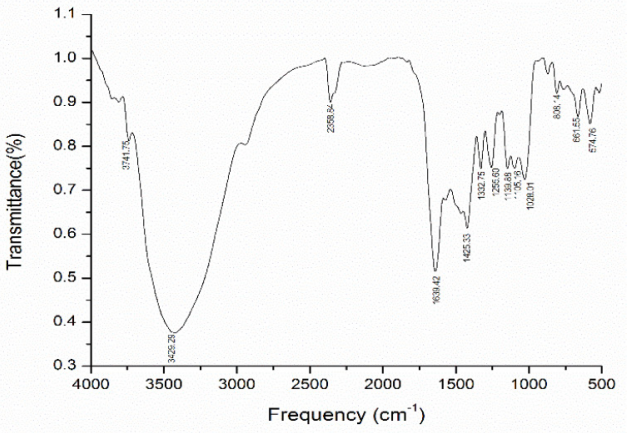


Figure 1: FT-IR Spectrum of *Enhalus acoroides.*

From the GC-MS analysis of ethanol extract of *E. acoroides* and *S. isoetifolium,* a total of each 10 compounds were identified. The chromatogram is presented in Figures 3 and 4. The chemical constituents with their retention time (RT), molecular formula

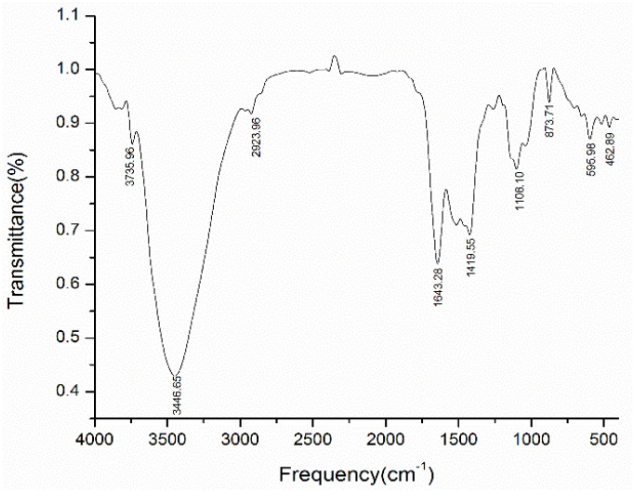


Figure 2: FT-IR Spectrum of *Syringodium isoetifolium*.

Table 3: FT-IR spectroscopy data of *E. acoroides* and *S. isoetifolium*.

Sl. No	Stretching Frequency cm^{-1}		Chemical bond	Phyto-constituents
	E. acoroides	*S. isoetifolium*		
1	3741	3735	O-H Stretch	Hydroxyl group
2	3429	3446	O-H Stretch	Phenolics/alcohol group
3	-	2923	C-H Stretch	Alkane
4	2358	-	NH^{+} Stretch	Tertiary amine salt
5	1649	1643	>NH bond	Secondary amine
6	1485	1419	C-C Stretch	Aromatic
7	1332	-	C-H rock	Alkane
8	1255	-	C-O Stretch	Ester, Ether
9	1139	-	C-N Stretch	Aliphatic amine
10	1105	1108	C-N Stretch	Aliphatic amine
11	1028	-	C-N Stretch	Aliphatic amine
12	808	873	C-H "oop"	Aromatic
13	661, 574	595, 462	C-Br Stretch	Alkyl halide

(MF), molecular weight (MW) concentration (%) and structure of bioactive compounds in ethanol extracts of *E. acoroides* and *S. isoetifolium* are presented in Tables 4 and 5. Through the GC-MS analysis of *E. acoroides*, n-Hexadecanoic acid, 9-Octadecenoic acid, Octadecanoic acid, Benzenepropanoic acid, 3,5-bis (1,1-dimethylethyl)-4-hydroxy, methyl ester, Benzene (1-Methyldodecyl)-, Cyclic octaatomic sulfur and Hexathane were established. Similarly, GC-MS analysis of *S. isoetifolium* were established to contain phytol, 2,4-Decadienal (E,E), Hexadecenoic acid, methyl ester, Undecane, Neophytadiene, Phthalic acid, hept-3-yl ester, Stigmasterol, Benzene, (1-methyldodecyl)- and

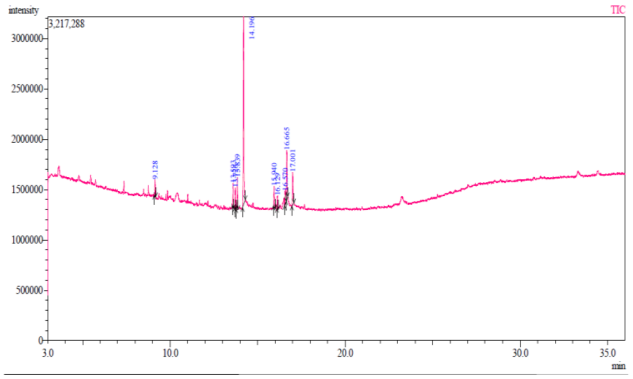


Figure 3: GC-MS Chromatogram of ethanol extract of *E. acoroides*.

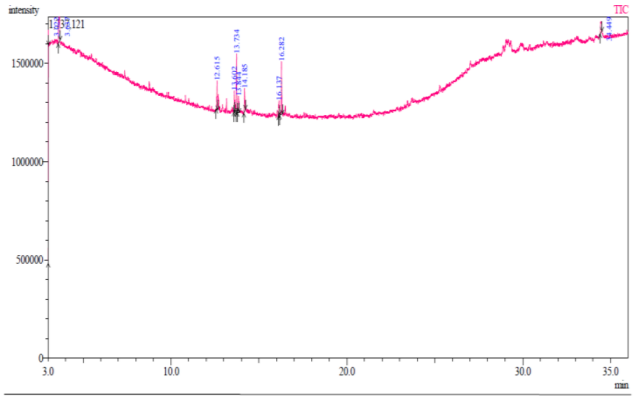


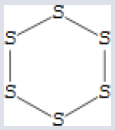
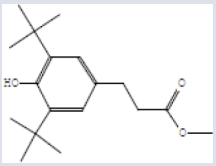
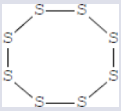
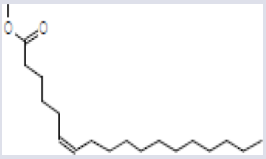
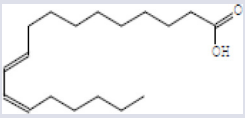
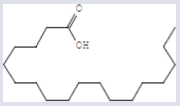
Figure 4: GC-MS Chromatogram of ethanol extract of *S. isoetifolium*.

Benzenepropanoic acid, 3,5,bis (1,1-dimethylethyl)-4-hydroxy and methyl ester.

DISCUSSION

The phytochemical screening of various solvent extracts of *E. acoroides* and *S. isoetifolium* proved the presence of phytocompounds that have been certified to have antioxidant and further activities. The notice of the phenolic compounds has raised all through the last decade because of their antioxidant power. Their free radical scavenging features helped in the prevention of chronic and oxidative stress-related disorders such as cancer, cardiovascular and neurodegenerative diseases.[14] Flavonoids are a significant group of naturally happening polyphenolic compounds and are regarded as health stimulating and disease evading dietary supplements. Many flavonoid compounds are shown to have an anti-oxidative activity, anti-inflammatory, anti-allergic free radical scavenging capacity, cardio-protective, anti-diabetic while some other flavonoid compounds show potential antiviral activities. More newly flavonoids are demonstrated to be the most effective as an anti-cancer

Table 4: Bioactive Compounds found in ethanol extract of *E. acoroides*.

Sl. No	R. Time	Name of the Compound	Molecular Formula	Molecular Weight	Peak Area %	Structure
1.	9.128	Hexathiane	S_6	192	3.32	
2.	13.593	Benzene,(1-methyldodecyl)-	$C_{19}H_{32}$	260	4.43	
3.	13.726	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	3.84	
4.	13.839	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-h	$C_{18}H_{28}O_3$	292	6.38	
5.	14.196	n-Hexadecanoic acid	$C_{17}H_{34}O_2$	270	49.96	
6.	15.940	Cyclic octaatomic sulfur	S_8	256	5.79	
7.	16.129	6-Octadecenoic acid, methyl ester, (Z)-	$C_{19}H_{36}O_2$	296	2.92	
8.	16.570	10(E),12(Z)-Conjugated linoleic acid	$C_{18}H_{32}O_2$	280	3.39	
9.	16.665	9-Octadecenoic acid, (E)-	$C_{18}H_{34}O_2$	282	11.45	
10.	17.001	Octadecanoic acid	$C_{18}H_{36}O_2$	284	8.53	

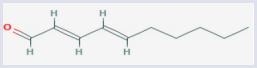
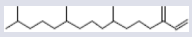
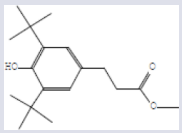
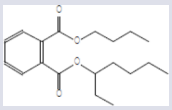
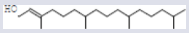
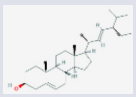
agent, through apoptosis by induction of cell cycle arrest and inhibition of key enzymes included in tumour promotion.[15-16]

Many studies were carried out to advance the result of catechin on the human baby and enhance its caring power against UV radiation. There are many instances of the positive anti-microbial, anti-inflammatory, anti-allergenic anti-viral, anti-oxidant, and anti-cancer effects of catechins.[17]

Saponins are triterpenoids glycosides shared in a large number of plants. Immunostimulant, permeability, hypocholesterolemic and anticancerogenic properties of saponins, widespread researches haves been carried out. Saponins also have the, anti-nocieptive, analgesic antioxidant activity, to damage the digestion of protein, to cause hypoglycaemia and to act as antifungal and antiviral agents.[18]

Tannin has significant nutraceutical properties. They are bioactive substance capable of producing helpful effects in the body, if they are consumed in the diet for long periods of time. Tannins are molecules supportive for the human health due to their antioxidant properties. Because of the cellular aging progression, they have the capability to protect the tissues from the action of

Table 5: Bioactive Compounds found in ethanol extract of *S. isoetifolium.*

Sl. No	R. Time	Name of the Compound	Molecular Formula	Molecular Weight	Peak Area %	Structure
1.	3.022	2,4-Decadienal (E,E)-	$C_{10}H_{16}O$	152	6.29	
2.	3.637	Undecane	$C_{11}H_{24}$	156	14.35	
3.	12.615	Neophytadiene	$C_{20}H_{38}$	278	7.20	
4.	13.602	Benzene, (1-methyldodecyl)-	$C_{19}H_{32}$	260	5.96	
5.	13.734	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	16.19	
6.	13.844	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy	$C_{18}H_{28}O_3$	292	5.49	
7.	14.185	Phthalic acid, butyl hept-3-yl ester	$C_{19}H_{28}O_4$	320	6.68	
8.	16.137	6-Octadecenoic acid, methyl ester, (Z)-	$C_{19}H_{36}O_2$	296	4.69	
9.	16.282	Phytol	$C_{20}H_{40}O$	296	17.11	
10.	34.449	Stigmasterol	$C_{29}H_{48}O$	412	6.03	

free radicals. They have also demonstrated anti-cancer properties, protecting agents in the urinary tract and the cardiovascular and immune system.[19-20]

The presence of these phytocompounds in different solvent extracts of *E. acoroides* and *S. isoetifolium* possibly indicates the numerous medicinal properties of seagrass such as anti-oxidant, , anti-viral, immuno stimulant anti-inflammatory, anti-cancer, anti-microbial and analgesic activities.

The study of infrared spectra involved the correlation of absorption bands in the spectrum of unknown compound with known absorption frequencies. For the identification of known and unknown compounds, it is important for the FTIR analysis are intensity (weak, medium or strong) sharp (broad or sharp) and position (cm^{-1}) in the spectrum. It facilitates to identify the chemical constitutes and expounds the chemical structure. Struggle was taken to understand the significance of such functional groups as bioactive components for the cure of various diseases.[21] The results of FT-IR analysis confirmed the presence of hydroxyl, phenolics/alcohol, alkane, tertiary amine salt, secondary amine, aromatic, ester, ether, aliphatic amine and alkyl halide.

Among the identified bioactive component of *E. acoroides,* n-Hexadecanoic acid has antiinflammatory, nematicide, antiandrogenic flavour, antioxidant, hypocholesterolemic, hemolytic and 5-alpha reductive inhibitor.[22-24] Hexadecanoic acid, methyl ester has antibacterial, antifungal, hypocholesterolemic and hemolytic activity.[25] 10(E), 12(Z)-conjugated linoleic acid has various antioxidant and antitumour activities.[26] Octadecanoic acid has antimicrobial, antiiflammatory and anticancer activity.[23] Similarly the identified bioactive components from *S. isoetifolium* phytol was reported with antioxidant, neuroprotective, antimicrobial, anticancer, antiinflammatory and antidiuretic activities.[15] Stigmasterol has thyroid inhibitory, antiperoxidative, hypoglycemic, anticancer and antimicrobial effects.[27] Undecane has antiallergic, antiinflammatory, antioxidant and antimicrobial activity.[28] Neophytadiene has

antiinflammatory, antioxidant, analgesic, antipyretic and antimicrobial activities.[29] Phthalic acid, butyl hept-3yl ester has antioxidant and antifungal activity.[30]

CONCLUSION

In the present study, *E. acoroides* and *S. isoetifolium* had shown to have various secondary metabolites which possess many pharmacological properties. The FT-IR analysis also confirmed the presence of different functional groups of secondary metabolites. The GC-MS analysis illustrated the presence of each 10 phytoconstituents which contribute to the activities like anti-oxidant, anti-cancer, anti-inflammatory, hypo-cholesterolemic, analgesic, hemolytic, anti-microbial and other activities. Therefore, the presence of phytochemicals is accountable for their therapeutic effects. Further isolation, characterization and elucidation of the structure of the bioactive compounds which are accountable for the medicinal values is essential.

ACKNOWLEDGEMENT

The authors acknowledge the Periyar University, Salem for providing FT-IR and GC-MS analysis facility.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

GC-MS: Gas chromatography-Mass spectrometry; **EPH:** Ethnopharmacology unit Herbarium; **FTIR:** Fourier transform infrared spectroscopy; **mL:** Millilitre; **µg:** Microgram; **g:** Gram; **RT:** Retention time; **MF:** Molecular Formula; **MW:** Molecular weight.

SUMMARY

The GC-MS analysis showed the presence of each ten phytochemical constituents from the ethanol extracts of *E. acoroides* and *S. isoetifolium*. The presence of various bioactive compounds justifies the use of the whole plants for treating various ailments. Some of the bioactive secondary metabolites identified may become commercially important phytopharmaceuticals. However, further studies are needed to ascertain their biological and pharmacological activity.

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