Full Paper

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GC-MS Analysis of n-hexane extracts of Marine Seagrass *Posidonia oceanica* leaves, rhizomes and roots Collected from Benghazi beach Libya

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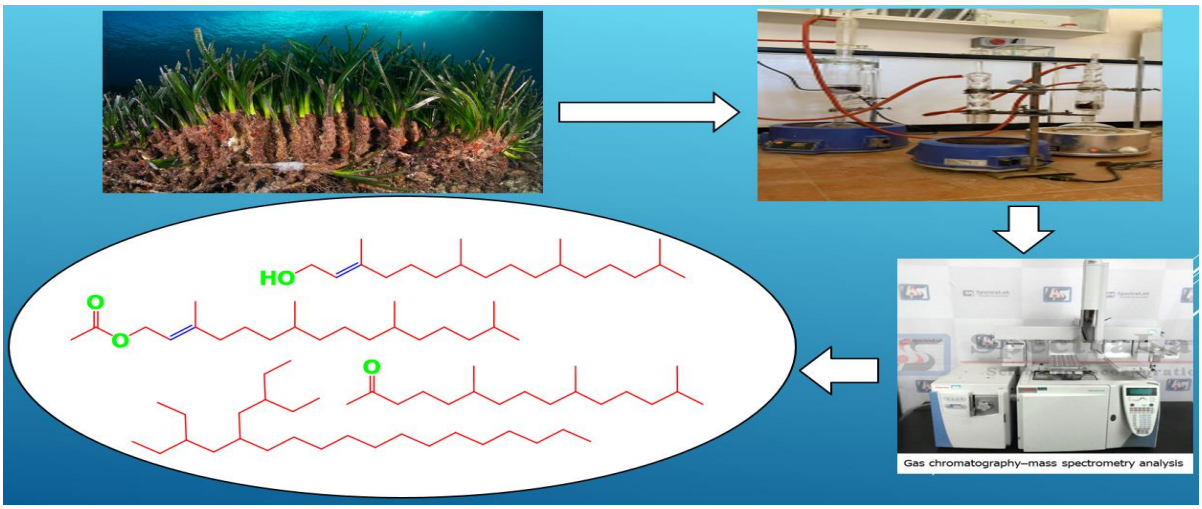

Abstract: *Posidonia oceanica* seagrass is endemic to the Mediterranean, and has very little information about volatile organic compounds. The plant was collected from Garyounis Beach in Benghazi, east of Libya, in September 2019. Plant parts, leaves, rhizomes, and roots were extracted using a Soxhlet extractor with Hexane. The compounds were characterized by gas chromatography–mass spectrometry. the extract's chemical constituents were de-convoluted using AMDIS software (www.amdis.net), and the mass spectra of the compounds spectra were explained by fragmentation pattern and matched to authentic standard spectra from Wiley and the NSIT Library database. The results revealed sixteen compounds, dominated by nine long-chain hydrocarbons,

three long-chain fatty acids, and a single long-chain ketone. This is the first discovery of 3-ethyl-5-(2ethyl-butyl-octadecane, 6,10,14-trimethylpentadecan-2-one, phytol, and phytyl acetate from this plant.

Keywords: Posidonia Oceanica, Seagrass, phytochemistry, GCMS, Libya

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Introduction

The Mediterranean Sea is across rode between there continents Africa Europe and Asia its almost enclosed the sea is only connected to the Atlantic Ocean Through The Strait of Gibraltar in the west and Marmara and the Black Sea Through the Dardanelles in the northeast it occupied area of 2,969,000 km2 and the deepest average from 1,460 m to 5,267 m, at maximum its rich biodiversity area about 17,000 marine species were reported about 27% Bacteria and Archaea 42% Animalia 14% invertebrate and 5% Plants with this unique diversity of marine life in and with 30% endemic species.[1] Seagrass are plants in the Alismatales order with more than 60 speices under four families Posidoniaceae, Zosteraceae, Hydrocharitaceae and Cymodoceaceae and they are the only marine angiosperms plants they distributed in 191 countries with meadows range from 177 000 to 600 000 km^2.[2] The angiosperm family in the plant kingdom known for anti-microbial properties [3] for Volatile organic compound there were about 1700 from this compounds reported from angiosperm Volatile organic compound are important in the plant for interaction with ecology by pollinator attraction and Plant reproduction [4] for Seagrass Posidonia oceanica not enough studies and in 2018 study was based around the Adriatic sea of Italy the Headspace-solid phase micro extraction (HSSPME)was the extraction method and compounds detected with Gas chromatography-mass spectrometry (GC-MS/FID) reported compounded are pentadecane heptadecane nonadecane and Dimethyl sulphide from the plant leaves [5] ,the literature review on the plant in investigation has shown that so far there are no enough published reports about Posidonia Oceanica Volatile organic compounds chemical components this study aimed to investigate the chemical components by first preparing the hexane of leaves, rhizomes, roots extracts by Soxhlet extractor and using GC-MS for compound separation and identification.

Results and discussion

The plant oil extraction shows diversity of reported compounds in the GC-MS analysis result for compound structure identification process the comparison method of authentic spectra mass molecular ion and fragmentation pattern from Wiley Spectral database library and National Institute of Standards and Technology the United States Department of Commerce Chemistry Web Book with the reported compounds spectra was used, Sixteen compounds were obtained from the plant part hexane crude (Table 1) the rhizomes are the major source of compounds six of this compounds only present in the rhizomes (Table 4) (heptacosane, methyl (Z)-octadec-10-enoate, 3-Ethyal-5-(2-ethylbutyl)octadecane, nonadecane, dotriacontane, pentatriacontane.For the roots seven compounds were reported but (heptadecane) is only present in the roots (Table 6), the only reports of terpenoids from leaves (phytol and phytyl acetate) (Table 2), the reported compounds divided to four categories in the first category are nine long chain hydrocarbons, in the second category three long chain fatty acids, and in the third category one ketone, and in the fourth category, one diterpene, in addition to its derivative.

Table 1. Compounds determined by GCMS.

Compound	Molecular formula	Molecular weight	Retention index	Source
Tetradecane	$C_{14}H_{30}$	198	1413	RT, RH
Hexadecane	$C_{16}H_{34}$	226	1612	RT, RH
Heptadecane	$C_{17}H_{36}$	240	1711	RT
Octadecane	$C_{18}H_{38}$	254	1810	RT, RH
Nonadecane	$C_{19}H_{40}$	268	1910	RH
Docosane	$C_{22}H_{46}$	310	2208	RT, RH
Heptacosane	$C_{27}H_{56}$	380	2705	RH
Dotrtacontane	$C_{32}H_{66}$	450	3202	RH
Pentatriacontane	$C_{35}H_{72}$	492	3500	RH
3-Ethyl-5-(2- ethylbutyl)octadecane	$C_{26}H_{54}$	366	2413	RH
Methyl hexadecanoate	$C_{17}H_{34}O_2$	270	1878	RT, RH
Methyl (Z)-octadec-9-enoate	$C_{19}H_{36}O_2$	296	2074.7	L, RT
Methyl (*Z*)-octadec-10-enoate	$C_{19}H_{36}O_2$	296	2085	RH
6,10,14-Trimethylpentadecan-2- one	$C_{18}H_{36}O$	268	1754	L
Phytyl acetate	$C_{22}H_{42}O_2$	338	2168	L
Phytol	$C_{20}H_{40}O$	296	2119	L

L=leaves, RH=Rhizomes, RT=Roots.

Table 2. Compounds determined by GC-MS from leaves

Compound	Molecular formula	Molecular weight	Retention Time [min]	Area%
6,10,14-Trimethylpentadecan-2- one	$C_{18}H_{36}O$	268	22.53	7.81
Methyl (Z)-octadec-9-enoate	$C_{19}H_{36}O_2$	296	28.49	3.14
3,7,11,15-Tetramethylhexadec- 2-en-1-ol	$C_{20}H_{40}O$	296	28.45	10.45

3,7,11,15-Tetramethylhexadec- 2-en-1-yl acetate	$C_{22}H_{42}O_2$	338	34.73	12.72

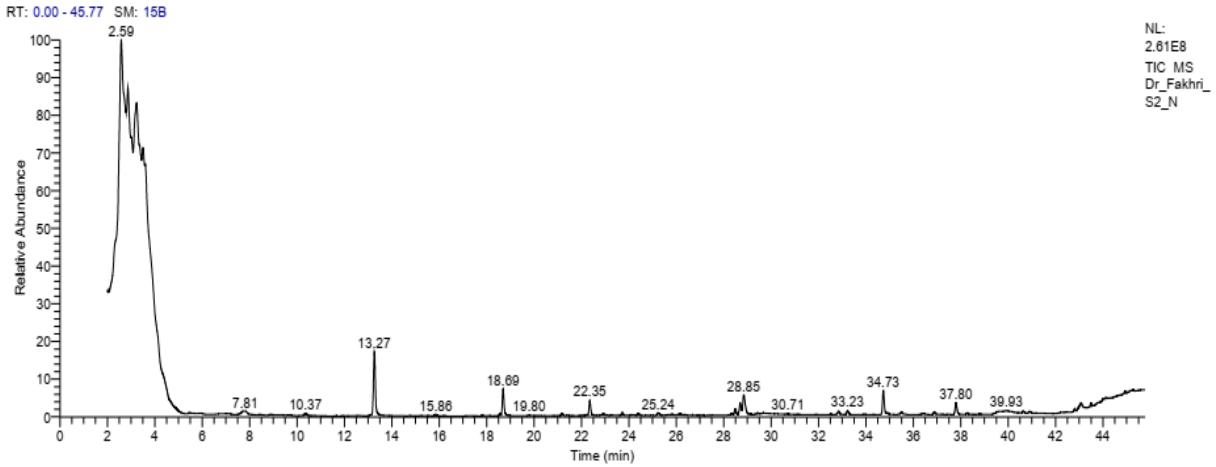

Fig 1. GC-MS Chromatogram of n-hexane extract of Posidonia oceanica leaves.

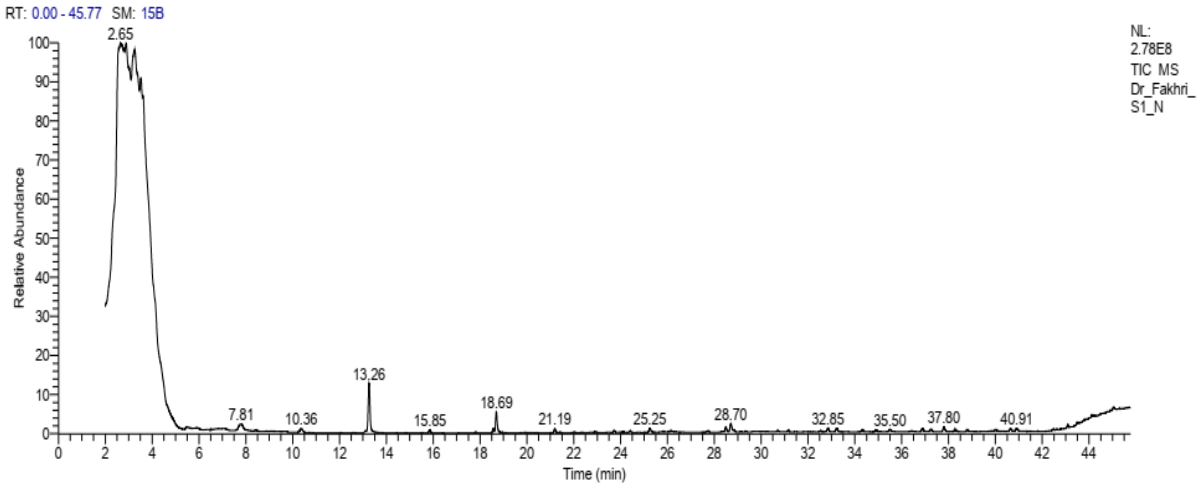

Fig 2. GC-MS Chromatogram of n-hexane extract of Posidonia oceanica rhizomes.

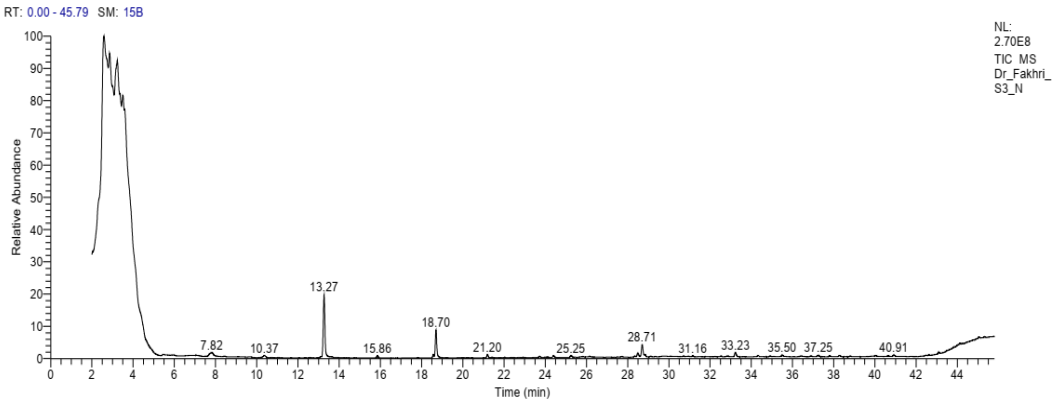

Fig 3. GC-MS Chromatogram of n-hexane extract of *Posidonia oceanica* roots.

Table 3. Mass fragmentations of compounds determined by GC-MS from leaves.

Compound	Molecular weight	Fragmentation ions [m/z]
6,10,14-Trimethylpentadecan-2- one	268	250, 225, 210, 165, 137, 95
Methyl (Z)-octadec-9-enoate	296	296, 265, 222, 180, 152, 123, 111, 97, 83, 71
3,7,11,15-Tetramethylhexadec-2-en-1-ol	296	193, 165, 123, 111, 95, 81, 71
3,7,11,15-Tetramethylhexadec-2-en-1-yl acetate	338	278, 263, 149, 207, 179, 123,109

Table 4. Compounds determined by GC-MS from rhizomes.

Compound	Molecular formula	Molecular weight	Retention time [min]	Area [%]
Tetradecane	$C_{14}H_{30}$	198	10.37	2.79
Hexadecane	$C_{16}H_{34}$	226	15.85	1.89
Octadecane	$C_{18}H_{38}$	254	21.19	2.47
Nonadecane	$C_{19}H_{40}$	268	22.82	1.28
Methyl hexadecanoate	$C_{17}H_{34}O_2$	270	24.40	1.05
Methyl (*Z*)-octadec-10-enoate	$C_{19}H_{36}O_2$	296	28.49	3.21
Docosane	$C_{22}H_{46}$	310	32.85	2.29
Heptacosane	$C_{27}H_{56}$	380	34.91	1.33
Pentatriacontane	$C_{35}H_{72}$	492	36.89	2.22
3-Ethyl-5-(2-ethylbutyl)octadecane	$C_{26}H_{54}$	366	38.80	1.29
Dotriacontane	$C_{32}H_{66}$	450	40.46	1.60

Table 5. Mass fragmentations of compounds determined by GC-MS from rhizomes.

Compound	Molecular weight	Fragmentation ions [m/z]
Tetradecane	198	198, 168, 141, 113, 99, 85, 71, 57
Hexadecane	226	226, 196, 169, 141, 113, 99, 85, 71, 57
Octadecane	254	254, 197, 169, 141, 113, 99, 85, 71, 57
Nonadecane	268	268, 211, 169, 141, 113, 99, 85, 71
Methyl hexadecanoate	270	270, 227, 185, 157, 143, 129, 97, 87, 74
Methyl (*Z*)-octadec-10-enoate	296	296, 254, 22, 180, 141, 111, 97, 85, 71
Docosane	310	324, 267, 223, 197, 169, 141, 113, 99, 85, 71
Heptacosane	380	281, 253, 207, 169, 141, 113, 99, 85, 71
Pentatriacontane	492	281, 253, 169, 141, 113, 99, 85, 71
3-Ethyl-5-(2-Ethylbutyl)octadecane	366	281, 253, 207, 169, 141, 113, 99, 85, 71
Dotriacontane	450	281, 253, 224, 207, 169, 141, 113, 99, 85, 71

Table 6. Compounds determined by GCMS from roots.

Compound	Molecular formula	Molecular weight	Retention Time [min]	Area [%]
Tetradecane	$C_{14}H_{30}$	198	10.37	1.85
Hexadecane	$C_{16}H_{34}$	226	15.85	1.59
Heptadecane	$C_{17}H_{36}$	240	18.56	1.56
Octadecane	$C_{18}H_{38}$	254	21.19	2.26
Docosane	$C_{22}H_{46}$	310	32.85	1.02
Methyl hexadecanoate	$C_{17}H_{34}O_2$	270	24.40	1.39
Methyl (Z)-octadec-9-enoate	$C_{19}H_{36}O_2$	296	28.49	2.99

Table 7: Mass fragmentations of compounds determined by GC-MS from roots.

Compounds	Molecular weight	Fragmentation ions [m/z]
Tetradecane	198	198,141,99,85,71,57
Hexadecane	226	226,182,149,113,99,85,71,57
Heptadecane	240	240,197,169,141,113,99,85,71,57
Octadecane	254	254,197,169,141,113,99,85,71,57
Docosane	310	268,183,155,127,113,99,85,71
Methyl hexadecanoate	270	270,227,199,171,143,129,97,87,74
Methyl (Z)-octadec-9-enoate	296	296,264,222,180,123,111,97,83,85,69

Material and methods

Collection of plant material

The plant was collected in September 2019 from Garyounis Beach in Benghazi City, east of Libya, and identified in the Botany Department by specialists. The plant was washed with water, and then the plant parts, including leaves, rhizomes, and roots, were separated from each other and washed with water twice to remove any remaining salts. Then, the plant parts were dried in a dark room using an air stream from a fan at 25OC for eight days.

Preparation of Plant Extracts

800 mg of each plant part (leaves, rhizomes, roots)were extracted with 750 ml of hexane in a Soxhlet extractor for 10 hours and splatted in two days The extracts were filtered and drying agent sodium sulfate anhydrase was used in the filtration process The extraction solvent was evaporated using an Airstream from a fan, and then the extracted oils were transferred and stored in dark bottles in a fridge until the determination with GC-MS.

GC-MS Analysis

The GC-MS analysis was carried out using gas chromatography-mass spectrometry instrument with the following specifications, Instrument: a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with a Thermo mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer).The GC-MS system was equipped with

a TR-5 MS column (30 m x 0.32 mm i.d., 0.25 µm film thickness) Analyses were carried out using helium as carrier gas at a flow rate of 1.0 mL/min and a split ratio of 1:10 using the following temperature program: 60 oC for 1 min; rising at 4.0 C/min to 240°C and held for 1min. The injector and detector were held at 210°C, Diluted samples (1:10 hexane, v/v) of 1 µL. of the mixtures were always injected, Mass spectra were obtained by electron ionization (El) at 70 eV, using a spectral range of m/z 40-450. The identification of the chemical constituents of the essential oil was de-convoluted using AMDIS software (www.amdis.net) and identified by its retention indices (relative to n-alkanes C8-C22), mass spectrum matching to (authentic standards (when available), Wiley spectral library collection and NSIT library database).

Conclusions

The analysis revealed the presence of sixteen compounds, with the most dominant compounds found in the rhizomes. Among the sixteen compounds, nine were identified as long-chain hydrocarbons, and three were long-chain fatty acids. Notably, this study reports the first discovery of 3-Ethyl-5-(2-ethylbutyl) Octadecane, phytol, phytyl acetate, and 6,10,14-Trimethylpentadecan-2-one from the plant. In contrast, these findings provide valuable insights into the chemical composition of *Posidonia oceanica* Seagrass, particularly in the Mediterranean region. The dominance of rhizomes in the reported compounds indicates their significance in the plant's volatile organic compound profile. This research contributes to the limited knowledge available on the volatile organic compounds of this seagrass species and sheds light on its potential ecological and pharmaceutical applications.

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