Preliminary Phytochemical Analysis and Characterization of *Phaseolus lunatus* Seed Extract in Methanol

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***Abstract*— Traditional treatments from plant resources have guided researchers in exploration for novel medications to develop healthy life for humans and animals. *Phaseolus lunatus* (PLS) selected for study belongs to Fabaceae family and the seeds appear normally white mottled with red and they possess good economic and cultural importance worldwide. GCMS studies and computational analysis were also carried out. Phytochemical screening revealed that seed extracts was found to be rich in saponins, amino acids, carbohydrates, flavonoids, glycosides and reducing sugar. Bioactive components of PLS have been evaluated using GCMS technique. GCMS spectrums of component compared with database of spectrum of known components stored in GCMS NIST (2008) library showed the identification of eleven compounds. GCMS used for analysis of extracts can be an important tool for investigation of amount of some active principles in plants. Report suggests that PLS is a potentially valuable seed that could utilized for therapeutic purpose in future.**

Keywords— ***Phaseolus lunatus* , GCMS, Fabaceae, Phytochemical Screening, Bioactive Components**

I. INTRODUCTION

Phytochemical constituents are essential resource for establishment of several pharmaceutical industries because phytoconstituents play a crucial role in identification of crude drugs. Most significant property of bioactive constituents is that they are more effective with no side effects in comparison to commonly used synthetic therapeutic agents. There is diversity of compounds present in plants, especially secondary metabolites that are isolated from plants and research work have shown that these active compounds have anticancer, antiinflammatory, antitumor, antibacterial, analgesic, antiviral and many other activities [1], [2]. In vitro screening techniques could provide preliminary interpretation required to select plant extracts with potentially important properties for further pharmacological investigations [3]. The aim of the present study was to carry out phytochemical screening and Gas chromatography and Mass spectroscopy (GCMS) for quantification and identification of bioactive compounds for *Phaseolus lunatus* seed (PLS) in methanolic extract.

II. MATERIALS AND METHODS

A. Collection and Identification of Plant Material

Phaseolus lunatus seed used for investigation was obtained from local market, Coimbatore District, Tamilnadu, India. The plant (Fig 1) was authenticated by Scientist, Botanical Survey of India, Tamil Nadu Agricultural University (TNAU) Campus, Coimbatore. Certification number is BSI/SRC/5/23/2019/Tech.-3428. Seeds were washed under running tap water, air dried, powdered in electric blender and stored in air tight container and used for further studies.

Fig 1. Phaseolus lunatus (Red Double Beans) plant

B. Sample Extraction

100 g of dried seed powder of PLS was soaked in 400 mL of methanol [4] for 48 h. After specified time exposure, extract was filtered using Whatmann filter paper no 40 to remove the sediments and traces in filter paper. Extract was evaporated to dryness at room temperature and stored in an airtight container for further use.

C. Phytochemical Screening

PLS / Methanolic extracts was subjected to preliminary phytochemical screening using standard methods (Table 1) of detection [5].

TABLE 1.
STANDARD PROCEDURE TO IDENTIFY PHYTOCHEMICAL GROUPS PRESENT IN SEED EXTRACTS

S. No	Group	Method of Identification	Inference
1	Carbohydrates	A mixture of 2 mL of Molisch's reagent and 2 mL of concentrated sulphuric acid was added to the extract.	Formation of a reddish ring.
2	Reducing Sugars	To 2 mL extract, Fehling's solution was added and boiled for 5 minutes.	Formation of a brick red precipitate.
3	Alkaloids	Extracts were treated with Mayer's reagent.	Formation of cream coloured precipitate.
4	Saponins	Extracts were diluted with 20 mL of distilled water and shaken in a graduated cylinder for 15 minutes.	Formation of 1cm layer of foam.
5	Tannins	To 2 mL of extract, few drops of 1% lead acetate were added.	Formation of yellowish precipitate.
6	Flavonoids	To a small quantity of the extract dilute sulphuric acid was added.	Appearance of orange colour.
7	Terpenoids	To 2 mL of extract, 2mL of acetic acid and sulphuric acid was added.	Formation of blue green ring.
8	Phlobotannins	The extract was boiled with 1% hydrochloric acid.	Deposition of red precipitate.
9	Coumarins	2 m L of extract with 10% of 3ml sodium hydroxide.	Yellow colour indicated the presence of coumarin.
10	Cycloglycosides	To 5 mL of extract, 2 mL of acetic acid, 1 drop of 1% ferric chloride and 1 mL of sulphuric acid was added.	Formation of brown violet and greenish ring.
11	Total Phenols	Extract with 3% ferric chloride.	Deep blue colouration.
12	Quinones	Extract with 5mL of hydrochloric acid.	Yellow precipitate.
13	Anthraquinones	2 mL of extract with 2 mL of 10% ammonium hydroxide.	Bright colour.
14	Steroids	2 mL of extract with 2 mL of chloroform, acetic acid and 1 mL of concentrated sulphuric acid.	Violet to blue green formation.

D. GCMS Analysis

Clarus 680 GC was employed for analysis with a fused silica column, packed along with Elite - 5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250µm df) and components were seggregated using Helium as carrier gas at a constant flow rate of 1 mL / min. 1µL of extract sample injected into instrument with oven temperature of 60 °C (2 min); followed by 300 °C at the rate of 10 °C min^{-1}. 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 spectrums of the components were compared with the database of spectrum of known components stored in the GCMS NIST (2008) library.

III. RESULTS AND DISCUSSION

A. Phytochemical Analysis

Phytochemical screening of PLS / M extract revealed presence of flavonoids, saponins, terpenoids, glycosides, amino acids, carbohydrates and reducing sugars that account for functional properties. Plants are rich in primary and secondary metabolites with different viable functions. Among the functional groups, saponins, amino acids and carbohydrates were copiously present in the seed extract (Table 2).

TABLE 2.
PHYTOCONSTITUENTS PRESENT IN PLS / METHANOL EXTRACT

S. No.	PHYTO COMPOUNDS	PLS / M
1.	Alkaloids	-
2.	Phenols	-
3.	Flavonoids	+
4.	Tannins	-
5.	Saponins	+++
6.	Steroids	-
7.	Glycosides	+
8.	Reducing Sugar	+
9.	Amino Acids	+++
10.	Terpenoids	-
11.	Carbohydrates	+++

Key: "+++" active compound copiously present, "++" active compound moderately present, "+" active compound present, "-" active compound absent.

B. GCMS Analysis

Natural plants are good resources of new drugs that could be used as modern medicines. These new active compounds have contributed many ingredients to control various disease conditions and illness. GC MS is used to identify constituents of long chain, branched chain hydrocarbons, alcohols, amino acids, volatile matter, esters, etc. Retention time and molecular formula were used for verification of phytochemical compounds. Active principles with their Retention time (RT), Molecular formula, Molecular weight (MW) are presented in Table 3. Extraction and analysis of plant material play a crucial role in development, modernization and quality management of herbal formulations.

GCMS chromatogram analysis of methanolic extract of *Phaseolus lunatus* (PLS) showed eleven peaks which indicate the presence of eleven phytochemical constituents. On comparison of spectrum database, eleven phytocompounds were characterized and identified using NIST library (Figure 2 - 5).

TABLE 3.

PHYTOCOMPONENTS IDENTIFIED IN METHANOLIC EXTRACT OF PLS / M USING GCMS

S. No	RT	Name of Compounds	M Wt	Molecular Formula
1	12.962	N-METHYL-N'-NITROGUANIDINE	118	$C_2H_6O_2N_4$
2	16.894	2,3-ANHYDRO-D-GALACTOSAN	114	$C_6H_8O_4$
3	18.270	BETA.-D-MANNOFURANOSIDE, METHYL	194	$C_7H_{14}O_6$
4	19.065	1-TETRADECYNE	194	$C_{14}H_{26}$
5	19.220	ALPHA-LINOLENIC ACID, TRIMETHYLSILYL ESTER	350	$C_{21}H_{38}O_2Si$
6	19.800	PROPANOIC ACID, 2-(AMINOOXY)	105	$C_3H_7O_3N$
7	20.846	BETA.-L-RHAMNOFURANOSIDE, METHYL-5-O-ACETYL	220	$C_9H_{16}O_6$
8	21.616	9,12-OCTADECADIENOIC ACID (Z,Z)-, TRIMETHYLSILYL ESTER	352	$C_{21}H_{40}O_2Si$
9	21.986	ISOTHIAZOLE-4-CARBONITRILE, 3,5-BIS[(2-DIMEMHYLAMINO)ETHYLTHIO]-	316	$C_{12}H_{20}N_4S_3$
10	25.733	SILANE, [[(3.BETA.)-GORGOST-5-EN-3-YL]OXY] TRIMETHYL	498	$C_{33}H_{58}OSi$
11	26.198	CYCLOPROPANE, 1,1-DIMETHYL-2-(1-METHYLETHOXY)-3-(3-METHYL-1-PENTYNYL)	208	$C_{14}H_{24}O$

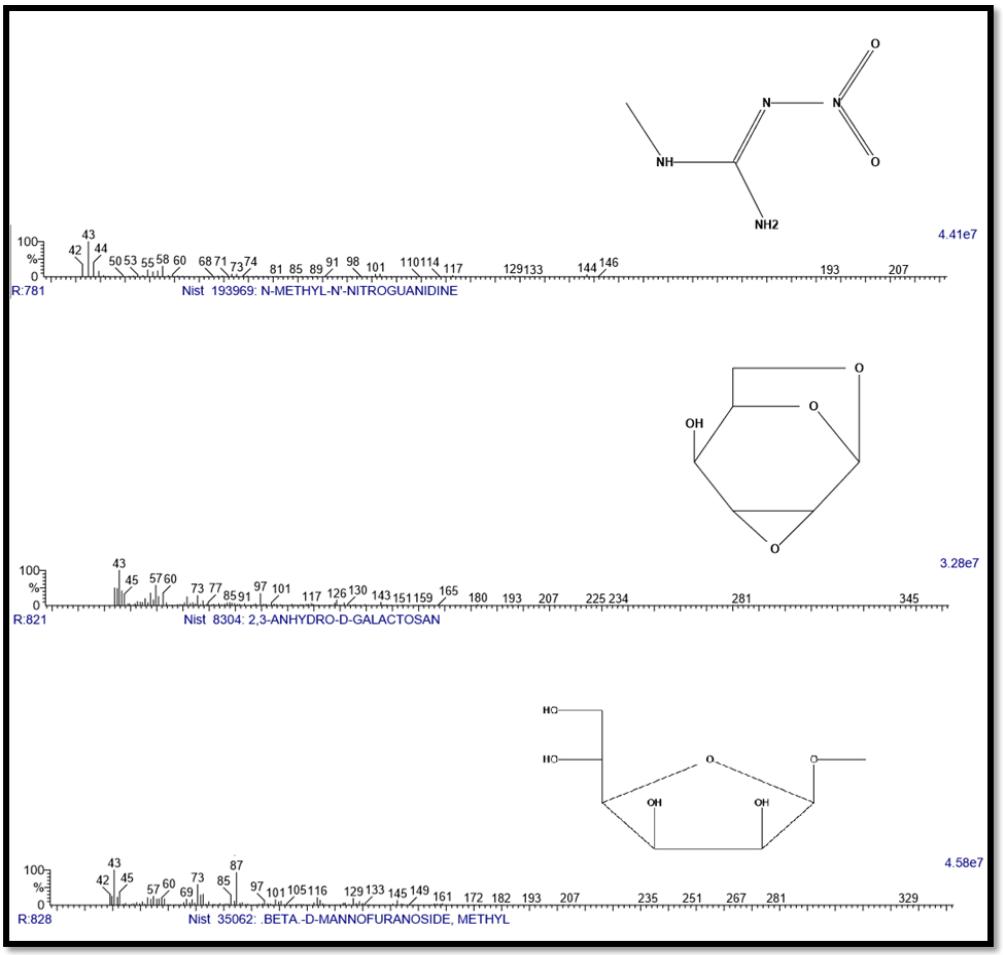
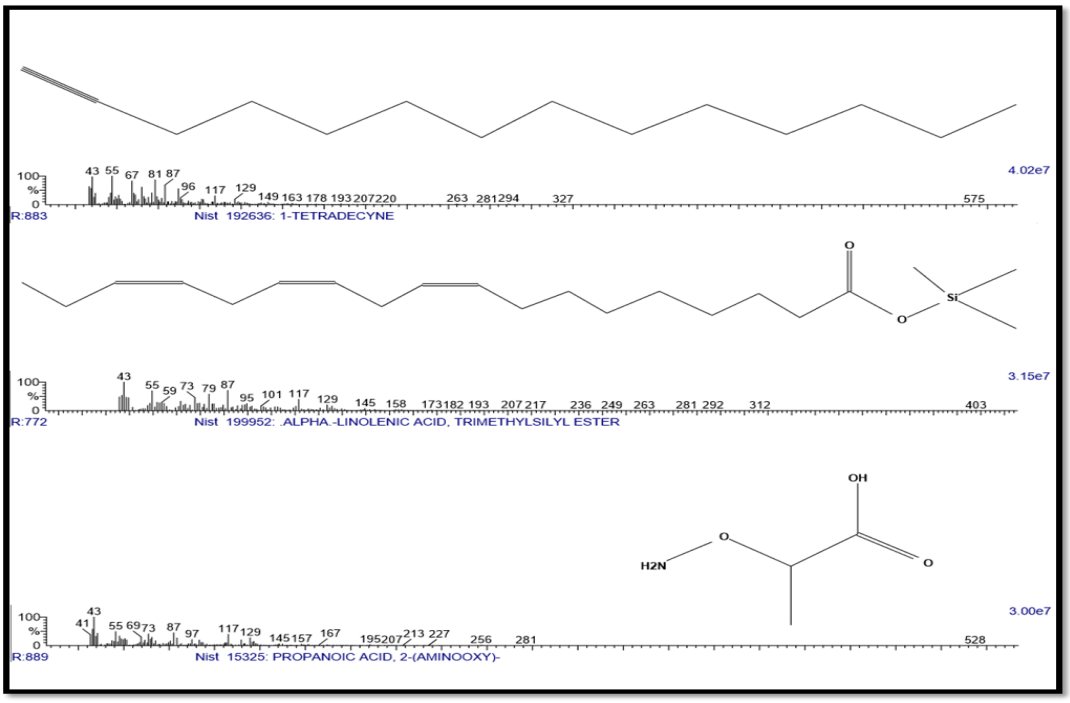


Figure 2. Mass Spectrum and Structure of Phytocomponents present in PLS / M Extract



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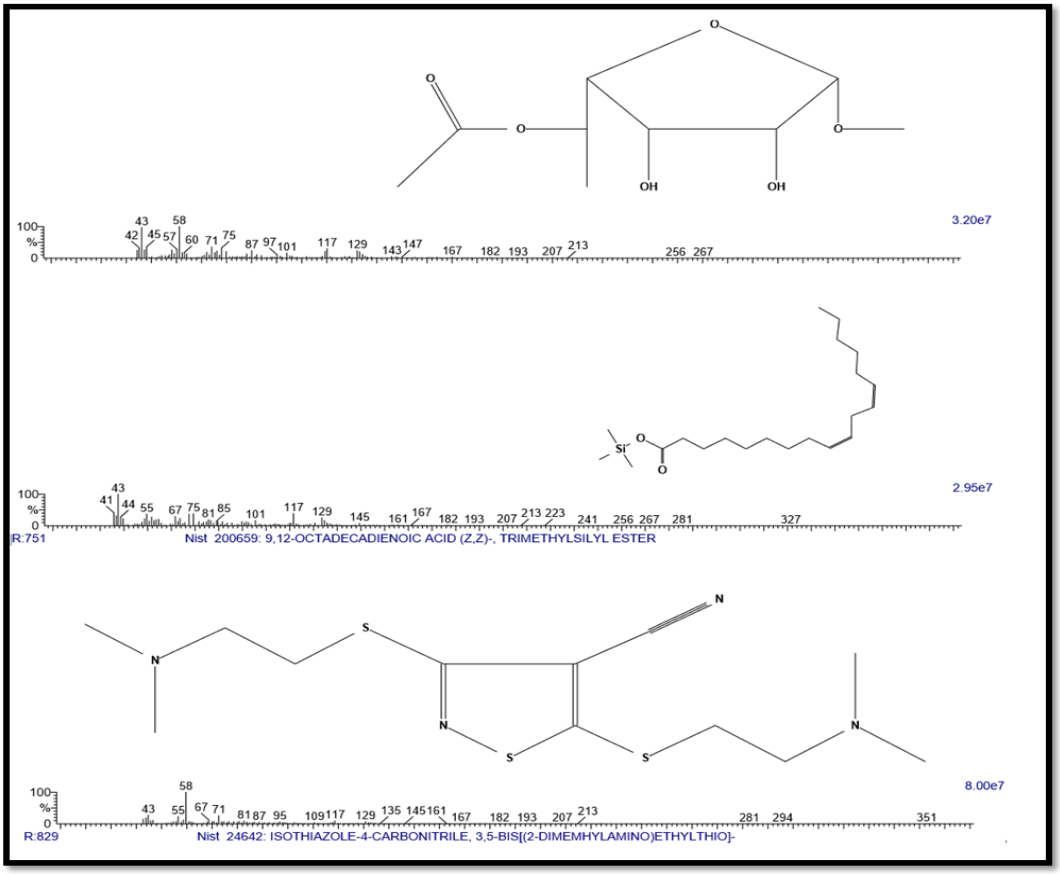

Figure 4. Mass Spectrum and Structure of Phytocomponents present in PLS / M Extract

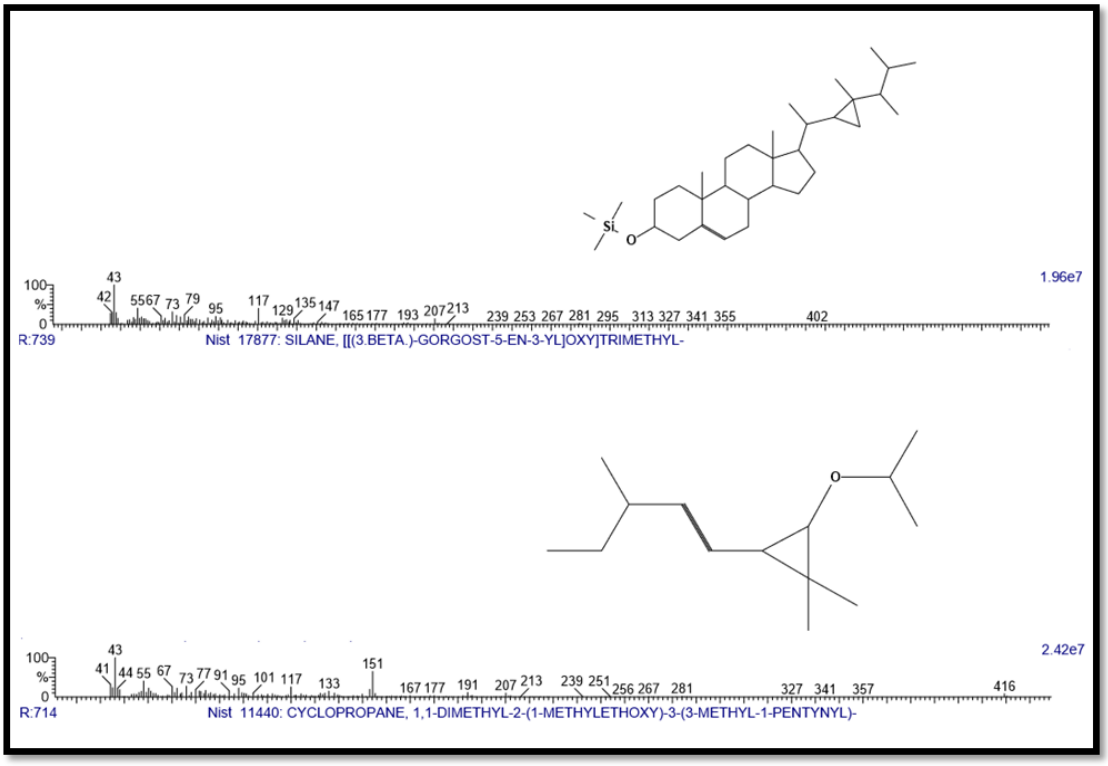

Figure 5. Mass Spectrum and Structure of Phytocomponents present in PLS / M Extract

The presence of bioactive compounds in methanolic extract of PLS justifies its use for various ailments by traditional practitioners. However, isolation of individual phytochemical compounds and subject active compounds to the biological activity will definitely provide good therapeutic agents. Therefore, it can be suggested as an application for phytopharmaceutical importance.

IV. Conclusions

The phytocompounds present in PLS / M suggests that seed extract was rich source of chemotherapeutic compounds. GCMS analysis of methanolic extract of seed extract revealed presence of medicinally active components like saponins, carbohydrates, steroids, amino acids, reducing sugar and flavonoids. Quantitative and GCMS analysis of these phytocompounds can be used for further *invivo* and *invitro* studies for application of PLS extract as therapeutic agent.

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