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In vitro Physico-chemical, Phytochemical and Fluroscence Assessment of Mucuna sps.

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Abstract: *Mucuna is commonly called as "Kapikacchu', which is known to have various medicinal properties and being used in Ayurveda since ages. Medicinal plants have bioactive compounds which are used for curing various diseases. The present investigation is aimed to screen the various bioactive compounds present in seeds of Mucuna pruriens (L.) DC., Mucuna cochinchinensis (Lour.)A. Chev. black seeds variety and Mucuna cochinchinensis (Lour.) A. Chev. white seeds variety. In the present study, an attempt was made to analyse and evaluate the presence of various phytoconstituents as well as fluorescent characteristics of seeds of Mucuna sps. Along with this, during the research work, physicochemical parameters were also determined. Qualitative and quantitative phytochemical analysis of the aqueous and methanolic extracts of seeds showed the presence of various phytoconstituents i.e. carbohydrates, reducing sugar, hexose sugar, proteins, amino acids, steroids, saponins, flavonoids, alkaloids, tannins and phenols. It was observed that all the extracts show more important chemical constituents for various pharmacological activities. In fluorescent analysis characteristic colours were observed with different chemical reagents in seed powder extracts under visible and UV light (short and long wavelength). The determination of these characters will aid future investigators in their Pharmacological analysis of this species. The phytochemical analysis of the plants is very important commercially and has great interest in pharmaceutical companies for the production of the new drugs for curing of various diseases.*

Keywords: *Kapikacchu, Fluorescence, Physicochemical analysis, Mucuna pruriens (L.) DC., Mucuna cochinchinensis (Lour.)A. Chev.*

I. Introduction

Kapikacchu (*Mucuna*, belongs to the family Fabaceae) is an ingredient of several commercial preparations that are being used as an aphrodisiac, in male sexual disorders. Seeds are astringent, laxative, anthelminitc, alexipharmic, hypotensive, spasmodic, hypocholestrolemic, antifungal, anti-inflammatory and nervine tonic. They are useful in gonorrhoea, sterility and general debility. In addition, *Mucuna* is also traditionally used in various other applications like, dye [1], treatment of pain and numbness of joints, and irregular menstruation [2].

The genus *Mucuna* belongs to the family Fabaceae (Leguminoceae) and includes about 150 species of annual and perennial legumes of pan-tropical distribution. India is one of the natural centers of origin of the *Mucuna* in the world [3]. To date fifteen species of *Mucuna* species are reported from India. Many species of the genus offer an excellent source as cover crop and green manure, in addition to their traditional use as feed and food [4, 5]. Almost all the species are reported to be used in the treatment of Parkinson's disease [6].

The literature survey showed that in India Kapikacchu species are regularly used to treat patients and getting good results. But it is not clearly mentioned the effectiveness of the particular species. Comparative chemical analysis of all the species is not worked out till date. It is highly essential to find out and report the comparative analysis of *Mucuna* species. In view of the above mentioned literature survey, it was necessary to conduct comprehensive research work for comparative assessment of pharmacologically active principles commonly occurring *Mucuna* sps in India.

Mucuna pruriens (L.) DC. commonly known as Kivach, Alkusi, Cowhage, Kaunch, Velvet bean is an economically important medicinal plant found in bushes and hedges and dry deciduous, low forests throughout the plains of India [7, 8]. It is reported to be native of China and Eastern India [9]. *M. pruriens* is a wild plant and it's every part is full of medicinal value. It's most important parts are seeds and roots which are good source of giving vital energies. Seeds are excellent source of L-DOPA (lavodopa 3,4- dihydroxyphenyl alanine) which is precursor of dopamine a neurotransmitter [10] used in the treatment of Parkinson's disease [11,12].

Mucuna cochinchinensis (Lour.)A. Chev. locally known as Lyon bean is an annual twining herb with white or pale purple flowers and glabrescent pods. It is widely distributed in the tropics and subtropics and cultivated mostly in Bengal and Bihar region of India for its edible pods and seeds. The fleshy and tender fruits of the plant are valued as vegetable [13]. The seeds of *M. cochienchinensis* contain carbohydrate 55.8%, protein 27.5% and fat 3.6%. The fruits yield 0.96% L-dopa [14].

The present study was aimed to assess and compare the physicochemical and phytochemical (qualitative as well as quantitative) parameters of *Mucuna pruriens* (L.) DC., *Mucuna cochinchinensis* (Lour.)A. Chev. black seeds variety and *Mucuna cochinchinensis* (Lour.) A. Chev. white seeds variety.

II. Materials and Methods

1. Collection and Processing

Seeds of *Mucuna pruriens* (L.) DC., *Mucuna cochinchinensis* (Lour.)A. Chev. black seeds variety and *Mucuna cochinchinensis* (Lour.) A. Chev. white seeds variety were collected from Medicinal Plant Garden of National Research Institute of Basic Ayurvedic Sciences, Kothrud, Pune, India during the month of January - February, 2013. Samples were taxonomically identified in the institute and were then deposited in the herbarium section for future reference. Samples were taxonomically authenticated by Plant Sciences Division of Agharkar Research Institute, Pune with voucher numbers S-159 (*Mucuna cochinchinensis* (Lour.)A. Chev. black seeds variety), S-161 (*Mucuna cochinchinensis* (Lour.)A. Chev. white seeds variety), and S-162 (*Mucuna pruriens* (L.) DC.).

2. Physicochemical Parameters

Physicochemical parameters like Soluble extractive values of dried seeds powder in Absolute Alcohol, Water, Methanol, Acetone, Ethyl acetate, Petroleum ether, Benzene, Chloroform, Carbon tetrachloride, Hydro-alcoholic; Total ash; Acid insoluble ash; Water soluble ash, Loss on drying (LOD); Foreign matter; Swelling factor, Specific gravity and pH value were determined using the standard protocols and guidelines.[15-17]

3. Extracts Preparation for Phytochemical analysis

Extraction of dried plant material was carried out using a method described by Harborne (1973) with minor modifications. The leaves were thoroughly washed under tap water followed by washing with distilled water [18]. Collected seeds as well as washed leaves were shade dried and powdered. The powdered leaves and seeds were kept overnight in different solvents systems like water (aqueous), methanol and hydro-alcohol. Extracts were filtered using Whatmann no. 42 (125mm) filter paper. The extracts were evaporated to reduce the volume to half (approximately) on a water-bath for 1-2 hrs. The plant extracts were cooled and then a part of the extract was used directly for qualitative determination of phytochemical parameters and remaining extract was subjected to lyophilization which was further taken to evaluate the quantitative parameters.

4. Qualitative Phytochemical Analysis

The following tests were carried out to determine the presence of various active phyto-constituents.

Carbohydrates: To 2-3 ml extract, few drops of Molisch reagent was added, mixed well and conc. H_2SO_4 was added from the sides of the test tube, violet ring formation at the junction of two liquids indicated the presence of carbohydrates.[19]

Sugars: Presence of Reducing sugar, Pentose sugar and Hexose sugar were determined using the Fehling's, Aniline acetate and Cobalt Chloride test respectively as described by Sadasivam and Manickam (1996) and Kokate (1996) [20,21].

Amino acids: Ninhydrin test was conducted for amino acids in general and presence of cysteine was checked by adding 40% NaOH and 10% lead acetate solution to extract. Appearance of black lead sulphate precipitate after boiling confirmed the test [20].

Proteins: Presence of proteins was determined by Biuret test, Million's test, Xanthoprotein test, heat test, Lead acetate test and Ammonium sulphate test [20].

Steroids: Salkowski test and Libermann-Buchard test were conducted for assessing the presence of steroids. While performing Libermann-Buchard test to 2 ml extract with chloroform.1-2 ml aceteic unhydride few drops cons. H_2SO_4 was added from the side of test tube. Steroids were indicated by reddish brown coloured ring at the junction of two layers [22].

Sakowski test: To 2 ml of extract 2 ml chloroform and 2 ml of conc. H_2SO_4 was added and mixed well. Chloroform layer appeared red and acid layer showed greenish yellow fluorescence [21].

Glycosides: To 2 ml extract glacial acetic acid, few drops of 5% $FeCl_3$ and conc. H_2SO_4 were added. Reddish brown colour at the junction of two liquid layers and upper layer appears bluish green indicating the presence of glycosides.[23,24] Presence of Cardiac Glycosides, Anthroquinone glycosides [25,26] and Coumarin glycosides[27] were determined using the protocols described by Sadasivam and Manickam (1996) and Kokate (1996) [20,21].

Saponins: Foam and lead acetate tests were carried out for indicating the presence of saponins. The layer of foam formation on vigorous shaking of extract and distilled water confirmed the presence of saponins in foam test and formation of white precipitate on the addition of lead acetate indicated the positive saponin test [28, 29].

Flavonoids: Shinoda test, Lead acetate test and alkaline reagent tests were conducted for the flavonoid determination [21, 28-30].

Shinoda Test: An alcoholic extract of plant material was treated with magnesium (dust) and conc. HCl. The appearance of Crimson red colour indicated the presence of flavonoids.
Lead Acetate test: To the small quantity of extract, lead acetate solution was added then yellow or reddish brown coloured precipitate determined the presence of flavonoids.
Alkaline Reagent test: The test solution was treated with sodium hydroxide which showed yellow coloration, indicating the presence of flavonoids.
Alkaloids: For the confirmation of alkaloids three tests were conducted [20, 21, 28].
Dragendroff's test confirmed the presence with formation of orange brown precipitate on addition of few drops of dragendroff's reagent onto the extract.
Mayer's test: Few drops of the Mayer's reagent was when added onto the extract, white - pale yellow precipitate was seen which indicated the presence of alkaloids.
Wagner's test**:** Extract was acidified with 1.5 % v/v of hydrochloric acid and a few drops of Wagner's reagent were added. A yellow or brown precipitate indicated the presence of alkaloids.
Tannins: Lead acetate test was performed for confirming the presence of tannins in the extract. To an aqueous solution of dried plant material, 5% lead acetate solution was added; appearance of white precipitate indicated the presence of tannins [20, 28, 31].
Mucilage: Mucilage test was conducted as per the protocols described in Kokate (1996). Powered drug material showed red color with ruthenium red [21].
Phenol: For the purpose of analysing the presence of phenol $FeCl_3$ test and Folin-ciocaltaue test was carried out [21, 32].
$FeCl_3$ Test: Small amount of extract of plant material was treated with neutral $FeCl_3$. Formation of blue or green colour indicated the presence of phenol [31].
Folin-ciocaltaue reagent test: When the extract was treated with a little residue of ammonia solution, a blue or blue to gray colour is formed indicating the phenols are present.
Starch: Iodine test was conducted for confirming the presence of starch in the extract. To 3 ml of extract few drops of dilute iodine solution was added and mixed. Appearance of blue colour which disappears on boiling and reappears on cooling indicated the presence of starch [20, 21].

5. Quantitative Phytochemical Analysis

For Quantification of various phytochemical constituents' standard procedures for estimation were performed. The parameters like Carbohydrates [16]; Flavanoids [33-35]; Tannins [35]; Proteins [36]; Glycosides [23,36]; Lipids [37] and Total Phenol Content[32,38] were quantified for all the *Mucuna* sps under study.

6. Fluorescence analysis of Seed extract

Fluorescence of the various extracts of *Mucuna* sps were checked in the day light and UV light (Short and long) [39,40]. Seed extracts which were used in the analysis were water, acetone, benzene, chloroform, carbon tetra chloride, ethanol, ethyl acetate, methanol and petroleum ether. All the extracts were observed under the visible and UV light after shaking for the visualization of characteristic colour reaction were compared with a standard colour chart and colours were noted [41].

III. Results

This study revealed the presence of physicochemicals and phytochemicals considered as active medicinal chemical constituents. The physicochemical constituents of *Mucuna* sps are presented in Table 1. Total ash was highest in *M. pruriens* (3.88%) Physicochemical parameter analysis is studied for understanding the nutritional value, quality and microbiological stability of the drug. Swelling factor was not found to be present in *M. pruriens.* Table 2, represents the specific gravity of *Mucuna* sps extract with different solvents. These results show that all the species almost same specific gravity in each solvent.

Extractive values for *Mucuna* sps in different solvents were determined and depicted in Table 3. The comparison between different solvents and *Mucuna* sps showed that seed powder has significant extractive values in water, methanol and hydro-alcohol extracts.

In qualitative analysis of *Mucuna* sps it was found that, methanolic and water extracts showed the presence of alkaloids (Wagner's test) and phenols. When protein presence was checked using lead acetate test only methanolic extract gave positive detection. Hexose sugar was found only in methanolic extract of M. pruriens. Carbohydrates, reducing sugar, saponins, flavonoids (lead acetate test), alkaloids (Mayer's test) and starch was detected when water extract of the samples were analyzed. Volatile oil, organic acids, mucilage glycosides, pentose sugar and Cysteine amino acid was not detected in any of the sample and extract (Table 4).

The screening and quantitative analysis using water and methanolic extracts of *Mucuna* sps for the carbohydrates, flavonoids, tannins, lipids, proteins, total phenolic content and glycosides is presented and expressed in Table 5 followed by respective graphs (Fig. 1-7).

The results of the quantitative phytochemical analysis showed significant amount of phytochemicals in methanolic extract of all the species under study. *M. cochienchinensis* white seed variety was found to be rich in carbohydrates and tannins whereas *M. cochienchinensis* black seed variety did not show much considerable quantity of phytoconstituents. In the quantification of lipid content, flavonoid and glycosides were present in maximum amount in *M. pruriens*.

The results of fluorescence analysis of *Mucuna* seed extracts with different solvents are expressed in Table 6. Fluorescence study is an essential and required parameter for first line standardization of crude drug. In this analysis most of the reactions resulted in colourless reaction mixture.

IV. Tables

Table 1: Physicochemical constituents of Mucuna sps

Sr. No.	Parameters	Mucuna pruriens (L.) DC.	Mucuna cochinchinensis (Lour.) A. Chev. (White Seed)	Mucuna cochinchinensis (Lour.) A. Chev. (Black Seed)
1	pH	7.40	7.65	7.43
2	Loss on Drying (LOD)	5.56 %	10.15 %	9.29 %
3	Total Ash	3.88 %	3.42 %	3.53 %
4	Acid Insoluble Ash	0.09 %	0.04 %	0.10 %
5	Water soluble Ash	0.14 %	0.05 %	0.07 %
6	Swelling factor	0.0 ml	1.0 ml	1.0 ml

Table 2: Specific gravity of Mucuna species extract with different solvents

Sr. No.	Solvents	Mucuna pruriens (L.) DC.	Mucuna cochinchinensis (Lour.) A. Chev. (White Seed)	Mucuna cochinchinensis (Lour.) A. Chev. (Black Seed)
1	Water	0.9831	0.9698	0.9739
2	Methanol	0.7801	0.5151	0.7669
3	Ethanol	0.7773	0.8160	0..7655
4	Acetone	0.7719	0.7935	0.7586
5	Ethyl acetate	0.8851	0.9001	0.8700
6	Petroleum ether	0.6518	0.6607	0.6452
7	Benzene	0.8570	0.8738	0.8574
8	Chloroform	1.4539	1.4454	1.4266
9	Carbon tetra chloride	1.5526	1.5521	1.5176

Table 3: Determination of Extractive values

Sr. No.	Extract	Mucuna pruriens (L.) DC. (in %)	Mucuna cochinchinensis (Lour.) A. Chev. (White Seed) (in %)	Mucuna cochinchinensis (Lour.) A. Chev. (Black Seed) (in %)
1	Absolute alcohol	9.61	13.46	14.08
2	Water	29.00	21.89	24.82
3	Methanol	19.05	12.32	15.83
4	Acetone	7.41	6.07	10.74
5	Ethyl acetate	5.02	5.17	9.60
6	Petroleum ether	5.34	5.17	11.41
7	Benzene	5.84	5.63	11.05
8	Chloroform	8.20	8.22	15.05
9	Carbon tetra chloride	8.20	7.61	11.12
10	Hydroalcoholic (1:1)	38.64	38.13	38.05

Table 4: Qualitative analysis for Phytochemicals

Sr. No.	Test	Test Method	Mucuna pruriens (L.) DC.		Mucuna cochinchinensis (Lour.) A. Chev. (White Seed)		Mucuna cochinchinensis (Lour.) A. Chev. (Black Seed)	
			ME	WE	ME	WE	ME	WE
1	Carbohydrates	Molish's test	-	+	-	+	-	+
2	Reducing sugar	Fehling's test	-	+	-	+	-	+
3	Pentose sugar	Phloroglucinol reag. Test	-	-	-	-	-	-
4	Hexose sugar	Tollen's Phloroglucinol test	+	-	-	-	-	-
5	Proteins	Biuret test	-	-	-	-	-	-
		Millon's test	-	-	-	-	-	-
		Xanthoprotein test	-	-	-	-	-	-
		Heat test	-	-	-	-	-	-
		5% Lead acetate	+	-	+	-	+	-
		5% Ammonium sulphate	-	+	-	-	-	-
6	Amino acid	a)Ninhydrin test	+	+	+	+	+	+
		b)Test for cysteine	-	-	-	-	-	-
7	Steroids	a)Libermann-Burchard Test	+	+	-	-	-	-
		b)Salkowski reaction	+	-	-	-	-	-
8	Glycoside	General test	-	-	-	-	-	-
9	Cardiac Glycosides	Legal test	-	-	-	-	-	-
		b)Keller-Killiani test	-	-	-	-	-	-
10	Anthroquinone glycoside	Borntrager's test	-	-	-	-	-	-
11	Cyanogenetic glycoside	a)sodium picrate test	-	-	-	-	-	-
		b)mercurous nitate test	-	-	-	-	-	-
12	Coumarin glycoside	a)No aromatic odor	-	-	-	-	-	-
		b)No green fluorescence	-	-	-	-	-	-
13	Saponins	a)Foam test	-	+	-	+	-	+
		b)Lead acetate test	-	-	-	-	-	-
14	Flavonoids	a)Shinoda test	-	-	-	-	-	-
		b)Lead acetate test	-	+	+	-	+	-
		c)Alkaline reagent test	-	-	-	-	-	-
15	Alkaloids	a)Dragendorff's test	-	-	-	-	-	-
		b)Mayer's test	-	+	+	-	+	-
		c)Wagner's test	+	+	+	+	+	+
16	Tannins	Lead acetate test	-	-	+	-	+	-
17	Mucilage		-	-	-	-	-	-
18	Phenol	a)$Fecl_3$ test	+	+	+	+	+	+
		b)Folin-ciocaltaue reagent	+	+	+	+	+	+
19	Starch	Iodine test	-	+	-	+	-	+
20	Organic acids	Calcium Chloride test	-	-	-	-	-	-
21	Volatile Oil		-		-		-	

ME: Methanolic extract; WE: Water extract; (+): Present; (-): Not present.

Table 5: Quantitative Phytochemical analysis

Sr. No.	Phyto-constituents	*Mucuna pruriens* (L.) DC. (µg/ml)		*Mucuna cochinchinensis* (Lour.) A. Chev. (White Seed) (µg/ml)		*Mucuna cochinchinensis* (Lour.) A. Chev. (Black Seed) (µg/ml)	
		WE	ME	WE	ME	WE	ME
1	Carbohydrates	57.4	89.8	146.6	112.6	275.8	206.9
2	Flavonoids	42.18	201.6	35.6	189.7	65.0	65.6
3	Tannins	203.6	168.9	306.2	362.7	576.7	509.1
4	Glycosides	56.28	221.9	10.16	112.9	32.8	56.8
5	Proteins	110.41	469.0	314	72.6	270.27	116.8
6	Total Phenolic content	10.42	149.6	80.68	90.8	40.00	266.8
7	Lipids	39.2	72.9	9.12	25.8	10.41	16.6

(ME: Methanolic extract; WE: Water extract)

Fig. 1: Graphical representation of quantitation of Carbohydrates

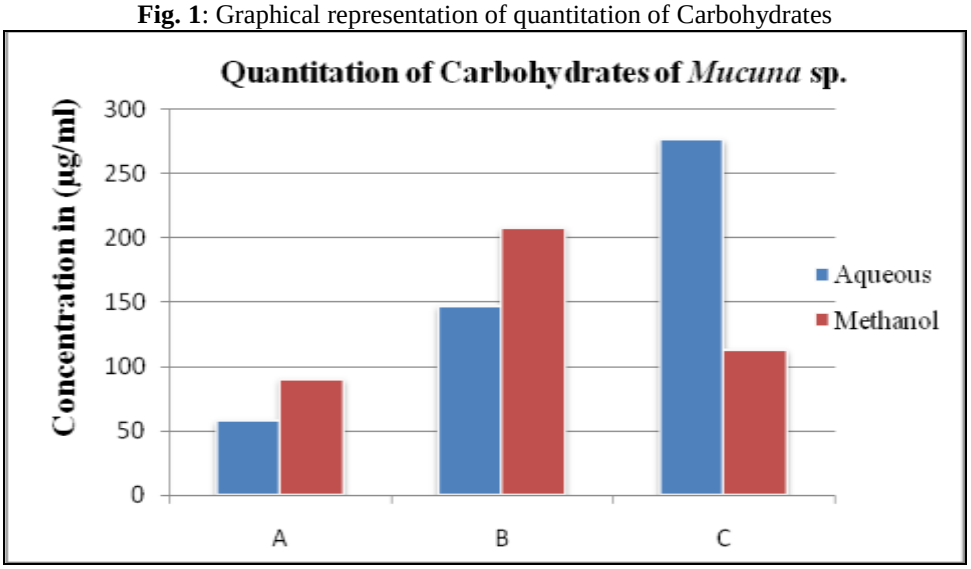


Fig. 2: Graphical representation of quantitation of Flavonoids

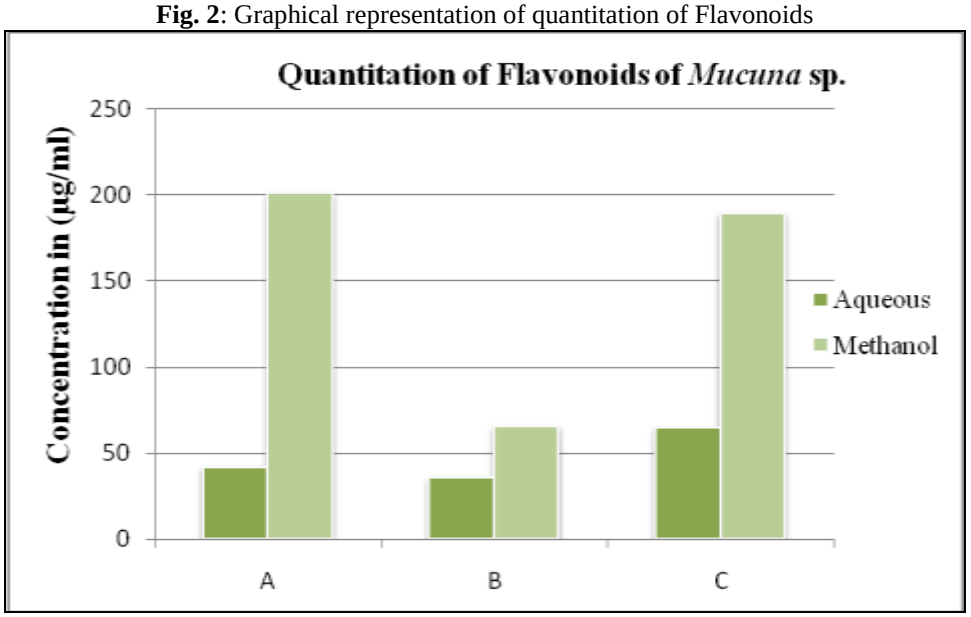


Fig. 3: Graphical representation of quantitation of Tannins

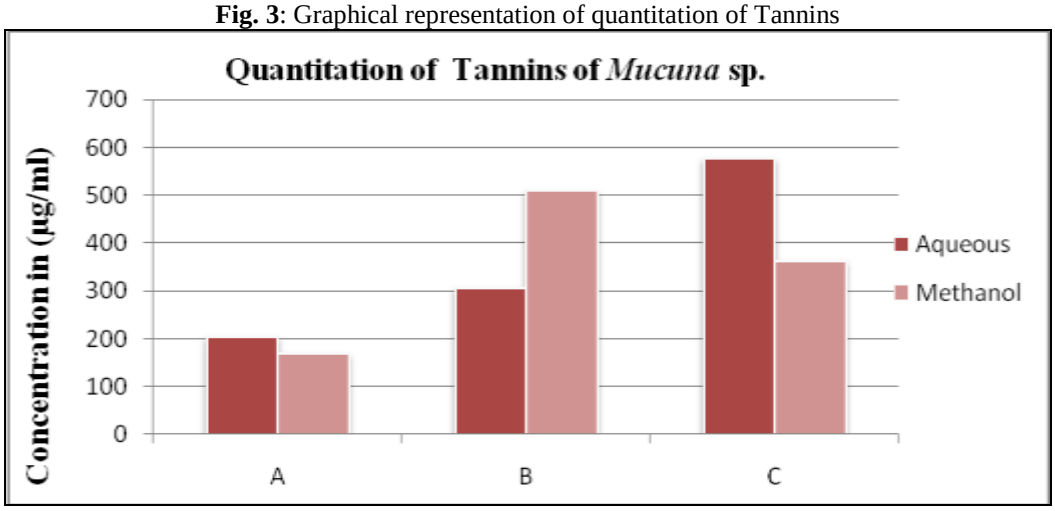


Fig. 4: Graphical representation of quantitation of Glycosides

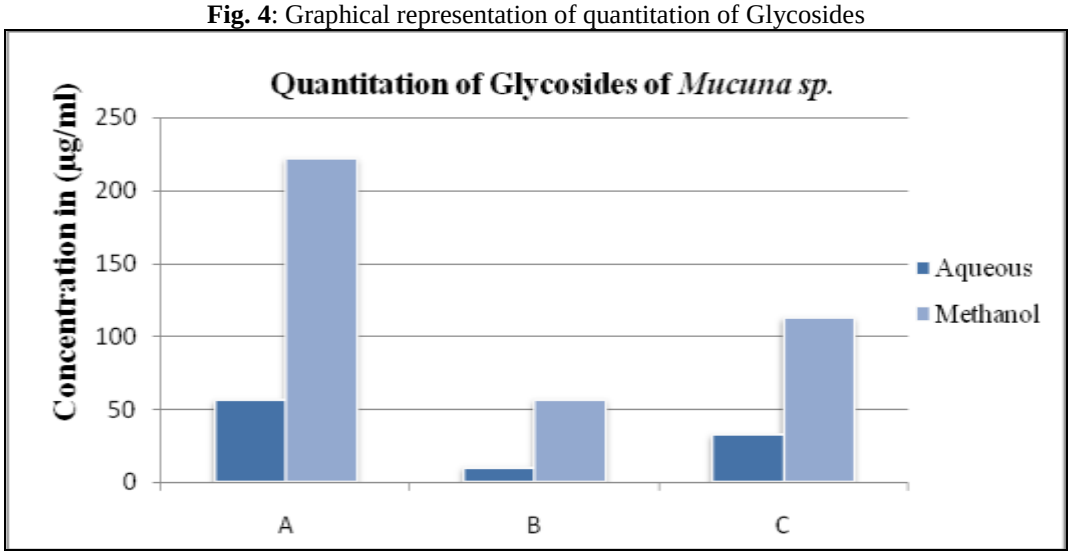


Fig. 5: Graphical representation of quantitation of Proteins

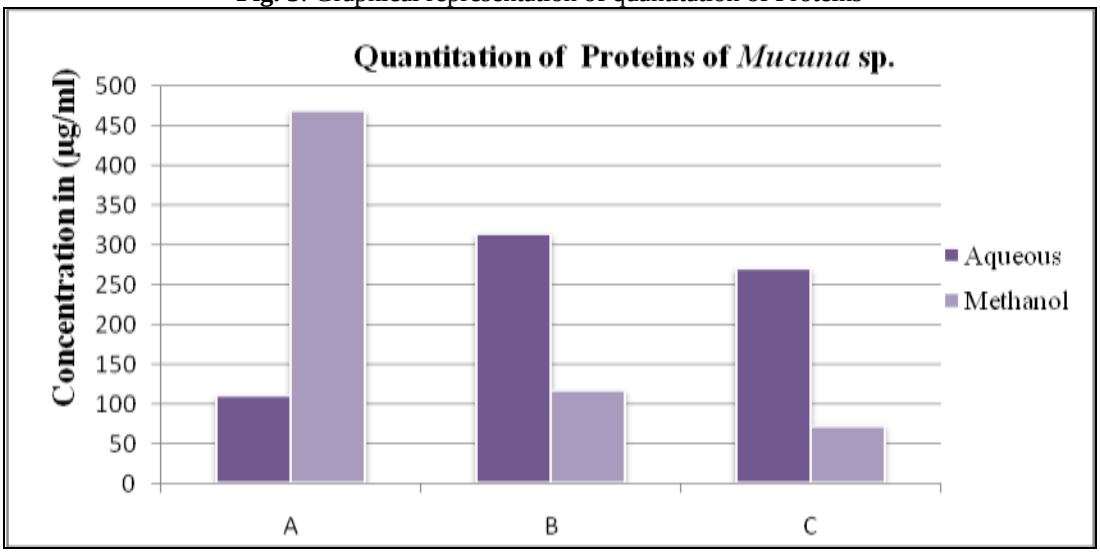


Fig. 6: Graphical representation of quantitation of Phenolic content

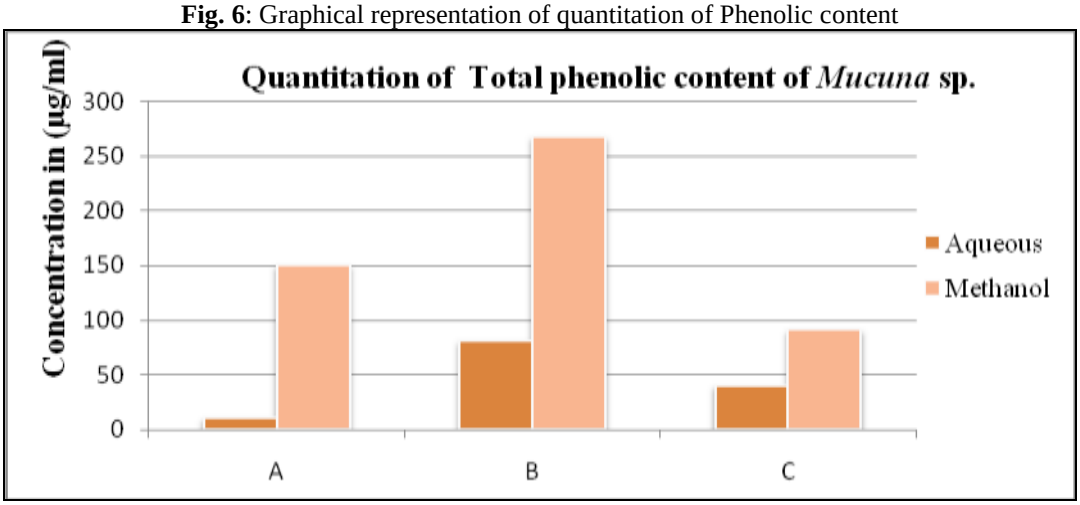
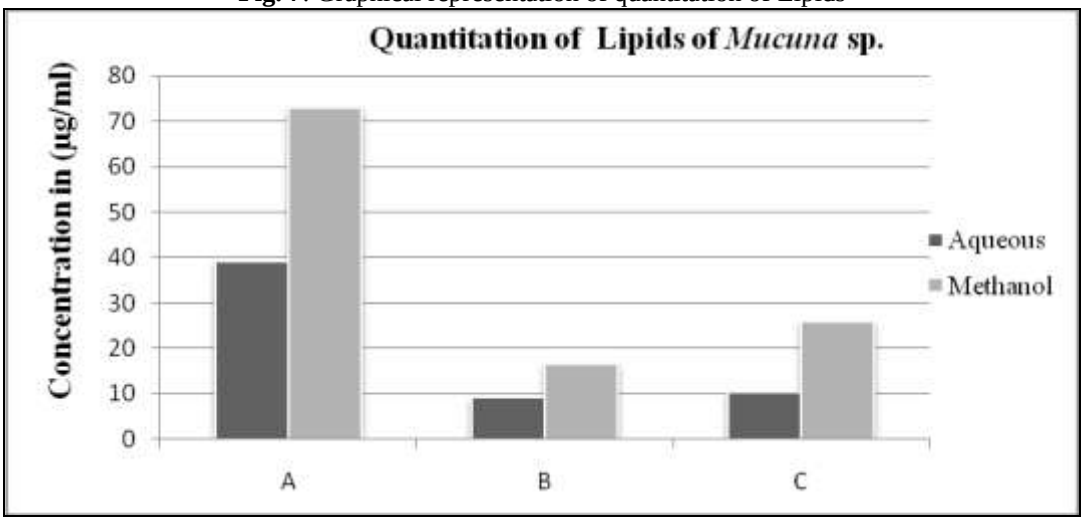


Fig. 7: Graphical representation of quantitation of Lipids



(In graphs: A*: *Mucuna pruriens*, B*: *M. cochinchinensis* (Black), C*: *M. cochinchinensis* (White))

Table 6: Fluorescence of Mucuna species with different solvents

Sr. No.	Solvents	Mucuna pruriens (L.) DC.			Mucuna cochinchinensis (Lour.) A. Chev. (White Seed)			Mucuna cochinchinensis (Lour.) A. Chev. (Black Seed)		
		Day light	Short UV	Long UV	Day light	Short UV	Long UV	Day light	Short UV	Long UV
1	Water	Light brown	Light umber	Light purple	Dark brown	Dark blackish green	Dark purple	Light brown	Chestnut	Dark purple
2	Acetone	Colourless	Colourless	Colourless	Colourless	Colourless	Colourless	Light yellow	Light yellow	Colourless
3	Benzene	Colourless	Colourless	Colourless	Colourless	Colourless	Colourless	Light yellow	Light yellow	Colourless
4	Chloroform	Colourless	Colourless	Colourless	Light yellow	Colourless	Colourless	Colourless	Buff	Colourless
5	Carbon tetra chloride	Colourless	Colourless	Colourless	Colourless	Colourless	Colourless	Very Light brown	Light yellow	Colourless
6	Ethanol	Light yellow	Colourless	Colourless	Colourless	Colourless	Colourless	Light yellow	Light yellow	Colourless
7	Ethyl acetate	Colourless	Colourless	Colourless	Colourless	Colourless	Colourless	Light yellow	Light yellow	Colourless
8	Methanol	Light yellow	Colourless	Colourless	Light pale yellow	Light pale yellow	Colourless	Light yellow	Light yellow	Colourless
9	Petroleum ether	Colourless	Colourless	Colourless	Colourless	Colourless	Colourless	Light yellow	Light yellow	Colourless

V. Conclusion

Phytochemical investigation of seed extracts showed the presence of alkaloids, phenols, carbohydrates, reducing sugar, saponins, flavanoids, starch, proteins and hexose sugar at least in one of the extract. These results are supportive with other studies perfomed with *Mucuna* sps, where same secondary metabolites were found [42]. Qualitative phytochemical screening is an essential step towards discovery of new drug as it provides the information regarding the presence of primary and secondary metabolites in the plant extract. The methods employed to isolate active substance are termed as extractive method. Crude extracts obtained from such extraction can be qualitatively tested to ascertain the presence of different types of components.

Volatile oil, organic acids, mucilage, glycosides, pentose sugar and cystine amino acid was not positive in any of the extract and *Mucuna* sps under study, indicating the absence of respective phytochemicals whereas, steroid was found to be present only in *M. pruriens* in less quantity (as the reactions were not very prominent) suggesting that the plant does not possess those phytochemical in abundance. Qualitative tests are used to detect the presence of functional groups, which play very important role in the expression of biological activity. These tests confirm the presence of certain types of phyto-constituents in the sample.

Correlation between the phyto-constituents and the bioactivity of plant is desirable to know for the synthesis of compounds with specific activities to treat various health ailments and chronic disease [43]. Owing to the significance in the above context, such preliminary phytochemical screening of plants is the need of the hour in order to discover and develop novel therapeutic agents with improved efficacy. Antitumor, anti inflammatory and antimicrobial properties are due to the presence of alkaloids [44]. Antiparasitic, antibacterial and antifungal activities are due to the presence of flavonoids [45, 46]. Tannins are used as tanning agents as they possess astringent, antioxidant and antimicrobial activities. Bactericidal and fungicidal properties are due to the presence of tannins [47-48].

In fluorescence the fluorescent light is always of greater wavelength than the exciting light. Light rich in short wavelengths is very active in producing fluorescence and for this reason ultraviolet light produces fluorescence in many substances which do not visibly fluoresce in daylight [49, 50].

The research work was carried out on *Mucuna* sps. seeds, which showed the presence of various phytochemical constituents and bioactive compounds in all the extracts in varying range and quantity. The quality and quantity of phytochemicals is based on the selection of solvent system and procedure selected for extraction. The selected plant is the source of secondary metabolites. The phytochemical constituents of *Mucuna.pruriens* and *Mucuna cochinchinensis* would be helpful in treating many diseases and the fluorescent analysis of powdered drug can play a remarkable role in the determination of quality and purity of the raw drug. These types of studies have vital role because of the commercial and research interest.

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