



Phytochemical profiling and GC-MS analysis of methanol extract of *Spinifexlittoreus* (Burm. f.) Merr

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Abstract:

Coastal sand dune plants are of great importance to researchers in the field of pharmacology, medicinal, industrial, nutritional, and ecosystem restoration. Nowadays most of the pharmaceutical industries depend on the medicinal plant for their raw materials for drug discovery. *Spinifexlittoreus* is a coastal plant under the family Poaceae growing on the sandy seashore and is well known for its medicinal properties. The present study was carried out to the phytochemical screening and estimated the bioactive components present in the methanolic extract of *Spinifexlittoreus* leaves. The phytochemical studies revealed the presence of flavonoids, saponins, alkaloids, terpenoids, tannins, and phenols. The GC-MS analysis revealed the presence of twenty bioactive compounds which include n-Hexadecanoic acid, 9,10-Secochola-5,7,10(19)-trien-24-al, 3-hydroxy-(3.beta.,5Z,7E), and 1,3-Benzenedicarboxylic acid, etc., The phytochemical and GC-MS profiling of the methanol extract of *Spinifexlittoreus* leaves revealed the presence of bioactive compounds with important medicinal properties. Hence, the presence of these phytochemicals could be responsible for the therapeutic effects of the plant.

Keywords: Coastal Sand Dune, *Spinifexlittoreus*, Phytochemicals, and GC-MS

Abbreviations:

CSD-Coastal Sand dune

GC-MS – Gas Chromatography mass spectrometry

NIST - National Institute of Standards and Technology

1. Introduction:

Coastal sand dune (CSD) plants are unique and vibrant ecosystems between marine and terrestrial realms (1). They are the coastal armoring with a bind of biota and sand grains. The coastal sand dune flora has ecological importance and socio-economic values. The CSD legumes have several value-added properties and applications. The usefulness of CSD legumes has been broadly divided into medicinal, pharmacological, industrial, nutritional, and ecosystem restoration. Most traditional applications are related to treating skin diseases and skin injuries (wounds, snake or spider bites). Some specific applications are directed to treat muscle sprains,

and gynecological problems and to improve immunological responses. Usually, topical applications are common to treat skin diseases or wounds and some involve oral administration (2). Many studies have exposed that the medicinal importance of plants is mainly due to the presence of secondary metabolites (3). Bioactive compounds and phytochemicals of CSD plant species have numerous pharmacological applications (4). Research in the areas of marine natural products has grown geometrically in the recent past (2). Sand-dune species, due to their understudied and unfamiliar stress adaptation mechanisms and strategies present a prospect for the discovery of new bioactive molecules (5). However, the reports on the bioactive substances possessed by coastal sand dune plant species are very scanty.

Spinifexlittoreus is a coastal plant under the family Poaceae growing on the sandy seashore of Asian countries such as Taiwan, Vietnam, Thailand, Malaysia, Indonesia, the Philippines, and India. It plays a vital role in dune stabilization and restoration (6). *Spinifexlittoreus* also called Ravan's Moustache. This plant is widely distributed along the coastal areas and used as a diuretic in folk medicine. Various Pharmacological studies on this plant reported anti-inflammatory, analgesic, and anti-microbial activities of its methanol and aqueous extract (7). But all these studies had done only in a crude extract of plant parts. The identification of secondary phytochemical compounds of plant is very less.

Kingdom : Plantae

Phylum : Tracheophyta

Class : Liliopsida

Order : Poales

Family : Poaceae

Genus : Spinifex

Species : *Spinifexlittoreus* (Burm f.) Merr.

Gas chromatography-mass spectroscopy (GC-MS) is a combined analytical technique used to determine and identify compounds present in a plant sample (8). GC-MS plays an essential role in the phytochemical analysis and chemotaxonomic studies of medicinal plants containing biologically active components(9). This study was mainly focused on Phytochemical screening and GC-MS profiling of methanol leaf extract of *Spinifexlittoreus*.

2. Materials & Methods:

Plant collection and Identification

Fresh leaves of *Spinifexlittoreus* were collected from Puthupettai beach, Cuddalore, TamilNadu, India. The leaves were identified and authenticated by Dr.T.Ramanathan, Associate professor, Faculty of Marine Sciences, Annamalai University, TamilNadu, India.

Preparation of plant material

Spinifexlittoreus leaves were air-dried at room temperature and pulverized into powder for extraction. Soxhlet extraction method was used. Methanol solvent was used in extraction process. 10g of a leave sample was put into a Soxhlet extraction with methanol for a maximum

of 6 hours. The resulting extract was evaporated, dried and their yield was assessed before being kept at 4°C for Phytochemical Screening and GC-MS analysis.

Preliminary phytochemical screening

Phytochemical profiling of methanol extract of *Spinifexlittoreus* leaves were carried out using the procedures as described (10,11& 12)

Gas chromatography-mass spectrometry (GC-MS) analysis

The Shimadzu GCMS QP 2020 was used in the analysis employed a fused silica column, packed with SH-Rxi-5Sil MS (30 m × 0.25 mm ID × 250µm df) and the components were separated using Helium as carrier gas at a constant flow of 1 ml/min. The injector temperature was set at 280°C during the chromatographic run. The 1µL of extract sample injected into the instrument the oven temperature was as follows: 40 °C (2 min); followed by 280 °C at the rate of 10 °C min⁻¹ and 280 °C, where it was held for 3 min. The mass detector conditions were: transfer line temperature 240 °C; ion source temperature 240 °C; and ionization mode electron impact at 70 eV, a scan time 0.2 sec and scan interval of 0.1 sec. The fragments from 40 to 550 Da.

Identification of compounds

Identification of components was achieved based on their retention indices and interpretation of mass spectrum was conducted using the database of National Institute of Standards and Technology (NIST). The database consists of more than 62,000 patterns of known compounds. The spectra of the unknown components of *Spinifexlittoreus* leaf obtained were compared with the standard mass spectra of known components stored in NIST (2017) library.

3. Results:

Preliminary Phytochemical screening of methanol crude extract of *Spinifexlittoreus* leaf revealed the presence of alkaloids, flavonoids, saponins, tannins, terpenoids and phenolsas shown in Table 1.

GC-MS profiling of *Spinifexlittoreus*

A total of 20 compounds were identified from the GC-MS analysis of methanol crude extract of *Spinifexlittoreus* leaves. The chromatogram is presented in Fig. 1, while the chemical constituents with their retention time (RT), molecular formula, molecular weight (MW), and concentration (%) are presented in Table 2.

The following bioactive compounds were present in the GC-MS analysis carried on methanol crude extract of *Spinifexlittoreus* leaves: Silane,dimethoxymethyl, 1,2,3-PROPANETRIOL, Dodecanoic acid, Tetradecanoic acid, Neophytadiene, 1,2-Benzenedicarboxylic acid, Hexadecanoic acid, Benzenepropanoic acid, n-Hexadecanoic acid, Hexadecadienoic acid, Phytol, 10(E),12(Z)-Conjugated linoleic acid, 7,10,13-Hexadecatrienal, Octadecanoic acid, 2-hydroxy-1-hydroxy,Bis(2-ethylhexyl) phthalate, 9,10-Secochola-5,7,10(19)-trien-24-al, 3-hydroxy-, (3.b, ERGOST-5-EN-3-OL, (3.BETA.), Octadecanoic acid,2,3-dihydroxypropyl ester, and 1,3-Benzenedicarboxylic acid,bis(2-ethylhexyl) ester.

4. Discussion:

Phytochemical screening of methanol crude extract of *Spinifexlittoreus* leaves revealed the presence of phytochemicals that have been proved to have antioxidant and other conditioning. Flavonoids have been shown to be largely effective scavengers of utmost oxidizing molecules, and diverse free radicals (13). Several studies have been reported the possible cardioprotective effects of flavonoids against Ischemia Reperfusion (14). Terpenoids gained important pharmaceutical value since primitive times, due to their broad spectrum of medical applications (15). Saponins may activate mucous membrane protective factors. Alkaloid reported to have high potent activity against gastric ulcers (16, 17). Tannins have been shown highly effective in antioxidative and antimicrobial (18). The presence of these phytochemicals in methanol crude extract of *Spinifexlittoreus* leaves possibly indicates its numerous medicinal properties such as anti-inflammatory, anti-ulcer, and anti-oxidative properties, among others.

Among the identified bioactive components, n-Hexadecanoic acid has highest percent peak area (32%). Following that 9,10-Secochola-5,7,10(19)-trien-24-al,3-hydroxy-, (3.β.,5Z,7E), 1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester or Diisobutyl phthalate, Octadecanoic acid or stearic acid, and cis,cis,cis-7,10,13-Hexadecatrienal are highest peak area respectively and 1,2,3-Propanetriol has lowest. n-Hexadecanoic acid has reported antioxidant, 5-α-reductase inhibitor, anti-fibrinolytic, antimicrobial activity, hypocholesterolemic and hemolytic properties (19). Phytol reported with antioxidant, neuroprotective, antimicrobial, anticancer, anti-inflammatory, and anti-diuretic activities (20, 21). Phenolic compounds like esters, and other major volatile compounds present which have antiulcer, anti-inflammatory, anti-arthritic, antidiabetic, hypolipidemic, and cytotoxic activities (21). Methyl palmitate has reported as antioxidant, hypocholesterolemic, hemolytic, and 5-α-reductase inhibitor (22)

5. Conclusion:

In the present study, methanol crude extract of *Spinifexlittoreus* leaves have shown to have various secondary metabolites which possess many pharmacological properties. The GC-MS analysis showed the presence of 20 phytochemical constituents which contribute the activities like antimicrobial, antioxidant, anticancer, hypercholesterolemic, anti-inflammatory, and other activities. Hence, the presence of phytoconstituents is responsible for their more therapeutic effects. Further investigation is required for promising development of novel drugs using some of the bioactive compounds found in *Spinifexlittoreus* leaves. This is the first study to report the GC-MS analysis of the leaf extract of *Spinifexlittoreus* and to identify the phytochemical compounds.

Phytoconstituents	Reagents used	Presence (+) or Absence (-)
Alkaloids	Wagner's reagent	+
Flavanoids	Alkaline reagent test	+
Terpenoids	Salkowski test	+
Steroids	Salkowski test	-
Tanins	Potassium dichromate test	+
Saponins	Foam test	+
Total phenol	Ferric chloride test	+

Table 1: Phytoconstituents of methanol extract of *Spinifexlittoreus* leaf

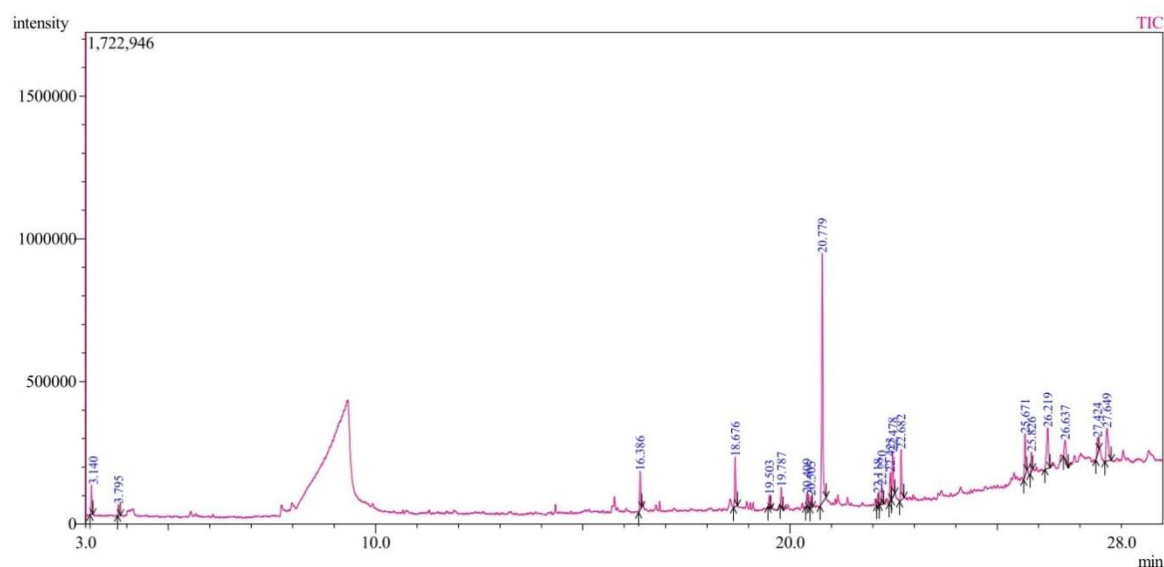
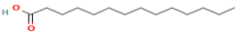
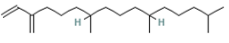
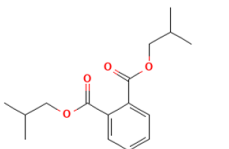
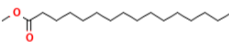
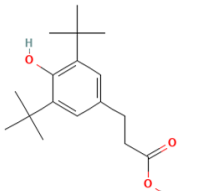
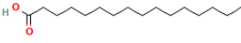
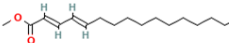
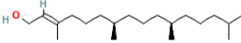
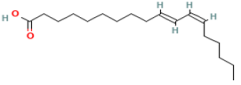
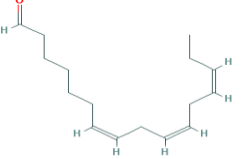
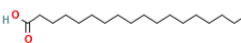
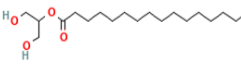


Figure. 1 GC-MS chromatogram of methanol extract of *Spinifexlittoreus* leaves

N o.	RT (min)	Name of the compound	Molecular formula	MW g/mol	Peak area %	Structure
1	3.140	Silane,dimethoxymethyl-	$C_3H_{10}O_2Si$	106.2	2.47	
2	3.795	1,2,3-PROPANETRIOL	$C_3H_8O_3$	92	1.20	
3	16.386	Dodecanoicacid or lauric acid	$C_{12}H_{24}O_2$	200	4.34	

4	18.676	Tetradecanoicacid or Myristic acid	$C_{14}H_{28}O_2$	228	5.52	
5	19.503	Neophytadiene	$C_{20}H_{38}$	278.50	1.30	
6	19.787	1,2-Benzenedicarboxylicacid, bis(2-methylpropyl)ester	$C_{16}H_{22}O_4$	278.34	1.84	
7	20.409	HEXADECANOICACID , METHYLESTER	$C_{17}H_{34}O_2$	270.5	1.33	
8	20.505	Benzenepropanoicacid,3,5-bis(1,1-dimethylethyl)-4-hydroxy-,methyl ester	$C_{18}H_{28}O_3$	292	1.12	
9	20.779	n-Hexadecanoicacid	$C_{16}H_{32}O_2$	256.42	32.92	
10	22.118	HEXADECADIENOICA CID, METHYLESTER	$C_{17}H_{30}O_2$	266	1.17	
11	22.220	Phytol	$C_{20}H_{40}O$	296.5	2.11	
12	22.423	10(E),12(Z)-Conjugatedlinoleicacid	$C_{18}H_{32}O_2$	280.4	3.45	
13	22.478	cis,cis,cis-7,10,13-Hexadecatrienal	$C_{16}H_{26}O$	234.38	6.01	
14	22.682	Octadecanoicacid	$C_{18}H_{36}O_2$	284.5	6.37	
15	25.671	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330	4.90	

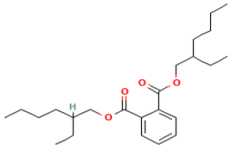
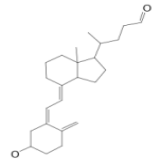
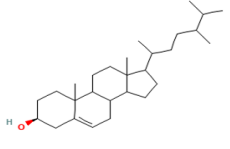
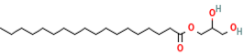
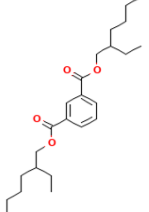
16	25.826	Bis(2-ethylhexyl)phthalate	C ₂₄ H ₃₈ O ₄	390.6	1.95	
17	26.219	9,10-Secochola-5,7,10(19)-trien-24-al,3-hydroxy-,(3.beta.,5Z,7E)	C ₂₄ H ₃₆ O ₂	356.5	8.06	
18	26.637	ERGOST-5-EN-3-OL, (3.BETA.)-	C ₂₈ H ₄₈ O	400.7	3.70	
19	27.424	Octadecanoic acid, 2,3-dihydroxypropylester	C ₂₁ H ₄₂ O ₄	358.6	2.32	
20	27.649	1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl)ester	C ₂₄ H ₃₈ O ₄	390.6	7.93	

Table 2: Phytochemical compounds found in methanol extract of *Spinifexlittoreus* leaves

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Competing Interest:

The authors have no relevant financial or non-financial interests to disclose

Author Contributions:

The first draft of the manuscript was written by RamamoorthyPurushothaman and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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7. References:

1. Saye, S.E. and Pye, K., 2007. Implications of sea level rise for coastal dune habitat conservation in Wales, UK. *Journal of Coastal Conservation*, 11(1), pp.31-52.

2. Yakkala, G.L., 2018. Physicochemical, Phytochemical and Antioxidant Properties of few South Indian Coastal Sand Dunes (CSD). *International Journal of Bio-Pharma Research*, 7(9), pp.2425-2435.
3. Jang, H.D., Chang, K.S., Huang, Y.S., Hsu, C.L., Lee, S.H. and Su, M.S., 2007. Principal phenolic phytochemicals and antioxidant activities of three Chinese medicinal plants. *Food chemistry*, 103(3), pp.749-756.
4. Morris, J.B., 1999. Legume genetic resources with novel value added industrial and pharmaceutical use. *Perspectives on new crops and new uses*, pp.196-201.
5. Beeby E, Magalhães M, Poças J, Collins T, Lemos MFL, Barros L, Ferreira ICFR, Cabral C, Pires IM. Secondary metabolites (essential oils) from sand-dune plants induce cytotoxic effects in cancer cells. *J Ethnopharmacol*. 2020 Aug 10;258:112803. doi: 10.1016/j.jep.2020.112803. Epub 2020 Apr 3. PMID: 32251759.
6. Padmavathy, K., Poyyamoli, G. and Balachandran, N., 2010. Coastal Dune Flora, Nallavadu Village, Puducherry, India. *Check list*, 6(2), pp.198-200.
7. Thirunavukkarasu, P., Ramanathan, T., Ramkumar, L. and Balasubramanian, T., 2010. Anti microbial effect of a coastal sand dune plant of *Spinifexlittoreus* (Burm. f.) Merr. *Current Research Journal of Biological Sciences*, 2(4), pp.283-285.
8. Uma, G. and Balasubramaniam, V., 2012. GC-MS analysis of *Nothapodytesnimmoniana* [J. Graham] mabberly leaves. *J Chem Pharm Res*, 4(9), pp.4417-4419.
9. Hethelyi, E., Tetenyi, P., Dabi, E. and Danos, B., 1987. The role of mass spectrometry in medicinal plant research. *Biomedical & environmental mass spectrometry*, 14(11), pp.627-632.
10. Harboume, J.B., 1973. *Phytochemical methods*. London: *Chapman and Hill*.
11. Harborne, A.J., 1998. *Phytochemical methods a guide to modern techniques of plant analysis*. Springer science & business media.
12. Soni, A. and Sosa, S., 2013. Phytochemical analysis and free radical scavenging potential of herbal and medicinal plant extracts. *Journal of Pharmacognosy and phytochemistry*, 2(4), pp.22-29.
13. Saeed, N., Khan, M.R. and Shabbir, M., 2012. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. *BMC complementary and alternative medicine*, 12, pp.1-12.

14. Njoku, U.O., Nwodo, O.F.C. and Ogugofor, M.O., 2017. Cardioprotective potential of methanol extract of *Costusafer* leaf on carbon tetrachloride-induced cardiotoxicity in albino rats. *Asian Journal of Pharmaceutical Research and Health Care*, pp.51-58.
15. Jaeger, R. and Cuny, E., 2016. Terpenoids with special pharmacological significance: A review. *Natural product communications*, 11(9), p.1934578X1601100946.
16. Rouibi, A. and Boukrita, S., 2018. Phytochemical characterization, anti-inflammatory and anti-ulcer activity of a spontaneous succulent *Delospermaressei*. *Universal J Agricultural Res*, 6(3), pp.113-117.
17. Sreeja, P.S., Arunachalam, K., Saikumar, S., Kasipandi, M., Dhivya, S., Murugan, R. and Parimelazhagan, T., 2018. Gastroprotective effect and mode of action of methanol extract of *Sphenodesmeinvolutrata* var. *paniculata* (CB Clarke) Munir (Lamiaceae) leaves on experimental gastric ulcer models. *Biomedicine & Pharmacotherapy*, 97, pp.1109-1118.
18. Chung, K.T., Wong, T.Y., Wei, C.I., Huang, Y.W. and Lin, Y., 2010. Tannins and human health. *a review*, 1998, p.38.
19. Starlin, T., Prabha, P.S., Thayakumar, B.K.A. and Gopalakrishnan, V.K., 2019. Screening and GC-MS profiling of ethanolic extract of *Tylophorapauciflora*. *Bioinformation*, 15(6), p.425.
20. Kumar, P.P., Kumaravel, S. and Lalitha, C., 2010. Screening of antioxidant activity, total phenolics and GC-MS study of *Vitexnegundo*. *African Journal of Biochemistry Research*, 4(7), pp.191-195.
21. Banjare, J., Salunke, M., Indapurkar, K., Ghate, U. and Bhalerao, S., 2017. Estimation of serum malondialdehyde as a marker of lipid peroxidation in medical students undergoing examination-induced psychological stress. *Journal of the Scientific Society*, 44(3), p.137.
22. Rajeswari, G., Murugan, M. and Mohan, V.R., 2012. GC-MS analysis of bioactive components of *Hugoniamystax* L.(Linaceae). *Research Journal of Pharmaceutical, Biological and chemical sciences*, 3(4), pp.301-308.

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