



ISSN No: 0975-7384
CODEN(USA): JCPRC5

J. Chem. Pharm. Res., 2011, 3(5):689-696

Phytochemical evaluation by GC-MS and antihyperglycemic activity of *Mucuna pruriens* on Streptozotocin induced diabetes in rats

¹Anusha Bhaskar*, ²Nithya V and ³Vidhya VG

¹Department of Biotechnology, PRIST University, Vallam, Thanjavur

²Department of Biotechnology, Srimad Andavan College of Arts and Science, Trichy

³Department of Biotechnology, Faculty of Science and Humanities, SRM University, Kattankulathur, Chennai

ABSTRACT

The aim of this study was to identify the phytochemical constituents of *Mucuna pruriens* extract by Gas Chromatography – Mass Spectrophotography (GC-MS) and to evaluate the hypoglycemic potential in streptozotocin induced diabetic rats. The rats were treated orally with the extract at 100 mg and 200 mg /kg b wt for 21 days. Biochemical parameters viz., fasting blood glucose (FBG), blood urea, serum creatinine and total cholesterol were analyzed. GC-MS analysis showed the presence of phytochemicals 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. *Mucuna pruriens* reduced the blood sugar of diabetic rats from 242.4 ± 9.2 mg/dl to 91.0 ± 5.2 mg/dl. Thus, *Mucuna pruriens* show a good potential of being used as alternate medicine in the treatment of diabetes mellitus.

Key words: diabetes mellitus, hypoglycemic effect, GC-MS, streptozotocin, *Mucuna pruriens*, squalene.

INTRODUCTION

Diabetes mellitus is considered to be a serious endocrine disorder. More than 150 million people suffer from it worldwide and this is likely to increase to 300 million by the year 2025 [1]. More than one-fifth of them are Indians and thus India has been declared the “Diabetic Capital of the World”. Thus there has been a pressing need to discover effective treatment of this disease and

current interests are being focuses towards herbal medicines which according to WHO accounts for 80% of the world's primary health care need [2]

Mucuna pruriens belongs (Fabaceae), commonly known as cowage plant. It is a popular Indian medicinal plant, which has long been used in Ayurvedic system of medicine. All parts of the *M. pruriens* possess valuable medicinal properties [3]. Roots, leaves and seeds of the plant are commonly used in the treatment of impotence [4], diabetes mellitus [5] and cancer [6]. The seeds in particular is used to manage several free radical mediated diseases, rheumatoid arthritis, diabetes, atherosclerosis, nervous disorders, analgesic, antipyretic activity and in the management of Parkinsonism.

In the light of the above information the present investigation was undertaken to evaluate the phytoconstituents by GC-MS and to assess the anti-diabetic effect of *M. pruriens* using Wistar rats as animal models.

EXPERIMENTAL SECTION

Plant material and preparation of extract

Mucuna pruriens seeds were collected from the forest garden, Theni District, Tamil Nadu, India, were authenticated by Dr. S. Kalavathy, Department of Botany, Bishop Heber College, Trichy. The seeds were shade dried and ground. The powder was suspended in water. The solvent was evaporated *in vacuo* at 50 °C giving the aqueous extract (yield: 20.5% w/w).

Chemicals

Streptozotocin (STZ) was obtained from Sigma Chemicals Co., St. Louis, MO, USA. All other chemicals were of analytical grade.

Preliminary screening

The presence of preliminary phytochemicals in the extract was estimated using colour reaction method [7].

Gas Chromatography – Mass Spectrum Analysis (GC-MS)

GC-MS was carried out at Indian Institute of Crop Processing Technology (IICPT) Thanjavur. This was carried out to study the phytochemical components present in the extract. 20 g of the powdered seed were soaked in 95% ethanol for 12 h. The extracts were then filtered through Whatmann filter paper No. 41 along with 2 g sodium sulphate to remove the sediments and traces of water in the filtrate. Before filtering, the filter paper along with sodium sulphate was wetted with 95% ethanol. The filtrate was then concentrated by bubbling nitrogen gas into the solution. The extract contained both polar and non-polar phytocomponents of the plant material used. 2µl of these solutions was employed for GC/MS analysis.

GC analysis

GC-MS analysis was carried out on a GC clarus 500 Perlin Elmer system comprising a AOC-20i autosampler and gas chromatograph interfaced to a mass spectrophotometer (GC – MS) instrument employing the following conditions: column Elite – 1 fused silica capillary column (30 x 0.25 mm ID x 1 EM df, composed of 100% Dimethyl polysiloxane), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1 ml/min

and an injection volume of 0.5 EI was employed (split ratio of 10:1 injector temperature 250 °C; ion-source temperature 280 °C. The oven temperature was programmed from 110 C (isothermal for 2 min). with an increase of 10 C/min, to 200 C then 5 C/min to 280 °C, ending with a 9 min isothermal at 280 °C. Mass spectra were taken at 70 eV; a scan interval of 0.5s and fragments from 40 to 550 Da.

Identification of components

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The 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.

Animals

Male Wistar rats (weighing 150 – 200 g) were obtained from Bharathidasan University and maintained under standard environmental conditions (12:12 h light dark cycles) and fed with a standard diet (Hindustan Lever, India). All the animal experimentations were premeditated and executed in compliance with the ethical norms approved by Ministry of Social Justices and Empowerment, Government of India and Institutional Animal Ethics Committee Guidelines (743/03/abc/CPCSEA dt. 3.3.03). All the studies were conducted in accordance with the National Institute of Health's guide for the care of Laboratory Animals [8].

Induction of diabetes

A freshly prepared solution of streptozotocin (45 mg/kg bw) in 0.1M citrate buffer, pH 4.5 was injected intraperitoneally to overnight fasted rats. After 3 days, blood was collected in vials from the tail vein of overnight fasted rats and was allowed to clot to separate serum. It is then centrifuged at 4000 rpm for 10 min to obtain clear serum. FBG level was estimated and PPG was checked regularly up to stable hyperglycemia, usually 1 week after streptozotocin injection. Animals having marked hyperglycemia (FBG>250 mg/dl) were selected for the study.

Experimental design

Animals were classified into 5 groups, of six animals each. Food and water were provided ad libitum to the animals. The groups are as follows:

Group I – Control rats treated with vehicle alone (Tween 80 – 0.2ml / 100g b wt.)

Group II – Diabetic controls

*Group III – Diabetic rats treated with *M pruriens* extract (100 mg/kg b wt)*

*Group IV – Diabetic rats treated with *M. pruriens* extract (200 mg/kg b wt)*

Group V – Diabetic rats treated with tolbutamide (250 mg/kg b wt)

Treatment with the *M. pruriens* extract was started from the 5th day after the STZ injection for 21 days till the end of the study. After 21 days of treatment fasting blood sample was collected from retro-orbital puncture technique under light ether anesthesia and used for biochemical analysis using standard enzymatic methods.

Statistical analysis

All experimental data were expressed as mean \pm S.D. The difference between test and controls were evaluated by Student's t-test.

RESULTS

The extract was found to contain flavonoids, saponins, tannins, steroids, phenols and alkaloids on preliminary screening. GC-MS study of the *M. pruriens* seed has shown the presence of a number of phytochemical constituents which contribute to the medicinal activity of the plant (Table 1). The Chromatogram is shown in Figure 1. The mass spectrum and the structure of n-Hexadecanoic acid, ascorbic acid and squalene are shown in figs 2, 3 and 4 respectively.

Table 2 shows the FBG level in control and experimental rats after the treatment for 21 days. The mean FBG level in control rats was 66.8 ± 3.6 mg/dl, in diabetic rats 242.4 ± 9.2 mg/dl, after 21 days of treatment with *M. pruriens* (200 mg/kg bw) it was found to be 91.0 ± 5.2 mg/dl. The group that received tolbutamide (250 mg/dl) showed a reduction in FBG to 98.8 ± 7.2 mg/dl. When the groups receiving 100 and 200 mg/kg bw were compared the later showed better hypoglycemic activity. The levels of total cholesterol blood urea and serum creatinine are also shown in table 2. In control groups the values were 59.1 ± 2.4 mg/dl (total cholesterol), 30.2 ± 1.15 mg/dl (blood urea) and 1.02 ± 0.12 mg/dl (serum creatinine). These values were significantly elevated in diabetic control group 124.5 ± 14.1 mg/dl in total cholesterol, 45.2 ± 1.65 mg/dl in blood urea and 2.15 ± 0.15 mg/dl in serum creatinine. The values were found to be lowered in groups administered with *M. pruriens*.

Table 1. Phytochemical components identified in the aqueous extract of seeds of *M. pruriens* by GC-MS.

RT	Name	Peak area%
2.99	Propane,1,1-diethoxy-2-methyl	1.00
3.08	4-Heptanol,3-methyl-	trace
4.13	1,2,3-Butanetriol	0.98
4.29	Hexanoic acid	0.37
6.06	Propane,1,1,3-triethoxy-	1.68
7.02	4-Dodecanol	0.25
8.12	4-Hepatanol, 2-methyl-	trace
13.94	3,4-Hexanediol, 2,5-dimethyl-	2.97
16.14	n-Decanoic acid	1.18
16.58	4-Ethoxybenzhydrazide	0.28
17.58	Undecanoic acid	1.54
21.99	Tetradecanoic acid	3.69
25.93	1-(+)-Ascorbic acid 2,6-dihexadecanoate	3.80
26.07	n-Hexadecanoic acid	48.21
26.60	Eicosanoic acid, ethyl ester	trace
29.30	9,12-Octadecadienoic acid (Z,Z)-	6.21
29.40	3-Methyl-2-(2-oxopropyl)furan	0.83
29.70	6,8-Dodecadien-1-ol (6Z,8E)	1.01
29.82	Oleic acid	7.62
32.35	Triarachine	2.41
33.72	Hexanedioic acid, bis(2-ethylhexyl)ester	4.05
36.11	1,2-Benzenedicarboxylic acid, diisooctyl ester	4.04
41.94	Squalene	7.87

Table 2 Effect of administration of aqueous extract of *Mucuna pruriens* on biochemical constituents in normal, diabetic and treated rats

Experimental groups	FBG 21 st day after induction (mg/dl)	Total cholesterol (mg/dl)	Blood urea (mg/dl)	Creatinine (mg/dl)
Group I	66.8 ± 3.6**	59.1 ± 2.4**	30.2 ± 1.15**	1.02 ± 0.12**
Group II	242.4 ± 9.2	124.5 ± 14.1	45.2 ± 1.65	2.15 ± 0.15
Group III	109.5 ± 6.2**	70.5 ± 1.3**	35.6 ± 1.6**	1.65 ± 0.29**
Group IV	91.0 ± 5.2**	61.5 ± 4.6**	34.8 ± 2.0*	1.40 ± 0.41**
Group V	98.8 ± 7.2**	69.2 ± 3.9**	33.5 ± 1.15**	1.10 ± 0.14**

Values are expressed as mean ± S.D. (n=6) statistically significance are ** $p < 0.001$ and * $p < 0.01$ when compared with Group II (Diabetic controls)

DISCUSSION

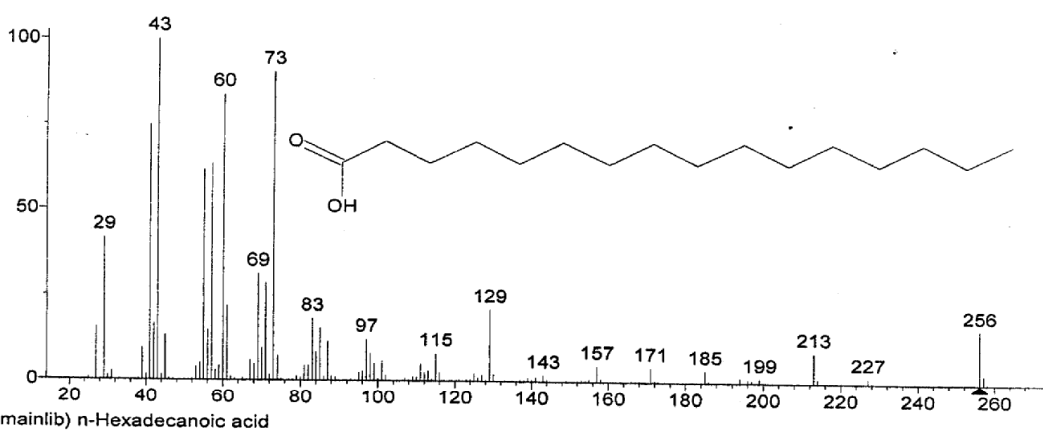
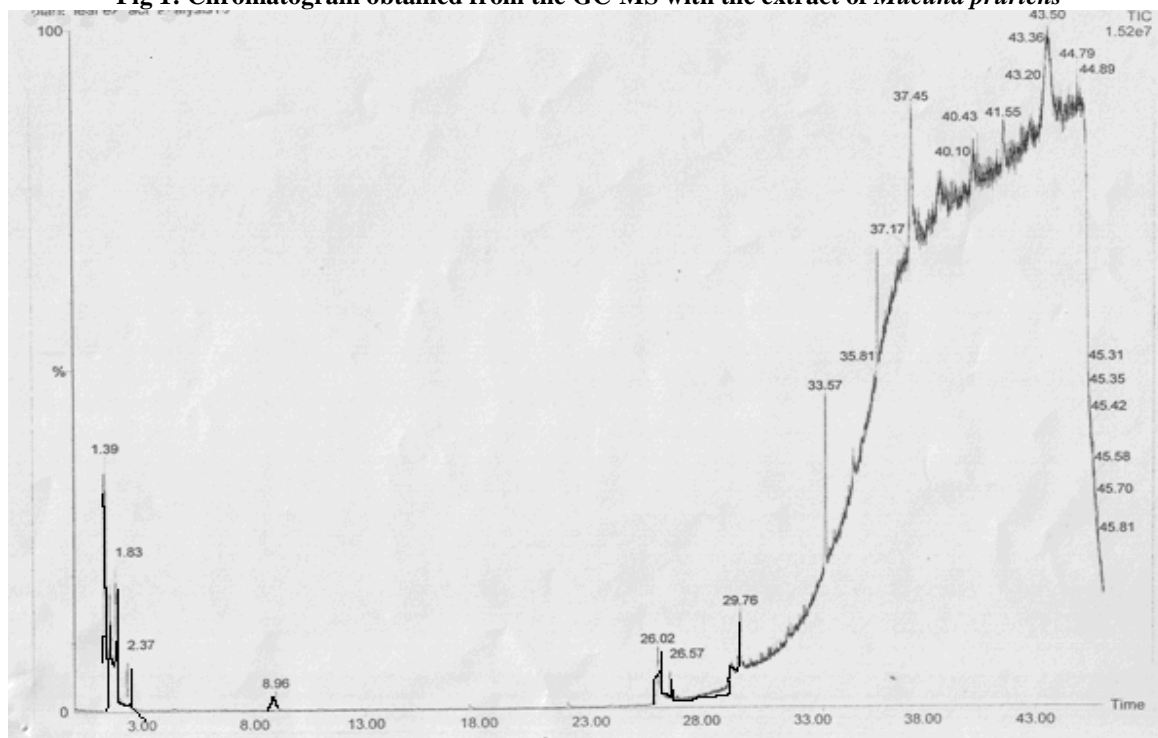
The aim of the present study was to test the effect of *Mucuna pruriens* aqueous extract on FBG, serum cholesterol and renal function in streptozotocin induced diabetic rats. STZ is well known for its selective pancreatic islet β -cell cytotoxicity and has been used extensively used to induce diabetes mellitus in animals. Oral administration of an aqueous extract of *Mucuna pruriens* seed has shown hypoglycemic effect against STZ- induced diabetes in rats a detailed study was already carried out [5].

It is well known that *Mucuna* is a rich source of dietary fiber (8.7 – 10.5 %) [9], it is also known to reduce plasma glucose in diabetic human subjects. Soluble dietary fiber with high viscosity is more effective in delaying glucose absorption than insoluble fiber. The aqueous extract might produce the hypoglycemic effect by an extra-pancreatic action [10].

The abnormally high concentration of cholesterol may be due to insulin deficiency or insulin resistance. Insulin deficiency causes an increase in free fatty acid mobilization from adipose tissue which results in increased production of cholesterol rich LDL particle. The level of total cholesterol in *M. pruriens* treated group was also decreased significantly this may be due to the presence of squalene [11] in the extract.

The levels of urea and creatinine levels were measured, as diabetes also causes renal damage due to abnormal glucose regulation. Significant reduction in these renal parameters in STZ-induced diabetic rats indicate the renoprotective role of *Mucuna pruriens* extract.

The results of the present investigation clearly indicate that the seed extract of *M. pruriens* have hypoglycemic effect on STZ-induced diabetic rats. The extracts were highly effective in managing the complications associated with diabetes mellitus such as hypercholesterolemia and impaired renal function.

Fig 1: Chromatogram obtained from the GC-MS with the extract of *Mucuna pruriens*

Name: n-Hexadecanoic acid

Formula: C₁₆H₃₂O₂

MW: 256 CAS#: 57-10-3 NIST#: 151973 ID#: 7874 DB: mainlib

Other DBs: None

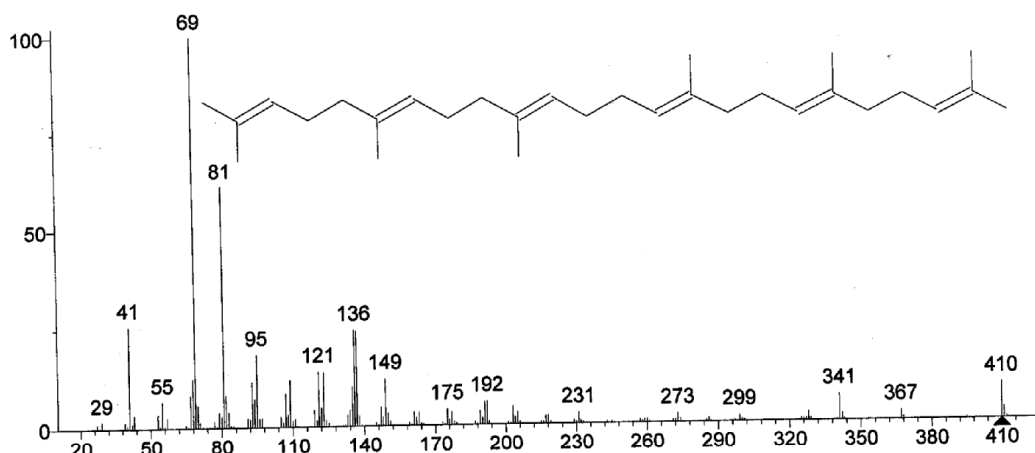
Contributor: Chemical Concepts

10 largest peaks:

43 999 | 73 905 | 60 838 | 41 749 | 57 634 | 55 616 | 29 414 | 69 310 | 71 285 | *61 218 |

Synonyms:

- 1.Hexadecanoic acid
- 2.n-Hexadecoic acid
- 3.Palmitic acid
- 4.Pentadecanecarboxylic acid
- 5.1-Pentadecanecarboxylic acid
- 6.Cetylic acid
- 7.Emersol 140
- 8.Emersol 143
- 9.Hexadecylic acid
10. Hexadecyl



(mainlib) Squalene

Name: Squalene

Formula: C₃₀H₅₀

MW: 410 CAS#: 7683-64-9 NIST#: 227620 ID#: 27655 DB: mainlib

Other DBs: None

Contributor: Japan AIST/NIMC Database- Spectrum MS-NW-8230

10 largest peaks:

69 999 | 81 612 | 41 257 | 136 243 | 137 240 | 95 184 | 121 139 | 123 137 | 68 124 | 149 119 |

Synonyms:

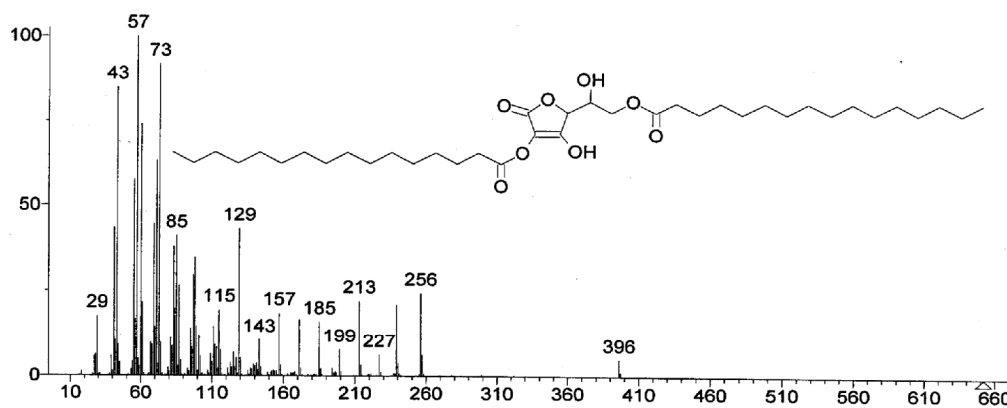
1.2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-

2.Skvalen

3.Spinacene

4.Supraene

5.(6E,10E,14E,18E)-2,6,10,15,19,23-Hexamethyl-2,6,10,14,18,22-tetracosahexaene #



(mainlib) I-(+)-Ascorbic acid 2,6-dihexadecanoate

Name: I-(+)-Ascorbic acid 2,6-dihexadecanoate

Formula: C₃₈H₆₈O₈

MW: 652 CAS#: 28474-90-0 NIST#: 233167 ID#: 20776 DB: mainlib

Other DBs: None

Contributor: Japan AIST/NIMC Database- Spectrum MS-NW-8860

10 largest peaks:

57 999 | 73 917 | 43 849 | 60 740 | 71 632 | 55 576 | 69 444 | 41 434 | 129 432 | 85 410 |

Synonyms:

no synonyms.

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