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Identification of Bioactive Components in the Hydroalcoholic Extract of *Syringodium Isoetifolium* and Assessment of its Biological Activity by Gas Chromatography – Masspectrometry

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ABSTRACT

Herbal plants show a growing interest in natural antioxidant, also play an important role in the treatment of muscle pain, stomach problems, wounds etc. About 75 to 80% of the world population uses herbal medicine. One such herb (Marine Plants) used in the treatment of cancer is *Syringodium isoetifolium.* In a previous study, *Syringodium isoetifolium* with three different extracts (Aqueous, ethanol and 70% hydroalcohol) proves the presence of phytocompound such as Tannin, Saponin, Flavonoids, Stroids, Terpenoids, coumarin etc and also has high antioxidant activity. The current study aims to identify the bioactive compounds from the hydroalcoholic extract of *Syringodium isoetifolium.* The photo components present in the hydroalcoholic extract was determined by GC-MS Analysis and the mass spectra of compounds present in the extract were matched with the National Institute of Standards and Technology library. 20 different phytocompounds were found to be present in the hydroalcoholic extract of *Syringodium isoetifolium* using GC-MS analysis. Some of the important phytocompounds are Hexanoic acid, Methyl ester, Octadecanoic acid, 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester, 1-Isopropylidene-3-methyl-3-vinylcyclobutane. The study reveals that the identified phytocompounds from the hydroalcoholic extract of *Syringodium isoetifolium* has essential biological activities such as Antibacterial, Antifungal, Antimicrobial, Antifouling and Anticancer properties. Further depth of the study will deal with the exact mode of action of the phytocompounds.

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INTRODUCTION

From the ancient period, natural herbs play a significant role in the treatment of diseases such as fever, stomach problems, muscle pains, wounds. But nowadays, we forget about traditional foods, as well as natural medicine. The marine sources are potent therapeutic drugs for various ailments (Lee and Jeon, 2013). Under the stress condition, the compounds present in the seagrass produce a defence mechanism due to the production of secondary metabolites (Subhashini *et al.*, 2013). One such seagrass, *Syringodium isoetifolium*, commonly called as Noodle grass, Neer pasi, Oosi korai, Nool pasi, Korai pasi, were found in the subtidal region, especially

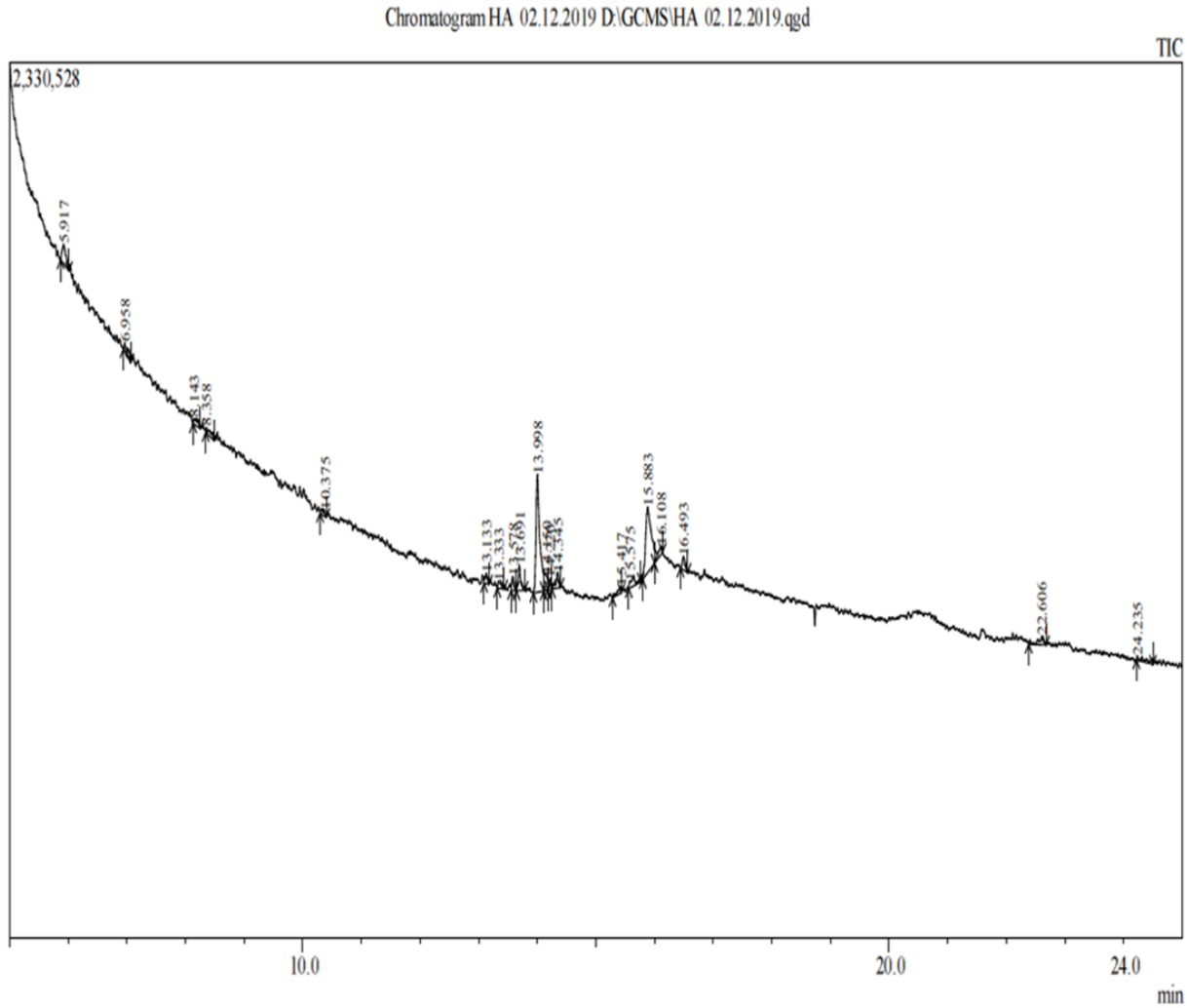


Figure 1: GC-MS Chromatogram of theHydro-alcoholic extract of *Syringodium isoetifolium* by GC-MS Analysis

in India richly found in the Andaman and Lakshwadeep islands (Kalaivani *et al.*, 2020).

Secondary metabolites such as flavonoids, alkaloids, terpenoids, tannins and steroids were present in *Syringodium isoetifolium*, which can be supplemented as a traditional medicine to human beings. The phytocompounds present in the seagrass *Syringodium isoetifolium* can be separated and identified by GC-MS Analysis. The phytocompounds can be interpreted and matched the spectrum with the National Institute of Standard and Technology (NIST) (Amudha *et al.*, 2018). The best technique to identify the long chain hydrocarbons, esters alkaloids, alcohols, nitro and amino compounds (Jadhav *et al.*, 2014).

The present study deals with the phytocompounds present in the hydroalcholic extract of *Syringodium isoetifolium* by GC-MS analysis and biological activities of the compounds were determined.

MATERIALS AND METHODS

Collection of seagrass

The fresh seagrass *Syringodium isoetifolium* were collected from Devapattinam, Ramanathapuram District, on June 2019 by sea divers. The sample has been identified and authenticated by Dr P.Jeyaraman, PhD., Director, Retd Professor, Presidency College, in Plant Anatomy Research Centre. The collected seagrass was washed with the tap water, shadow dried and powdered in the herbal grinder. The powdered sample was stored in an air-tight container.

Extraction

With three different conical flasks, 20 grams of powdered sample were added and the flask contains different solvent such as aqueous, ethanol and 70% hydroalcohol in the measurement of 1000ml. By the rotator shaker, they were shaked vigourously for one hour and the mixture was kept for 24

Table 1: Identification of Bioactive components in the Hydro-alcoholic extract of *S.isoetifolium* using GC-MS Analysis

Peak	Retention Time	Area %	Height %	Molecular Formula	Molecular Weight	Name of the compounds
1	5.917	4.91	5.12	$C_{10}H_{16}$	136	1-Isopropylidene-3-methyl-3-vinylcyclobutane
2	6.958	2.00	2.03	C_3H_4	40	1-propyne (cas) propyne
3	8.143	1.85	1.46	C_3H_4	40	1,2-propadiene (cas) allene
4	8.358	2.14	1.55	C_3H_4	40	1-propyne (cas) propyne
5	10.375	1.26	1.48	$C_{17}H_{26}O_2$	262	1,1'-bibicyclo(2.2.2)octyl-4-carboxylic acid
6	13.133	1.64	2.75	$C_{20}H_{26}O_4$	330	1,2-Benzenedicarboxylic acid, dicyclohexyl ester
7	13.333	1.48	1.79	C_3H_4	40	1-propyne (cas) propyne
8	13.578	2.09	3.72	$C_7H_{14}O_2$	130	Hexanoic acid, methyl ester
9	13.691	4.18	6.75	$C_{14}H_{22}N_2O$	234	Xylocaine
10	13.998	29.99	31.73	$C_{18}H_{36}O_2$	284	Octadecanoic acid
11	14.150	3.46	4.20	$C_{16}H_{22}O_4$	278	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester
12	14.220	2.29	3.07	$C_8H_{11}NO$	137	7-hydroxy-5,6,7,8-tetrahydroindoliziaine
13	14.345	3.03	4.20	$C_{15}H_{30}O_2$	242	Oxirane, [(dodecyloxy)methyl
14	15.417	1.59	1.69	$C_{22}H_{13}NO_4$	355	Ethyl 1-hexyl-4-hydroxy-2(1h)-oxo-3-quinolinecarboxylate
15	15.575	3.05	1.93	$C_{22}H_{13}NO_4$	355	Ethyl 1-hexyl-4-hydroxy-2(1h)-oxo-3-quinolinecarboxylate
16	15.883	24.50	17.53	$C_{18}H_{34}O_2$	282	9-Octadecenoic acid
17	16.108	3.18	2.16	$C_{12}H_{12}F_6O_2$	302	8,9,9,10,10,11-hexafluoro-4,4-dimethyl-3,5-dioxatetracyclo[5.4.1.0(2,
18	16.493	2.74	3.48	$C_{15}H_{30}O_2$	242	Oxirane, [(dodecyloxy)methyl]-
19	22.606	2.52	2.21	$C_{15}H_{14}N_2O_2$	254	4-(methoxymethyl)-6-methyl-2-phenoxynicotinonitrile
20	24.235	2.08	1.14	C_3H_4	40	1-propyne (cas) propyne

S.isoetifolium: *Syringodium isoetifolium*,GC-MS: Gas chromatography-Mass spectrometry

hours. Then the different extracts were filtered through Whattman no.1 filter paper and the filterate used for further studies. Hydroalcoholic extract of *Syringodium isoetifolium* shows more Secondary Phytochemicals in the previous preliminary studies and hence it has been used in this study.

GC-MS Analysis

GC-MS analysis was carried out following conditions: column RTX 5Ms with column diameter is 0.32mm; column thickness 0.50μm, column length is 30m, operating in electron impact mode at 70eV. Carrier gas used in this analysis is the helium gas (99.999%) with a constant flow of 1.73 ml /min. Injection volume employed was 0.5 μI was employed (split ratio of 10:1) with an injector temperature of 270^{o}C and an ion-source temperature is 200^{o}C. The temperature of the column was programmed from 40^{o}C (isothermal for 2 min), with an increase of 8^{o}C/min, to 150^{o}C, then 8^{o}C/min to 250^{o}C and finally ends with 20min isothermal at 280^{o}C. At 70eV, the Mass spectra were measured at an interval of 0.5 seconds and fragments from 40 to 450 Da. The total running time of GC are 51.25min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The phytocomponents were identified with the software adopted to handle mass spectra. And chromatograms was a Turbo Mass Ver 5.2.0 (Srinivasan *et al.*, 2013).

Identification of components

Table 2: Biological activities of the identified compounds in Hydro-alcoholic extracts of *S.isoetifolium* by GC-MS Analysis

Peak	R. Time	Name of the compounds	Biological activities
1	13.998	Octadecanoic acid	Cosmetic, Flavor, Hypocholesterolemic, Lubricant, Perfumery, Propecic, Suppository, Lower LDL Cholesterol level Kanthal *et al.* (2014)
2	15.883	9-Octadecenoic acid	Antihypertensive, Allergenic, Anti-inflammatory, Anticancer, Antiandrodenic, Flavour, Irritant Increase HDL and decrease LDL Cholesterol, Velayutham and Karthi (2015)
3	13.578	Hexanoic acid, methyl ester	Antibacterial, Antifungal
4	14.150	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	Antimicrobial and Antifouling Kanthal *et al.* (2014)
5	22.606	4-(methoxymethyl)-6-methyl-2-phenoxynicotinonitrile	Anticancer, Mucolytics, Drugs for the genital disorder Tyagi and Agarwal (2017)
6	15.417	Ethyl 1-hexyl-4-hydroxy-2(1h)-oxo-3-quinolinecarboxylate	Antimicrobial
7	16.493	Oxirane	Natural mediators for allergic Asthma Rao *et al.* (1983)
8	13.333	1-propyne (cas) propyne	Antidepressive or Antihypertensive agents Maycock *et al.* (1976)

S.isoetifolium:*Syringodium isoetifolium*,GC-MS: Gas chromatography-Mass spectrometry

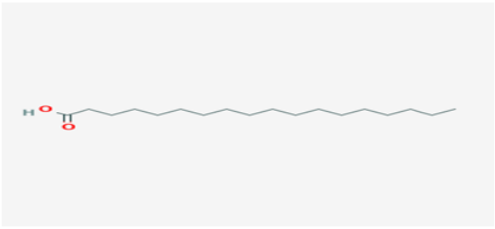
The phytocomponents present in the hydro alcoholic extract of *Syringodium isoetifolium* was identified by comparing spectrum with a database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The name of the compound, molecular formula, structure and also the retention time were determined. The spectrum of the unknown and known components was compared and stored in the NIST library.

RESULTS AND DISCUSSION

Identification of Bioactive components in Hydroalcoholic extract of *Syringodium isoetifolium* by GC MS Analysis

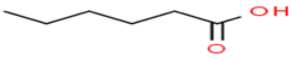
The compounds such as esters, alkaloids, steroids and alcohols present in the test sample can be identified by the combination of Gas Chromatography and Mass Spectrometry technique (Saravanan *et al.*, 2014). GC-MS also used to analyse a specific test that can identify the forensic substances (Wagner *et al.*, 1984). Twenty compounds were identified in the hydro-alcoholic extract of *Syringodium isoetifolium* by GC-MS analysis. The active principles with their concentration (%), retention time (RT), molecular formula and molecular weight (MW) are presented in Table 1. The chromatogram of the hydro-alcoholic extract of seagrass *Syringodium isoetifolium* was shown in Figure 1.

The prevailing compounds are Octadecanoic acid, Hexanoic acid methyl ester, 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester and 9-Octadecenoic acid were found in seagrass *Syringodium isoetifolium.* Octadecanoic acid shows the highest peak with a retention value of 13.998 when compared to the other compounds. And the second peak value was shown by the compound 9-Octadecenoic acid (Oleic acid) with the retention time of 15.883. The other compounds are 1-Isopropylidene-3-methyl-3-vinylcyclobutane (5.917), 1-propyne (cas) propyne (6.958), 1,2-propadiene (cas) allene (8.143), Xycaine (13.691), Oxirane, [(dodecyloxy)methyl (14.345). However, isolation of the individual phytochemical constituents and subjecting to its biological activity will give fruitful results. The biological activities of the compounds present in the hydro-alcoholic extract *Syringodium isoetifolium* was given in Table 2 and the structure of some of the compounds was shown in Figure 2.

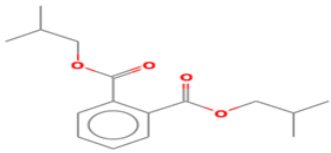


Octadecanoic acid

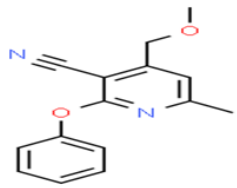
9-Octadecenoic acid



Hexanoic acid, methyl ester

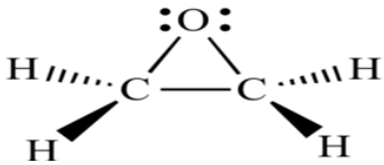


1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester



4-(methoxymethyl)-6-methyl-2-phenoxynicotinonitrile

1-propyne (cas) propyne



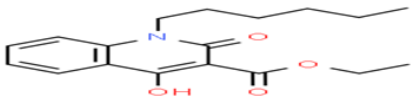


Figure 2: Structure of the compounds identified by GC-MS analysis

Octadecane are the long chain alkanes and acts as a lubricant and anticorrosion agents. The highest peak value shown by Octadecanoic acid has the following biological activities as Antimicrobial, Anticancer, Hepatoprotective, Anti-arthritic, Anti-asthma and Diuretic (Kanthal *et al.*, 2014). 4-(methoxymethyl)-6-methyl-2 has anticancer activity and they also used as a drug for the genital disorder. 2-Benzenedicarboxylic acid, diethyl ester was used in Plasticizers. 1,2-Benzenedicarboxylic acid reduces oxidative stress and able to cure neurodegenerative disorders (Tyagi and Agarwal, 2017). Oxirane are the natural mediators for allergic asthma and they also act as a biogenetic precursor of leukotrienes (Rao *et al.*, 1983). Oxirane also exhibits antibacterial, insect antifeedant and antioxidant activities (Thirunarayanan and Vanangamudi, 2011). The bioactive compound 1-propyne (cas) propyne acts as antidepressive or antihypertensive agents (Maycock *et al.*, 1976). Xylocaine compound has anti-pyretic activity and also acts as a local anesthetic (Chakraborty *et al.*, 2010). 9-Octadecenoic acid has many biological activities such as Antiandrogenic, Allergenic, Anti-inflammatory, Antileukotrienes, Anticancer (Velayutham and Karthi, 2015). This paper reveals the importance and goodness of *Syringodium isoetifolium.* Based on the phytocompounds present, the biological activities of earlier studies have been reported. Hence the *Syringodium isoetifolium* is a natural medicine without any side effects and also cost effective.

CONCLUSIONS

In the present investigation, nearly twenty compounds have been identified from the hydroalcoholic extract of *Syringodium isoetifolium* using GC-MS analysis. The presence of bioactive compounds in the seagrass *Syringodium isoetifolium* proved pharmaceutical importance. The various bioactive compounds present in the hydroalcoholic extract of *Syringodium isoetifolium* justify its use for various ailments by traditional practitioners. Further studies and investigation may proceed with the mode of action of each compound.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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