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IDENTIFICATION OF BIOACTIVE COMPONENTS IN *ENHALUS ACOROIDES* SEAGRASS EXTRACT BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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ABSTRACT

Objective: This study deals with the determination of possible phytochemicals present in the ethyl acetate extract of *Enhalus acoroides* using gas chromatography-mass spectroscopy (GC-MS) technique.

Methods: Using GC-MS technique the phytochemicals present in the ethyl acetate extract of *E. acoroides* whole seagrass was investigated, and the mass spectra of the compounds found in the extract were matched with the National Institute of Standards and Technology library.

Results: GC-MS analysis of *E. acoroides* extract revealed the existence of several phytochemicals which includes 1-nonadecene (17.15%), n-tetracosanol-1 (11.48%), 1-octadecene (10.06%), 2-pentadecanone (7.87%), behenyl alcohol (7.33%), 17-pentatriacontene (4.84%), triacontane (4.25%), tetratetracontane (4.17%), and butylated hydroxytoluene (2.09%).

Conclusion: *E. acoroides* possess distinct phytochemicals such as 1-nonadecene and n-tetracosanol-1 which possess antioxidant property, triacontane which has antibacterial, antidiabetic and antitumor activities. Further studies need to elute novel bioactive compounds and toxicity profile through animal models.

Keywords: *Enhalus acoroides*, Gas chromatography-mass spectroscopy, Phytochemicals, Biological activity, 1-Nonadecene, n-tetracosanol-1.

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INTRODUCTION

Traditional customs of practicing the medication plants is composed of skills and practical knowledge and also followed on theoretical knowledge, belief in treatment and various traditions which are practiced to maintain the physical fitness and also the medicinal plants help to prevent the diseases, diagnostic purposes or to cure physical, as well as mental illness [1]. Various kinds of medicinal plants used traditionally, and other herbal practices known as complementary or alternative medicine are nowadays implementing in developing countries and also in developed countries. World Health Organization has defined herbal plants possesses biological components which can be used for many medication purposes and also to synthesize secondary metabolites which can be used to produce essential drugs [2].

Nowadays, medication plants become more of importance in improving the analysis and quality control section along with many improvements in the field of clinical research have become prominent in treating various diseases [3]. Over the past century, the phytochemicals in herbs play an important role in pharmaceutical industries [4]. Phytochemicals are natural bioactive compounds seen in whole parts of the plants such as root, stem, bark, leaves, seed, and fruits. Many traditionally used plants are economically benefitted, for example, oils, resins, tannins, natural rubber, gums, waxes, dyes, pharmaceuticals, and pesticides [5].

Analytical techniques have shown a drastic improvement from the past decade such as thin-layer chromatography, ultraviolet, nuclear magnetic resonance, and GC-MS that were promising techniques for separation, identification, and structural determination of phytochemicals. GC-MS, a powerful tool and also a consistent technique and most commonly used the procedure for identification and quantification of phytochemicals. The unknown phytochemicals in a complex phytochemical mixture can also be determined by interpretation, and

the spectrum was matched with the National Institute of Standards and Technology (NIST) [6].

Marine plants are a good source of nutraceuticals and potent drugs in curing many human diseases. Approximately 80% of the diverse plant as well as animal species are seen in oceans [7]. There are approximately 57 species of seagrass worldwide [8]. *Enhalus acoroides* L.C. Rich ex Steud, belonging to the monotypic marine genus *Enhalus* in the family Hydrocharitaceae is commonly seen in the coasts of Indian Ocean and also tropical parts of Western Pacific [9]. Gillan *et al.* showed that *E. acoroides* leaves contain sterol as well as fatty acid components. The crude extract of *E. acoroides* possesses scavenging free radicals since it contains active secondary metabolites such as phenolics, flavonoids, and tannins and this justified its uses in folkloric medicines [10]. However, the isolation and bioactivity of other secondary metabolites from *E. acoroides* are not reported [11].

Literature studies reveal that the information on secondary metabolites using GC-MS analysis of *E. acoroides* is inadequate. Hence, the current study is focussed on the dealing of phytochemicals present in ethyl acetate fraction of *E. acoroides*.

METHODS [12]

Collection and authentication of *E. acoroides*

The fully matured *E. acoroides* were taken from Devipattinam, Ramanathapuram District during the period of June 2016 and authenticated in ICAR by Dr. N. Kaliaperumal M.Sc., Ph.D., Scientist-in-charge, CMFRI.

Preparation of extracts

The collected *E. acoroides* leaves washed with distilled water carefully to remove the traces of impurities. The leaves were shade dried, mechanically grinded and powdered. The grinded powder was initially

soaked into 1:2 ratio ethyl acetate (semi-polar) for 3 days with mild shaking. After 3 days, the ethyl acetate was decanted, and the collected extract was stored at room temperature in a dark place, and the ethyl acetate solvent was evaporated. Finally, solid material was collected and stored at 4°C until used.

GC-MS analysis [13]

GC-MS analysis was carried out on GC-MS - 5975C (AGILENT) under the following conditions. DB-5ms Agilent (30.0 m × 0.25 mm × 0.25 μm) was used. Using helium as carrier gas (99.9995% purity) at a constant flow rate of 1.51 mL/min and an injection volume of 2 μL was employed in a split mode. The injector temperature was maintained at 240°C, and the column temperature was programmed to 70°C (isothermal for 2 min) with increasing temperature of 10°C/min to 300°C (isothermal for 9 min). 200°C for ion source temperature and 240°C for interface

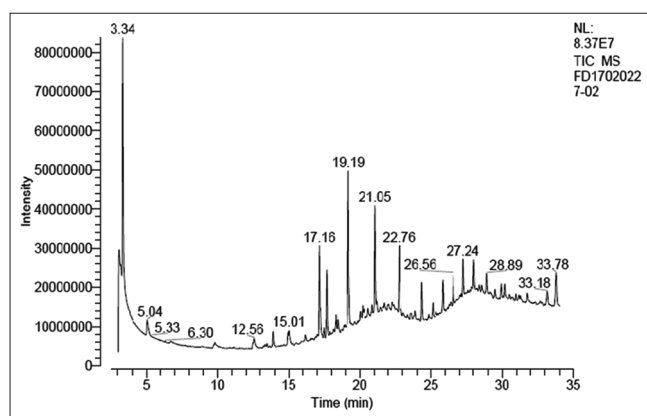


Fig. 1: Chromatogram of ethyl acetate extracts of *Enhalus acoroides*

temperature were maintained. The mass spectra were obtained through ionization energy of 70 eV in the EI mode. Total 30 min need to run GC-MS. The phytocompounds were identified by comparison of mass spectra with the national libraries (NIST - 11).

Identification of components

The phytocomponents present in the ethyl acetate extract was identified by comparing the spectrum with the database of National Institute of Standard and Technology (NIST) which has 62,000 patterns. The compound name, retention time, molecular formula, as well as structure were determined. Each components percentage of area was calculated by comparing its average peak area to the total areas. The unknown compounds spectrum was compared with the spectrum of the known compound stored in the NIST library.

RESULTS AND DISCUSSION

GC-MS is a technique which combines the properties of gas chromatography-mass spectrometry which identifies various substances within the test sample which includes hydrocarbons, alcohols, acids, esters, alkaloids, steroids, amino and nitro compounds, and so on [14]. GC-MS also helps to find the traces in materials. GC-MS has been commonly called as “gold standard” for forensic substance identification since it can be used to analyze a specific test [15,16].

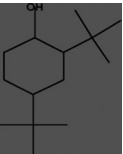



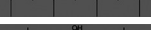

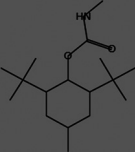


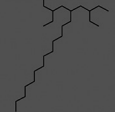

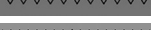
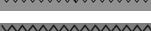



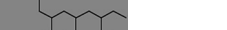
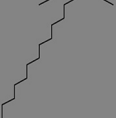
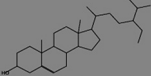
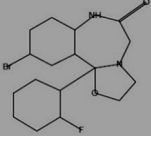

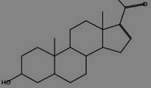
Nearly 30 phytocompounds were found in *E. acoroides* by GC-MS analysis. The active phytocompounds with their retention time, molecular formula, molecular weight, and concentration (%) are presented in Table 1 and Fig. 1. The prevailing compounds were 1-nonadecene (17.15%), n-tetracosanol-1 (11.48%), 1-octadecene (10.06%), 2-pentadecanone (7.87%), behenyl alcohol (7.33%), 17-pentatriacontane (4.84%), triacontane (4.25%), tetratetracontane (4.17%), and butylated hydroxytoluene (2.09%). The biological functions of these phytocompounds were identified in the ethyl acetate extracts of the *E. acoroides* tabulated in Table 2.

Table 1: GC-MS analysis and mass spectral data of ethyl acetate fraction from the seagrass *E. acoroides* showing molecular formula, molecular weight, mass peak, retention time, and structure

Retention time	Name of the compound	Molecular formula	Molecular weight	Peak%
5.04	o-Xylene	C ₈ H ₁₀	106	3.16
13.89	Phenol, 2,4-bis (1,1-dimethylethyl)-	C ₁₄ H ₂₂ O	206	1.94
17.16	1-Nonadecene	C ₁₉ H ₃₈	266	10.06
17.16	1-Octadecene	C ₁₈ H ₃₆	252	10.06
17.16	1-Hexadecanol	C ₁₆ H ₃₄ O	242	10.06
17.67	2-Pentadecanone, 6,10,14-trimethyl-	C ₁₈ H ₃₆ O	268	7.87
18.33	Butylated hydroxytoluene	C ₁₅ H ₂₄ O	220	2.09
18.33	Phenol, 2,6-bis (1,1-dimethylethyl)- 4-methyl-, methyl carbamate	C ₁₇ H ₂₇ NO ₂	277	2.09
19.19	1-Heneicosanol	C ₂₁ H ₄₄ O	312	17.15
19.19	1-Docosene	C ₂₂ H ₄₄	308	17.15
20.20	Heptadecane, 9-hexyl-	C ₂₃ H ₄₈	324	1.57
20.20	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	C ₂₆ H ₅₄	366	1.57
21.05	n-Tetracosanol-1	C ₂₄ H ₅₀ O	354	11.48
21.05	Behenyl alcohol	C ₂₂ H ₄₆ O	326	11.48
24.35	17-Pentatriacontene	C ₃₅ H ₇₀	490	4.84
24.35	Cyclopentane, (4-octyldodecyl)-	C ₂₅ H ₅₀	350	4.84
25.13	Heptadecane, 9-hexyl-	C ₂₃ H ₄₈	324	1.57
25 0.13	Pentadecane, 2-methyl-	C ₁₆ H ₃₄	226	1.57
25.85	Tetratetracontane	C ₄₄ H ₉₀	618	4.17
25.85	Heptacosane	C ₂₇ H ₅₆	380	4.17
26.56	Pentacosane	C ₂₅ H ₅₂	352	3.24
27.24	Heneicosane	C ₂₁ H ₄₄	296	4.25
28.01	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	C ₂₆ H ₅₄	366	3.97
30.16	â-Sitosterol	C ₂₉ H ₅₀ O	414	1.85
33.18	Haloxazolam	C ₁₇ H ₁₄ BrFN ₂ O ₂	376	2.70
33.18	Lanosterol	C ₃₀ H ₅₀ O	426	2.70
33.78	Pregn-16-en-20-one, 3-hydroxy-, (3â,5â)-	C ₂₁ H ₃₂ O ₂	316	6.34
33.78	Nitralin	C ₁₃ H ₁₉ N ₃ O ₆ S	345	6.34
33.78	Benzamide, N, N'-1,4-phenylenebis-	C ₂₀ H ₁₆ N ₂ O ₂	316	6.34

E. acoroides: *Enhalus acoroides*, GC-MS: Gas chromatography-mass spectrometry

Table 2: Bioactivity of phytochemicals identified in the ethyl acetate extracts of *E. acoroides* by GC-MS

Name of the compound	Structure of the compound	Biological activities
Phenol, 2,4-bis (1,1-dimethylethyl)-		Antibacterial and anti-inflammatory [17]
1-Nonadecene		Antituberculosis, anticancer, antioxidant, antimicrobial [18,19]
1-Octadecene		Not intended for a therapeutic purpose
1-Hexadecanol		Antioxidant [20]
2-Pentadecanone, 6,10,14-trimethyl-		Hypocholesterolemic, antioxidant, and lubrication [21]
Butylated hydroxytoluene		Antioxidant [22]
Phenol, 2,6-bis (1,1-dimethylethyl)-4-methyl, methyl carbamate		Treatment for Huntington's disease [23]
1-Heneicosanol		Antifungal [24]
Heptadecane, 9-hexyl-		Antifungal [25]
Octadecane, 3-ethyl-5-(2-ethyl butyl)-		Not intended for a therapeutic purpose
n-Tetracosanol-1		Antioxidant [26]
Behenyl alcohol		Hair conditioners, moisturizers, and lubricating oils [27]
17-Pentatriacontene		Not intended for a therapeutic purpose
Tetratetracontane		Antioxidant and cytoprotective activities [28]
Heptacosane		Not intended for a therapeutic purpose
Triacontane		Antibacterial, antidiabetic, and antitumor activities [29]
Heneicosane		Not intended for a therapeutic purpose
Octadecane, 3-ethyl-5-(2-ethylbutyl)-		Antimicrobial and antifungal agents [30]
á-Sitosterol		Antimicrobial anticancer anti-inflammatory anti-asthma, and diuretic antiarthritic [31]
Haloxazolam		Not intended for a therapeutic purpose
Lanosterol		Regulators of cholesterol biosynthesis [32]
Pregn-16-en-20-one, 3-hydroxy-, (3á,5á)-		Not intended for a therapeutic purpose

E. acoroides: *Enhalus acoroides*, GC-MS: Gas chromatography-mass spectroscopy

The most abundant phytochemicals identified in *E. acoroides* are 1-nonadecene (17.15%) and n-tetracosanol-1 (11.48%), respectively. 1-Nonadecene is long-chain fatty acids showed

antituberculosis activity as well as antifungal activity, respectively. n-Tetracosanol-1 is an alcoholic compound which possesses antibacterial activity [18].

Alam et al. [33] found antibacterial activity in methanol and hexane extracts of *E. acoroides*. 1-Hexadecanol, an alcoholic compound which showed antioxidant activity. Antioxidant safeguards the body from degenerative diseases [34] and the phytoconstituent 2-pentadecanone, 6, 10, 14-trimethyl- which has hypocholesterolemic activity, antioxidant, and lubrication activity [21]. Butylated hydroxytoluene has antioxidant properties [22] and phenol-2,6-bis(1,1-dimethylethyl)-4, methyl carbamate could be used to synthesize phenol-4-[2-(aminomethyl)-4-thiazolyl]-2,6-bis(1,1-dimethylethyl) monohydrochloride which can be used to treat Huntington's disease [23].

Phenol-2, 4-bis (1, 1-dimethylethyl), and cyclic compound are unsaturated and hence plays an important role in free radical scavenging. The unsaturation degree for cyclic compounds is greater and also a good antioxidant [35].

Heptadecane, 9- hexyl and Ethyl iso-allochololate are most effective plant extract which possesses antifungal activity. Tetratetracontane possesses antioxidant as well as cytoprotective activities. Heptadecane and octadecane, 3-ethyl-5-(2- ethyl butyl)- are effective antimicrobial and antifungal agents.

Steroid compounds are derived from lanosterol, a tetracyclic triterpenoid. One biological system that can be used to produce oxygenated derivatives of cholesterol and lanosterol [36,37]. This proves that oxysterols may be natural regulators of cholesterol biosynthesis in the intact cell.

This paper reveals the goodness of *E. acoroides* which has various medicinal properties and can be highly commended as a plant of phytopharmaceutical importance. Based on the phytoconstituents present, biological properties of earlier studies have been reported. Hence, *E. acoroides* can be used as an important medication plant in the folklore medicine.

CONCLUSION

The present study revealed that the ethyl acetate extract of *E. acoroides* of GC-MS analysis proves the presence of numerous active phytoconstituents responsible for various pharmacological activities and justifies the medicinal use of this plant in folklore medicine. According to the literature survey that we believe this is the first report of GC-MS analysis of seagrass extract. Hence, *E. acoroides* might be utilized for finding new drugs, and further investigation needs to elute novel bioactive compounds and toxicity profile through *in vitro* and *in vivo* models.

AUTHOR'S CONTRIBUTION

No author's contribution.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this article.

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