International Journal of Chemical and Physical Sciences, ISSN:2319-6602
IJCPS Vol. 4 Special Issue – NCSC Jan-2015
www.ijcps.org

GC-MS analysis of the liverwort *Plagiochasma appendiculatum* Lehm. *et* Lindenb.

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Abstract

Bryophytes are the most primitive, non-vascular and first terrestrial plants which pioneered the process of conquest on the land. It is second largest group in the plant kingdom after angiosperms. Bryophytes can be found in any habitat globally where photosynthesis is possible. They are divided in three classes – mosses, liverworts and hornworts. An important direction in bryophyte studies is analysis of their biologically active substances, particularly the secondary metabolites. The Bryophyte Plagiochasma appendiculatum is a wavy, evergreen, thalloid liverwort commonly found in Melghat forest and known for antimicrobial, antifungal and antifeedent activities. Studies on biologically active compounds in bryophytes are rapidly growing, resulting in identification of a large number of specific substances with high biological activity. The methanolic extract obtained from this plant and subjected to Gas Chromatography and Mass Spectroscopy for the determination of bioactive volatile compounds. Quantitative determinations were made by relating respective peak areas to TIC areas from the GC-MS. Chemical composition showed compounds like Caryophyllene, Phytol, hexacosane and Heneicosane in the analysis. Volatile organic compounds emitted from plants can originate from biogenic and or anthropogenic sources. Many plants emit substantial amounts of phytogenic volatile organic compounds which include Alkanes, Alkenes, Alcohols, Aldehydes, Ethers, Esters and Carboxylic acids.

Introduction

Most of the bryophytes used as medicinally important plants due to characteristics pharmacognostic compounds present in the thalli. Asakawa (1981, 1984) reported the presence of terpenoids and lipophillic aromatic compounds in liverworts as potent source of antibiotics. Using advanced Gas Chromatography and Mass Spectroscopy (GC-MS) techniques compounds like monoterpenoids, sesquiterpenoids, diterpenoids, bicarbocyclic diterpenoids, triterpenoids, sterols, flavonoids, phenolic compounds and fatty acids can be found out (Banerjee, 2001). The characteristic adour, earthy and pungent smell of bryophytes is due to presence of various chemical constituents present in the thalli. Asakawa *et al.,* (2013) suggested that Bryophytes emit volatile terpenoids of simple aromatic compounds responsible for intense terpenic, mushroomy, sweet woody, sweet mossy, seaweed like or carrot like adour.

Material and Methods

The plant material get collected from the Melghat forest during rainy season in sealed container to avoid interaction with air nitrogen. The material get cleaned, washed, shade dried and powdered by the blender.

The fraction of methanolic extract obtained from this powder and subjected to Gas Chromatography and Mass Spectroscopy for the determination of bioactive volatile compounds. GC-MS analysis of the sample was carried out using Shimadzu Make QP-2010 with non polar 60 M RTX 5MS Column. Helium was used as the carrier gas and the temperature programming was set with initial oven temperature at 40^0C and held for 3 min and the final temperature of the oven was 480^0C with rate at 10^0C [min.sup.1]. A 2-µL sample was injected with split less mode. Mass spectra was recorded over 35 - 650 amu range with electron impact ionization energy 70 eV. The chemical components from the methanolic extract of plant was identified by comparing the retention times of chromatographic peaks using Quadra pole detector with NIST Library to relative retention indices. Quantitative determinations were made by relating respective peak areas to TIC areas from the GC-MS.

Results and discussion:

To trace the possible chemical compounds, Gas Chromatography and Mass Spectroscopic analysis of crude methanolic extract done. The compound obtained from GC-MS were identified with compare with mass spectral analysis of the liverwort *Plagiochasma appendiculatum* (Fig:1) and also revealed the presence of compounds like Caryophyllene, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, n-Hexadecanoic acid, Phytol, Hexacosane and Heneicosane. (Table: 1)

Fig:1 GC-MS chromatogram of *Plagiochasma appendiculatum*

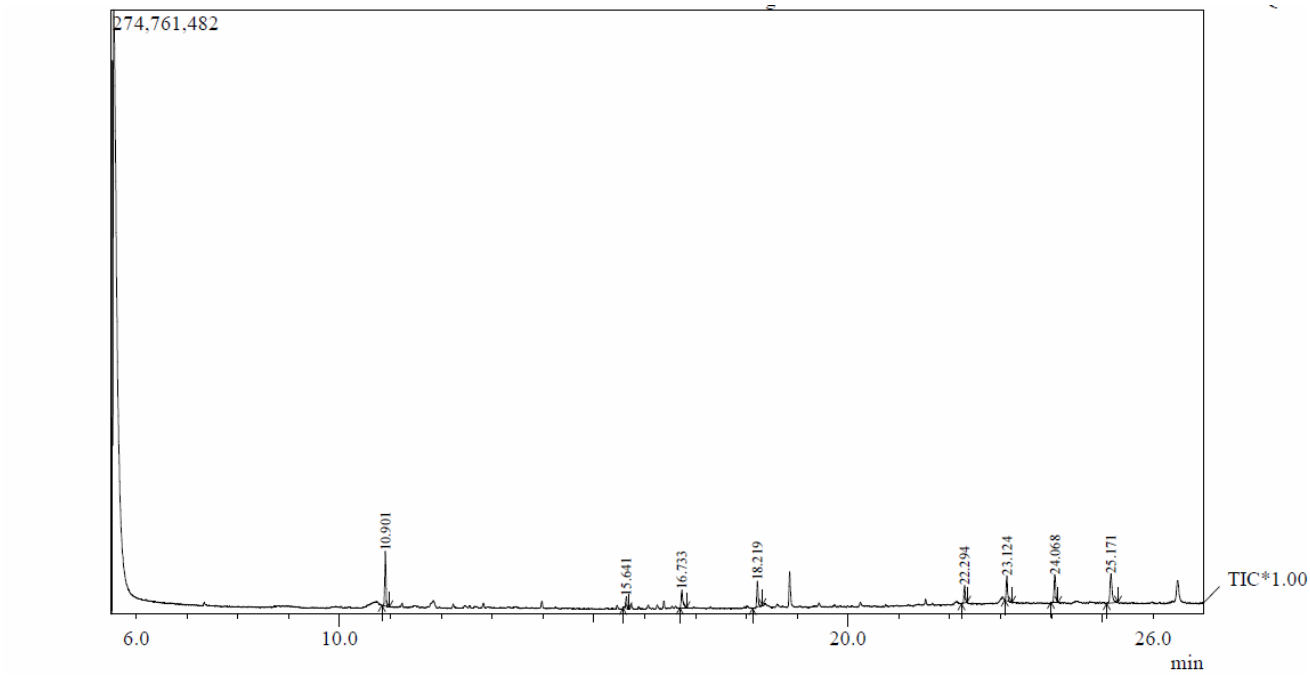

Table : 1 : compounds analyzed from mass spectrum

Sr. No.	Retention time	% area of the peak	Compound analyzed	Molecular formula	Molecular weight	Common Name	Activity reported*
1	10.90	20.70	Caryophyllene	$C_{15}H_{24}$	204	Sesquiterpene	Antibacterial, Antifungal and Cytotoxicity
2	15.64	5.19	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	Terpene alcohol	Anti-inflammatory Antibacterial, and Antifungal
3	16.73	9.80	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	Palmitic acid	Antioxidant, Hypocholesterolemic Nematicide, Pesticide, Lubricant,
4	18.21	10.15	Phytol	$C_{20}H_{40}O$	296	Diterpene	Antimicrobial Anti-inflammatory Anti-cancer, Diuretic
5	22.29	7.72	Hexacosane	$C_{26}H_{54}$	366	Alkane	Anti-inflammatory
6	23.12	10.44	Heneicosane	$C_{21}H_{44}$	296	Alkane	Anti-inflammatory
7	24.06	14.89	Heneicosane	$C_{21}H_{44}$	296	Alkane	Anti-inflammatory
8	25.17	21.11	Hexacosane	$C_{26}H_{54}$	366	Alkane	Anti-inflammatory

* http://www.ars-grin.gov/duke/ Dr. Duke's Phytochemical and Ethnobotanical Databases**,** Jim Duke, Green Pharmacy Garden, 8210 Murphy Road, Fulton, MD 20759.

The chemical most sTable compound found in *Plagiochasma appendiculatum* is Caryophyllene. This shows a natural bicyclical sesquiterpene which is a sTable constituent of many essential oil like clove and pepper. This compound is noTable for having cyclobutane ring i.e. a rarity in nature (Chopra and Kumar,1988). Claude, (2013) found that on insects attacks, these terpenoids will attract nematodes, that will destroy the larvae of these herbivorous insects. Interestingly, the author observed that the thallus of this plant species never infected by any pathogens rather than provides shelter to many invertebrates, earthworms around the thalli and soil. A typical aromatic smell always rendered during collection of these plants across Melghat region. Ozturk *et al.*, (2009) reported the antimicrobial activity of such essential oil against *P. aeruginosa* and *S. aureus*. Xian *et al.*, (2006) reported that Caryophyllene from *Marchantia convoluta* with cytotoxic effect of leaf extracts to human liver and lung cancer cells. Hence, this naturaly occurring compounds have potential medicinal properties.

3, 7, 11, 15-Tetramethyl-2-hexadecen-01-ol is a diterpene alcohol. It is by-product from the production of chlorophyll and essential material to produce vitamin K1 and vitamin E. It commonly occurs in bryophytes species *P. appendiculatum.* Lalifa (2012), reported antibacterial, anti-fungal and anti inflammatory activity of essential oil like hexadeconoic acid, methyl ester against, *E. coli, S. aureus, C. albicans* and *P. aeruginosa* extracted from plant *Carduus pychocephalus* L. The bryophytes like *P. appendiculatum* showed the presence of chemical compound like n-Hexadecanoic acid or Palmitic acid. This is most common saturated fatty acids either found in plants, animals and even microorganisms. It mainly occurs as its ester in triglycerides (Fats).

Phytol is an acyclic diterpene alcohol that can be used as precursor of synthetic forms of vitamin E and vitamin K. Yoshihiro *et al.,* (2005) showed that phytol is a constituent of chlorophyll and had unique antibacterial activity to inhibit pathogen like *S. aureus*. This activity was reported in present work especially in species *P. appendiculatum*. Moreover, Venci and Morton, (1998) reported that *Sumac Flea beetle* use phytol and its metabolites as a shield defense or as chemical deterrents against predation. The species *Plagiochasma appendiculatum* also showed the presence of unique compounds like Hexacosane and Heneicosane. These compounds are higher alkanes with higher number of carbon atoms. Agnihotri *et al.* (2010) reported the anti-inflammatory properties of these compounds in traditional medicines.

Conclusion

The GC-MS data obtained from the plant extract revealed the presence of chemical constituents like sesquiterpenoids i.e. caryophyllene, the terpenes like phytol 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-01, the hydrocarbons group of alkanes compounds like hexacosane and heneicosane in the analysis. The present state of knowledge in this matter reveals that the bryophytes in future may prove to be a rich store-house of hitherto unknown drugs.

Acknowledgement

The authors are thankful to common facility centre at Shivaji University Kolhapur, M.S. for the help in GC-MS analysis of the plant extract.

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