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Research Article

GC-MS ANALYSIS OF BIOACTIVE COMPOUNDS IN WILD LEAF AND CALLUS EXTRACTS OF *BIOPHYTUM SENSITIVUM* (L)

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ABSTRACT

The Phyto pharmacological screening of medicinal plants and their extracts will reveal their presence of valuable compounds and provide insight into new ways of treatment with new drugs. *Biophytum sensitivum* is an important medicinal plant widely used in traditional systems of medicine. In the present work, we reported the FTIR and GC-MS analysis with wild plant leaf extracts and correlated the presence of compounds with that of the callus extracts. The in vitro callus was grown on MS medium supplemented with BA (0.2 mg/l) and NAA (0.5, 1.0 mg/l) with leaf explants. The phyto profile of callus extracts of *Biophytum sensitivum,* fluorescence UV-Vis and FT-IR were analysed and GC-MS analysis revealed twenty five phytocompounds. The most prevailing major compounds were Octadecanoic acid, methyl ester, 1,2-Benzenedicarboxylic acid, butyl octyl ester, Pentanoic acid, 4-methyl-Decane, 1-bromo- 1H-Purine-2,6-dione, 3,7-dihydro-8-(hydroxymethyl)-1,3,7-trimethyl-, 9-Dodecenoic acid, methyl ester, (E)-, 6-Nonenoic acid, methyl ester, Hexadecanoic acid, methyl ester, Phenol, 2,4-bis(1,1-dimethylethyl)- 1-Decanol, Z,Z-10,12-Hexadecadien-1-ol acetate. The phytochemicals from these extracts are a potential source of bioactive compound that opens vista to explore various pharmacological activities.

Keywords: *Biophytum sensitivum,* Leaf extracts, callus extracts, FT-IR and GC-MS analysis.

INTRODUCTION

A large number of medicinal plants are explored from the natural flora for the commercial production of drugs. The accumulation of phytochemicals in the plant cell cultures has been studied for more than thirty years, and the generated knowledge has helped in the realization of using cell cultures for production of desired phytochemicals.[1] Although very few plant cell processes are operating commercially, the most successful commercial pharmaceuticals produced from undifferentiated cell cultures are anti-biotic compounds.[2] In the past few decades, increasing scientific interest was noticed in both growth of the plant tissue culture and the commercial development of this technology as means of producing valuable phytochemicals.[3] So, cell suspension culturing is considered one of the best approaches for studying the biosynthesis of natural products, and calli are the richest sources of cell mass when establishing such cultures.[4] The tissue culture system is a very useful tool for both studying and producing economically important secondary metabolites.[5]

Biophytum sensitivum is commonly called as life plant, little tree plant and sensitive plant, Attapatti, Chumi, Jala puspa in Telugu. It possesses a wide spectrum of medicinal properties including positive effects in treating inflammatory diseases.[6,7] Diabetic drink formulation from this plant is effective in treating diabetes. It possesses a wide spectrum of medicinal properties namely antiseptic properties, asthma[8] including positive effects in inflammatory diseases.[9]

MATERIAL AND METHODS

Callus culture

Fresh, young leaf material were collected and washed thoroughly under running tap water to remove dust particles. Leaf explants was excised aseptically and cultured on MS

(Murashige & Skoog) [10] medium supplemented with BA (Benzyl adenine) (0.2 mg/l) and NAA (Naphthalene acetic acid) (0.5 and1.0 mg/l).

Extraction from Callus Cultures

6 - 8 week-old callus derived from the shoot cuttings were collected and dried in an oven at 40 ± 1º c for 5 hours. 25g of shoot callus powder were extracted with 150 ml of solvent ethyl acetate for 24 h by using Soxhlet apparatus. 100 mg / ml were prepared by re dissolving the extracted powder in the same solvent which was used in the extraction. This callus extracts was used for FTIR and GC-MS analysis.

Spectroscopic analysis

For UV-Vis and FT-IR spectrophotometer analysis, the crude extracts and wild plant leaf extracts were centrifuged at 3000 rpm for 10 min and filtered through Whatmann No. 1 filter paper by using high pressure vacuum pump. The sample is diluted to 1:10 with the same solvent. The extracts were scanned in the wavelength ranging from 1100-3000 nm using Perkin Elmer Spectrophotometer and the characteristic peaks were detected. FT-IR analysis was performed using Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks and their functional groups. The peak values of the UV-Vis and FT-IR were recorded. Each and every analysis was repeated twice for the spectrum confirmation.

Procedure

The GC-MS analyses were carried out in a GC Model: 7890A GC System, MS: 5975C Inert MSD with Triple Axis Detector, gas chromatograph fitted with a DB1 (methylphenylsiloxane, 30 m × 0.25 mm i.d.) capillary column. 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 35min. and Mass Scan Range: 30 to 600m/z, eV voltage 3000 volts. The concentrated extract is injected into the GC/MS instrument (HP-5MS 30m*0.25um*0.25 Agilent Technologies Part No: 19091S-433).

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. 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.

Compound Identification

Components of the extracts were identified by comparison of their mass spectra and retention indices with those published in the literature and contained in The National Institute of Standard and Technology (NIST) library database (Library Version: 2.0 MS computer library).

RESULTS

The FTIR (Fourier Transform Infrared Spectrometer) was performed from the plant callus extract of *Biophytum sensitivum* to analyse the functional group. In *Biophytum sensitivum* extract IR spectrum showed strong absorption peaks at 1651.07 cm-1, 1112.93 cm-1, 1735.93cm-1, 2040.69cm-1, 2216.21cm-1, 2524.82 cm-1, 2835.36 c m-1, 2947 cm-1, 3302 cm-1 which corresponds to alkene (C=C) and ether (C-O) groups (Table. 1 and Fig. 1).

The bioactive compounds present in the ethyl acetate with leaf callus extract of *Biophytum sensitivum* were identified by GC-MS analysis (Table. 2 Fig. 2). Nineteen compounds were detected in the callus ethyl acetate extract of *Biophytum sensitivum*. The active principles with their retention time, molecular formula, molecular weight and concentration (%) in the ethyl acetate extracts of leaf callus of *B. sensitivum* are presented in Table 2, and the total running time was 35 min. The spectra of the compounds were matched with National Institute of Standards and Technology libraries (Version 2.0).

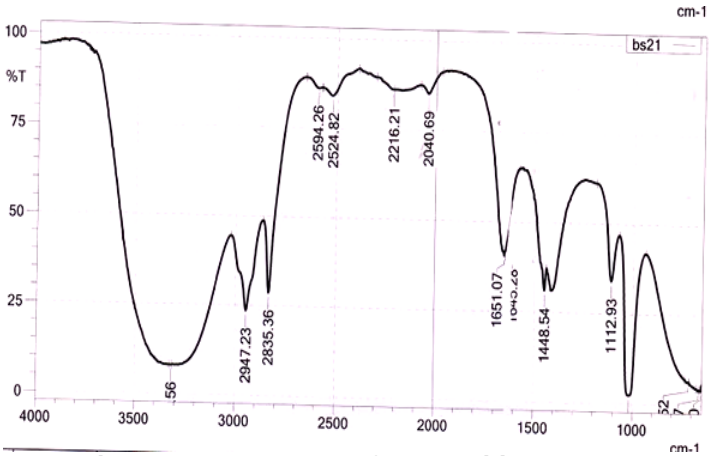

Fig.1: FTIR spectrum of *B. sensitivum* extracts

Table 1: FT-IR peak values with functional groups in *B. sensitivum* extracts

Peak	Functional Group
1112.93	Aliphatic amines (C-N Stretch) Alcohols, carboxylic acid, esters, ethers (C-O Stretch)
1448.54	Aromatics (C-C Stretch (in-ring)
1651.07	Alkenes(-C=C-Stretch) Aldehydes, saturated aliphatic (C=O Stretch)
1735.93	Esters, saturated aliphatic(C=O Stretch) Carboxylic acids, Carbonyls (general)
2040.69	Alkanes (C-H Stretch); Alkanes (terminal)(-(triple bond) (C-H:C-H stretch)
2216.21	Primary, secondary amines, amides (N-H Stretch)
2835.36	Alkanes(C-H Stretch); Alkanes (terminal)(-(triple bond) (C-H:C-H stretch); Primary, secondary amines, amides (N-H Stretch)
3302.13	Alcohols, phenols (O-H Stretch, H-bonds) carboxylic acids (O–H stretch)

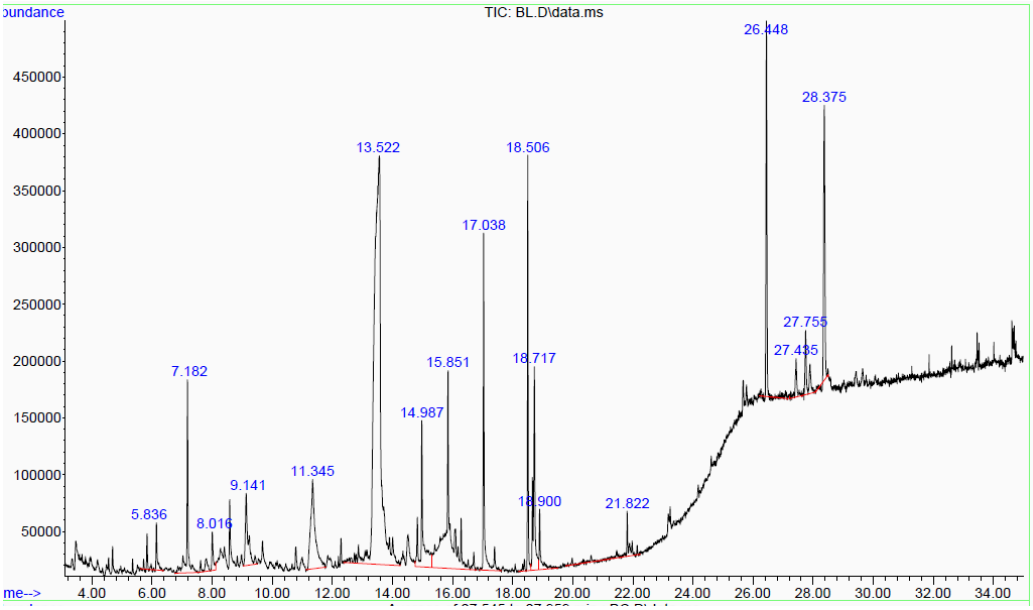

Fig. 2: GC MS analysis of leaf extracts of *Biophytum sensivitum*

Table 2: GC MS analysis of leaf extracts of *Biophytum sensivitum*

No	RT	Name of the compound	Molecular formula	Molecular Weight	Peak area %
1	5.83	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	C6H8O3	128	6.0
2	6.30	Maltol	C6H6O3	128	7.0
3	7.18	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-	C6H8O4	144	7.16
4	8.01	6-methyl-	C12H14Cl2N2O	272	8.02
5	9.04	Acetamide, N-(3,5-dichlorophenyl)-2-(1-	C5H10O4	134	8.62
6	9.14	pyrrolidinyl)-	C8H14O3	158	9-20
7	11.34	1,2,3-Propanetriol, 1-acetate	C10H14O	150	9.55
8	13.52	Methyl 6-oxoheptanoate	C12H26OSi	214	10.22
9	14.96	3,5-Heptadienal, 2-ethylidene-6-methyl-	C11H14N4O5	282	11.64
10	15.05	4-Methyl(trimethylene)silyloxyoctane	C7H12O6	192	12.22
11	17.03	9-[2-Deoxy-β-d-ribohexopyranosyl]purin-6(1H)-one	C10H12O3	180	12.68
12	18.50	(1R,3R,4R,5R)-(-)-Quinic acid	C11H19NO	181	13.52
13	18.71	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	C20H40O	296	14.98
14	18.90	Oxazole, 5-hexyl-2,4-dimethyl-	C20H40O	296	15.85
15	21.82	Phytol	C17H34O2	270	17.03
16	26.48	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C16H32O2	256	18.50
17	26.49	Hexadecanoic acid, methyl ester	C12H18O3	210	18.71
18	27.75	n-Hexadecanoic acid	C18H32O2	280	18.90
19	27.45	trans-(2-Octenyl)succinic anhydride	C18H30O2	278	21.82
20	27.37	9,12-Octadecadienoic acid (Z,Z)-	C18H36O2	284	26.44
21	28.22	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	C19H38O4	330	27.75
22	28.48	Octadecanoic acid	C29H50O2	430	27.88
23	27.38	Hexadecanoic acid, 2,3-dihydroxypropyl ester	C29H50O	414	27.66
24	28.88	Vitamin E	C29H50O2	430	27.45
25	28.32	γ-Sitosterol	C29H50O	414	28.37

The methanol leaf extracts of *Biophytum sensitivum* revealed twenty five bioactive compounds such as 2,5-Dimethyl-4-hydroxy-3(2H)-furanone, Maltol, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-,Acetamide, N-(3,5-dichlorophenyl)-2-(1-pyrrolidinyl)-, 1,2,3-Propanetriol, 1-acetate, Methyl 6-oxoheptanoate, 3,5-Heptadienal, 2-ethylidene-6-methyl-, 4-Methyl(trimethylene) silyloxyoctane, 9-[2-Deoxy-β-d-ibohexopyranosyl]purin-6(1H)-one, (1R,3R,4R,5R)-(-)-Quinic acid, 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol, Oxazole, 5-hexyl-2,4-dimethyl-, Phytol, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, Hexadecanoic acid, methyl ester, n-Hexadecanoic acid, trans-(2-Octenyl)succinic anhydride, 9,12-Octadecadienoic acid (Z,Z)-, 9,12,15-Octadecatrienoic acid, (Z,Z,Z)-, Octadecanoic acid, Hexadecanoic acid, 2,3-dihydroxypropyl ester, Vitamin E, γ-Sitosterol.

Pawar and Vyawahare reported the presence of phenolic and polyphenolic compounds, saponins, essential oil, polysaccharides and pectin in the whole plant extracts of *B. sensitivum.*[11] Two biflavones (cupressuflavone and amentoflavone), three flavonoids (luteolin 7-methyl ether, isoorientin and 3'-methoxyluteolin 7-O-glucoside) as well as two acids (4-caffeoylquinic acid and 5-caffeoylquinic acid) were isolated from the aerial parts of *Biophytum sensitivum*[12] 3', 8"biapigenin proanthocyanidins and also some phenolic compounds. In addition, presence of orientin, isovitexin, isoorientin 7-O-glucoside, isoorientin 2"-O-rhamnoside, (–)-epicatechin and proanthocynidin B2 have also been reported.[13] The essential oil of the air-dried plant, *Biophytum sensitivum* was analyzed using gas chromatographic-spectroscopic (GC-FID and GC-MS) and olfactoric methods and found to contains benzene derivatives, such as 1,4-dimethoxy benzene (24.9%), 1,2-dimethoxy benzene (10.6%) and 2-methoxy-4-methyl phenol (3.5%), the monoterpenes (Z)-linalool oxide (8.1%), (E)-linalool oxide (5.2%) and linalyl acetate (3.4%) as well as 1-octen-3-ol (9.5%) and isophorone (3.1%) as main constituents. [14]

Rajeswari *et al*., (2015) reported the whole plant of *B. sensitivum* with methanol extracts using the GC-MS analysis, eighteen compounds are identified, 9,12-Octadecadienoic acid (Z,Z) (50.36%), n-Hexadecanoic acid (17.42%), 9,12-Octadecadienoic acid, methyl ester (E,E) (6.34%), 2-Pentadecanne,6,10,14-trimethyl- (3.31%), *n*-hexadeconic 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. [15] Kumar *et al.,* (2017)[16] reported five phenolic compounds Caffeic acid, ferulic acid, gallic acid, chlorogenic acid and rutin revealed in the whole plant of *B. sensitivum*. Our results coincided with these reports in the presence of the n-Hexadecanoic acid, 9,12-Octadecanoic acid in the plant and callus extracts of *B. sensitivum*.

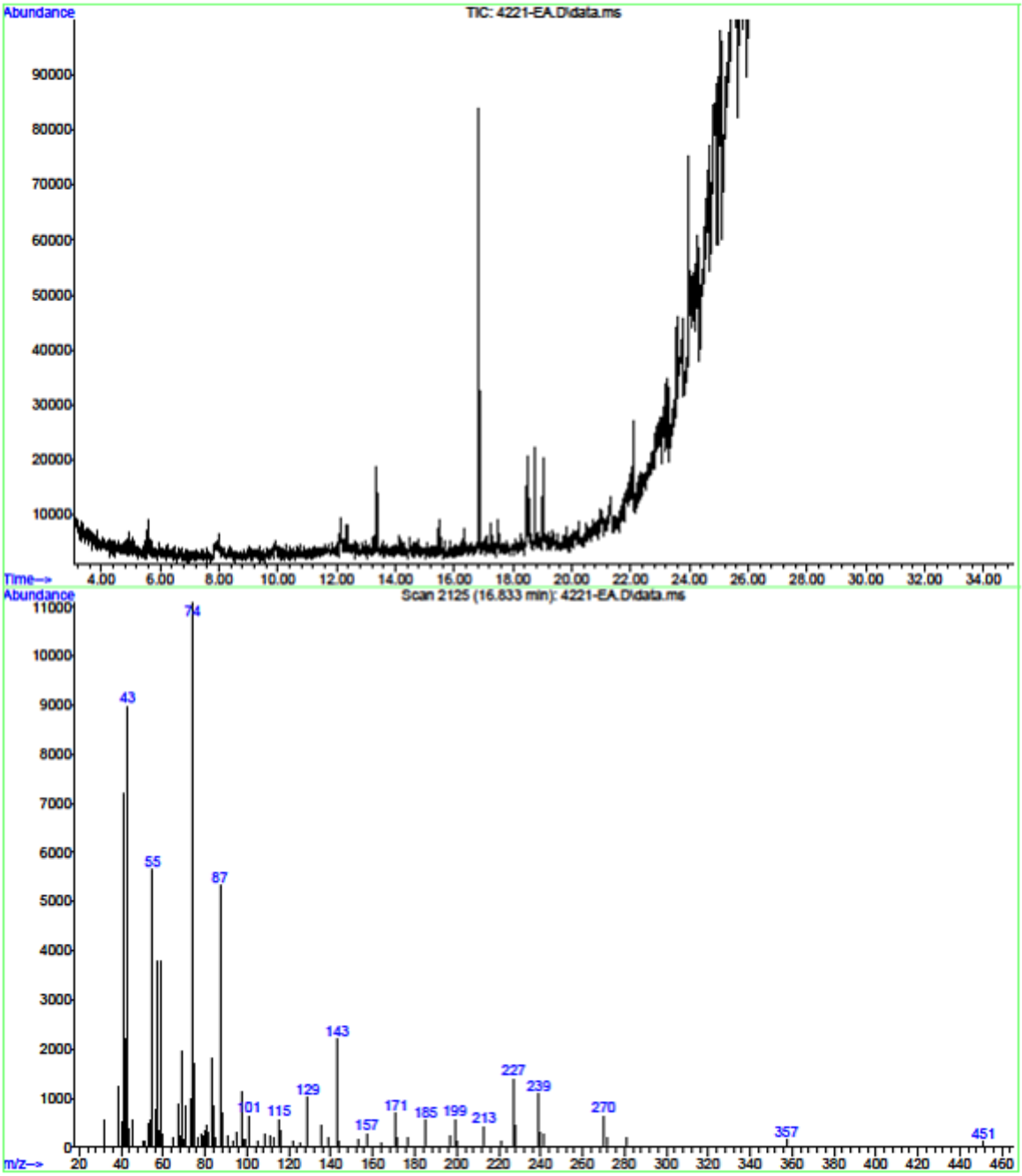

Fig. 3: GC-MS analysis of callus extract of *B. sensitivum*

Table 3: GC MS analysis of callus extracts of *Biophytum sensivitum*

No	RT	Name of the compound	Molecular formula	Molecular Weight	Peak area %
1	16.832	1,5-Cyclooctadiene, 1,5-dimethyl	C10H16	136	7.152
2	25.727	Phenol, 2,4-bis(1,1-dimethylethyl)-	C14H22O	206	5.066
3	16.288	Cyclohexane	C10H16	136	8.796
4	27.711	Diethyl Phthalate	C12H14O4	222	4.881
5	16.228	A-Pinene	C10H16	136	7.845
6	27.787	1-Decanol	C10H22O	158	5.783
7	30.865	1-Dodecanol	C12H26O	186	5.203
8	31.036	Decane, 1-bromo-	C10H21Br	220	5.881
9	31.472	Hexadecanoic acid, methyl ester	C17H34O2	270	5.805
10	31.594	1, 2-Benzenedicarboxylic acid, butyl	C20H30O4	334	4.758
11	31.818	octyl ester	C6H12O2	116	8.457
12	31.935	Pentanoic acid, 4-methyl-	C18H32O2	280	10.058
13	32.355	Z, Z-10,12-Hexadecadien-1-ol acetate	C19H34O2	294	13.15
14	34.056	9,12-Octadecadienoic acid, methyl ester,	C19H36O2	296	16.19
15	34.318	(E,E)-Octadecenoic acid (Z)-, methyl ester	C13H24O2	212	5.998
16	34.328	9-Dodecenoic acid, methyl ester, (E)-	C10H18O2	170	5.553
17	34.393	6-Nonenoic acid, methyl ester	C19H38O2	298	5.288
18	34.905	Octadecanoic acid, methyl ester	C9H12N4O3	224	5.255
19	34.922	1H-Purine-2,6-dione, 3,7-dihydro-8-(hydroxymethyl)-1,3,7-trimethyl-3-Naphthalen-2-yl-3-piperidin-1-yl-	C18H23NO	269	9.056

The most prevailing compounds from *Biophytum sensitivum* from the methanolic callus extracts include 1,5-Cyclooctadiene, 1,5-dimethyl, Phenol, 2,4-bis(1,1-dimethylethyl)-,Cyclohexane,Diethyl Phthalate, A-Pinene, 1-Decanol, 1-Dodecanol, Decane, 1-bromo-, Hexadecanoic acid, methyl ester, 1, 2-Benzenedicarboxylic acid, butyl octyl ester, Pentanoic acid, 4-methyl-, Z, Z-10,12-Hexadecadien-1-ol acetate, 9,12-Octadecadienoic acid, methyl ester, (E,E)--Octadecenoic acid (Z)-, methyl ester, 9-Dodecenoic acid, methyl ester, (E)- 6-Nonenoic acid, methyl ester, Octadecanoic acid, methyl ester,1H-Purine-2,6-dione, 3,7-dihydro-8-(hydroxymethyl)-1,3,7-trimethyl-,5 3-Naphthalen-2-yl-3-piperidin-1-yl-propan-1-ol.

GC-MS is one of the most efficient technology platforms to approach complex mixtures of organic compounds based on a combination of MS database search and the use of calculated RI values.

DISCUSSION

The major compounds revealed in the callus extracts of *Biophytum sensitivum* include Octadecanoic acid, methyl ester, 1,2-Benzenedicarboxylic acid, butyl octyl ester, Pentanoic acid (93.45%), 4-methyl-Decane, 1-bromo-, 1H-Purine-2,6-dione, 3,7-dihydro-8-(hydroxymethyl)-1,3,7-trimethyl-, 9-Dodecenoic acid, methyl ester, (E)-, 6-Nonenoic acid, methyl ester, Hexadecanoic acid, methyl ester, Phenol, 2,4-bis(1,1-dimethylethyl)-. 1-Decanol, Z,Z-10,12-Hexadecadien-1-ol acetate and minor components were 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, commonly called as Linoleic acid, an essential fatty acid that must be consumed for proper health. The phenolic compounds reported as antioxidant agents positively correlated in the treatment of cardiovascular diseases[17] and these phenolic compounds are known to be synthesized by plants in response to microbial infection, therefore possible that they can act as effective antimicrobial substances against a wide array of microorganisms.

Hexadecanoic acid, methyl ester exhibited antioxidant, hypocholesterolemic, anti androgenic, hemolytic, alpha reductase inhibitor activities. Dodecanoic acid present in callus extracts of *Biophytum sensitivum*, revealed the antimicrobial activities, increase HDL level. Our results coincided with the GC-MS analysis of Muthusamy et al., 2015.[18] Hexadecanoic acid can be an antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant and hemolytic inhibitor.[19,20] Hexadecanoic acid, the major compound of GC-MS analysis was present in both leaf and callus extracts of *Biophytum sensitivum.* Our results matched with leaf extract of *Cleistanthus collinus,* [24] *Goniothalamus umbrosus, Kigelia pinnata.*[21, 22] and *Melissa officinalis*[23] *Euphorbia longan* leaves with the presence of n-hexadecanoic acid and 9, 12-Octadecadienoic acid. The methanolic extracts of *Biophytum sensitivum* revealed the existence of phytol compound and it exhibits more biological properties like Antimicrobial, Anticancer, Anti inflammatory and Diuretic.[25] The most prevailing major compounds are Phenols showed the biological activities like

antioxidant, anti carcinogenic, anti-inflammatory and presence of the in 9-Octadecanoic acid consist of antihypertensive, increase HDL and decrease LDL Cholesterol. The 9-Octadecanoic acid is a saturated fatty acid, and exhibit decreasing of Lower LDL Cholesterol level. Unsaturated fatty acids are important for normal growth, especially of the blood vessels and nerves to keep the skin and other tissues young and healthy.[26] On the other hand octadecanoic acid have the antibacterial activity. In the present study octadecanoic acid is present in the both leaf and callus extracts of *Biophytum sensitivum*.

CONCLUSION

GC-MS is frequently applied to characterize the chemical complexity of analytical samples based on its separation and identification capacity. Recent developments in GC-MS technology have facilitated global metabolic approaches in order to approach biological functions and perturbations of biological systems, and for diagnostics and quality assessment purposes. However, one should be aware of the limitations of global GC-MS metabolite profiling. Processing, automated sample handling and analysis conditions need to be strictly defined and controlled in order to minimize data variation and allow for quantitative calculations. In the methanol extracts of *Biophytum sensitivum* (Oxalidaceae) callus, nineteen compounds have been identified by (GC) and Mass Spectroscopy (MS) method. 9, 12, -Octadecatrienoic acid (Z, Z) and n-Hexadecanoic acid were the most abundant of fatty acid identified in the fatty acid fraction. Further research is in progress for the pharmacological evaluation of callus extracts of *Biophytum sensitivum.* The identification of various bioactive compounds confirms the therapeutic application of *Biophytum sensitivum* for a variety of diseases.

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REFERENCES

1. Castello MC, Phatak A, Chandra N and Sharon M. Antimicrobial activity of crude extracts from plant parts and corresponding calli of Bixa orellana L. Indian J Exp Biol. 2002;40:1378-1381.
2. Sirigiri Chandra Kala, Kokkanti Mallikarjuna and Patchala Aruna. An efficient protocol devised for rapid callus induction from leaf explants of Biophytum sensitivum (Linn). International Journal of Phytopharmacy. 2014;4 (1): 20-24.
3. Havkin-Frankel D, Dorn R and Leustek T. Plant tissue culture for production of secondary metabolites. Food Technol. 1997; 51: 56-61.
4. Edeoga HO DE and Mbaebie BO. Phytochemical constituents of some Nigerian Medicinal Plants. African J Biotechnol. 2005; 4:685-688.
5. Yamin Bibi, Muhammad Zia, Sobia Nisa and Darima Habib. Abdul Waheed and Fayyaz M Chaudhary. Regeneration of Centella asiatica plants from non- embryogenic cell lines and evaluation of antibacterial and antifungal properties of regenerated calli and plants. J. Biological Engg. 2011;5:13.
6. Mitra AP and Ambasta SP. The Wealth of India (Raw Materials). New Delhi: Council of Scientific and Industrial Research, Publication and Information Directorate. 1998;6:151-152.
7. Kirtikar KR and Singh BSMP. Dehradun, Indian Med. Plants. 1984; I:440-441.
8. Pullaiah T. Medicinal Plants in India, Volume 1, Regency Publications, New Delhi. 2002;96-97.
9. Puri D, Baral N and Upadhyaya BP. Indigenous plant remedies in Nepal used in heart diseases. J Nepal Med Assn. 1997;36:334-337.
10. Murashige T and Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Plant Physiol. 1962;150: 473-497.
11. Bharati AC and Sahu AN. Ethnobotany, phytochemistry and pharmacology of Biophytum sensitivum DC. Pharm Rev. 2012; 6(11):68-73.
12. Lin YL and Wang WY. Chemical constitutents of Biophytum sensitivum. Chin Pharm J. 2003; 55(1):71-5.
13. Bucar F, Jachak SM, Kartnig T and Schubert-Zsilavecz M. Phenolic compounds from Biophytum sensitivum. Pharm. 1998; 53(9):651-3.
14. Leopold J, Gerhard B, Andrea W, Mohamed SP and Beena J. Medicinally used plants from India: analysis of the essential oil of air-dried Biophytum sensitivum (L.) DC Sci Pharm. 2004; 72(1):87-96.
15. Devi Raja Rajeswari P, Aruna K, Prabu K, Chidambaram R and Raja

Sankar S. GC-MS analysis of the ethanol extract of Biophytum sensitivum (L.) DC (Oxalidaceae). Int J Pharm Phytopharmacol Res. 2015; 5(1):13-17.
16. Bhanu Kumar, Pushpendra Kumar Shukla, Abhishek Niranjaran, Ankita Misra, Ajay Kumar Singh Rawat and Sharad Srivastava. RP-HPLC quantification of five phenolic compounds in Biophytum sensitivum (L). DC. (Oxalidaceae) and their biological evaluation. Indian Journal of Traditional Knowledge. 2017;16 (2):319-324.
17. Nugroho AE, Abdul Malik and Suwidjiyo Pramono. Total phenolic and flavonoid contents, and in vitro antihypertension activity of purified extract of Indonesian cashew leaves (Anacardium occidentaleL.). Int Food Res J. 2013; 20:299-305.
18. Karthikeyan Muthusamy, Gopal V and Velavan Sivanandham. GC-MS analysis of bioactive components of Karisalai karpa chooranam- a siddha poly herbal formulation. International Journal of Phytopharmacology. 2015; 6(3):131-136.
19. Praveen Kumar P, , Kumaravel S and Lalitha C. Screening of antioxidant activity, total phenolics and GC-MS study of Vitex negundo. Afr J Biochemistry Res. 2010; 4(7):191-195.
20. Devi P, Nagarajan M, Christina AJM, Meera R and Merlin NJ. GC-MS analysis of Euphorbia longan leaves. Int. J. of Pharmaceutical Res and Development. 2009; (8):1-4.
21. Grace OM, Light ME, Lindsey KL, Moholland DA, Staden JV and Jader AK. Antibacterial activity and isolation of antibacterial compounds from fruit of the traditional African medicinal plant, Kigelia africana. 2002; 68:220-222.
22. Siddiq Ibraham A, Ahmad Bustamam A, Manal Mohammed E Syam, MI Mohamed Yousif M, Abdelbasit Adam, Alhaj NA and RasedeeAbdullah. GC-MS determination of bioactive components and antibacterial properties of Goniothalamus umbrosus extracts. Afr. J.Biotech 2009; 8(14):3336-3340.
23. Sharafzadeh S, Khosh-Khui M and Javidnia K. Aroma profile of leaf and stem of Lemon Balm (Melissa Officinalis L.) grown under Greenhouse Conditions. Advan. Environmental Biol. 2011; 5(4):547-550.
24. Parasuraman S, Raveendran R and Madhavrao C. GC-MS analysis of leaf extracts of Cleistanthus collinus Roxb. (Euphorbiaceae). Int J Ph Sci. 2009;1(2):284-286.
25. Purushoth Prabhu.T, Panneerselvam P, Suresh R, Clement Atlee W and Balasubramanian S. GC-MS analysis of ethanolic extract of Canthium parviflorum Lamk Leaf. Journal of Applied Pharmaceutical Science. 2013;3(2):166-168.
26. Igwe OU and Donatus Ebere Okwu. GC-MS evaluation of bioactive compounds and antibacterial activity of the oil fraction from the seeds of Brachystegia eurycoma. HARMS) Asian Journal of Plant Science and Research. 2013;3-2.