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Research Article

DETERMINATION OF BIOACTIVE COMPONENTS OF *MYXOPYRUM SERRATULUM* A.W. HILL (OLEACEAE) STEM BY GC-MS ANALYSIS

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ABSTRACT

The present study is to evaluate bioactive compounds of ethanol extract of *Myxopyrum serratulum* stem using GC-MS analysis. The chemical compositions of the ethanol extract of stem of *Myxopyrum serratulum* were investigated using Perkin-Elmer Gas Chromatography-Mass Spectrometry, while the mass spectra of the compounds found in the extract were matched with the National Institute of Standard and Technology (NIST) library. Thirty two compounds were identified. The prevailing compounds in ethanol extract were 2-Propenoic acid, 3-(4-methoxyphenyl)-, α-Amyrin, 1-(2-Hydroxy-ethyl)-2-methyl-1H-benzoimidazole-5-carboxylic acid methyl ester, n-Hexadecanoic acid, Pyrazolidine-3,5-dione and 4-phenyl-, 5-Hydroxymethylfurfural. The compounds identified through the GC-MS analysis were used in various applications as antimicrobial, antiinflammatory, hepatoprotective and cancer preventive.

Keywords: *Myxopyrum serratulum*, GC-MS, Phytocomponent, Phytol, 5-Hydroxymethylfurfural, 2-Propenoic acid, 3-(4-methoxyphenyl)-, α-Amyrin.

INTRODUCTION

There is an increasing interest in the phytochemical compounds, which could be relevant to their nutritional incidence and their role in health and disease[1]. In the recent years, the interest for the study of the organic compounds from plants and their activity has increased. A lot of extraction methods and analytical methods like spectrophotometry, high performance liquid chromatography, capillary electrophoresis (HPLC), gas chromatography-flame ionization detection (GC-FID), gas chromatography-mass spectrometry (GC-MS) are developed for the study about plant active compounds[2,3]. The aim of the present study is to develop a rapid method for quantitative determination of organic compounds in herbs by GC-MS technique.

Myxopyrum serratulum (Oleaceae) commonly known as "Chaturamulla" is a large woody climbing shrub. The leaves are used as astringent, acrid, sweet, thermogenic, anodyne, febrifuge and tonic. They are useful in vitiated conditions of kapha and vata, cough, asthma, rheumatism, cephalalgia, nostalagia, consumption, fever, otopathy, neuropathy and cuts and wounds[4]. Taking into consideration of the medicinal importance of this plant, the ethanol extract of stem of *M. serratulum* was analysed for the first time using GC-MS. This work will help to identify the compounds of therapeutic value. A majority of the rich diversity of southern region of Western Ghats Tamil Nadu medicinal plants are yet to be scientifically evaluated for such properties. With this background, the present study was aimed to identify the phytoconstituents present in *M. serratulum* using GC-MS analysis. 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[5].

MATERIALS AND METHODS

Collection of plant sample

The stem of *Myxopyrum serratulum* A.W. Hill were collected from Pechiparai, Kanyakumari District, Tamil Nadu. With the help of local flora, the specimens were identified and preserved in the Ethnopharmacology Unit, Research Department of Botany, V.O. Chidambaram College, Tuticorin, Tamil Nadu.

Preparation of plant extract

The stem of *Myxopyrum serratulum* were cleaned, shade dried and pulverized to powder in a mechanical grinder. Required quantity of powder was weighed and transferred to stoppered flask, and treated with ethanol until the powder is fully immersed. The flask was shaken every hour for the firsts 6 hours and then it was kept aside and again shaken after 24 hours. This process was repeated for 3 days and then the extract was filtered. The extract was collected and evaporated to dryness by using vacuum distillation unit. The final residue thus obtained was then subjected to GC-MS analysis.

GC-MS Analysis

GC-MS analysis of ethanol extract was performed with GC clarus 500 Perkin Elmer system and Gas chromatograph interfaced to a Mass spectrometer (GC-MS) equipped with a Elite – 1 fused silica capillary column (30 mm x 0.25 mm 1D x 1 um df, composed of 100% Dimethyl poly siloxane). For GC-MS detection, and electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1 ml / min and an injection volume of 2 ul was employed (Split ratio of 10:1); Injector temperature 250^0C; ion-source temperature 280^0C. The oven

temperature was programmed from 110^0C (isothermal for 2 min) with an increase of 10^0C/min, to 200^0C, then 5^0C/min to 280^0C, ending with a 9 min isothermal at 280^0C. Mass spectra were taken at 70 eV, a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 36 minutes. The relative % 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.

Interpretation on mass spectrum of GC-MS was done using the database of National Institute of Standard and Technology (NIST) having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

RESULTS

The results pertaining to GC-MS analysis led to the identification of number of compounds from the GC fraction of the ethanol extract of *Myxopyrum serratulum* stem. These compounds were identified through mass spectrometry attached with GC. The compounds present in the ethanol extract of *M. serratulum* stem identified by GC-MS analysis are shown in Figure 1. The active principles with their retention time (RT), molecular formula, molecular weight, (MW), and concentration (%) in the ethanol extract of *M. serratulum* stem are presented in table 1. The prevailing compounds in ethanol extract were 2-Propenoic acid, 3-(4-methoxyphenyl)- (25.87), α-Amyrin (13.71), 1-(2-Hydroxy-ethyl)-2-methyl-1H-benzoimidazole-5-carboxylic acid methyl ester (10.96), n-Hexadecanoic acid (7.63), Pyrazolidine-3,5-dione, 4-phenyl- (5.82), 5-Hydroxymethylfurfural (4.66), (3-Nitrophenyl) methanol, isopropyl ether (3.68), 9-Octadecenoic acid, (E)- (3.35), 9,12-Octadecadienoic acid (Z,Z)- (3.16), β-Amyrin (2.68), Phytol (2.59), Isoquinoline, 1,2,3,4-tetrahydro-1-allyl-6,7-dimethoxy-3,3-dimethyl- (2.13), 5-Hydroxy-6-methyl-12,13-dioxa-tricyclo[7.3.1.0(1,6)]tridecane-8-carboxylic acid, methyl ester (1.80), Ethyl α-d-glucopyranoside (1.79), 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol (1.41) respectively. Figure 2,3,4,5,6,7,8,9,10,11,12,13 and 14 show the mass spectrum and structures of D-Mannopyranose, Ethyl hydrogen succinate, (3-Nitrophenyl) methanol, isopropyl ether, Pyrazolidine-3,5-dione, 4-phenyl-, 1-(2-Hydroxy-ethyl)-2-methyl-1H-benzoimidazole-5-carboxylic acid methyl ester, Galactitol, Phytol, 9-Octadecenoic acid, (E)-, Oleic Acid, Betulin, β-Amyrin, α-Amyrin, 9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, acetate, (3β,4α,5α). Table 2 listed the major phytocomponents and their biological activities obtained through the GC-MS study of *M. serratulum* stem.

Table 1: Components detected in *M. serratulum* stem

RT	Name of the compound	Molecular Formula	Molecular Weight	Peak Area %
4.47	D-Mannopyranose	$C_6H_{12}O_6$	180	0.63
4.72	Ethyl hydrogen succinate	$C_6H_{10}O_4$	146	0.75
5.83	5-Hydroxymethylfurfural	$C_6H_6O_3$	126	4.66
6.06	7-Ethyl-4-decen-6-one	$C_{12}H_{22}O$	182	0.17
6.48	3-Hydroxydecanoic acid	$C_{10}H_{20}O_3$	188	0.52
9.14	(3-Nitrophenyl) methanol, isopropyl ether	$C_{10}H_{13}NO_3$	195	3.68
9.77	Pyrazolidine-3,5-dione, 4-phenyl-	$C_9H_8N_2O_2$	176	5.82
10.59	Methyl trans-4-methylcinnamate	$C_{11}H_{12}O_2$	176	0.60
11.63	Ethyl α-d-glucopyranoside	$C_8H_{16}O_6$	208	1.79
12.53	2-Propenoic acid, 3-(4-methoxyphenyl)-	$C_{10}H_{10}O_3$	178	25.87
12.83	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	$C_{10}H_{12}O_3$	180	1.41
14.00	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	0.87
15.16	1-(2-Hydroxy-ethyl)-2-methyl-1H-benzoimidazole-5-carboxylic acid methyl ester	$C_{12}H_{14}N_2O_3$	234	10.96
15.62	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	7.63
15.76	Isoquinoline, 1,2,3,4-tetrahydro-1-allyl-6,7-dimethoxy-3,3-dimethyl-	$C_{16}H_{23}NO_2$	261	2.13
16.47	Galactitol	$C_6H_{14}O_6$	182	0.22
17.69	Phytol	$C_{20}H_{40}O$	296	2.59
18.01	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	3.16
18.11	9-Octadecenoic acid, (E)-	$C_{18}H_{34}O_2$	282	3.35
18.44	Octadecanoic acid	$C_{18}H_{36}O_2$	284	1.02
18.50	Oleic Acid	$C_{18}H_{34}O_2$	282	0.32
19.55	5-Hydroxy-6-methyl-12,13-dioxa-tricyclo[7.3.1.0(1,6)]tridecane-8-carboxylic acid, methyl ester	$C_{14}H_{22}O_5$	270	1.80
20.74	E)-13-Docosenoic acid	$C_{22}H_{42}O_2$	338	0.36
24.53	Betulin	$C_{30}H_{50}O_2$	442	0.62
26.16	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{21}H_{40}O_4$	356	0.11
27.22	β-Amyrin	$C_{30}H_{50}O$	426	2.68
29.15	α-Amyrin	$C_{30}H_{50}O$	426	13.71
30.54	Quinoline, 3-dodecyl-2-methyl-4-[(4-methoxyphenyl)methoxy]-	$C_{30}H_{41}NO_2$	447	1.00
32.96	dl-α-Tocopherol	$C_{29}H_{50}O_2$	430	0.24
34.37	1b,4a-Epoxy-2H-cyclopenta[3,4]cyclopropa[8,9]cycloundec[1,2-b]oxiren-5(6H)-one, 7-(acetyloxy)decahydro-2,9,10-trihydroxy-3,6,8,8,10a-pentamethyl-	$C_{22}H_{32}O_8$	424	0.20
34.86	6,7-Epoxypregn-4-ene-9,11,18-triol-3,20-dione, 11,18-diacetate	$C_{25}H_{32}O_8$	460	0.70
35.75	9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, acetate, (3β,4α,5α)-	$C_{32}H_{52}O_2$	468	0.39

Table 2: Activity of phytocomponents identified in the ethanol extract of *M. serratulum* stem

No.	Name of the compound	Compound Nature	**Activity
1.	D-Mannopyranose	Sugar moiety	Preservative
2.	Ethyl hydrogn succinate	Succinic acid compound	Antimicrobial
3.	5-Hydroxymethylfurfural	Aldehyde compound	Antimicrobial, Antiinflammatory
4.	7-Ethyl-4-decen-6-one	Ketone compound	No activity is reported
5.	3-Hydroxydecanoic acid	Alcoholic compound	Antimicrobial
6.	(3-Nitrophenyl) methanol, isopropyl ether	Nitrogen compound	Antimicrobial
7.	Pyrazolidine-3,5-dione, 4-phenyl-	Alkaloid	Antimicrobial, Anti-inflammatory
8.	Methyl trans-4-methylcinnamate	Cinnamic acid compound	Antimicrobial, Anti-inflammatory
9.	Ethyl α-d-glucopyranoside	Sugar moiety	Preservative
10.	2-Propenoic acid, 3-(4-methoxyphenyl)-	Aromatic compound	No activity reported
11.	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	Phenolic compound	Antimicrobial, Anti-inflammatory Antioxidant, Analgesic
12.	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Terpene alcohol	Antimicrobial, Anti-inflammatory
13.	1-(2-Hydroxy-ethyl)-2-methyl-1H-benzoimidazole-5-carboxylic acid methyl ester	Amino compound	Antimicrobial, Anti-inflammatory
14.	n-Hexadecanoic acid	Palmitic acid	Antioxidant, Hypocholesterolemic Nematicide, Pesticide, Lubricant Antiandrogenic, Flavor, Hemolytic
15.	Isoquinoline, 1,2,3,4-tetrahydro-1-allyl-6,7-dimethoxy-3,3-dimethyl-	Alkaloid	Antimicrobial, Anti-inflammatory
16.	Galactitol	Sugar alcohol	Leads to cataract
17.	Phytol	Diterpene	Antimicrobial, Anti-inflammatory Anticancer, Diuretic
18.	9,12-Octadecadienoic acid (Z,Z)-	Linoleic acid	Antiinflammatory, Hypocholesterolemic Cancer preventive, Hepatoprotective, Nematicide, Insectifuge, Antihistaminic , Antieczemic, Antiacne, 5-Alpha reductase inhibitor Antiandrogenic, Antiarthritic, Anticoronary, Insectifuge
19.	9-Octadecenoic acid, (E)-	Oleic acid compound	Cancer preventive, Flavor Hypocholesterolemic, 5-Alpha reductase inhibitor, Antiandrogenic, Perfumery Insectifuge, Anti-inflammatory Anemiagenic, Dermatitigenic, Choleretic
20.	Octadecanoic acid	Stearic acid	No activity reported
21.	Oleic Acid	Oleic acid compound	Cancer preventive, Flavor, Hypocholesterolemic 5-Alpha reductase inhibitor, Antiandrogenic Perfumery, Insectifuge, Anti-inflammatory, Anemiagenic, Dermatitigenic, Choleretic
22.	5-Hydroxy-6-methyl-12,13-dioxa-tricyclo[7.3.1.0(1,6)]tridecane-8-carboxylic acid, methyl ester	Ester compound	No activity reported
23.	E)-13-Docosenoic acid	Unsaturated fatty acid	No activity reported
24.	Betulin	Triterpene	Antibacterial, Antioxidant, Antitumor Cancer preventive, Antiviral, Antiflu Cytotoxic, Anti HIV, Anticarcinomic Anti-inflammatory, Antifeedant
25.	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	Oleic acid compound	No activity reported
26.	β-Amyrin	Triterpene	Antibacterial, Antioxidant Antitumor, Cancer preventive Immunostimulant, Chemo preventive Lipoxygenase-inhibitor, Pesticide
27.	α-Amyrin	Triterpene	Antibacterial, Antioxidant Antitumor, Cancer preventive Immunostimulant, Chemo preventive Lipoxygenase-inhibitor, Pesticide
28.	Quinoline, 3-dodecyl-2-methyl-4-[(4-methoxyphenyl)methoxy]-	Alkaloid	Antimicrobial, Anti-inflammatory
29.	dl-α-Tocopherol	Vitamin E compound	Antiageing, Analgesic, Antidiabatic, Antiinflammatory, Antioxidant, Antidermatitic, Antileukemic, Antitumor, Anticancer, Hepatoprotective, Hypocholesterolemic, Antiulcerogenic, Vasodilator, Antispasmodic, Antibronchitic, Anticoronary

30.	1b,4a-Epoxy-2H-cyclopenta[3,4]cyclopropa[8,9]cycloundec[1,2-b]oxiren-5(6H)-one, 7-(acetyloxy)decahydro-2,9,10-trihydroxy-3,6,8,8,10a-pentamethyl-	Oxiren compound	No activity reported
31.	6,7-Epoxypregn-4-ene-9,11,18-triol-3,20-dione, 11,18-diacetate	Ketone compound	No activity reported
32.	9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, acetate, (3β,4α,5α)-	Steroid	Antimicrobial, Anti-inflammatory Anticancer, Antiasthma Hepatoprotective , Diuretic

**Source: Dr.Duke's Phytochemical and Ethnobotanical Databases

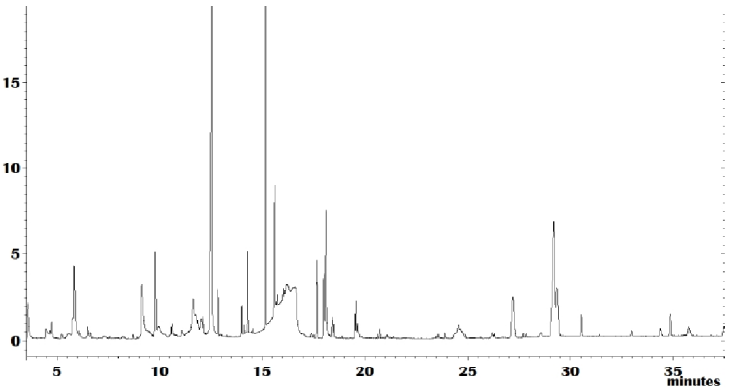

Figure 1: GC-MS chromatogram of the ethanol extract of *M. serratulum* stem

Mass Spectrum of *Myxopyrum serratulum* stem

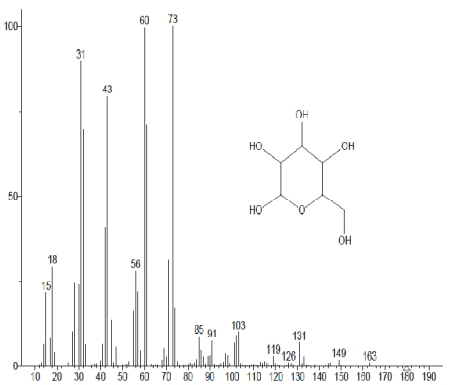

Figure 2: D-Mannopyranose

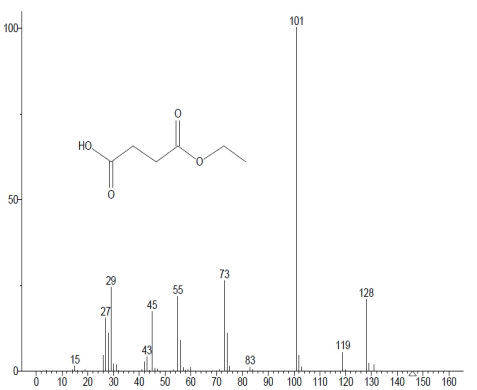

Figure 3: Ethyl hydrogn succinate

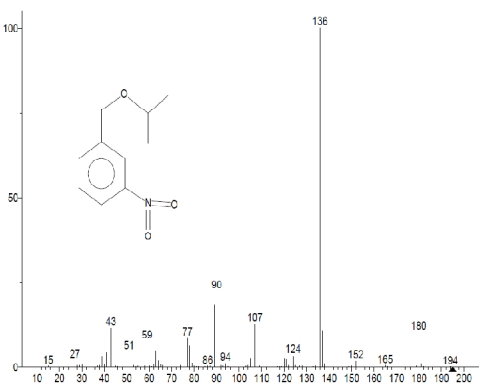

Figure 4: (3-Nitrophenyl) methanol, isopropyl ether

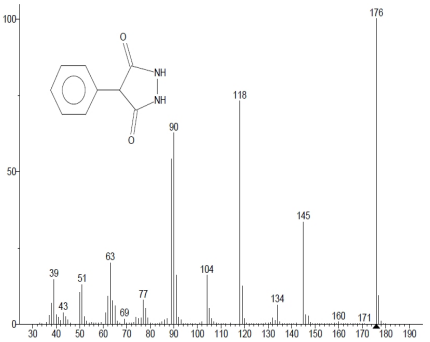

Figure 5: Pyrazolidine-3,5-dione, 4- phenyl

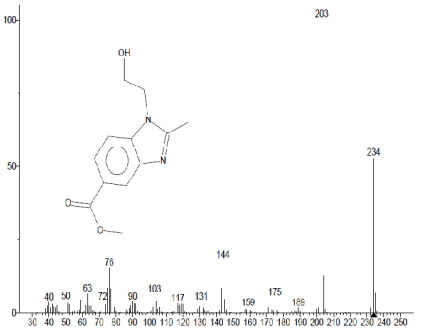

Figure 6: 1-(2-Hydroxy-ethyl)-2-methyl-1H- benzoimidazole-5-carboxylic acid methyl ester

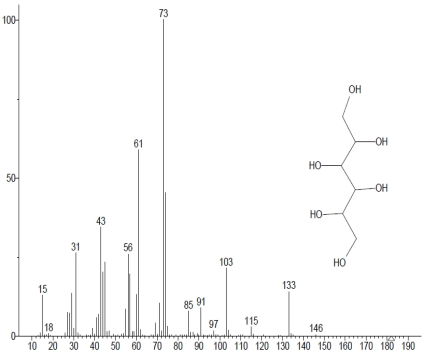

Figure 7: Galactitol

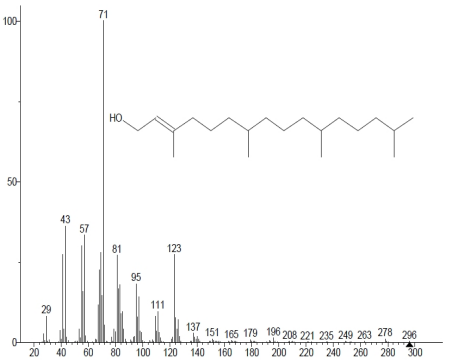

Figure 8: Phytol

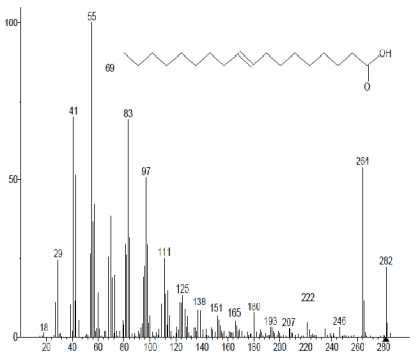

Figure 9: 9-Octadecenoic acid, (E)

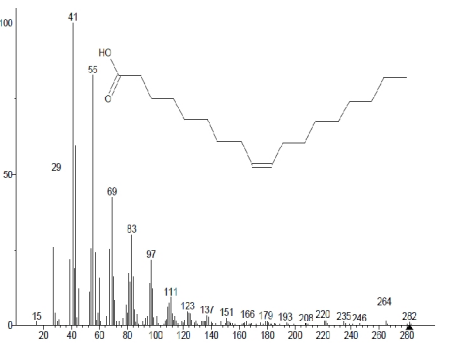

Figure 10: Oleic acid

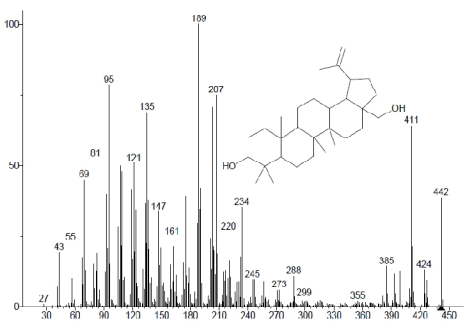

Figure 11: Betulin

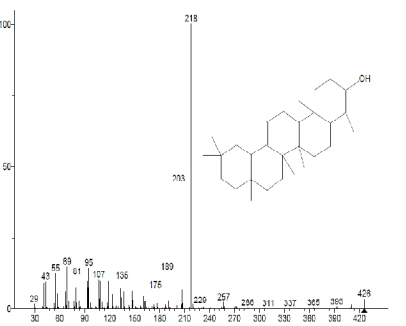

Figure 12: β-Amyrin

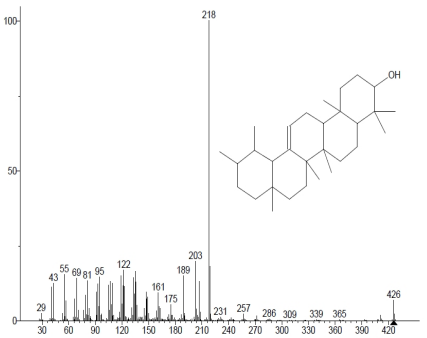

Figure 13: α-Amyrin

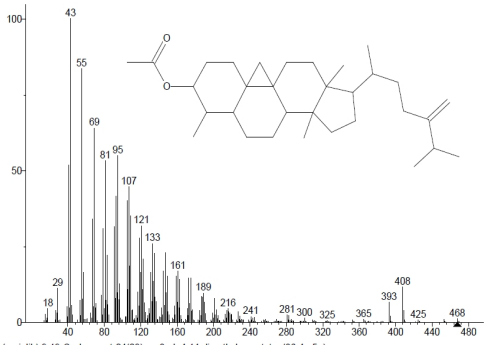

Figure 14: 9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimsethyl-, acetate, (3β,4α,5α)-

DISCUSSION

Among the identified phytochemicals, 9,12-Octadecadienoic acid (Z, Z) have the property of antiinflammatory and antiarthritic as reported by the earlier workers[6-8]. n-Hexadecanoic have the property of antioxidant and antibacterial activty[9]. n-Hexadecanoic acid has shown cytotoxicity to human leukemic cells, MOLT-4 and also showed *in vivo* antitumor activity in mice[10,11]. Biological screening tests showed that 2-propenoic acid, 3-(4-methoxypheneyl)- have significant activity against different bacteria and fungi[12]. 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol, was used as antimicrobial agents[13]. Pyrazolidine-3, 5-dione derivative has been developed as antiinflammatory and antibacterial agents[14]. Oleic acid may hinder the progression of adreno leuko dystrophy (ALD), a fatal disease that affects the brain and adrenal glands[15]. Oleic acid also keeps cell membranes soft and fluid allowing helpful antiinflammatory substances like omega-3 fatty acid to penetrate the cell membrane move easily and preventing the negative effects of bad cholestrol[16]. It has been reported that 5-hydroxymethylfurfural has antioxidant and anti-ischemic properties, and can enhance erythrocyte deformation and alter blood haemorheology in rats[17]. 5-Hydroxymethylfurfural was also found to improve acute liver injury in mice[18] and could inhibit the oxidative damage to hepatocytes caused by H_2O_2 in human LO_2 hepatocytes[19]. All these suggest the anti- apoptosis mechanism of 5-hydroxymethylfurfural. A mixture of α- and β-amyrin isomers protects liver against acetaminophen-induced injuries[20] and ameliorates l-arginine-induced acute pancreatitis[21]. α-Amyrin esters were also reported to exhibit significant biological activity, namely cytotoxic[22] and antiinflammatory[23]. Phytol is detected in *M. serratulum* stem which was also found to be effective in different stages of arthritis. Phytol is a key acyclic diterpene alcohol that is a precursor for vitamins E and K_1. It was found to give good as well as preventive and therapeutic results against arthritis. The results show that reactive oxygen species promoting substances such as phytol constitute of promising novel class of pharmaceuticals for the treatment of rheumatoid arthritis and possibly chronic inflammatory disease[24].

CONCLUSION

The presence of various bioactive compounds justifies the use of this plant for various ailments by traditional practitioners. However, isolation of individual phytochemical constituents and subjecting its biological activity will definitely give fruitful results. It could be concluded that *Myxopyrum serratulum* stem contains various bioactive compounds. So it is recommended as a plant of phytopharmaceutical importance. However, further studies are needed to undertake its bioactivity and toxicity profile.

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