ISSN (Online) 2249-6084 (Print) 2250-1029

International Journal of Pharmaceutical and Phytopharmacological Research (eIJPPR)
[Impact Factor – 0.852]

Journal Homepage: www.eijppr.com

Research Article *Article ID: 391*

GC-MS analysis of the ethanol extract of *Biophytum sensitivum* (L.) DC (Oxalidaceae)

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Article info

Article History:
Received 1 June 2015
Accepted 29 June 2015

Keywords:
Ruminal artery, Nitric oxide, sGC, cGMP.

Abstract

The medicinal plant *Biophytum sensitivum* (L.) DC possess a wide spectrum of medicinal properties and are therefore used in the treatment of for asthma, snakebites, stomachalgia and phthisis, positive effects in inflammatory diseases, anti-tumor activities and antioxidant activity. In the present study, ethanol extracts from *Biophytum sensitivum* were subjected to GC-MS analysis to study the important phytochemical constituents responsible for the above reported pharmacological activities. The crude extracts of ethanol were obtained by soxlet method. The GC-MS analysis of ethanol extract from *Biophytum sensitivum* revealed the presence of eighteen phytocompounds. The compounds were identified by comparing their retention time and peak area with that of literature and by interpretation of mass spectra. The most prevailing major compounds were 9, 12-Octadecadienoic acid (Z,Z) (50.36%), n-Hexadecanoic acid (17.42%), 9,12-Octadecadienoic acid, methyl ester (E,E) (6.34%) and 2-Pentadecanne,6,10,14-trimethyl- (3.31%).

1. INTRODUCTION

The use of local plants in folk medical practices has a long history. The resource base of the traditional medical practices prevalent in rural and tribal villages of India and abroad is mainly the plants. Medicinal plants are used to maintain and promote healthy life, prevent disease and cure ailments. It has been estimated that even today, 80% of the world population rely on herbal traditional medicine for their primary health care. Traditional knowledge of medicinal plants has always guided the search for new cures. In spite of the advent of modern high throughput drug discovery and screening techniques, traditional knowledge systems have given clues to the discovery of valuable drugs[1]. Standardization is an essential measure of quality, purity and authenticity. The standardization of crude drug is an integral part for establishing its correct identity. Also, WHO has emphasized the need to ensure the quality of medicinal plants products using modern controlled technique and applying suitable standards[2]. Gas chromatography-mass spectrometry (GC-MS) is the best technique to identify the bioactive constituents of long chain hydrocarbons, alcohols, acids, esters, alkaloids, steroids, amino acid and nitro compounds [3, 4, 5]. Therefore, characterization of extracts of medicinal plants is necessary due to its numerous benefits to science and society. Review of literature divulges that information on the GC-MS analysis of *Biophytum sensitivum* (L.) DC belonging to the family Oxalidaceae is totally lacking. Hence, the objective of the present study is to identify the phytochemical constituents with the aid of GC-MS technique. This work will help to identify the compounds of therapeutic value.
Pharmacologically, the *Biophytum* plant has been investigated for its hypoglycemic[6], hypocholesterolemic[7], and anti-cancer effect[8]. It is a known traditional remedy for the treatment of diabetes[9] and anti-tumor activities[10].

1.1 Plant description

The little plant grows up to maximum of 20 cm and possess unbranched woody erect stem. Leaves abruptly pinnate, leaflets opposite, 6 to 12 pairs, and each leaflet is up to 1.5 cm long, the terminal pair is the largest. The flowers are many and crowded at the apices of the numerous peduncles, normally yellow, white, or orange with red streak in the center of each of the five petals. The sepals are subulate-lanceolate, striate, and about 7 mm long. Fruits are ellipsoid capsules which are shorter than the persistent calyx[11].

2. MATERIALS AND METHODS

2.1 Plant material

The medicinal plant *Biophytum sensitivum* (L.) DC was collected from Tirunelveli District, Tamil Nadu, India. The identified plant species was confirmed with Voucher specimen available in the Survey of Medicinal Plant Unit (SMP), Govt. Siddha Medical College, Palayamkottai, Tirunelveli, Tamil Nadu. The taxonomic features of the plant confirmed with the Flora of Presidency of Madras [12] and The Flora Tamil Nadu Carnatic[13].

2.2 Soxhlet extraction

About 60 g dried sample was refluxed with 250 ml of the ethanol for 5 hour on a steam bath. The extract was collected and concentrated.

2.2.1 Procedure

The GC - MS analyses were carried out in a Shimadzu GC – MS - QP 2010 gas chromatograph fitted with a DB1 (methylphenylsiloxane, 30 m × 0.25 mm i.d.) capillary column. Carrier gas, helium with a flow rate of 1 ml/min; column oven temperature 70^{o} C, 5 min in 180°C, 180-260°C at 3°C/min, 5 min in 260°C, 260-280°C at 0.2°C/min, and finally 5 min in 280°C; injector temperature, 280°C detector temperature, 290°C, volume injected, 2 µL: Split ratio, 10:1. The MS operating parameters were as follows: ionization potential 70 eV; ion source temperature 200°C; quadrupole 100°C, solvent delay 6.0 min , scan speed 2000 amu/s, total MS running time 36min. and scan range 30-600 amu, eV voltage 3000 volts.

The concentrated extract is injected into the GC/MS instrument (Hewlett Packard 5890 GC/MS with Mass Selective Detector with Turbo mass gold-perkin Elmer). The sample is volatilized at the injection port and eluted through a capillary column under increasing temperature. As the sample moves through the column, various components are separated due to their affinity for the stationary phase of the column and can be identified by retention time (the time it takes for a compound to pass through the column and gas chromatograph system). Each chemical component in a sample has a distinct retention time measured in minutes, shown in a peak on a graph which measures abundance on the ordinate against retention time on the abscissa. The integrated peak is correlated to the concentration of the chemical. A mass selective detector breaks up each chromatographic component into fragment ions, which are shown by their abundance, with each ion represented as a vertical line in increasing molecular weight. The height of each line corresponds to the abundance of that ion. The resulting mass spectrum is unique to that chemical. This mass spectrum forms a ‘‘fingerprint’’ that can identify the compound by a computer search of mass spectra. A computer search of the mass spectra corresponding to all the chromatographic peaks for a sample should yield a statistical match for nicotine at a 12·9 min retention time value if they were present two modes of GC/MS were possible with this instrumental method. First, there is a ‘‘Scan’’ mode which looks at all the constituents of a sample, listing whatever chemical components are present.

2.2.2 Compound Identification

Components of the methnolic extracts were identified by comparison of their mass spectra and retention indices with those published in the literature and contained in the NIST 2005 MS computer library (Wiley).

3. RESULTS

The bioactive compounds present in the ethanolic extract of leaf of *Biophytum sensitivum* were identified by GC-MS analysis (Figure 1). Eighteen compounds were detected in the ethanolic extract of *Biophytum sensitivum*. The active principles with their retention time, molecular formula, molecular weight and concentration (%) in the ethanol extracts of leaf of *B. sensitivum* are presented in Table 1, and the total running time was 36 min. The spectra of the compounds were matched with Wiley 9.0 and National Institute of Standards and Technology libraries. The most prevailing major compounds were 9,12- Octadecadienoic acid (Z,Z) (50.36%), n-Hexadecanoic acid (17.42%), 9,12-Octadecadienoic acid, methyl ester (E,E) (6.34%) (Fig: 2), 2-Pentadecanne,6,10,14-trimethyl- (3.31%), *n*-hexadec Trans-3-Caren-2ol (3.16%), *etc.*, and minor components were 2-Cyclopentene -1-undecanoic acid(+) (0.39%), Oleic acid (0.99%), and 9-Octadecenal (0.70 %). 9,12- Octadecadienoic acid is a unsaturated fatty acid, occurring widely in plant glycosides. It is an essential fatty acid in mammalian nutrition and is used in the biosynthesis of prostaglandins and cell membranes. It is commonly called as Linoleic acid. It is an essential fatty acid that must be consumed

for proper health. It can be used to show the antioxidant effect of natural phenols. Unsaturated fatty acids are important to every cell in the body for normal growth, especially of the blood vessels and nerves and to keep the skin and other tissues youthful and supple trough their lubricating quality[14]. Linolenic acid possesses anti-inflammatory, insectifuge, hypocholesterolemic, cancer preventive, nematicide, hepatoprotective, antihistaminic, antieczemic, antiacne, 5-alpha reductase inhibitor, antiandrogenic, antiarthritic and anticoronary properties[15]. n-Hexadecanoic acid can be an antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant activities and hemolytic 5-alpha is a reductase inhibitors [16, 17]. *Euphorbia longan* leaves mainly contained n-hexadecanoic acid and 9, 12-Octadecadienoic acid. Hexadenoic acid has earlier been reported as a component in alcohol extract of the leaves of *Kigelia pinnata*[18] and *Melissa officinalis*[19]. 17 compounds with n-Hexadecanoic acid and Octadecanoic acid as the major compounds in the leaves of *Cleistanthus collinus* [20.] GC-MS analysis of ethyl acetate extract of *Goniothalamus umbrosus* revealed the presence of n-Hexadecanoic acid[21].

Table: 1 GC MS analysis of *Biophytum sensivitum*

No.	RT	Name of the compound	Molecular formula	Molecular Weight	Peak area %
1.	2.06	À-Pinene	$C_{10}H_{16}$	136	1.72
2.	2.32	Trans-3-Caren-2ol	$C_{10}H_{16}O$	152	3.16
3.	2.52	1,4-Cyclohexadiene, 1-methyl-1-4-(1-methylethyl)-(ǫ_terpinen)	$C_{10}H_{16}$	136	1.27
4.	11.05	7-Octen-1-ol, 3,7-dimethyl-(S) – (à-Citronellol)	$C_{10}H_{20}O$	156	2.05
5.	11.15	2-Pentadecanne,6,10,14-trimethyl-	$C_{18}H_{36}O$	268	3.31
6.	11.37	E-7-Tetradecenol	$C_{14}H_{28}O$	201	0.54
7.	12.79	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	17.42
8.	14.02	2-Cyclopentene -1-undecanoic acid(+)	$C_{16}H_{28}O_2$	252	0.39
9.	14.85	9,12-Octadecadienoic acid, methylester (E,E)	$C_{19}H_{34}O_2$	294	6.34
10.	15.27	9,12- Octadecadienoic acid (Z,Z)	$C_{18}H_{32}O_2$	280	50.36
11.	19.39	7-Hexadecenal(Z)-	$C_{16}H_{30}O$	238	1.11
12.	19.97	9-Octadecenal	$C_{18}H_{34}O$	266	0.70
13.	22.17	3-Hexadecyloxycarbonyl 1-5-(2-hydroxyethyl) -4-methylimidazolium ion	$C_{24}H_{45}N_2O_3$	409	1.88
14.	23.71	2,6,10-Decacatrien-1-ol,3,7,1,-trimethyl,(E,E)-	$C_{15}H_{26}O$	222	2.14
15.	24.69	Pentanoic acid, 10-undecentryl ester	$C_{16}H_{30}O_2$	254	1.42
16.	27.27	Oleic acid	$C_{18}H_{34}O_2$	282	0.99
17.	27.40	1-Nonadecanol	$C_{19}H_{40}O$	284	2.77
18.	28.00	Vitamin E	$C_{29}H_{50}O_2$	430	2.44

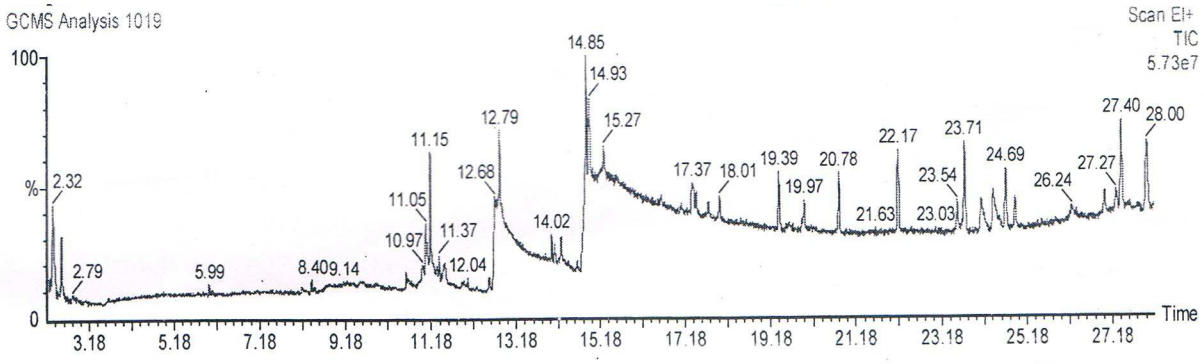
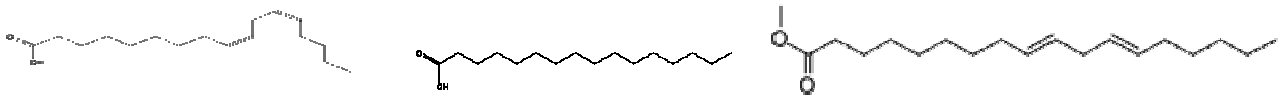


Fig: 1 Chromatogram of *Biophytum sensitivum* ethanol extract



9,12-Octadecatrienoic acid (Z,Z) **n-Hexadecanoic acid** **9,12-Octadecadienoic acid, methyl ester (E,E)**

Fig 2: Structure of major compounds

4. CONCLUSION

In the ethanol extracts of plant *Biophytum sensitivum* (Oxalidaceae) eighteen compounds have identified by (GC) and Mass Spectroscopy (MS) method. Components of the methnolic extracts were identified by comparison of their mass spectra and retention indices with those published in the literature and contained in the NIST '2005 MS computer library (Wiley). 9, 12, - Octadecatrienoic acid (Z,Z) and n-Hexadecanoic acid were the most abundant of fatty acid identified in the fatty acid fraction. Further experiments, are planned to establish the influence of the components of these mixtures on the pharmacological activity.

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