Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2016, 8(8):403-409

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Analysis of bioactive components from ethyl acetate and ethanol extract of *Mucuna pruriens linn* seeds by GC-MS technique

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ABSTRACT

GC-MS analysis was carried out to analyze the bioactive constituents in seeds of Mucuna pruriens Linn. Seven compounds were identified in ethyl acetate extract and eight ompounds were detected in ethanol extract by GC-MS analysis. This study reveals that Hexadecanoic acid, methyl ester, n- Hexadecanoic acid, 9, 12-Octadecadienoic acid, (Z,Z)- and Pyrrolidine,1-(1-oxo-7,10-hexadecadienyl)-were present on both extract. 1,2,3- Propanetriol,1-acetate, 9,12-Octadecadienoic acid, methylester, (E,E)-, and Deoxyspergualin were analyzed only on ethyl acetate extract. While Palmidrol, Hexadecanoic acid, 2-hydroxy-1-(hydroxyl methyl) ethyl ester, 9, 12-Octadecadienoic acid (Z,Z)-2-hydroxy-1-(hydroxymethyl) ethyl ester and 13-Docosenamide,(Z)- were found to be present only on ethanol extract. The presence of these bioactive constituents shows the pharmaceutical value of this plant.

Keywords: *Mucuna pruriens Linn*, bioactive constituents, seeds, GC-MS analysis.

INTRODUCTION

Since primeval times herbal drugs have been prepared from medicinal plants. Different parts of plant (leaves, flowers, stem, roots, seeds, bark etc.) could be used as herbal drugs in peculiarly or in combinations with each other. Medicinal value of the plants is due to the presence of various bioactive phytochemical constituents that produce specific physiological action on the human body [1]. These phytochemicals could be classified into two categories viz. primary and secondary according to their function in the plant body. Primary metabolites such as sugars, amino acids, proteins, lipids, chlorophyll etc are necessary for growth while secondary metabolites such as alkaloids, essential oils, flavanoids, tannins, terpenoids, saponins, phenolic compounds, cardiac glycosides etc. play a vital role in plant defence against herbivory [2] and other interspecies defences. Secondary metabolites extensively form the backbone of the modern medicine [3]. Humans use secondary metabolites as medicines, flavorings and drugs and for other purposes.

Now a days, gas chromatography–mass spectrography (GC–MS) a new hyphenated technique has been adapted precisely to analyze the structures of different phytoconstituents in plant extracts and biological samples with great success [4,5]. GC–MS is a conscientious and high-principled technique to diagnosticate the constituents of volatile matter, long-chain branched hydrocarbons, alcohols, acids, and esters [6]. Usually, the highest concentration of octadecadienoic acid, hexadecanoic acid, and oleic acid was detected with GC–MS.

The *Mucuna pruriens Linn* the most attractive drug in Ayurvedic system of medicine is commonly known as Kewanch or Kaunch and Velvet bean [7-8]. It belongs *to* Fabaceae family. This climbing annual legume plant is habitually present in tropical and sub-tropical regions of the world. Various parts of *Mucuna* plant possesses valuable medicinal properties [9].The seed of *Mucuna pruriens* exhibit various pharmacologiacal activities such as antimicrobial, anti-inflammatory, antidiabnetic, aphrodisiac, antioxidant, neuroprotective, antiprotozoal, analgesic, antivenom, Anthelmintic , anti-parkinsonism activity etc [10-11] .

In present work different bioactive chemical constituents present in ethyl acetate and ethanol extract in seeds of *Mucuna pruriens Linn* has been analyzed by GC-MS.

EXPERIMENTAL SECTION

Collection of plant material

Mature seeds of *Mucuna pruriens* were collected from the tribal region of Mandla district, Madhya Pradesh. The Plant material was thoroughly cleaned, shade dried and powdered with the help of blender. 100g of the dried plant material was used for extraction.

Solvent extraction

100g of powdered material was filled in thimble and sequentially extracted in soxhlet Apparatus with solvents of increasing polarity starting from petroleum ether, ethyl acetate, ethanol, and finally with water. Extracts were filtered by Whatman filter paper. Filtrate was then concentrated under reduced pressure and preserved at 5^{o}c in air tight bottle.

Sample preparation for GC-MS analysis

50 µl of sample was dissolve in 2ml of methanol and kept in ultrasonic bath for 15 min and centrifuged for 10 min at 6000 rpm and supernatant was injected in GC-MS for analysis.

GC-MS analysis

GC-MS of methanol extract was performed using Agilent 7890A. Compounds were separated on Agilent 1909S-433: 2065.49541 HP -5MS. 5% phenyl methyl silox column (30m x 250µm x 0.25µm). Oven temperature was programmed as follows: isothermal temperature of 50o C for 2 min then increased to 150oC at the rate of 5oC /min and held for 1.75 min then increased to 280oC at the rate of 8oC /min and kept constant for 5min. The run time was 45 min. ionization of sample components were performed on EI mode (70 eV). The carrier gas was helium at 1.0ml/min flow rate. 0.5 ml of sample was injected insplit mode of 20:1.The mass spectrum scan range was set at 29.0 to 500(m/z).

Identification of compounds

Interpretation of mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST). The mass spectrum of phytochemicals was compared with the spectrum of known compounds stored in the NIST library.

RESULTS AND DISCUSSION

Phytochemical present in the seed of *Mucuna pruriens* along with molecular formula and molecular weight were presented in Table 1 and 2. GC-MS chromatogram of ethyl acetate and ethanol extract of seed of *Mucuna pruriens* along with their retention time (RT) are shown in the Fig 1 and 2.

The GC-MS chromatogram of ethyl acetate extract shows many peaks out of which 7 major peaks were characterized and identified on comparision of mass spectra with NIST library. Phytochemical identified in ethyl acetate extract were 1,2,3- Propanetriol,1- acetate, Hexadecanoic acid, methyl ester, n- Hexadecanoic acid, 9,12-Octadecadienoic acid, methylester, (E,E)-, 9,12-Octadecadienoic acid, (Z,Z)-, Pyrrolidine,1-(1-oxo-7,10-hexadecadienyl)- and Deoxyspergualin. Retention time of phytochemicals analyzed in ethyl acetate extract was found to be 5.248, 7.849, 8.103, 8.6495, 8.948, 9.997 and 15.2935.

GC-MS analysis of Ethanol extract showed the presence of 8 compounds. Retention times of these phytochemicals were 7.849, 8.07, 8.948, 9.953, 10.0745, 11.6705, 13.962 and 15.277.These compounds were detected as Hexadecanoic acid, methyl ester, n- Hexadecanoic acid, 9,12-Octadecadienoic acid, (Z,Z)-, Pyrrolidine,1-(1-oxo-7,10-hexadecadienyl)-, Palmidrol, Hexadecanoic acid, 2-hydroxy-1-(hydroxyl methyl) ethyl ester, 9,12-Octadecadienoic acid (Z,Z)-2-hydroxy-1-(hydroxymethyl) ethyl ester and 13-Docosenamide,(Z)-.

Table 1: Phytochemicals identified in Ethyl acetate Extract of Seeds of *Mucuna pruriens Linn*

S.No.	RT	Name of Compound	MF	MW
1	5.248	1,2,3- Propanetriol,1- acetate	C5H10O4	134
2	7.849	Hexadecanoic acid, methyl ester	C17H34O2	270
3	8.103	n- Hexadecanoic acid	C16H32O2	256
4	8.6495	9,12-Octadecadienoic acid, methylester, (E,E)-	C19H34O2	294
5	8.948	9,12-Octadecadienoic acid, (Z,Z)-	C18H32O2	280
6	9.997	Pyrrolidine,1-(1-oxo-7,10-hexadecadienyl)-	C20H35NO	305
7	15.2935	Deoxyspergualin	C17H37N7O3	387

Table 2: Phytochemicals identified in Ethanol Extract of Seeds of *Mucuna pruriens Linn*

S.No.	RT	Name of Compound	MF	MW
1	7.849	Hexadecanoic acid, methyl ester	C17H34O2	134
2	8.07	n- Hexadecanoic acid	C16H32O2	270
3	8.948	9,12-Octadecadienoic acid, (Z,Z)-	C18H32O2	256
4	9.953	Pyrrolidine,1-(1-oxo-7,10-hexadecadienyl)-	C20H35NO	294
5	10.0745	Palmidrol	C18H37NO2	280
6	11.6705	Hexadecanoic acid, 2-hydroxy-1-(hydroxyl methyl) ethyl ester	C19H38O4	305
7	13.962	9,12-Octadecadienoic acid (Z,Z)-2-hydroxy-1-(hydroxymethyl) ethyl ester	C21H38O4	354
8	15.277	13-Docosenamide,(Z)-	C22H43NO	387

Table 2: Biological activities of phytochemical compounds identified in Seeds of *Mucuna pruriens Linn*

S.No.	Name of the compound	Biological Activity
1	1,2,3- Propanetriol,1- acetate	Bacterial inhibiting effect [12].
2	Hexadecanoic acid, methyl ester	Antifungal,Antioxidant,Hypocholesterolemic, Nematicide, Pesticide, Antiandrogenic, Flavour, Haemolytic, 5-Alpha Reductase Inhibitor, Potent Antimicrobial Activity [13].
3	n- Hexadecanoic acid	Pesticide, Lubricant, Antiandrogenic, Antifungal, Antioxidant, Hypocholesterolemic, Nematicide, Anti-Androgenic, Flavour, Haemolytic, 5-Alphareductase Inhibitor, Potent Antimicrobial Agent, Antimalarial And Antifungal [14].
4	9,12-Octadecadienoic acid, methylester, (E,E)-	Hepatoprotective, antihistaminic, hypocholesterolemic, antieczemic
5	9,12-Octadecadienoic acid, (Z,Z)-	Antiinflammatory, Hypocholesterolemic, Cancer Preventive, Hepatoprotective, Nematicide, Insectifuge, Antihistaminic, Antieczemic, Antiacne, 5-Alpha Reductase Inhibitor, Antiandrogenic, Antiarthritic, Anticoronary, Insectifuge [15].
6	Pyrrolidine,1-(1-oxo-7,10-hexadecadienyl)-	Cytotoxic
7	Deoxyspergualin	Cytoprotection, Immunomodulation
8	Palmidrol	Antiinflammatory, anticonvulsant
9	Hexadecanoic acid, 2-hydroxy-1-(hydroxyl methyl) ethyl ester	Hemolytic, pesticide, flavor, antioxidant
10	9,12-Octadecadienoic acid (Z,Z)-2-hydroxy-1-(hydroxymethyl) ethyl ester	Hypocholesterolemic, Nematicide, Antiarthritic, Hepatoprotective, Antiandrogenic, Hypocholesterolemic, 5-Alpha reductase inhibitor, Antihistaminic, Anticoronary, Insectifuge, Antieczemic, Antiacne
11	13-Docosenamide,(Z)-	Antimicrobial

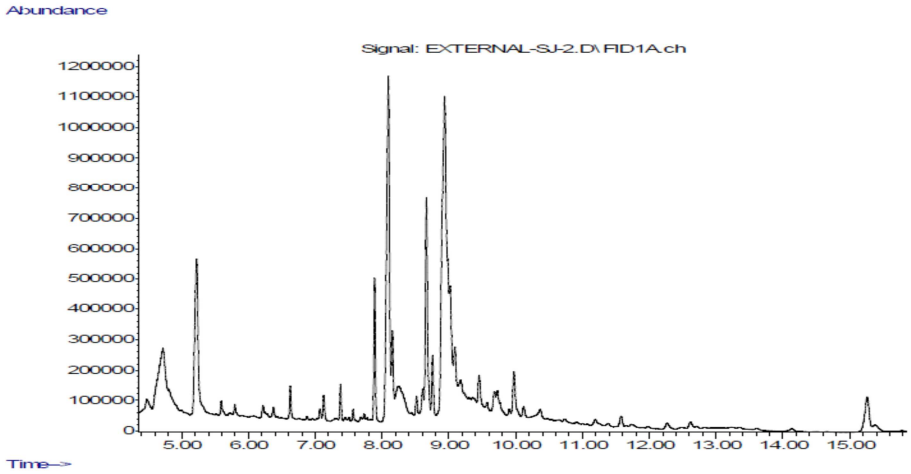


Fig. 1: GC-MS Chromatogram of Ethyl acetate Extract of *Mucuna pruriens Linn*

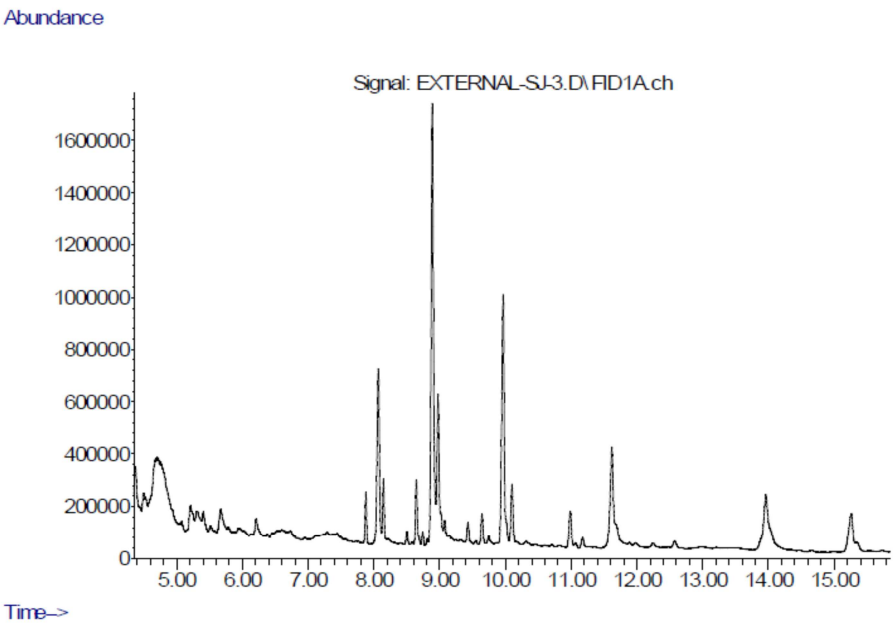
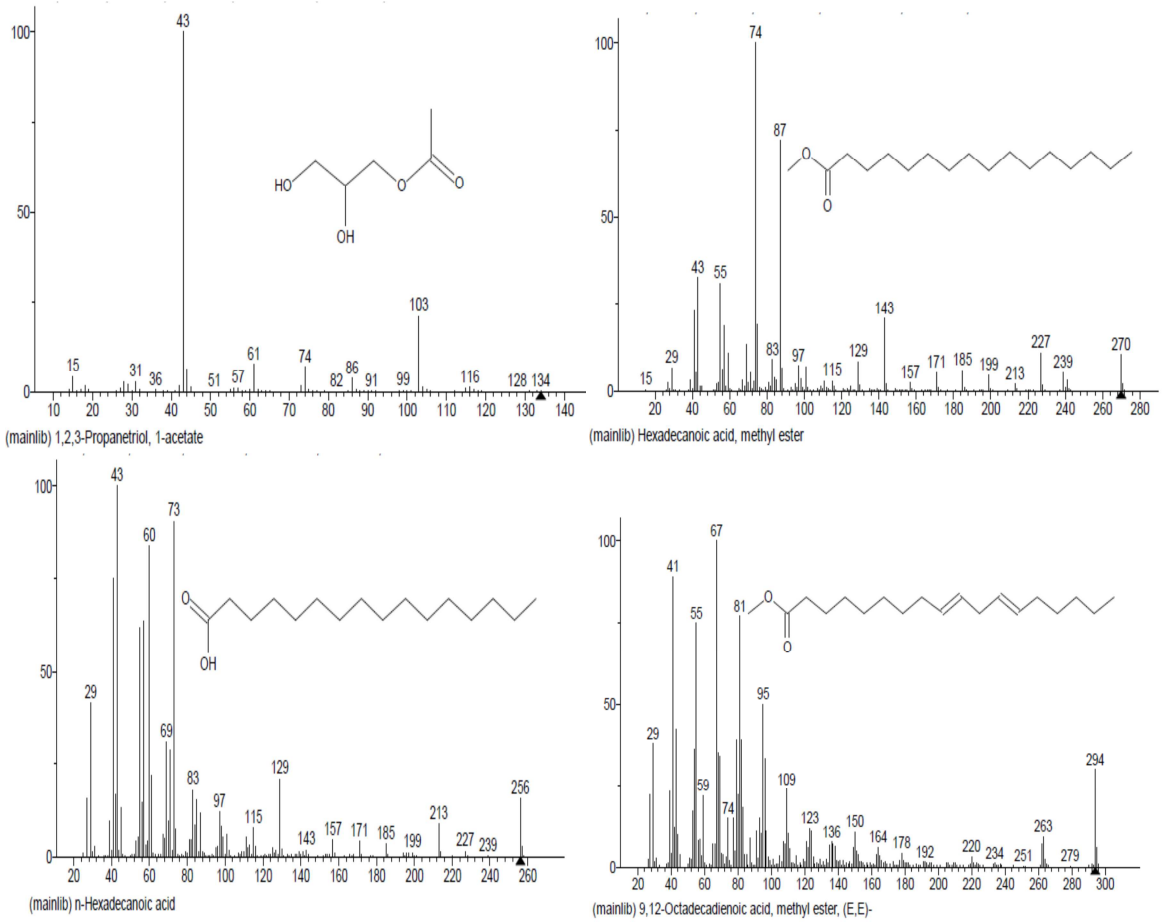


Fig. 2: GC-MS Chromatogram of Ethanol Extract of *Mucuna pruriens Linn*



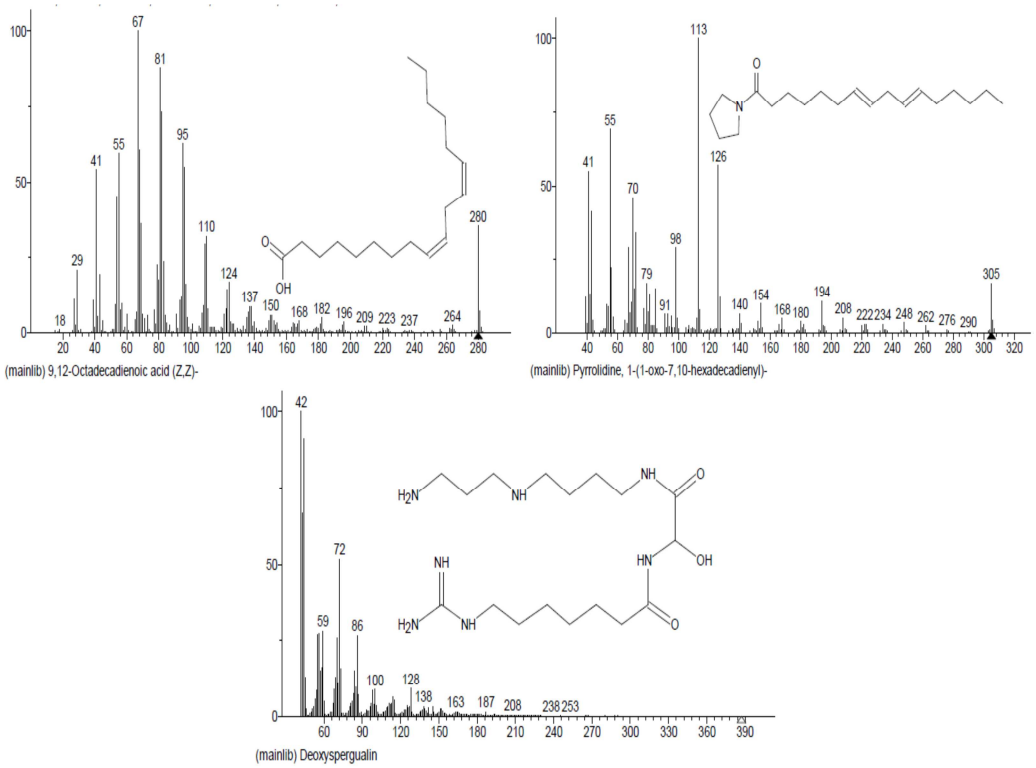
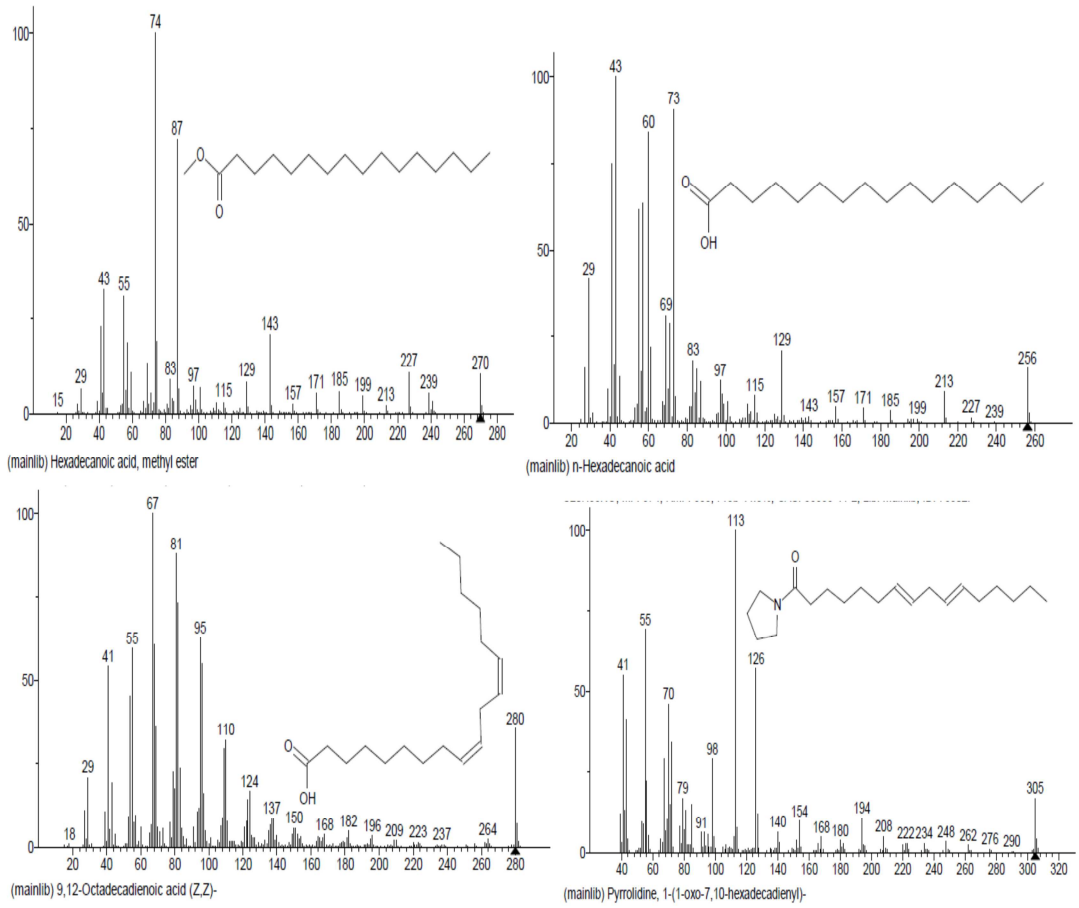


Figure 3: Mass spectrum of Phytochemicals identified in Ethyl acetate Extract of *Mucuna pruriens Linn*



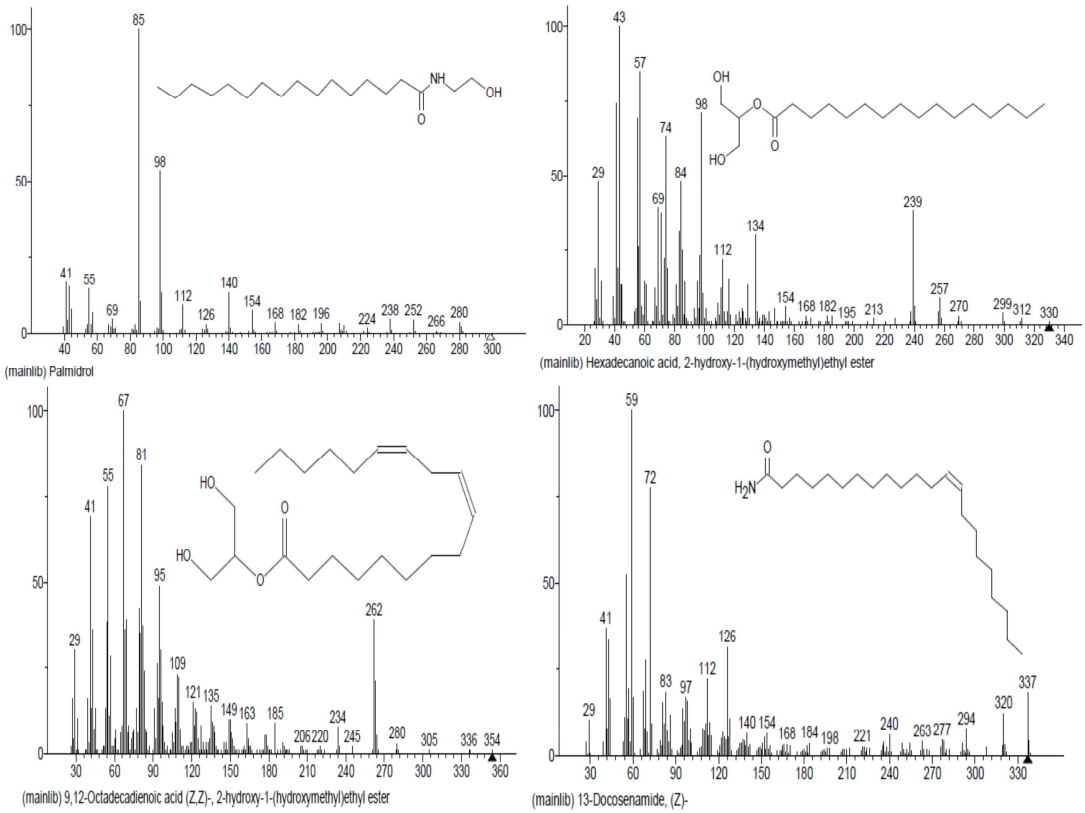


Figure 4: Mass spectrum of Phytochemicals identified in Ethanol Extract of *Mucuna pruriens Linn*

The GC-MS analysis of ethyl acetate and ethanol extract revealed the presence of 7 and 8 compounds respectively. Hexadecanoic acid, methyl ester, n- Hexadecanoic acid, 9, 12-Octadecadienoic acid, (Z, Z)- and Pyrrolidine,1-(1-oxo-7,10-hexadecadienyl)-were common on both extract. 1,2,3- Propanetriol,1- acetate, 9,12-Octadecadienoic acid, methylester, (E,E)-, and Deoxyspergualin were present only on ethyl acetate extract. However Palmidrol, Hexadecanoic acid, 2-hydroxy-1-(hydroxyl methyl) ethyl ester, 9, 12-Octadecadienoic acid (Z, Z)-2-hydroxy-1-(hydroxymethyl) ethyl ester and 13-Docosenamide,(Z)- were distinguished only on ethanol extract.

The identified compounds exhibit many biological properties. Among the identified phytochemicals Hexadecanoic acid, methyl ester and n- Hexadecanoic acid are very potent biological compound and possess many important biological activities like antimicrobial, antioxidant, antifungal, 5-Alpha reductase inhibitor and hypocholesterolemic. 1, 2, 3- Propanetriol,1- acetate shows bacterial inhibiting effect. 9, 12-Octadecadienoic acid, (Z,Z)- is investigated for Antiinflammatory, Hypocholesterolemic, Cancer Preventive, Nematicide activity. Deoxyspergualin is considered for Cytoprotection, Immunomodulation. Palmidrol is used as Antiinflammatory, anticonvulsant agent.

CONCLUSION

GC-MS analysis of the ethyl acetate and ethonol extract of Seeds of *Mucuna pruriens* reveals the presence of phytoconstituents which possess significant biological activity. Thus it gives a clear picture of the pharmaceutical value of this plant. The presence of various bioactive compounds justifies the use of this plant for various ailments by traditional practitioners. Further By isolation and identification of these individual compounds could be carried out for formulation of new drugs to treat different diseases.

Acknowledgements
The first author gratefully acknowledges Rajiv Gandhi National Fellowship University Grants Commission, New Delhi, India award letter no. F1-17.1/2014-15/RGNF-2014-15-SC-MAD-59875 /(SA-III/Website) for providing financial support for the research work.

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