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 phytocompounds present in the ethyl acetate extract of Enhalus acoroides using gas chromatography-mass spectrometry (GC-MS) technique.

Methods: Using GC-MS technique the phytocompounds 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 phytocompounds 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%), tetracontacontane (4.17%), and butylated hydroxytoluene (2.09%).

Conclusion: E. acoroides possess distinct phytocompounds 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 spectrometry, Phytocompounds, Biological activity, 1-Nonadecene, n-tetracosanol-1.
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 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 phyto-compounds were identified by comparison of mass spectra with the national libraries (NIST - 11).

**Identification of components**

The phyto-compounds 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, alkalioids, 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 phyto-compounds were found in *E. acoroides* by GC-MS analysis. The active phyto-compounds 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-tetracontane-1 (11.48%), 1-octadecene (10.06%), 2-pentadecanone (7.87%), behenyl alcohol (7.33%), 17-pentatriacontane-1 (4.84%), triacontane (4.25%), tetratetracontane (4.17%), and butylated hydroxytoluene (2.09%). The biological functions of these phyto-compounds 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 (min) | Name of the compound | Molecular formula | Molecular weight | Peak% |
|----------------------|----------------------|-------------------|-----------------|-------|
| 5.04                 | 2,6-Dimethylphenol    | C₆H₆O             | 106             | 3.16  |
| 13.89                | 1-Nonadecene          | C₁₀H₂₂O          | 206             | 1.94  |
| 17.16                | 1-Octadecene          | C₁₉H₃₆          | 266             | 10.06 |
| 17.16                | 1-Hexadecan          | C₁₉H₃₈          | 242             | 10.06 |
| 17.67                | 2-Pentadecanone-6,10,14-trimethyl- | C₂₀H₃₆ | 268             | 7.87  |
| 18.33                | 1-Octadecane          | C₁₉H₃₈          | 220             | 2.99  |
| 18.33                | 1-Octadecane-4-methyl- | C₁₀H₁₉NO₂  | 277             | 2.09  |
| 19.19                | 1-Heneicosanol        | C₂₁H₴₀          | 312             | 17.15 |
| 19.19                | 1-Docosene           | C₂₃H₴₄          | 308             | 17.15 |
| 20.20                | Heptadecane, 9-hexyl- | C₂₀H₴₀          | 324             | 1.57  |
| 20.20                | Octadecane, 3-ethyl-5-(2-ethylbutyl)- | C₂₁H₴₄ | 366             | 1.57  |
| 21.05                | n-Tetracontane-1     | C₃₂H₶₄          | 354             | 11.48 |
| 21.05                | Behenyl alcohol      | C₃₂H₶₄          | 326             | 11.48 |
| 24.35                | 17-Pentacontane-1    | C₃₂H₶₄          | 490             | 4.84  |
| 24.35                | Cyclopentane, (4-octyldodecyl)- | C₃₂H₶₄ | 350             | 4.84  |
| 25.13                | Heptadecane, 9-hexyl- | C₂₁H₴₀          | 324             | 1.57  |
| 25.13                | Pentadecane, 2-methyl- | C₂₁H₴₀          | 226             | 1.57  |
| 25.85                | Tetratetracontane-1  | C₃₄H₷₂          | 618             | 4.17  |
| 25.85                | Heptacosane          | C₂₃H₴₄          | 380             | 4.17  |
| 26.56                | Pentacosane          | C₂₅H₵₀          | 352             | 3.24  |
| 27.24                | Heneicosane          | C₃₀H₶₄          | 296             | 4.25  |
| 28.01                | Octadecane, 3-ethyl-5-(2-ethylbutyl)- | C₂₁H₴₄ | 366             | 3.97  |
| 30.16                | á-Storsterol         | C₂₃H₴₄          | 414             | 1.85  |
| 33.18                | Haloxazolam          | C₂₃H₴₄BrFN₄O₂  | 376             | 2.70  |
| 33.18                | Lanosterol           | C₂₅H₵₀          | 426             | 2.70  |
| 33.78                | Pregn-16-en-20-one, 3-hydroxy- | (3á,5á)- | C₂₃H₴₄ | 316     | 6.34 |
| 33.78                | Nitratin             | C₂₃H₴₄NO₅S    | 345             | 6.34  |
| 33.78                | Benzamid e, N, N-1,4-phenylenebis- | C₂₃H₴₄NO₅ | 316     | 6.34  |

*E. acoroides: Enhalus acoroides; GC-MS: Gas chromatography–mass spectroscopy*
The most abundant phytocompounds 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].

### Table 2: Bioactivity of phytocomponents 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)-phenol | | 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- | | Hypcholesterolemic, antioxidant, and lubrication [21] |
| Octylated hydroxytoluene | | Antioxidant [22] |
| Phenol, 2,6-bis (1,1-dimethylethyl)-4-methylz, 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 |
| Triaccontane | | 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- (3α,5α)- | | Not intended for a therapeutic purpose |
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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 phytopharmaceutical *Z- pentadecane*, 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-dimethylbenzyl)-4, methyl carbamate could be used to synthesize phenol-1-(2-[(aminomethyl)-4- thiazoyl]-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-allocholate are most effective plant extract which possesses antifungal activity. Tetraetracontane possesses antioxidant as well as cytotoxic 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 phytocomponents 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 need 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.

REFERENCES

1. Tapsell LC, Hemphill I, Cobial L. Health benefit of herbs and species. The past, the present, the future. Med J Australia 2006;188(4):24-24.
2. Amudha P, Bharathi NP, Vanitha V. *Caesalpinia bonducella*— a review on pharmacological and phytochemical activity of seeds. Int J Pharm Bio Sci 2010;1:41-5.
3. Tabacco E, Borreani G, Crovetto GM, Galassi G, Colombo D, Cavallarin L, et al. Effect of chestnut tannin on fermentation quality, proteolysis, and protein rumen degradability of alfalfa silage. J Dairy Sci 2006;89:4736-46.
4. Amudha P, Varadaraju V. Phytochemical and pharmacological potential of *Annona* species: A review. Asian J Pharm Clin Res 2017;10:1-8.
5. Christopher B. RHSIA-Z Encyclopedia of Garden Plants. 3rd ed. London: Dorling Kindersley, 2003. p. 738-51.
6. Hites AR. Gas Chromatography Mass Spectroscopy: Handbook of Instrumental Techniques for Analytical Chemistry. New Jersey: Prentice Hall PTR; 1997. p. 609-11.
7. Jayalakshmi M, Amudha P, Vanitha V. Assessment of minerals from shrimp shell waste. Res Pharm Sci 2017;8:194-7.
8. Arsal A, Bujang JS, Zakaria MH. Distribution and Significance of Seagrass Ecosystems in Malaysia. Malaysian: Aquatic Ecosystem Health and Management Society, 2006. p. 203-14.
9. Hartog CD, Kuo J. Taxonomy and biogeography of seagrasses. In: Larkum AW, Orth RJ, Duarte CM, editors. Seagrass. Springer, Berlin: Biology, Ecology and Conservation; 2006. p. 691.
10. Amudha P, Vanitha V. Phytochemical analysis and in vitro antitoxin screening of sea grass-Enhalus acoroides. Int J Res Pharm Sci 2017;8:1-8.
11. Gillan FT, Hogg RW, Drew EA. The sterol and fatty acid compositions of seven tropical seagrasses from North Queensland, Australia. Phytochemistry 1984;23:2917-21.
12. Hemalatha S, Amudha P, Bharathi NP, Vanitha V. Determination of bioactive phytocomponents from hydroethanolic extract of *Annona squamosa* (Linn.) by leaf by GC-MS. Int J Pharm Sci 2017;8:2539-44.
13. Mathi P, Nikiil K, Das S, Roy P, Bokka VR, Botlagunta M. Evaluation of in vitro anticancer activity and GC-MS analysis from leaf of *Sophora Interrupta* Bedd. Int J Pharm Pharm Sci 2015;7:303-8.
14. Saravanan P, Chandramohan G, Mariajancynari J, Shannugasundaram P. GC-MS analysis of phytochemical constituents in ethanolic bark extract of *Ficus religiosa* Linn. Int J Pharm Pharm Sci 2014;6:457-60.
15. Harbourne JB. Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis. 2nd ed. London: Chapman and Hall; 1984. p. 4-120.
16. Wagner H, Bladi S, Zainski EM. Plant Drug Analysis. Berlin: Springer-Verlag. 1984. p. 298-334.
17. Amaral AC, Comes LA, Sila IR, Fernandes, AFR, Ferreira JL, Ade SR, Mdo SR, et al. Liposonal formulation of tumericine-rich hexane fractions from *Curcuma longa* enhances their antiinflammation activity. Biomed Res Int 2014; doi: 10.1155/2014/694934.
18. Rukachaisirirakul T, Srivathanakrit P, Sukcharoenphol K, Wongvein C, Ruttanawang P, Ruttanawang P, et al. Chemical constituents and antiactivity of *Piper sarmentosum*. J Ethnopharmacol 2004;93:73-16.
19. Lee YS, Kang MH, Cho SY, Jeong CS. Effects of constituents of *Anomomum cantonioides* on gastritis in rats and on growth of gastric cancer cells. Arch Pharm Res 2007;30:436-43.
20. Mishra PM, Sree A. Antibacterial activity and GC-MS analysis of the extract of leaves of *Finlaysonia obovata* (A mangrove plant). Asian J Plant Sci 2007;6:168-72.
21. Kumar PP, Kuravarele S, Lalitha C. Screening of antioxidant activity, total phenolics and GC-MS study of *Vitex negundo* Ander. J Biochem Pharmacol 2010;4:191-5.
22. Yehye WA, Rahman NA, Ariffin A, Hamid SB, Alhadi AA, Kadir FA, et al. Understanding the chemistry behind the antioxidant activity of butylated hydroxytoluene (BHT): A review. Eur J Med Chem 2015;101:295-312.
23. Committee for Orphan Medicinal Products. Public Summary of Opinion of Orphan designation. Phenol, 4- (2-(amino-methyl)-4- thiazoyl)-, 6-bis (1, 1-dimethylethyl) Monohydrochloride for the Treatment of *Huntington’s Disease*; 2015.
24. Ananchicha LA, Naspi CV, Pucci GN, Arce ME., Colloca CB. Biological activity of 1-heneicosanol isolated from *Senecio colubraupiensis*, an endemic species from Patagonia. Pharm Chem J 2016;3:73-7.
25. Abubacker MN, Devi PK. In vitro antifungal potentials of bioactive compounds heptadecane, 9- hexyl and Ethyl iso-allocholate isolated from *Lepidagathis cristaeta* Wild. (Acanthaceae) leaf. BBF 2015;3:336-43.
26. Lakshmi M, Nair BR. GC-MS analysis of the chloroform extract of bark of *Terminalia Travancorensis* Wight and Arn. (Combretaceae). J Pharm Sci Res 2013;5:794-8.
27. Arunachalam LA, Yilmaz MA, Firat M. Chemical profile by LC-MS/MS, GC/MS analysis of the chloroform extract of *Terminalia travancorensi* L. Combretaceae. *Combretum*; 2015; doi:10.1155/2014/694934.
28. Mammen D, Daniel M, Sane RT. Seasonal and geographic variations of *Combretum* and *Combretaceae*. Adv Chem 2014; doi.org/10.1155/2014/143948.
29. Ertas A, Yilmaz MA, Firat M. Chemical profile by LC-MS/MS, GC/MS analysis of the chloroform extract of *Terminalia travancorensi* L. Combretaceae. *Combretum*; 2015; doi:10.1155/2014/694934.
30. Rao MR, Phillips S, Kumar MH, Saranya Y, Divya D, Prabhu B. GC-MS analysis, antimicrobial, antioxidant activity of an ayurvedic medicine, *Salmuli nayya*. J Chem Pharm Res 2015;7:131-9.
31. Jeyageevaram P, Alihau NM, Kumara S. Identification of pesticide compounds of *Cynodon dactylon* by GC-MS analysis. Int J Pharm Bio Sci 2014;5:604-8.
32. Kandutsch AA, Chen HW, Heiniger HJ. Biological activity of some oxygenated sterols. Science 1978;201:498-501.
33. Alam MS, Chopra N, Ali M, Niwa M. Oleanen and stigmasterol derivatives from Ambroma augusta. Phytochemistry 1996;41:1197-200.
34. Chandramohan A, Divya RS. Comparison of antioxidant activity in Gracilaria edulis and Hypnea valentiae. IJARIIT 2017;3:294-6.
35. Prakash A, Saneetha V. Punica granatum (Pomegranate) rind extract as a potent substitute for L- ascorbic acid with respect to the antioxidant activity. Res J Pharm Biol Chem Sci 2014;5:597.
36. Nelson JA, Steckbeck SR, Spencer TA. Biosynthesis of 24,25-epoxycholesterol from squalene 2,3:22,23-dioxide. J Biol Chem 1981;256:1067-8.
37. Panini SR, Sexto RC, Rudney H. Regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase by oxysterol by-products of cholesterol biosynthesis. Possible mediators of low density lipoprotein action. J Biol Chem 1984;259:7767-71.