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Research Article

Gas Chromatography-Mass Spectrum (GC-MS) Analysis of Bioactive Components of the Methanol Extract of Halophyte, *Sesuvium portulacastrum* L.

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ABSTRACT

Sesuvium portulacastrum L., (family: Aizoaceae), commonly known as "sea purslane" has long been used as food and traditional medicine. Traditionally plants are used in the treatment of epilepsy, conjunctivitis, dermatitis, haematuria, leprosy, purgative, fever, kidney disorders, and scurvy and also used to therapy toothache. Leaves have acidulous flavor of sorrel as well as antiscorbutic. The present investigation deals with the identification of phytochemical constituents of the methanol extracts by GC-MS analysis. Number of underivatized constituents identified in the pet methanol extracts, by comparing with the reference spectra's of NIST & WILEY libraries, were found to be twelve chemical constituents. The major chemical constituents were hentriacontane (26.26), pyrrolo[1,2-a]pyrazine-1, 4-dione, hexahydro-3-(2-methylpropyl) (22.22), l-(+)-ascorbic acid 2,6-dihexadecanoate (18.14), phenol, 2,4-bis(1,1-dimethylethyl) (13.15) and octadecanoic acid (19.97). These compounds have been found to possess antimicrobial, antioxidant, anticancer, and antiulcerogenic activities.

Keywords: Halophyte; *Sesuvium portulacastrum*, Methanol Extract, GC-MS, Hentriacontane.

1. INTRODUCTION

The pharmaceutical, cosmetic and food industries are constantly being faced with the challenge of identification, isolation and characterization of volatile compounds of medicinal importance in plant materials. Medicinal plants have been used by human being since ages in traditional medicine due to their therapeutic potential and the search on medicinal plants have led to the discovery of novel drug candidates used against diverse diseases. According to the World Health Organization (WHO) in 2008, more than 80% of the world's population relies on traditional medicine for their primary healthcare needs[1]. Plants are a rich source of secondary metabolites with interesting biological activities. In general, these secondary metabolites are an important source with a variety of structural arrangements and properties[2].

A knowledge of the chemical constituents of plants is desirable not only for the discovery of therapeutic agents, but also because such information may be of great value in disclosing new sources of economic phytocompounds for the synthesis of complex chemical substances and for discovering the actual significance of folkloric remedies[3]. Hence a thorough validation of the herbal drugs has emerged as a new branch of science emphasizing and prioritizing the standardization of the natural drugs and products because several of the phytochemicals have complementary and overlapping mechanism of action. Mass spectrometry, coupled with chromatographic separations such as Gas chromatography (GC/MS) is normally used for direct analysis of components existing in traditional medicines and medicinal plants. In recent years GC-MS studies have been increasingly applied for the analysis of medicinal plants as this technique has proved to be a valuable method for the analysis of non polar components and volatile essential oil, fatty acids, lipids[4] (Jie and Choi, 1991) and alkaloids[5](Betz *et al.*, 1997).

Halophytes grow in a wide variety of saline habitats, from coastal sand dunes, salt marshes and mudflats to inland deserts, salt flats and steppes[6]. These plants are characterized by a high physiological plasticity not only for their salt tolerance limits, but also for the climatic zone from which they originate. A geographical classification differentiates between hydrohalophytes, typical from brackish wetlands, and xerohalophytes, that are particularly well-adapted to deserts and low-moisture environments[7]. Recently, works on halophyte species, native of saline biotopes characterised by a high physiological plasticity, showed that these plants display strong antimicrobial activity, powerful scavenging capacity against free radicals and high polyphenol levels[8, 9].

Sesuvium portulacastrum (L.), a member of the family Aizoaceae, is an important halophyte in the category of ‘‘salt accumulator’’ plants which accumulates high salt concentration in their cells and tissues and overcomes salt toxicity by developing succulence. This plant is used as a fodder for animals and has an ornamental value since it blooms throughout the year in the barren areas[10]. Due to its survival in adverse environmental conditions, the plant is recognized as a promising candidate for the environmental protection [10, 11, 12, 13, 14, 15, 16, 17, 18].Medicinally and economically, Sesuvium containing secondary metabolites has shown a great potential as a substitute for some synthetic raw materials in the food, perfumery, cosmetic and pharmaceutical industries[19]. This plant is used in traditional medicine as a remedy for fever, kidney disorders and scurvy[20]by the indigenous people in Africa, Latin America and in Asian countries such as India, China, Pakistan and Japan. The plant is used on the Senegal coast as a haemostatic and a decoction of it is considered to be the best known antidote for stings of venomous fish. Leaves have acidulous flavor of sorrel as well as antiscorbutic[21]. *S. portulacastrum*expresses fatty acid methyl esters (FAME extract) which can be used in medicine as a potential antimicrobial and antifungal agent[22].The essential oil from the fresh leaves of *S. portulacastrum* exhibited antibacterial, antifungal and antioxidant activity[23].Thus, the aim of the present work was to identify the phytochemical constituents with the aid of GC-MS technique.

2. MATERIALS AND METHODS

2.1. Collection of plant material

The Fresh leaves and stems of *Sesuvium portulacastrum* were collected from Vellar estuary (Lat. 11^0 29’N and Long.79^0 49’E), Parangipettai, Tamilnadu, India in December 2012. The collected specimens were identified based on the manual by Kathiresan[24].Withered leaves and stems were rinsed under running tap water to eliminate dust. After that samples were washed several times with distilled water and air-dried at 25-30^0C for about 3-5 days. The dried samples were ground to fine powder using mortar and pestle. The powder was passed through a sieve of 22 mm mesh size.

2.2. Preparation of extract

The air dried plant powder (10g) was extracted with 100ml of methanolsolvent in 1:10 ratio. This mixture was kept in mechanical shaker up to 72 hours for separation of bioactive compounds. The extract was filtered through filter paper (Whatmann No. 1) and allow to evaporation in a room temperature. Weigh the extract obtained with each solvent and calculate its percentage of the dried weight of the plant material. The obtained extracts were stored then subjected to further analysis.

2.3. Identification of phytochemicals through GC - MS analysis

GC-MS technique was used in this study to identify the components present in the extract. GC-MS technique was carried out at VIT University, Vellore, Tamil Nadu. GC-MS analysis of this extract was performed using a Perkin Elmer GC Claurus680 system and gas chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with Elite-5MS column (30.0m, 0.25mmID, 250µm df). For GC-MS detection, an electron ionization energy system with ionization energy of 70eV was used. Helium gas (99.99%) was used as the carrier gas at a constant flow rate of 1ml/min. and an injection volume of 1µl was employed (Split ratio of 10:1). Injector temperature was 250ºC. The oven temperature was programmed from Initial temp 60°C for 2 min, ramp 10°C/min to 300°C, hold 6 min. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 50 to 600 Da. Total GC running time was 32 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a Turbomass Ver5.4.2.

2.4. Identification of Components

Interpretation on mass spectrum of GC-MS was conducted using the data base of National Institute Standard and Technology (NIST) and Wiley spectra Libraries. Spectrum of the unknown component was compared with the spectrum of known components stored in the NIST Library having more than 62,000 patterns. The molecular weight, molecular formula and the number of hits used to identify the name of the compound from NIST and Wiley spectra Libraries were recorded.

Table 1
PHYTOCOMPONENTS IDENTIFIED IN THE METHANOLIC EXTRACTS OF THE WHOLE PLANT OF *SESUVIUM PORTULACASTRUM*BY GC-MS

S. No	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1.	13.158	PHENOL, 2,4-BIS(1,1-DIMETHYLETHYL)-	C14H22O	206	18.571
2.	16.904	PYRROLO[1,2-A]PYRAZINE-1,4-DIONE, HEXAHYDRO-3-(2-METHYLPROPYL)-	C11H18O2N2	210	6.789
3.	17.279	BUTANOIC ACID, PYRROLIDIDE	C8H15ON	141	3.799
4.	17.865	L-PROLINE, N-VALERYL-, HEXADECYL ESTER	C26H49O3N	423	3.403
5.	18.030	PYRROLO[1,2-A]PYRAZINE-1,4-DIONE, HEXAHYDRO-3-(2-METHYLPROPYL)-	C11H18O2N2	210	6.853
6.	18.140	L-(+)-ASCORBIC ACID 2,6-DIHEXADECANOATE	C38H68O8	652	33.988
7.	18.295	HEPTACOSYL HEPTAFLUOROBUTYRATE	C31H55O2F7	592	4.114
8.	19.975	OCTADECANOIC ACID	C18H36O2	284	9.943
9.	22.221	PYRROLO[1,2-A]PYRAZINE-1,4-DIONE, HEXAHYDRO-3-(PHENYLMETHYL)-	C14H16O2N2	244	5.751
10.	24.887	HENTRIACONTANE	C31H64	436	1.723
11.	25.588	HENTRIACONTANE	C31H64	436	1.911
12.	26.263	HENTRIACONTANE	C31H64	436	1.605

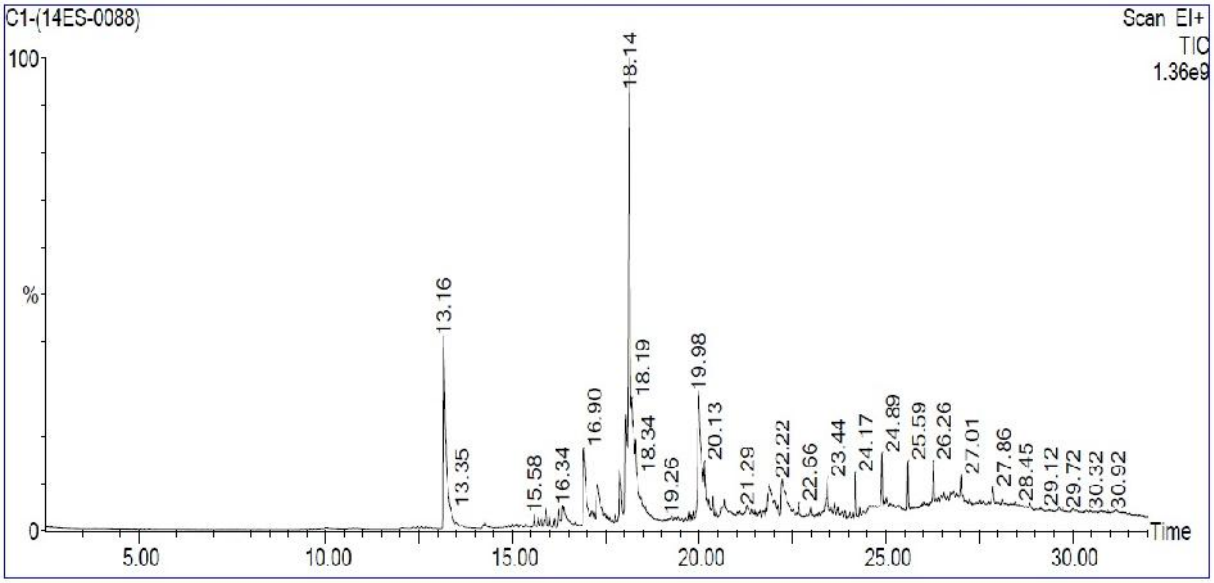

Figure 1
GC-MS pattern of Phytoconstituents obtained from *Sesuvium portulacastrum*

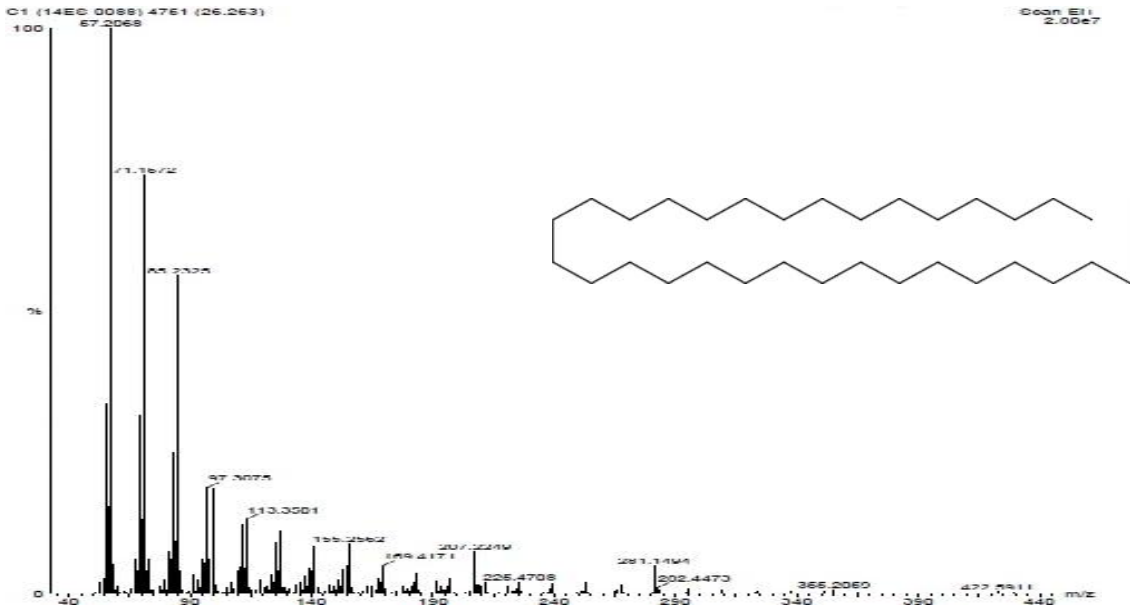

Figure 2
The mass spectrum analysis and structure of Hentriacontane

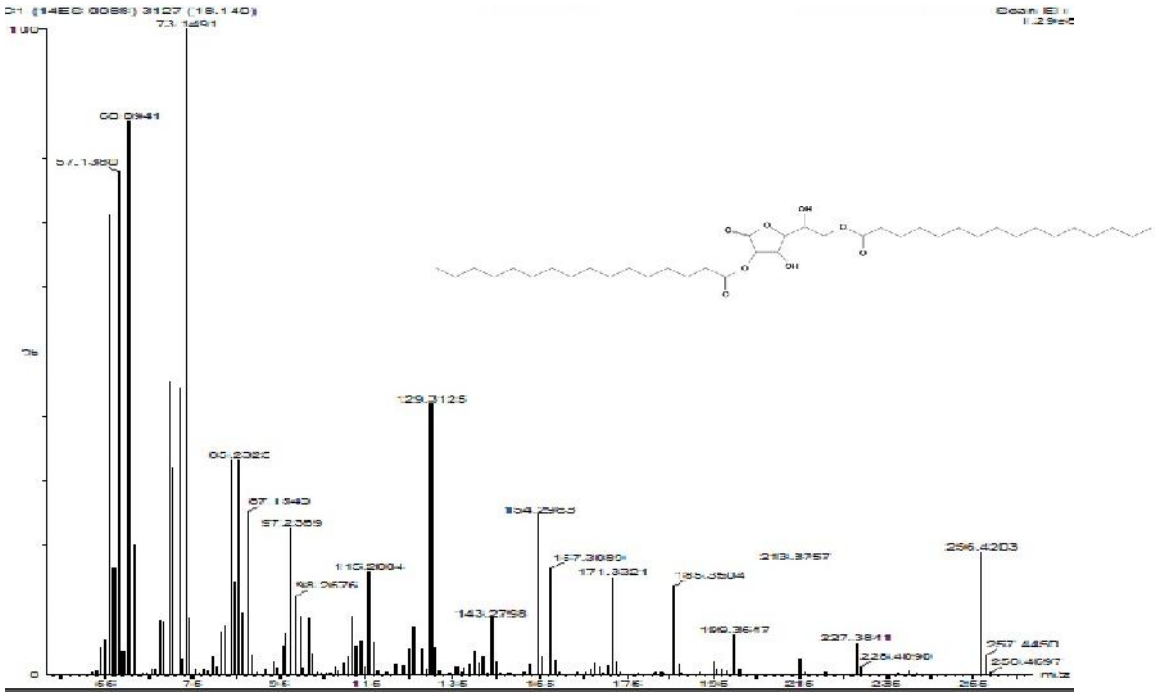

Figure 3
The mass spectrum analysis and structure of L-(+)-ascorbic acid, 2-6-dihexadecanoate

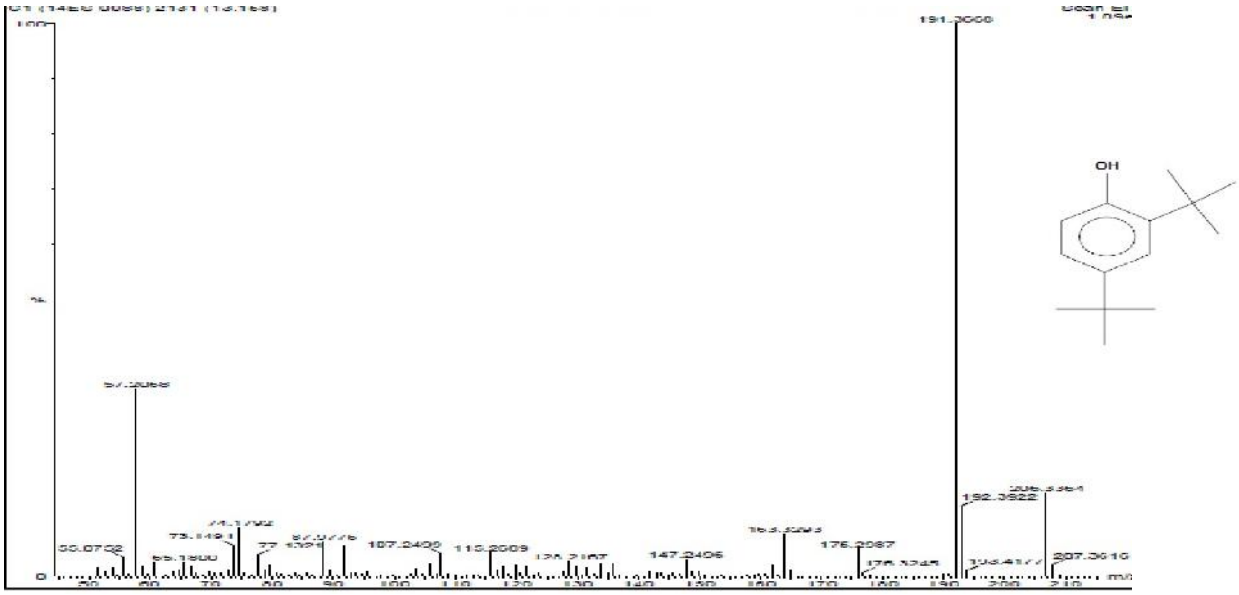

Figure. 4
The mass spectrum analysis and structure of phenol, 2,4-bis(1,1-dimethylethyl)

3. RESULTS AND DISCUSSION

The more precise information in qualitative analysis can be obtained by gas-chromatography coupled with mass spectrometry[25]. For quantitative determination, gas-chromatography with flame ionization detector (GC-FID) and GC-MS are preferred[26, 27, 28].The compounds present in the methanolic extract of *Sesuvium portulacastrum were* identified by GC-MS analysis (Figure 1).

On comparison of the mass spectra of the constituents with the NIST library, thirteen peaks were obtained out of which twelve phytoconstituents were characterized and identified.

The active principle Molecular Weight (MW), concentration (%), molecular Formula (MF), and retention time (RT) is presented in Table 1.

Among the twelve compounds identified after GC-MS, one of compoundsHentriacontane, a saturated hydrocarbon, the major compound was identified has been isolated from*Scabiosacomosa*. It is reported to be responsible for its uptake in the soil by plant and shown to be involved with stimulation of fungal spore germination. Hentriacontane has also been isolated from spinach leaves, and discovered to be unsaponifiable and shown to have possible anti-tumour activity[29]. Other compound phenol, 2, 4-bis (1, 1-dimethylethyl), theiranti-inflammatory activity, in comparison with indomethacin and vitamin E[30] (Costantino*et.al.*,1993). L-(+)-ascorbic acid, 2-6-dihexadecanoate which is a derivative of ascorbic acid, vitamin C, is present in the essential oil. Vitamin C is an antioxidant and belongs to the class of compounds identified to enhance sperm quality and prevent sperm agglutination, thus making them more motile with forward progression and hence promote male fertility[31, 32]. L-(+)-ascorbic acid, 2-6-dihexadecanoate has also been isolated from *Ipomoea pes-caprae (L.) R.Br* leaves[33].

They were identified as three major phytochemical constituent's mass spectra are presented in Figure 2-Figure 4. They were identified as Hentriacontane, L-(+)-ascorbic acid, 2-6-dihexadecanoate and phenol, 2,4-bis(1,1-dimethylethyl).

4. CONCLUSION

Therefore, GC-MS method is a direct and fast analytical approach for identification of phytocompoundsand only few grams of plant material is required. The importance of the study is due to the biological activity of some of these compounds. The present study, which reveals the presence of components in *Sesuvium portulacastrum* suggest that the contribution of these compounds on the pharmacological activity should be evaluated. Thus the plant studied can be used as a potential source of new useful drugs. The phytochemical characterization of the extracts, the isolation of responsible bioactive compounds and their biological activity are necessary for future studies.

5. ACKNOWLEDGEMENT

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