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## PHYTOCHEMICAL ANALYSIS OF METHANOLIC EXTRACT OF SEEDS OF *MUCUNA PRURIENS* BY GAS CHROMATOGRAPHY MASS SPECTROMETRY

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**ABSTRACT:** *Mucuna pruriens* is a climbing legume used in the treatment of various ailments. It is commonly known as Cowitch or velvet bean. Traditionally, it was used in treating male infertility. The main aim of this study was to identify the bioactive materials present in the methanol extract of *Mucuna pruriens* seeds by Gas Chromatography Mass Spectrometry (GC-MS) technique. The analysis by GC-MS reveals the presence 5 major compounds namely, Pentadecanoic acid, 14-methyl-, methyl ester, Dodecanoic acid, 9,12-Octadecadienoic acid (Z,Z)-, methyl ester, 9,12-Octadecadienoic acid and 2-Myristynoyl-glycinamide. By comparing with the references of earlier studies, it was clear that these major compounds played a major role in its neuroprotective, antioxidant, anti-inflammatory, anticancer, hepatoprotective, and antimicrobial effects. The presence of antioxidants has been linked with neurogenesis in the brain. The presence of these compounds may authenticate the scientific evidences of many of its proposed therapeutic potentiality of the seeds of *Mucuna pruriens* (MP).

**INTRODUCTION:** Indigenous herbs are used as remedies against various diseases in the traditional system of medicine. For the past few decades, compounds from natural sources have been gaining importance because of their vast therapeutic benefits. This has led to phenomenal increase in the demand for the herbal medicine in the last two decades in spite of the development in modern medicine <sup>1</sup>. Compared to modern medicine, traditional medicine like Siddha and Ayurveda are easily available at a low cost and this enables them to be widely spread than modern medicine <sup>2</sup>. *Mucuna pruriens* is an herbaceous twinning plant found throughout India.

It belongs to the Fabaceae family. All parts of the plant have been used for therapeutic purpose. The plant is mainly found in South East Asia in countries like India, Bangladesh, Sri Lanka and Malaysia. Apart from Asia, it is also found in certain parts of America and Africa <sup>3</sup>. In India it is grown throughout the entire length of the tropical plains <sup>4</sup>. The plant has got various common names in different languages. It is commonly called the cowitch, velvet bean, buffalo bean. In Tamil it is called as Punaikkali, Poonakkate <sup>5</sup>. It is usually cultivated in any soil type but more favourable in acidic soil with pH between 5 and 8 and with an annual temperature between 19 ° - 27 °C <sup>6</sup>.

Traditionally, the roots are used as a blood purifier, diuretic, for asthma, cholera and elephantiasis. It is also being used in fever, gout, renal stones, and cataract and to relieve rheumatism <sup>7</sup>. It is also used in the treatment of nervous system disorders <sup>6</sup>. The leaves and pods are used as aphrodisiac, nervine tonic and diuretic.

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It is also used in the treatment of diabetes, rheumatic disorders, gout, and tuberculosis<sup>8</sup>. The seeds are used in Parkinsonism, cough, muscular pain, snakebite and also used for male virility<sup>9, 10</sup>. The earlier phytochemical studies and acute toxicity studies conducted by us revealed the presence of Carbohydrates, Proteins and amino acids, flavonoids, saponins, alkaloids, Phytosterols and phenolic compounds. The LD<sub>50</sub> value was found to be 2000 mg/kg and was found to be safe to use. The present study was done to evaluate the Phytochemical compounds present in the methanol extract of *Mucuna pruriens* by GC-MS to validate its therapeutic effects.

## MATERIALS AND METHODS:

**Plant Collection and Extract Preparation:** The seeds of the *Mucuna pruriens* were procured from a local market and were authenticated. Methanol extract of the seed was prepared by Soxhlet method<sup>11</sup>.

## Gas Chromatography Mass Spectrometry

**Analysis:** Gas Chromatography Mass Spectrometry is a very effective method for the separation and recognition of composite mixtures of phytochemicals. In this method, the components of a mixture are first separated using gas chromatography and each separated component is analysed individually using mass spectrometry<sup>12</sup>.

This method helps in the identification of compounds even less than 1 mg, where the sample is first injected into the injection port of the Gas Chromatography (GC) device which vaporizes the sample and then separates and analyses the various components<sup>13</sup>. Each component ideally produces a specific spectral peak that may be recorded on a paper chart electronically. The time elapsed

between injection and elution is called the "retention time." The retention time can help to differentiate between some compounds. The peak is measured from the baseline to the tip of the peak<sup>14</sup>. GC analysis of the plant extract was performed using a Shimadzu GC 17A - QP5050A gas chromatograph. The oven temperature was programmed to be 290.00 °C at a rate of 10 °C/min; the carrier gas was helium with a flow rate of 1ml/min. The sample was injected using the split sampling technique in the ratio of 1:10.

**Identification of the Components:** The database of National Institute Standard and Technology (NIST) which has more than 62,000 patterns was used for the interpretation on the mass spectrum GC-MS. The fragmentation pattern spectra of the unknown components were compared with those of the known components stored in the NIST library. The relative percentage amount of each bio-component was calculated by comparing its average peak area to the total area. The name, molecular weight, molecular formula and structure of the components of the extract were ascertained.

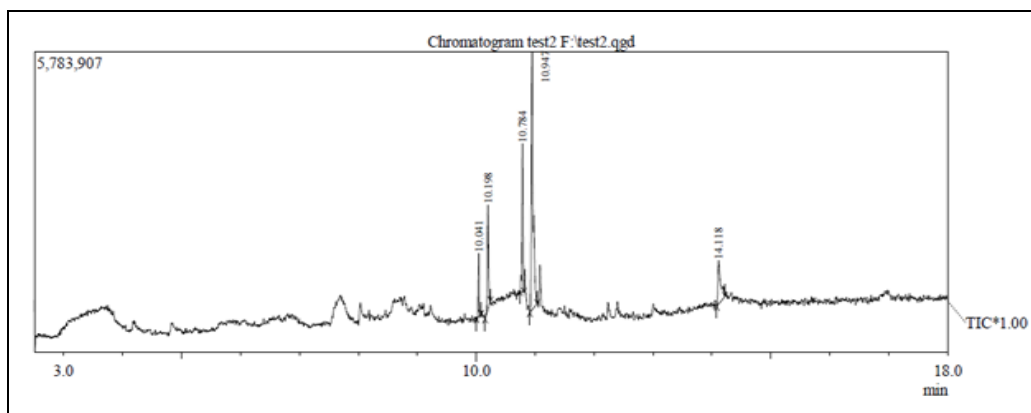
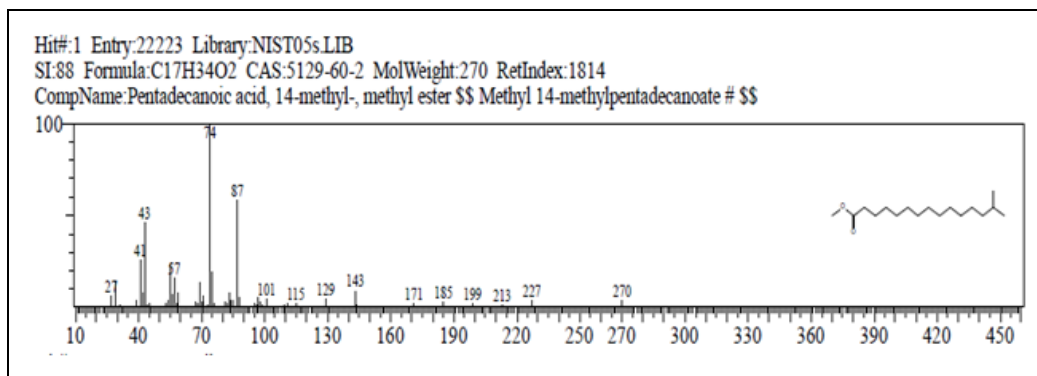
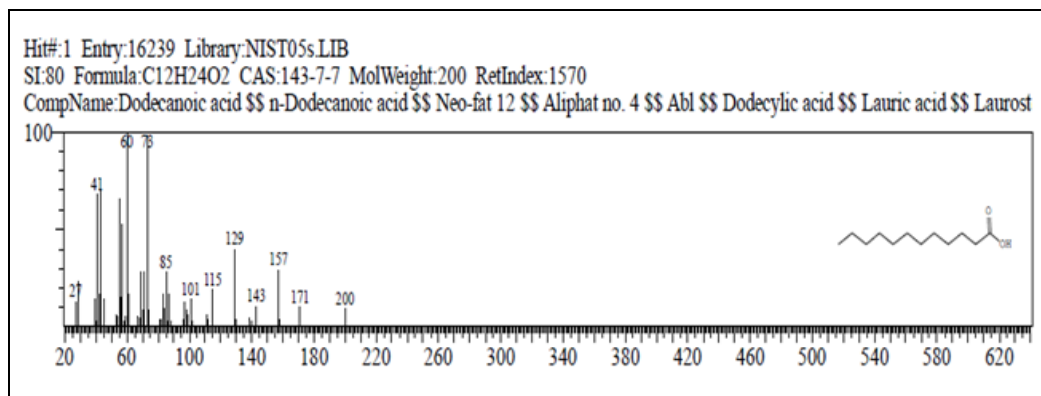
**RESULTS AND DISCUSSION:** The GC-MS analysis of the methanolic extract of MP seeds revealed the presence of five different phyto-compounds viz Pentadecanoic acid, 14-methyl-, methyl ester, Dodecanoic acid, 9, 12-Octadecadienoic acid (Z,Z)-methyl ester, 9,12-Octadecadienoic acid and 2-Myristinoyl-glycinamide. The gas chromatogram of the extract is shown in **Fig. 1** and the mass spectra of the compounds are presented in **Fig. 2A, 2B, 2C, 2D** and **2E**. The retention time, name of the compound, compound nature, molecular formula, molecular weight are discussed in **Table 1** and the therapeutic uses of each of the compound is discussed in **Table 2**.

**TABLE 1: BIOACTIVE COMPOUNDS IN MUCUNA PRURIENS IDENTIFIED BY GAS CHROMATO-GRAPHY MASS SPECTROMETRY (GCMS)**

S. No	RT (min)	Name of the compound	Nature of the compound	Molecular formula	Molecular weight (g/mol)
1	10.042	Pentadecanoic acid, 14-methyl-methyl ester	Fatty acid ester	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270.45
2	10.208	Dodecanoic acid/lauric acid	Fatty acid	C <sub>12</sub> H <sub>24</sub> O <sub>2</sub>	200.322
3	10.783	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	Polyenoic fatty acid	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	294.47
4	10.950	9,12-Octadecadienoic acid/linoleic acid	Fatty acid	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	280.452
5	14.117	2-Myristinoyl-glycinamide	Amino compound	C <sub>16</sub> H <sub>28</sub> N <sub>2</sub> O <sub>2</sub>	280.412

**TABLE 2: THERAPEUTIC USES OF THE BIOACTIVE COMPOUNDS IN *MUCUNA PRURIENS* IDENTIFIED BY GAS CHROMATOGRAPHY MASS SPECTROMETRY (GCMS)**

S. No	Name of the compound	Therapeutic uses
1	Pentadecanoic acid, 14-methyl-, methyl ester	Catechol-O-Methyl-Transferase-Inhibitor, Methyl-Guanidine-Inhibitor
2	Dodecanoic acid/lauric acid	Antibacterial, antifungal, Anticancer activity. Antioxidant, cox-1 and cox-2 inhibitor, antiviral, hypocholesterolemic, candidicide
3	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	Hepatoprotective, antihistaminic, hypocholesterolaemic, antieczemic, Catechol-O-Methyl-Transferase-Inhibitor, Methyl-Guanidine-Inhibitor
4	9,12-Octadecadienoic acid/linoleic acid	Anti-inflammatory, Nematicide, Insectifuge, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Antihistaminic, Antiacne, Antiarthritic, Antieczemic,
5	2-Myristynoyl-glycinamide	5-Alpha reductase inhibitor, Anticoronary Antimicrobial

**FIG. 1: GAS CHROMATOGRAM OF METHANOLIC EXTRACT OF *MUCUNA PRURIENS* SEED****FIG. 2A: MASS SPECTRA OF METHYL 14-METHYLPENTADECANOATE****FIG. 2B: MASS SPECTRA OF DODECANOIC ACID**

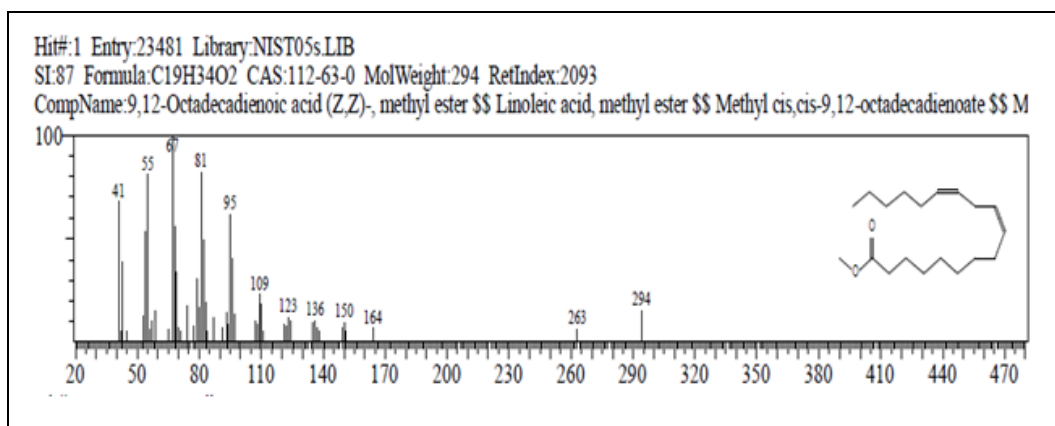


FIG. 2C: MASS SPECTRA OF 9, 12-OCTADECADIENOIC ACID, METHYL ESTER

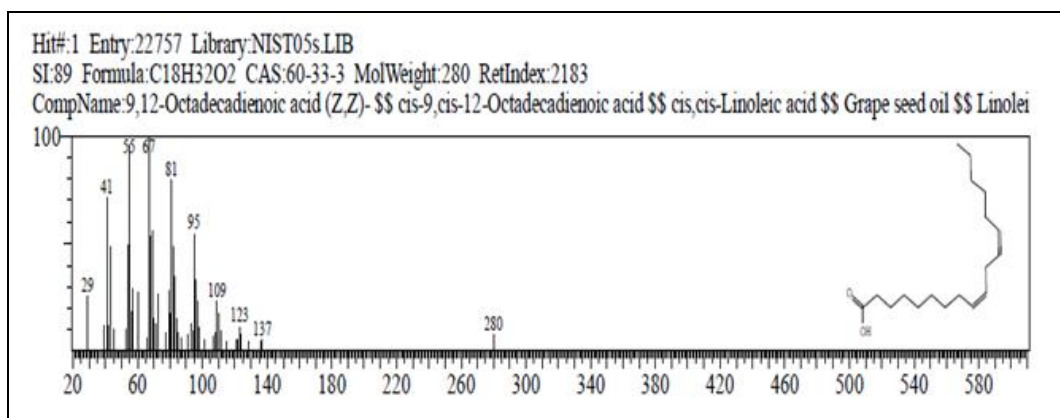


FIG. 2D: MASS SPECTRA OF 9, 12-OCTADECADIENOIC ACID

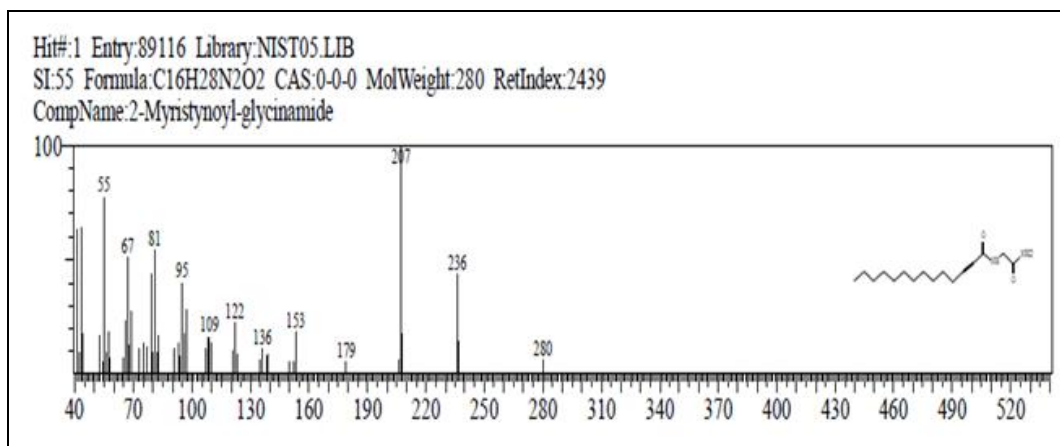


FIG. 2E: MASS SPECTRA OF 2-MYRISTOYL-GLYCINAMIDE

Pentadecanoic acid, 14-methyl- methyl ester and 9, 12-Octadecadienoic acid (Z, Z)-, methyl ester found in the extract is known to have catecholamine O methyl transferase inhibitor activity and methyl guanidine inhibitor activity<sup>15</sup>. Catecholamine O methyl transferase (COMT) is an enzyme that catalyses degradation of the catecholamines- adrenaline, nor adrenaline and dopamine. Thus COMT inhibitor opposes the degradation of the catecholamines which act as neurotransmitters. Out of these,dopamine is an

important neuro-transmitter in basal ganglia and hence COMT inhibitors can be used in the treatment of Parkinson's disease. Methyl guanidine (MG) is a known nephrotoxin and neurotoxin<sup>16</sup>. It is formed from creatinine (CRN) *via* reactive oxygen species (ROS) in particular the hydroxyl radicals<sup>17</sup>.

Thus inhibition of hydroxyl radical mediated MG synthesis from CRN is indicative of antioxidant activity of the said compound. MG has also been

implicated in brain dysfunctions, such as epilepsy<sup>18, 19</sup>. MG inhibitors may be useful clinically in the treatment of epilepsy, renal failure etc. Dodecanoic acid or lauric acid is a saturated fatty acid which has been reported to have antibacterial, anti-inflammatory<sup>20, 21</sup> and anti-cancer activity<sup>22</sup>. It is also a proven antioxidant<sup>23</sup>, cox-1 and cox-2 inhibitor, antiviral, hypo-cholesterolemic and candidicide<sup>24</sup>. Lauric acid increases total HDL cholesterol than any other saturated or unsaturated fatty acid<sup>25</sup>. Lower HDL cholesterol is found to be associated with a decrease in the risk of atherosclerosis<sup>26</sup>. 9,12-Octadecadienoic acid/linoleic acid is a polyunsaturated essential fatty acid which is found to have Antiinflammatory, Nematicide, Insectifuge, Hypocholesterolemic, Cancer preventive, Hepato-protective, Antihistaminic, Antiacne, Antiarthritic, Antieczemic, 5-Alpha reductase inhibitor, Anti-coronary activities<sup>27</sup>. It also has antibacterial effects and inhibits bacterial enoyl-acyl carrier protein reductase (FabI), an essential component of bacterial fatty acid synthesis, which has served as a promising target for antibacterial drugs<sup>28</sup>. 2-Myristynoyl-glycinamide is an amino compound which has antimicrobial properties<sup>29,30</sup>.

**CONCLUSION:** The pursuit towards medicinal herbs to cure various ailments is a never ending quest. There are various indigenous plants with different bioactive compounds that can be used for therapeutic purposes. *Mucuna pruriens* is one such plant with promising medicinal value especially the seeds of the plant. The presence of various bioactive compounds in the seed extract of MP validates its therapeutic uses and further isolation of individual phytochemical constituents may pave way for the development of new drugs and treatment strategies.

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