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Metabolite Profiling and Principle Component Analysis of a Mangrove Plant Aegiceras Corniculatum L (Blanco)

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Abstract

Metabolite profiling is the multi-parallel relative quantification of a mixture of compounds or compound classes using chromatography and universal detection technologies (GC-MS, LCMS) for biological samples. Metabolic profiling could provide the link between traditional herbal medicines and molecular pharmacology. Aegiceras corniculatum is one of the important species of black river mangrove plant. Present studies explaining the strategies of metabolite profiling of polar as well as non-polar compounds from leaves and bark extracts of plant Aeiceras corniculatum by using GC-MS. The range of solvent from non-polar to polar viz. petroleum ether, chloroform, ethyl acetate, methanol and water separately used for extraction. The GC-MS profile revealed the presence of phenolics, carbohydrates, steroids, alkaloids, hydrocarbons, fatty acids and esters, amines etc. In parallel with GC-MS statistical analytical tools such as principal component analysis used to yield significant and precise results. In present study the biologically active compounds-Plectoxanxthin, N-deacetyl Colchicine, Cyclopentaneundecanoic acid, Thiirane, Gamolenic acid, 8,11,14-Eicosatrienoic acid, Lupol, Rhodopine were reported from different extracts of plant.

Keywords: Mangrove; GC-MS; Principle component analysis; Biologically active compounds; Alkaloids.

Introduction

Metabolite profiling is an analytical method for relative quantitation of a number of metabolites from biological samples [1]. Commonly, these samples have been collected from a specific tissue or a part of a tissue of interest or either from a larger mixture of different organs (such as whole-shoots) or conversely on a micro scale from single cells or purified organelles. The metabolite profiling has two important approaches namely targeted metabolite analysis and untargeted or global metabolite analysis. Metabolomics is a fast-becoming approach of choice across broad range of sciences including system biology, drug discovery, molecular and cell biology and other medical and agriculture sciences. Plant metabolome are much more complex as compare to mammalian metabolome. The diversity of plant secondary metabolite evolved through continuous interaction with environment coupled with characteristics species and agronomic conditions. These metabolites generally confer specific biological activity related to their biochemical structure [2]. Metabolic profiling could provide the link between traditional herbal medicines and molecular pharmacology. The

metabolomics analysis of natural resources especially medicinal plants has great potential in development of new phototherapeutics and nutraceuticals. In addition, analysis of wide spectrum of composition with varied concentrations is crucial foundation to quality control and elucidation the pharmacological effectiveness of plant extract. The well-known chemotherapeutic agents such as champtothecine, paclitaxel and podophyllotoxin are derived from plant secondary metabolite analysis [3]. In traditional methods, the identification and characterization of such natural metabolites is done by thin layer chromatography. In traditional methods, thin layer chromatography (TLC) is generally used for the analysis of botanical raw materials.

Currently increased depth of metabolome coverage can only be possible by combination of analytical technologies like gas chromatography, liquid chromatography coupled with mass spectrometry and nuclear magnetic resonance are common technologies used in metabolic profiling. GC-MS can facilitate the identification and robust quantification of few hundred metabolites within a single plant extract. GC-MS has a relatively broad coverage of compound classes, including organic and amino acids, sugars, sugar alcohols, phosphorylated intermediates and lipophilic compounds. The main advantages of this technology that are it have long been used for metabolite profiling and thus there are stable protocols for machine set-up and maintenance, and chromatogram evaluation and interpretation [4].

Plant synthesizes a vast range of metabolites that are divided in two groups' primary metabolites and secondary metabolites. Primary metabolites are involved in fundamental body processes like photosynthesis, respiration, growth and development. While secondary metabolites are not directly involved in fundamental life processes, but they are produced as result of interaction of plants and their environment. These are alkaloids, phenolics, terpenoids, steroids, tannins, saponins etc. These secondary metabolites are used in preparation of dyes, fibers, glues, oils, waxes, flavoring agents and perfumes. They are important source of insecticides, herbicides, antibiotics and discovery of new drugs [5].

Aegiceras corniculatum is a small shrub that grows in the swamps of Asia and Australia. It has been traditionally used in treatment of diabetes, asthma, inflammation and rheumatism. Realizing the pharmacological importance of *Aegiceras corniculatum* the several efforts have been carried out to identify bioactive compounds from extracts of leaves, bark, and root of plant. The earliest pharmacologically studies revealed cytotoxicity, itchy toxicity, anti-inflammatory, antioxidant, antimicrobial and tyrosine phosphate 1B inhibitory activity [6]. A number of saponins, triterpenes, sterol and hydroquinone's have been previously reported from this plant and it was reported that hydroquinone 5-O-methyl embelin shows fish toxicity [7,8].

Aegiceras corniculatum belongs to family Myrsinaceae. The members of family Myrsinaceae characteristically show presence of secondary metabolite embelin which is a one of the phenolics lipid (benzoquinone). It is mainly synthesized in plants as well as microorganisms like bacteria and fungi both during normal development and in response to stress such as infection, wounding, salt stress etc. [9]. The embelin is promising biologically active compound showing antimicrobial, anti-inflammatory, antioxidant activity. It shows cytotoxic mechanism via apoptosis and cell cycle arrest caused by topoisomerase II mediated DNA damage [10]. The earlier reports on this compound demonstrated its anti-androgenic activity and are considered as anti-fertility agent in males [11]. From the bark of *Aegiceras corniculatum* compounds like embelin and 5-0-methyl embelin were isolated which are potent inhibitor of hepatitis C virus protease [12] and also exhibits antioxidant and anti-inflammatory action.

Materials and Methods

The plant material was collected from Pawas region of Ratnagiri district of Maharashtra state (south-west costs of India) in 2011-12 and 2012-13. The collected plant material was first separated, cleaned, dried in shade, and ground into fine powder. The five solvents were selected with increasing polarity viz. petroleum ether, chloroform, ethyl acetate, methanol and water were selected for extraction procedure.

5 gm of powdered plant material (leaves and bark) was mixed with 50 ml of solvent separately. The mixture was sonicated at 33 KHz for 40 min and allowed to stand for at least 12 h. The extraction was carried out repeatedly until solvent get colorless. The extracts were filtered and concentrated in rotary evaporator to dryness. Out of ten extracts (WB, MB, EB, CB, PB, WL, ML, EL, CL, PL) mostly all soluble in ethyl acetate. Therefore, for GCMS samples were suspended in ethyl acetate (ultra-pure) and for another samples methanol (ultra-pure) were used. GC-MS analysis was performed using gas chromatographer (Shimadzu OP

2000).

Injection temperature was 2700^{0}C. Sample volume was 20 µl. The oven temperature was programmed to rise from 1000^{0}C to 2000^{0}C at 100 C/min for 10 min and then 2000^{0}C to 2600^{0}C at 100 C/min for 10 min with total run time of 45 min. Helium gas was used as carrier gas at a flow rate of 1 ml/min. Retention time, % peak area and M/z values were recorded.

Identification of components was established by comparison of their mass spectra with those reported in NIST library. Principal component analysis was used to reduce data and hierarchal cluster analysis for classification of data. PCA and HCA were carried out by using software Biodiversity professional (version 2).

Result and Discussion

The GC-MS profile revealed the presence of phenolics, carbohydrates, steroids, alkaloids, hydrocarbons, fatty acids and esters, amines etc.

GC-MS spectra used for identification metabolites. Following samples of GC-MS spectra of two extracts (Figures 1 and 2). The total 105 different type of compounds were identified in GC-MS analysis from different extracts.

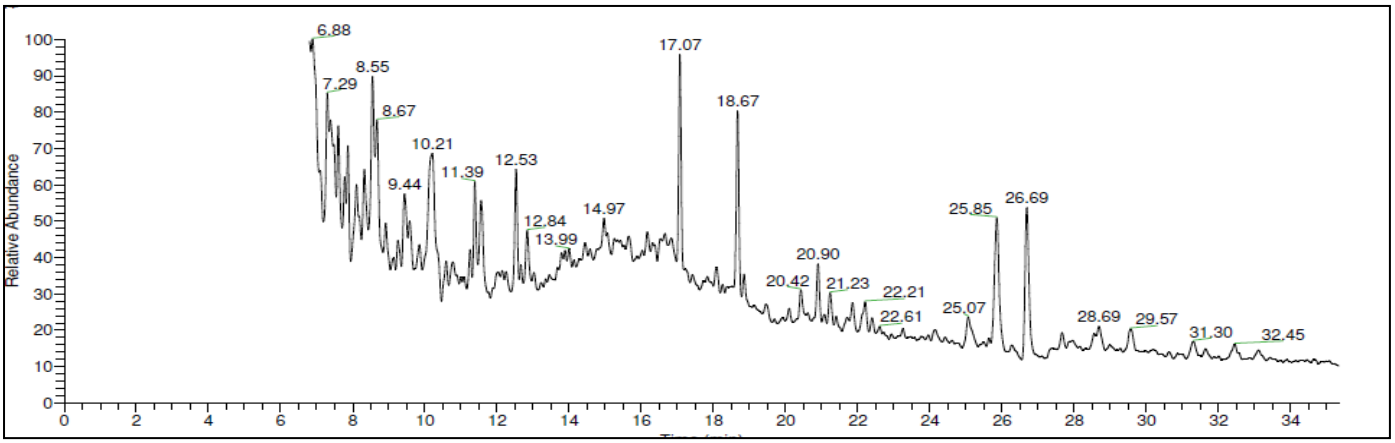

Figure 1: Gas chromatographic profile of ethyl acetate extracts of leaves (EL)

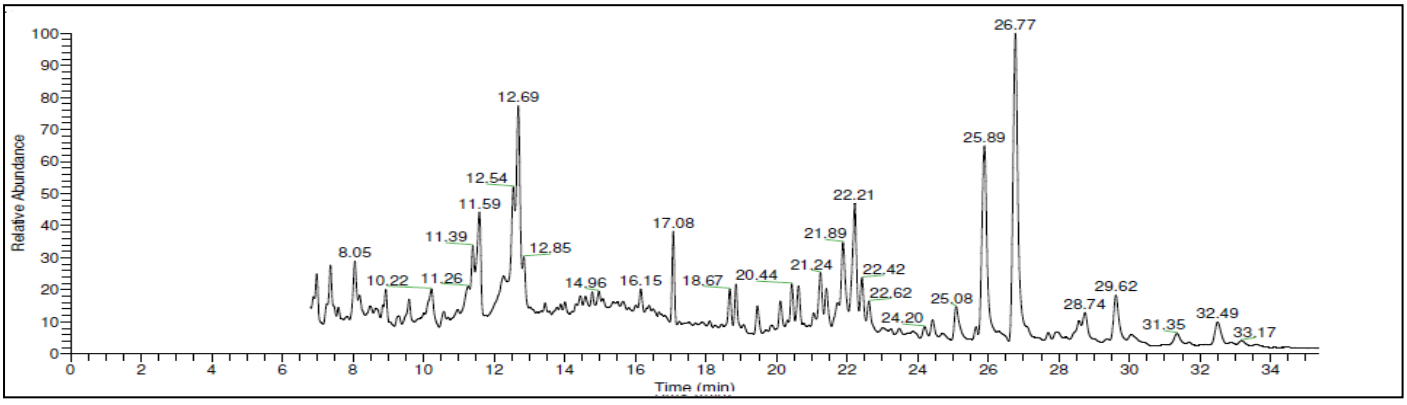

Figure 2: Gas chromatographic profile of chloroform extracts of bark (CB)

The compounds have been listed in Table 1.

Table 1: List of metabolites identified from GC-MS

Name of compound	Name of extract									
	WS	MS	ES	CS	PS	WL	ML	EL	CL	PL
Alkaloids										
N-deacetyl, Colchicine	+	-	-	-	-	-	-	+	-	-
3-acetyl-pancracine	-	-	-	+	-	-	-	-	-	-
Quinoline-4-ol,2-tetradecyl, N-oxide	-	-	-	+	-	-	-	-	+	-
11-hydroxy-Cephalotaxine	-		+	-	-	-	-	-	-	-
1,2-dihydro-3-(2-hydroxyethyl)-4-methoxy-2-oxoquinoline	-	-	+	-	-	-	-	-	-	-
Colchicine	-	-	-	-	-	-	+			
Thiocolchicine									+	
Thieno (2,3-b) quinoline-3-amine,5,6,7,8-tetrahydro -2-octylsulfonyl	-	-	-	-	-	-	+	-	-	-
3,11-epoxy Cephalotaxine	-	-	-	-	-	+	-	-	-	-
Lumicolchicine		+	-	-	-	-	-	-	-	-
Dihydrohistrionicotoxin 285e	-	-	-	-	-	-	-	-	-	+
3-Acetyl-pancracine	-	-	-	-	-	-	-	-	+	-
Carbostyril,3-ethyl-4-hydroxy-7-methoxy	-	-	-	-	-	-	-	-	+	-
Terpens/ Terpenoids										
Rhodopin	+	-	-	-	-	-	-	-	-	-
(S)Plectozanthin	+	-	-	-	-	-	-	-	-	-
3-Buteneitrile,4-anilino-3,4-bis (4-quinolyl)	-	-	-	-	-	-	-	-	-	-
Benzo(a,i)quinolisin -5-one,1,2,3,5-tetrahydro-6-cyclohexyl-7-hydroxy	-	-	-	+	-	-	-	-	-	-
Luponolacetate	-	-	+	-	-	-	-	-	-	-
Lupeol	-	-	-	-	-	-	-	-	--	+
Caretenoids										
Rubixanthin acetate	-	-	-	-	-	-	-	-	-	+
Beta,psi-Carotene,3,4-dihydro-1,2-dihydroxy-	+	-	-	-	-	-	-	-	-	-
Hydrocarbons										
3-Hydroxy-1-(4-(13-(4(3-hydroxy-3-phenylacryloyl) phenyl-3-phenylprop-2-ene	+	-	-	-	-	-	-	-	--	-
Z,Z,Z-4,6,9-Nanadecatriene	-	-	-	-	+	-	-	-	-	-
6-Propyltetrahydro-2H-thiopyran-2-one	-	-	-	-	-	+	-	-	-	-
0 (z,z)-3,6-Nonadienal	-	+	-	-	-	+	+	-	-	-
6-Propyltetrahydro-2H-thiopyran-2-one	-	-	-	-	-	+	-	-	-	-
12-Azabicyclo (9,2,2) pentadeca-1(14),11(15)-dien-13-one	-	-	-	+	-	-	-	-	-	-
1-Methoxymethoxy-hexa-2,4-diene	-	-	-	-	-	-	-	+	-	-
9,10-Phenanthrenedione,1,2,3,4,4a,10a-hexahydro -6-hydroxy-1,1,4a-trimethyl-7-(1-methylethyl-	-	-	--	-	-	-	-	+	-	-
17,18-diphenyl, paracyclophan-17-ene	-	-	-	-	-	-	-	+	-	-
1,1-dibutyl Germacyclobutane	-	-	-	-	-	-	-	+	-	-
4H-cyclopropa (5,6) benz (1,2:7,8) azuleno (5,6-b) oxiren-4-one,8,8a-bis(acetyloxy)-2a (acetyloxy)methyl	-	-	-	-	-	-	+	-	-	-

5,6,7,8,9,10-Hexahydro-9-benzyl-spiro(2H-1,3benzoxazine-4,1-cyclohexane)-2-thion	-	-	-	-	-	-	+	-	-	-
4H-Benzopyran-4-one,2-(3,4-dimethoxyphenyl-5,6,7-trimethoxy	-	-	-	-	-	+	-	-	-	-
5,6Dihydroxyingol-3,7,8,12-tetraacetate	-	-	-	-	-	-	-	-	+	-
Alcohols and Phenols										
Alpha methyl -2-Pyridineethanol	-	+	-	-	+	-	-	-	-	-
2,2-Dithioethanol	-	-	-	-	-	+	-	-	-	-
3-ethyl-2,4-pentadien-1-ol	-	-	-	-	-	-	-	+	-	-
9H-Fluren-9-one,3-nitro-2,7-bis(2-(1-piperidinyl) ethoxy	-	-	-	-	-	-	-	-	-	+
Allo-inositol tri-n-octaneboronate	-	-	-	-	-	-	-	-	+	-
Fatty acids and Esters										
Tetradecanoic acid	+	-	-	-	-	-	-	-	-	-
Decatrienoic acid	+	-	-	-	-	-	-	-	-	-
Acetic Acid, hydroxyl ethyl ester	+	-	-	-	-	-	-	--	-	-
Dithiodiacetic acid ester	-	-	-	-	+	-	-	-	-	-
Mesoprophyrinix dimethyl ester	-	-	-	-	-	-	--	-	+	-
Calconcarboxylic acid	-	-	-	-	-	+	-	-	-	-
9,12,15-octadecatrienoic acid,2-phenyl-1,3-dioxan-5-yl eser	-	-	-	+	-	-	-	-	-	-
11-Acetamidooctadecanoic acid	-	-	-	+	-	-	-	-	+	-
8,11,14-Eicosatrienoic acid, methyl ester	-	-	-	-	-	-	+	-	-	-
Heptadeca-1,6,11,16-tetraene-9-carboxylic acid,9-cyano-methyl ester	-	-	-	+	-	-	-	-	-	-
Phenylalanine,4-amino-N-t-butyoxycarbonyl-t-butyl ester	-	-	-	-	-	-	-	+	-	-
Phorbo-12,13-dihexanoate	-	-	-	-	-	-	-	+	-	+
3- Penten-1-ol,2,2,4-trimethyl	-	-	-	-	-	-	-	+	-	-
Cyclohexanecarboxylicacid,2-hydroxy-methyl ester	-	-	+	-	-	-	-	+	-	-
Heptadeca-1,6,11,16-tetraene-9-carboxylic acid,9-cyno-methyl ester	-	-	-	-	-	-	-	-	+	-
Nonanoic acid,2-oxo-methyl ester	-	+	-	-	-	-	-	-	-	-
1H-Pyrrol-3-propanoic acid,2-(ethoxycarbonyl)-4-(ethoxycarbonylmethyl)-5-(hydroxymethyl)-	-	-	-	-	-	-	-	-	-	+
2-Hexyldecanoic acid	-	-	+	-	-	-	-	+	-	-
Gamolenic acid	-	-	+	-	-	-	-	+	+	-
Cyclopentaneundecanoic acid	-	+	-	-	-	-	-	-	-	-
Methyl-5,2(undecylcyclopropyl) pentanote	-	+	-	-	-	-	-	-	-	-
6-Aminocaproic acid,n-isobutoxy carbonyl, ethyl ester	-	-	-	-	-	-	-	-	+	-
5-chloro-3-phenyl-2,1-Benzisoxazole-6-carboxylic acid	-	-+	-	-	-	-	-	-	-	-
9,12,15-octadecatrienoic acid,2-phenyl-1,3-dioxan-5-yl ester	-	-	-	-	-	-	-	-	+	-
Ethers										
Bromo methyl methyl ether	-	-	+	-	+	-	-	-	-	-
Dihydromorphin,di(trimehylsilyl)ether	-	-	-	-	-	+	-	-	-	-
Bromomethyl methyl ether	-	-	-	-	-	-	-	+	-	-
Tert-amyl methyl ether	-	-	-	-	-	-	-	-	+	-
Sulphids/disulphide										

Disulphide,bis(2-sulfhydrylethyl)	-	-	-	-	+	-	-	-	-	-
Methoxycarbonyl methoxythiomethyldisulphide	-	-	-	-	+	+	-	-	-	-
2-bromoethyl methyl sulfide	-	-	+	-	-	-	-	+	-	-
Thiirane	-	-	+	-	-	-	-	+	-	-
Steroids										
Stigmast-7-en-3-ol	-	-	-	+	-	-	-	-	+	-
2,3-epoxy-2-methyl cholestrane	-	-	-	+	-	-	-	-	-	-
4-Ethylthio-3,20-dioxo-4-pregene-17alpha-yl acetate	-	-	-	-	-	-	+	-	-	-
Cholestan-26-oic acid,3,7,12-tri hydroxyl	-	+	-	-	-	-	+	-	-	-
Ethylmorphine,triethyl ether	-	+	-	-	-	-	-	-	-	-
-Cholestrane ,2,3-epoxy-2-methyl	-	-	-	-	-	-	-	-	+	-
17a-Allyl-3-beta-methoxy-17a-aza-D-homoandrost-5-ene-17-one	-	-	-	-	-	-	-	-	+	-
Amines/Amides										
4,4(Sulfonylbis(4,1-phenyleneoxy) Benzamines	-	-	-	-	+	-	-	-	-	-
Sulfonyl diamine	-	-	-	+	-	-	-	-	+	-
Pyrrole-3-carbonitril,2-animo-1-butyl-(1,1-dimethylethyl)	-	-	-	-	-	-	+	+	-	-
Pyrroline,5-butyl-2-(1,3-heptadienyl)	-	-	-	-	-	-	-	+	-	-
Hexadecanamide,N,N-bis(2-(2-butoxyethoxy)ethoxy)carbonylmethyl	-	-	+	-	-	-	-	+	-	-
2,6-diisopropylaniline,N,N-di (trifluoroacetyl)-	-	-	-	-	-	-	-	+	-	-
Pperidine,1-(1,7,7-trimethylbicyclo (2.2.1)hept-2-en-2-yl)	-	-	-	-	-	-	+	-	-	-
3-methoxybenzylamine,N,Ndiheptyl	-	-	-	-	-	-	+	-	-	-
1-(1-(2-Nitro-phenyl)-2,5-dixo-pyrrolidine-3-yl)-piperidine-4-carboxylic acid amide	+	+	-	-	-	-	-	-	-	-
Ethoxymethylthioethane	-	-	-	-	-	-	-	-	-	+
Benzenamine,4,4-[sulfonylbis(4,1-phenyleneoxy)] bis-	-	-	-	-	+	-	-	-	-	-
Others										
Cobalt,bis(1,3-disopropylcyclo pentadienyl	-	-	-	-	+	-	-	+	-	-
2-(p(phenylazo)phenyl)hydrazine sulfonic acid	-	-	+	-	-	-	-	+	-	-
Androst-5-en-3-one,19-acetoxy-4,4-dimethyl-,oxime	-	-	+	-	-	+	-	+	-	-
Cyclopentadienyl-trichlorogermyl-dicacarbonyl-timethylphosphan-molybdan	-	-	+	-	-	-	-	+	-	-
O,O-bis (triethylsilyl)	-	-	-	-	-	-	-	-	-	+
7-methoxy-3,4,5,6-tetramethyl-2,1-benzisoxazole	-	-	-	-	-	-	+	-	-	-
Silane,dimethyl(2-napthoxy)octadecyloxy	-	-	-	-	-	-	+	-	-	-
Methane,(methylsulfinyl)(methylthio)	-	+	-	-	-	+	-	-	-	-
6-Nitroundec-5-ene	-	+-	-	-	-	-	-	-	-	-
1-desmethyl-1-benzyl-Eserine	-	+	-	-	-	-	-	-	-	-
Cyclobuta(1,2-b:3,4-b)dithophene, octahydro-1,1,4,4-tetrahydro-	-	-	-	-	-	-	-	-	-	+
N-Acridin-9-yl-N'-(7-methyl-thieno[3,2-d]pyrimidin-4-yl)-hydrazine	+	-	-	-	-	-	-	-	-	-
Benzenamine,N-(6-bromo-n-hexanoyl)-4-[(2-thiazolyl)amino]sulfonyl	-	-	-	-	-	-	-	-	+	-
2,6-Diisopropylaniline,N,N-di(trifluroacetyl)	-	-	-	-	-	-	-	-	+	-

7-methoxy-3,4,5,6-tetramethyl-,2,1-benzisoxazole	-	-	-	+	-	-	-	-	-	-
1,2,3,4-Tetrahydroisouinoline,2acetyl-6,7-dimethoxy-1-phenmethylene	-	-	+	-	-	-	-	-	-	-

The metabolic profile of Petroleum ether, chloroform, ethyl acetate, methanol, and water extracts of leaves and bark tissue generated from GC-MS data were compared by using PCA.

The analysis of PCA components generated in data sets showed the principal components of water, Methanolic extracts of leaves and bark (WB, WL, MB, ML) were clustered together while the Petroleum ether components (PB, PL) were clustered together. Chloroform extracts (CB, CL) and ethyl acetate extracts (EB, EL) were separated distinctly showing metabolite difference between them (Figures 3 and 4).

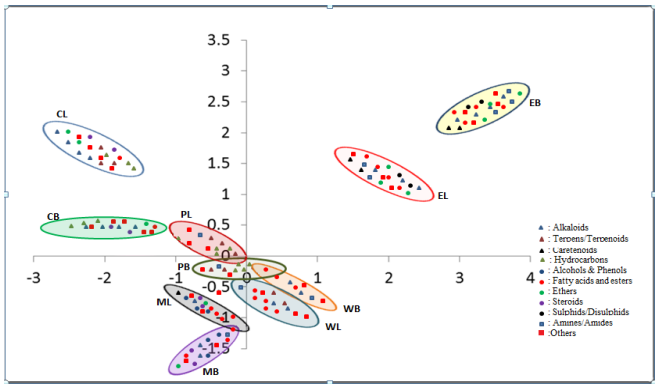

Figure 3: Scatter plots of PCA factor scores for different extract.

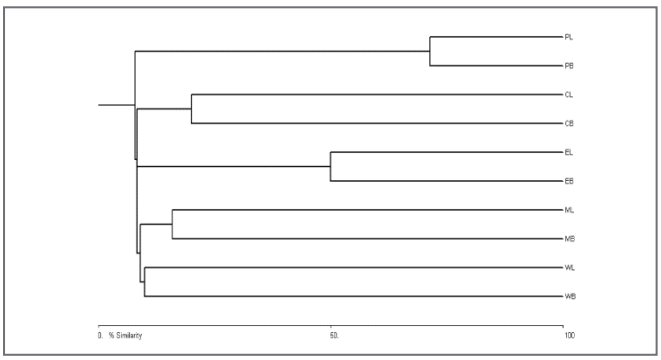

Figure 4: HCA analysis for different extracts.

HCA analysis based on metabolic profiling data obtained through GC-MS analysis showed clear distinction between extracts of polar solvent, medium polar solvent and low polarity solvents. From following dendrogram it was clear that the petroleum ether (PB, PL) extracts forming a separate cluster from other samples. On the contrary methanolic (MB, ML), water (PB, PL) and ethyl acetate (EB, EL) extracts were very close to each other. The water extracts (WB, WL) and methanolic extracts (MB, ML) showed maximum similarity. The extracts of different tissues (leaves and bark) but extracted with same solvent were clustered together forming group and showed similarity (Figure 4).

The metabolites screened from GC-MS analysis showed the wide range of biological activities. Literature serve showed that these compounds showing antimicrobial, anti-oxidant, anti-HIV, anti-cancer activity, anti-inflammatory activity and enzyme inhibitory activity. (S)-Plectoxanxthin [13], Rhodopin [14], Gamolenic acid [15], Tert-Amyl methyl ether, Rubixanthin acetate [16], Tetradecanoic acid, n-hexadecanoic acid [17] are promising antioxidant compounds.

While Methyl-5,2 (undecylcyclopropyl) pentanote, 2,3-epoxy-2-methyl, cholestran [18], Carbostyril, 3-ethyl-4-hydroxy-7-methoxy [19], 2,2,4-trimethyl-3-Penten-1-ol [20] were reported to have good antimicrobial activity. In present study several anticancer compounds were also reported viz. N-deacetyl Colchicine [21], 11-hydroxy, Cephalotaxine [22], Tetradecanoic acid [17], Thiocholchicine [23]. Some compounds like Cyclopentaneundecanoic acid [24] having Alpha amylase inhibitory activity, while Quinoline-4-ol,2-tetradecyl, n-oxide [25] having inhibitor activity of photosystem II and cytochrome b6 complex were also reported in GC-MS studies.

Some multidisciplinary compounds like Lupol [26], showing antiprotozoal, antimicrobial, anti-inflammatory and antitumor activity were also present. Further the separation and purification of reported compounds needed for pharmaceutical uses.

Conclusion

Present studies showed that metabolomics approach in herbal medicine research has application in identification of plant species, quantification of metabolites and identification of bioactive compounds and their stability [27]. The combination metabolomics approaches along with bioactivity assay in mammalian

system may helpful to identify novel chemical compounds and their use in future development of new drugs.

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Conflict of Interest

The authors have no actual or potential conflicts of interest to report.

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