COMPARATIVE ANALYTICAL STUDY OF DIFFERENT EXTRACTS ON *MUCUNA PRURIENS*

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ABSTRACT

Mucuna pruriens is an under-utilized wild legume, which is widespread in tropical and sub-tropical regions of the world. The test was carried out in Mucuna pruriens seed 'Magic bean' a species of Fabaceae family is a pot of medicinal uses. Preliminary phytochemical analysis was carried in Mucuna pruriens seed and confirmed the presence of alkaloids, reducing sugar, proteins, carbohydrates, saponins and tannins. Three extracts were prepared namely Oil, Aqueous, Alcohol extracts from Mucuna pruriens for GC-MS analysis. Oil extract of Mucuna pruriens Seed through GC- MS analysis identified about 17 components and the main component is N-Hexadecanoic acid. Water extract of Mucuna pruriens Seed shows Ethane, 1,1-Diethoxy-, 2-Methoxy-1,3-Dioxolane, 1-Butanol, 3-Methyl, Formic Acid, 1-Methylethyl Ester, 3,3-Diethoxy-1-Propanol as main compounds. Ethanolic extract shows 1,2-ethanediamine, n-methyl, tuaminoheptane, 2,2-dimethoxybutan as major compounds. Among the three extracts tested, Ghee extract contains toxic- free compounds analysed by GC-MS and found to be safe for consumption. Oil extract can be recommended for the preparation of Ayurvedic medicine for treating various disease conditions.

Keywords: *Mucuna pruriens,* Phytochemical analysis, GC-MS analysis

1. Introduction

Ayurveda the holistic medicine, is a treasure of various formulation which gives a multitude application of medicine in wide range of disease in the form of different in different extracts. From time immemorial plants are the core source of medicines. Herbal based drugs have become the basis of medicinal plants and play an essential role in disease management. In AYUSH numerous plants and their products have been used against stress, general resistance, against infection, eventual improvement of male sexual function, alleviating mental sickness.

The mode of application or consumption of medicines are very important as the medicine itself. In Ayurveda, there is an ample of forms for taking the medicine. In Ayurveda drugs are rarely consumed alone. Usually, they are taken along with different constituents like honey, water, ghee etc. Some of the Different forms of traditional Ayurvedic medicines include Powered form (Choorna), simple juice extract (Swarsa), Herbal oil or ghee extract (Gritham), water decoction (Kashaya)etc. [1] *Mucuna pruriens* is an ascent legume that grows 3-18m tall of family Fabaceae. Its endemic to tropical regions particularly African, India and West indies. Its widely found in bushes hedges and the dry deciduous low forest.

The largest subfamily of the legumes, are the Faboideae, early known as the Papilionoids.*Mucuna pruriens* a drug which is known as the 'Magic Bean' belongs to the family Fabaceae, also called Leguminosae, Leguminous plants contain in vitamins A, B, C, phosphorus, iron, calcium, and potassium. Edible legumes provide nutrients for human consumption to help world hunger and malnutrition. *Mucuna* comes under the subfamily Faboidacea which is the largest group of legumes. The mutual relationship between Rhizobium and the plant, which takes place in root nodules and "fixes" atmospheric nitrogen is most strongly developed in Papilionoidea legumes [2]. On the other hand, it also contains various anti-nutritional factors, such as protease inhibitors, total phenolic, oligosaccharides (raffinose, stachyose, verbascose), and some cyclitols with anti-diabetic effects. In fact, all parts of the *Mucuna* plant is medicinally very important. The main phenolic compound is L-dopa (5%), and *M. pruriens* seeds contain some components that are able to inhibit snake venom. [4]

Ayurvedic herbal dosage forms are formulated on the basis of transference of active components of different drugs by different manufacturing processes. Bhashajya Kalpana is a branch of Ayurveda deals with the preparation of medicines in various forms like Asava Arishta Gulika, etc. [1] While preparing a formulation there are so many aspects has to be considered for getting the expected efficacy from a formulation. A detail understanding and analysis is needed for each method of preparation because a plant contains many active principles. These active principles may be either water soluble, Fat soluble or alcohol soluble. In Ayurveda the different preparation is explained in the form of Panchavidha Kashya Kalpana, Sandhana Kalpana, (Fermented Preparations) Sneha Kalpana (Fat Preparation), Choorna, Gutika Etc. [5] Sneha Kalpana/Paka is a unique dosage form which is aimed for transferring the active principal s of a drug in to aqueous and to a fat soluble medium [6].

In Ayurvedic medicine, Kashaya is made from decoction of herbs by boiling in water. Kashaya refers to water decoction or water extract of a or group of herbs. Aristas are Ayurvedic

medicines prepared by boiling mechanical herbs in water and then fermenting. Arista is considered as best formulation in Ayurveda because they possess better quality the decoction Fat preparation, is also known as Gritham (medicated clarified butter) which is considered as a precious base for preparing medicine in Ayurveda processing of ghee with plant ingredients is renowned for enhancing their therapeutic efficiency. It is very essential to know which extract is better for consuming with almost effect.

Present study focuses on the comparison of most effective formulation in name of maximum components which has more health benefits. Aqueous, ghee, and alcohol extracts are taken to compare in the experiment to find the better form of preparation. So that it could be a valuable contribution for the manufacturing industry to design a formulation. *Mucuna pruriens* seed is a well-known drug which is widely used in the treatment of parkinsonism due to the presence of L-Dopa. [6] Hence the study emphasized in which formulation the more components are extracted by using GC-MS technique.

The objectives of the present study are as follow

- To evaluate physical analysis of *Mucuna pruriens* seed in powdered, fat and aqueous extracts
- To evaluate *invitro* Phytochemical analysis of *M. pruriens* seed
- To determine phytochemicals in oil aqueous and ethanolic extracts of *Mucuna pruriens*.by GC-MS analysis

2.Materials and Method

Mucuna pruriens is collected from local market and authentified in Dravya Guna department. The seeds are washed properly, dried and powdered and kept for raw drug analysis. The phytochemical analysis where conducted in approved labs of Santhigiri Ayurveda Medical College.

2.1 Preparation of Extracts

a. Alcohol extract of *Mucuna pruriens* seed

10gm of seed of *Mucuna pruriens is* added with 100ml of ethanol and macerated well for 6 hrs and filtrate was taken 18hrs. The extract was taken for the analysis [7].

b. Water extract of *Mucuna pruriens seed*

100 gm of coarse powder of *Mucuna pruriens is* added with 800 ml of water and then reduce to ¼ the left-over decoction was taken for the analysis [7].

c. Ghee preparation of *Mucuna pruriens seed*

100gm of seed powder is made into a paste and it is added with 400ml of pure ghee and added with 1600ml of water and is then reduced to 400ml [8].

2.2 Physico-chemical Analysis and Phytochemical Analysis

Physico-chemical Analysis

a. Loss On Drying

Place 2 gm of accurately weighed sample in a tarred evaporating dish. It is allowed to dry at 105 C for 3 hrs. Then dried sample is again weighed.

Percentage of LOD =

$$\frac{\text{Loss in weight}}{\text{Weight of sample}} * 100 \quad (1)$$

b. Water soluble extractive

Weight about 5gm of sample in Iodine flask. Add 100 ml chloroform water. After macerated for 6 hrs it is kept undisturbed for 18 hrs. After it is filtered and 25ml of filtrate is collected in pre weighed Petri dish and place in hot air oven for 1 hr. Then weighed and calculated.

Water soluble extractive value =

$$\frac{\text{Weight of Extract} * \text{Volume Chloroform}}{\text{Weight of Sample} * \text{Volume of Filtrate}} * 100 \quad (2)$$

c. Alcohol soluble extractive value

Weigh about 5 gm of sample in an iodine flask and add 100 ml of alcohol and shake for 6 hrs and kept undisturbed for 18 hrs. Filter the content and collect 25ml of filtrate in a pre -weighed Petri dish. Then it is placed in hot air oven for 1 hr.

Alcohol soluble extractive value =

$$\frac{\text{Weight of extract} * \text{volume of alcohol}}{\text{Weight of sample} * \text{volume of filtrate}} * 100 \quad (3)$$

d. Specific Gravity

Take appropriate sample of material to be tested. Sample should be free from moisture and any other impurity. Carefully wash and clean the specific gravity bottle and dry the interior with a current of air. Weigh the bottle and note the weight. Fill the bottle with distilled water

and record the weight. The again clean the bottle and dry well. Fill the bottle with the sample. Weight must be recorded. Divide the weight of the material by the weight of the water.

$$\text{Specific gravity} = \frac{\text{Density of the liquid sample}}{\text{Density of water}} \quad (4)$$

Preliminary Phytochemical Analysis

Phytochemical screenings are tests conducted to detect the presence of both primary and secondary metabolites in an extract. The preliminary screening of selected plants with methanolic extract were analysed by standard method. Several qualitative analyses described were used to detect the presence of carbohydrates, protein, amino acid, glycosides, steroids, flavonoids, alkaloids, saponins, tannins, terpenoids. [9]

2.3 GC-MS Analysis:

The Clarus 680 GC was used in the analysis employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250μm df) and the components were separated using Helium as carrier gas at a constant flow of 1 ml/min. The temperature was set at 260°C during the chromatography. The 1μl of extract sample injected into the instrument the oven temperature was as follows: 60 °C (2 min); followed by 300 °C at the rate of 10 °C min−1; and 300 °C, where it was held for 6 min. The mass detector conditions were: transfer line temperature 240 °C; ion source temperature 240 °C; and ionization mode electron impact at 70 ev, a scan time 0.2 sec and scan interval of 0.1 sec. The fragments from 40 to 600 Da. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS NIST (2008) library.

3. RESULT AND DISCUSSION

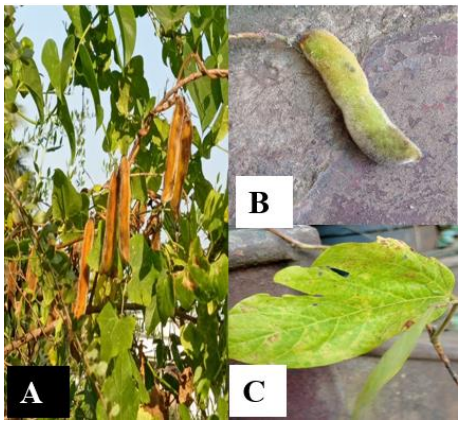

Figure 1. A-Habit, B-Seed Pod and C- Leaf

Systematic position:

Botanical name -*Mucuna pruriens*

Kingdom -Plantae

Division -Magnoliophytes

Class -Magnoliopsida

Order -Fabales

Family -Fabaceae

Trib –Phaseoleae

Genus -Mucuna

Species - pruriens

Parts used – Seed [10]

Mucuna pruriens is an herbaceous twinning annual. Leaves trifoliate, grey silky below: Leaflets elliptic, broadly ovate or rhomboid ovate, unequal at the base. Flowers- In axillary pendulous racemes, Purple. Fruits- Pods, curved, longitudinally ribbed, 5 to 10 cm, densely clothed with grey or pale brown bristles. Seeds 4 to 6 ovoid. Hairs covering the seed pod are vermifuge which are locally stimulant and mild vesicant. [11] Uses of the *M. pruriens* seed are pods are covered with stiff hairs which produce an intense irritation of the skin if incautiously handled. A vinous infusion of the pods is good remedy for dropsy. Hairs of seed pods mixed with honey have been used as a vermifuge in round worms which are expelled. Seeds are prescribed in the form of powdering doses of 20 to 40 grains in leucorrhoea, spermatorrhea etc and in cases requiring an aphrodisiac action. Inolic bases derived from *M. Pruriens* showed anti spasmodic action on smooth muscle preparations against spasms induced by acetyl choline, histamine, serotonin. [12]

Physical Parameters

Results of foreign matter analysis, loss on drying, extractive values in different solvents, pH determination are given in (Table I, II, III). pH is an important quantity that reflects the chemical conditions of a solution. The pH can control the availability of nutrients, biological functions, microbial activity, and the behaviour of chemicals. pH of the dry seed powder of *M. pruriens* is found to be 4.68 which is alkaline in nature. Loss on drying is used to determine the moisture content of the drug. The moisture content of seed powder of *M. pruriens* was found to be 0.6829% (Table I). Water-soluble extractive and alcohol soluble extraction value plays an important role in evaluation of crude drugs. Less extractive value indicates addition of exhausted material, adulteration or incorrect processing during drying or storage or formulating. Water soluble extraction value and alcohol soluble extraction value of seed powder showed 40.3933% and 61.837% respectively (Table II). Loss on drying of ghee extract of *M. pruriens* seed powder is found to be 0.6234%. Specific gravity is defined as the ratio of material's density to the density of water at 23°C. Specific Gravity of ghee extract found to be

0.9180%. pH of analysis of aqueous extract of *M. pruriens* seed powder was found to be 4.07. Amount of total soluble solid present in the unit volume of solution is 0.127%. (Table III)

Table I

PHYSICAL ANALYSIS OF *M. PRURIENS* SEED POWDER

S. No	PHYSICAL ANALYSIS	VALUE
1.	pH	4.68
2.	Loss of drying	0.6829%
3.	Water soluble extraction value	40.3933%
4.	Alcohol Soluble Extraction Value	61.8367%

Table II

ANALYSIS OF GHEE EXTRACT OF *M. PRURIENS* SEED POWDER

S. No	PHYSICAL ANALYSIS	VALUE
1.	LOD	0.6234%
2.	Specific Gravity	0.9180
3.	Ghee Acid Value	1.06

Table III

ANALYSIS OF AQUEOUS EXTRACT OF *M. PRURIENS* SEED POWDER

S. No	PHYSICAL ANALYSIS	VALUE
1.	pH	4.07
2.	Total Soluble Solids	0.127%

The phytochemical results were tabulated in the Table IV. Phytochemical analysis of *Mucuna pruriens* was attempted in the present study. The seed was shade dried and powdered and were subjected to phytochemical screening. Evaluation of ethanolic extract was prepared as per the standard method of extraction. For this grams of powder were macerated with ethanol

and the extract was taken for identification of alkaloids, glycosides, tannin, etc. Table IV shows the results of *Mucuna pruriens* seeds. From the result, we could observe that, *Mucuna pruriens* contains alkaloids, reducing sugar, proteins and moderate amount of tannin and carbohydrates, lesser amount of saponins. Flavonoids and terpenoid were found to be below the quantification limit.

The presence of this constitutes are very important for a medicinal drug formulation. Alkaloids are tested by adopting the method of Iyengar 1995, orange brown colour confirmed the presence of alkaloid with high intensity. Alkaloids are anaesthetics, cardio protective, and anti-inflammatory agent. Tannin was also tested and blue colour confirmed the presence. Tannins promote rapid healing and the formulation of new tissues on wound and inflamed mucosa. Protein was tested and precipitate dissolves giving red precipitate. Proteins are important part of healthy diet. Body uses amino acids to build and repair muscles and bones and to make hormones and enzymes. Reducing sugar also was tested and brick red precipitate shows the presence. Reducing sugar intake lowers specifically the risk of developing over weight and obesity and in turn developing diabetes. Presence of saponins was tested and frothing appearance confirmed saponins presence. Saponin decrease blood lipids lower cancer risks, and the lower blood glucose response.

The results were compared with the standards from the previous studies. For the phytochemical evaluation ethanolic extract was prepared as per the standard method of extraction. For this 5gm of powder was macerated with ethanol and the extract was taken for identification of alkaloids, glycosides, tannins. Flavonoids are absent in the previous tests and steroid content were present(Krishnaveni,2017) [13].

TABLE IV
QUALITATIVE ANALYSIS OF THE PHYTOCHEMICAL PRESENT IN POD CONSTITUENT EXTRACT OF *MUCUNA PRURIENS*

S.NO	TEST	RESULT
1	Test for Alkaloids	+++
2	Test for Tannins	++
3	Test for Glycosides	-
4	Test for Carbohydrates	++
5	Test for Protein	++
6	Test for Saponins	+
7	Test for Flavonoid	-
8	Test For Reducing Sugar	++

+++ Stronger, ++ Moderate, + Mild reaction, -Absent

GC-MS analysis is used to find the chemical constituents and can be able to find better extract. So far the work is not done regarding the comparison of various extracts of *Mucuna pruriens.* The GC-MS analysis of Oil, Aqueous extract and Alcohol extract were tabulated in Table- V, VI, and VII respectively.

GC-MS data of oil prepared with *Mucuna pruriens* seed. Sample were analysed and 17 components were identified and the main components are Tetradecanoic Acid, Tridecanoic Acid, N-Hexadecanoic Acid, Octadecanoic Acid, Pentadecanoic Acid, Undecanoic Acid, N-Decanoic Acid, Eicsanoic Acid, Dodecanoic Acid, Oleic Acid, Z-8- Methyl -9- Tetra Decanoic Acid, (E)-13-Docosenoic acid, 1,19-Eicosadiene, Pentanoic Acid, 13-Octadecadien-1-Ol, Z-7-Tetradecenoic Acid. Figure I shows the spectrum of oil extract on *Mucuna pruriens.*

FIGURE I
SPECTRUM OF OIL EXTRACT ON *MUCUNA PRURIENS*

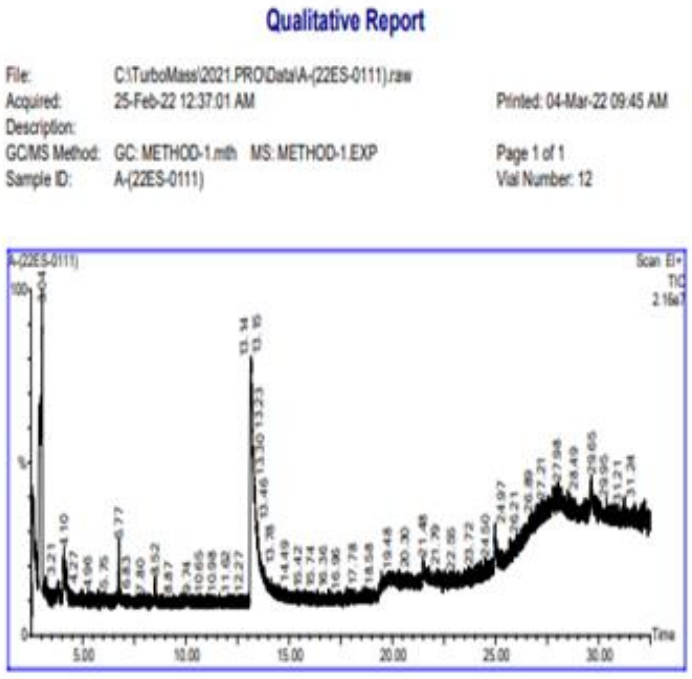

TABLE V

GC MS ANALYSIS OF *MUCUNA PRURIENS* OIL EXTRACT

SL. NO.	NAME OF THE COMPONENTS	USES
1.	Tridecanoic acid	It mainly acts as a metabolic agent
2.	N-hexadecanoic acid	It inhibits the activity of bacteria and inflammation.
3.	Octadecanoic acid	It has properties of anti-inflammatory, anti-arthritic, and anti-acne.
4.	Pentadecanoic acid	It is used to decrease inflammation and the severity of anaemia.
5.	Undecanoicacid	It is often used as an antifungal agent.
6.	N- Decanoic acid	It is used in the production of lubricants, dyes, and various drugs that treat schizophrenia.
7.	Eicosanoid acid	It supports the inhibition of inflammation, and allergy, and controls blood pressure.
8.	Dodecanoic acid	It is used for treating viral fevers and herpes.
9.	Oleic acid	It supports the decrease of LDL cholesterol and reduces the risk of coronary heart disease.
10	Z-8- Methyl	It supports antibacterial activity and acts as an antioxidant.
11.	-9- Tetra decanoic acid	It positively impacts cardiovascular health and strengthens the immune system.
12.	(E)-13-Docosenoic acid	It consists the property of anti-inflammation and precursor of acids that are important to the nervous system.
13.	1,19-eicosadiene	It supports the anti-bacterial activity.
14.	Pentanoic acid	It is used in various drugs that treat epilepsy and mental health problems such as depression and bipolar.
15.	13-octadecadien-1-ol	It increases the pheromone activity in females.

GC-MS data of Aqueous extract of *Mucuna pruriens* seed. Main components identified was Ethane, 1,1-Diethoxy, Ethane, 1,1-Diethoxy-, 2-Methoxy-1,3-Dioxolane, Propane, 13,13-Dimethyl-3,6,9-trioxa-13-silatetradecan-1-ol,(Methoxymethyl)Trimethylsilane,Trimethylsilylmethanol, Ethanol, 2-(2-Ethoxyethoxy) ethanol, Propanoic Acid, 2-Hydroxy-, Ethyl Ester, (S)-,1-Butanol, 3-Methyl- 1-Pentanol.Figure II shows the spectrum of aqueous extract on *Mucuna pruriens*.

FIGURE II: SPECTRUM OF AQUEOUS EXTRACT MUCUNA *PRURIENS*

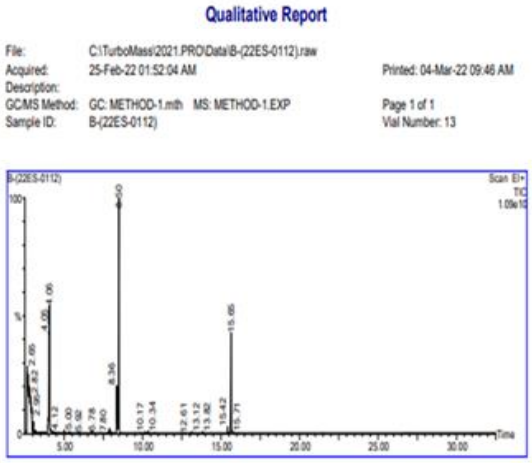

TABLE VI

GC MS ANALYSIS OF MUCUNA *PRURIENS* AQUEOUS EXTRACT

Sl. No.	Name of the components	Uses
1.	1-butanol	It is used in the manufacture of antibiotics and vitamins.
2.	3-methyl	It works as an antimicrobial and antioxidant agent.
3.	Formic acid	It is used as a preservative and acts as an antimicrobial agent.
4.	1-methylethyl ester	It contains properties such as anti-inflammatory, anti-microbial, and antioxidant.
5.	1-(1-Ethoxyethoxy,	It has metabolic properties.
6.	13,13-Dimethyl-3,6,9-Trioxa-13-Silatetradecan-1-Ol	It has antioxidant properties and antimicrobial properties.
7.	(Methoxymethyl)Trimethylsilane	It is used in drugs that treat ailments due to ischemic.
8.	Ethanol, 2-[2-(2-Ethoxyethoxy) Ethoxy]	It is used as a solvent in various objects.
9.	Propionic Acid 2-Hydroxy-, Ethyl Ester	It is used as a food additive and in several pharmaceuticals.
10.	(S)-, 1-Butanol	It acts as a protic solvent and a metabolite in humans.

GC-MS data on Ethanolic extract of *Mucuna pruriens* seed. Main component are 1,2-Ethanediamine,N-Methyl, Alanine,(R)-(-)-2-Amino-1-propanol, O-Methylisourea Hydrogen Sulfate, 1,2-Ethanediamine, N-Methyl, Alanine,(R)-(-)-2-Amino-1-Propanol Hydrogen Sulfate,Trimethyl [4(1,1,3,3- Tetramethyl Silo butyl)Phenoxy]Silane,1,2vbenzenediol, 3,5-Bis(1,1-Dimethylethyl),Paredrine ,Tms, Benzene , 2 [(Tert-Butyldimethylsilyl)Oxy]-1-Isopropyl-4-Methyl, BUTYRONITRIL,2-(trimethylsilyloxy)-(3S)-(t-Butoxycarbonyl)amino ,Carbamic Acid, N-Aminocarbonylmethyl-, Isobutyl(Ester), 1-Decylamino-2-Trimethylsilyloxyethane,Trimethyl(4-Tert.-Butylphenoxy)Silane,, 1-(2-Amino-Propyl)-4-Benzoyl-3-Hydroxy-5-Phenyl-1,5-Dihydro-Pyrrol-2.Figure III shows the spectrum of alcohol extract on *Mucuna pruriens.*

FIGURE III: SPECTRUM OF ALCOHOL EXTRACT OF *MUCUNA PRURIENS*

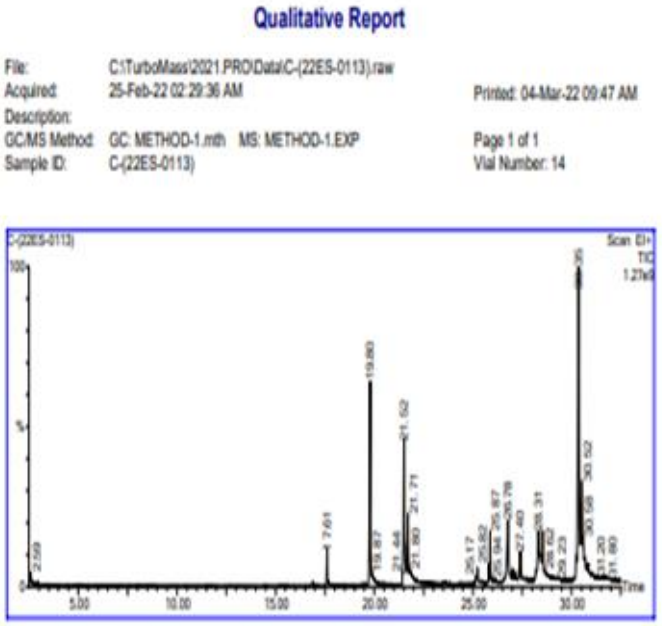

TABLE VII : GC MS ANALYSIS OF *MUCUNA PRURIENS* ALCOHOL EXTRACT

Sl. No.	Name of the components	Medical Use
1.	1,2-ethanes diamine	It is used as a drug to treat respiratory and skin diseases
2.	Alanine	It acts as a strengthening agent for the immune system and an energy source for the nervous system.
3.	(R) (-)-2-Amino-1-Propanol	It is used as an additive in health care products and a synthesizer in pharmaceutical products.
4.	O-MethylisoureaHydrogen Sulphate	It has been used as a reagent for antiarrhythmic activity and a synthesizer for microbial activity in various drugs.
	Hydrogen Sulphate	It has several therapeutic purposes for various diseases such as hypertension, inflammatory diseases, Parkinson's disease, and Alzheimer's disease.
5.	1,2benzenediol	It is used in pesticides and perfumes.
6.	3,5Bis(1,1Dimethylethyl)	It can be used as an antioxidant and for anti-cancer treatments.
7.	Paredrinetms	It is used for the treatment of hypotension that is induced by spinal anaesthesia.
8.	Benzene	It is mainly used as a solvent and can be used for the treatment of a few blood disorders.
9.	CarbamicAcid	It is commonly used as an insecticide.
10.	Trimethyl(4-Tert Butylphenoxy)Silane	The component supports the antibacterial activity.

GC-MS analysis showed the presence of photo chemicals like n-Hexadecanoic acid (48.21%), squalene (7.87%), Oleic acid (7.62%), ascorbic acid (3.80%) and Octadecanoic acid (6.21%) were present in the extract. The seed also two tetrahydroquinoline alkaloids namely (-) 3-methoxy-1, 1- dimethyl-6, 7- dihydroxy-1,2,3. 4- tetrahydroquinoline and (-) 3- methoxy-1, 1-dimethyl-7,8-dihydroxy-1,2,3.4- tetrahydroquinoline. It also contains serotonin (5 hydroxy tryptamine, 5-HT), 5-hydroxy tryptophan (5- HTP), nicotine, N, N-dimethyltryptamine (DMT), Bufotenine, and 5- imethoxy- N, N-dimethyl tryptamine (5-meo-DMT) 5- imethoxy-N, N-dimethyltryptamine (5-MeO-DMT). The mature seeds of the plant contain about 3.1-6.1% L-DOPA, with trace amounts of serotonin, nicotine, Bufotenine, and beta-carboline. [10]

4. CONCLUSION

There are umpteen medicinal herbs in the world. In present study, phytochemical and GC-MS analysis of *Mucuna pruriens* was attempted. Fat preparation is absolutely safe as a medicine based on the bioactivity of the components present in the preparation when compare to other extracts. In nutshell there more than ten types of different constituents found in ghee prepared with *Mucuna pruriens* which has different kinds of pharmacological action with minimal toxic constituents. In contrast the constituents found in the alcoholic, aqueous extract contains substance which are toxic in nature. The exact action of the constituents in alcoholic and aqueous extract is not yet explored. Based on these facts it is concluded that *Mucuna pruriens* is most effective while preparing in the lipid medium. Therefore, an Oil-based preparation is safer and more suitable for Ayurvedic drug formulation. Further, HPLC and male spermatogenesis studies can be employed in order to know the possible in future.

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