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Investigational Screening of Phytochemicals in Various Extracts of Mucuna Utilis

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Abstract: ***Introduction: The present paper deals to identify the phytochemical constituents and acute oral toxicity of petroleum ether, ethyl acetate, chloroform and methanolic extracts of seeds of Mucuna Utilis. Fresh mature seeds were shade dried at room temperature, coarse powdered and extracted with petroleum ether, ethyl acetate, chloroform and methanol by soxhlet's extraction method. Thereafter, the extracts was concentrated using rotary flash evaporator to obtain semisolid crude extracts. Mucuna Utilis seed extracts was investigated for the presence of phytochemical constituents. The preliminary phytochemical evaluation of the Mucuna Utilis seed extracts revealed the presence of steroids, alkaloids, tannins, carbohydrates, amino acid, resins and starch. Methods: Seeds of Mucuna Utilis were washed twice using tap water and then washed again in distilled water to remove the dust. The seeds were shade dried for 7-12 days at room temperature, until they were free from the moisture and then pulverized into coarse powder. The powdered material was extracted with petroleum ether, ethyl acetate, chloroform and methanol by soxhlet's extraction method. Thereafter, the extracts were concentrated using evaporator to obtain semisolid crude extracts. The extract was stored in airtight container in refrigerator below 10°C. Desired concentration of stock solution was prepared using distilled water for the following studies and then preliminary phytochemical investigations were done. Results: These results obtained in the present study are in good consonance with the earlier reports of Mucuna Utilis. Carbohydrates, proteins & amino acid, glycosides, fixed oils & fats, saponins, tannins, phytosterols, alkaloids and flavonoids were present in Ethyl acetate & methanolic extracts of seeds of Mucuna Utilis. The medicinal values of the seeds may be related to their constituent phytochemical. Conclusion: In conclusion, the findings of the present study suggest that carbohydrates, proteins & amino acid, glycosides, fixed oils & fats, saponins, tannins, phytosterols, alkaloids and flavonoids were present in ethyl acetate & methanolic extracts of seeds of Mucuna Utilis.***

Keywords: Mucuna Utilis, Extracts, Carbohydrates, proteins & amino acid, glycosides, fixed oils & fats, saponins, tannins, phytosterols, alkaloids and flavonoids

1. Introduction

Nature always stands as a golden mark to exemplify the outstanding phenomenon of symbiosis. The biotic and abiotic elements of nature are all interdependent. The quest for long, healthy and happy life is as old as man himself. Nature has provided a complete storehouse of remedies to relieve the ailments of mankind. The consistent effects have resulted in many effective means of ensuring health care. The seers of Ayurveda were able to understand and record the various aspects regarding the drugs that even today are difficult to understand with modern available parameters.[1] The medicinal plant products, which are derived from plant parts such as stem bark, leaves, fruits and seeds have been part of phytomedicine that produce a definite physiological action on the human body. The most important of these natural bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds.[2] Itching bean *Mucuna Utilis* an underutilized legume species grown predominantly in Asia, Africa and in parts of America.[3]

Mature seeds, seeds from unripe pods and young pods of itching bean, *Mucuna Utilis* are soaked and boiled/roasted and eaten as such or mixed with salt by the North-East Indian tribes; North-Western parts of Madhya Pradesh tribes; South Indian tribes.[4] To make this less-known legume palatable, tribal people follow a special processing method of continuous boiling and draining for about eight times until the boiled water changes from black to milky white. Consumption of improperly boiled seeds of itching bean is known to cause increase in body temperature and skin eruptions.[5] It is attributed to the presence of high levels of 3, 4-dihydroxy-L-phenylalanine, L-Dopa, the aromatic non-protein amino acid.[6]

2. Material and Methods

2.1 Plant Materials

The seeds of *Mucuna Utilis* were purchased from hansraj and sons dry fruit merchant, sadar market, delhi and identified by NISCAIR, Delhi (voucher specimen no. NISCAIR/RHMD/Consult/-2008-09/1030/61).

2.2 Preparation of Extract

Seeds were washed twice using tap water and then washed again in distilled water to remove the dust. The seeds were shade dried for 7-12 days at room temperature, until they were free from the moisture and then pulverized into coarse powder. The powdered material was extracted with petroleum ether, ethyl acetate, chloroform and methanol by soxhlet's extraction method. Thereafter, the extracts were concentrated using evaporator to obtain semisolid crude extracts. The extract was stored in airtight container in refrigerator below 10°C. Desired concentration of stock solution was prepared using distilled water for the following studies and then preliminary phytochemical investigations were done.

2.3 Preliminary Phytochemical Screening

Petroleum ether, ethyl acetate, chloroform and methanolic extracts of *Mucuna Utilis* were subjected to qualitative tests for the identification of various active constituents' viz.

Carbohydrate, glycoside, alkaloid, amino acids, flavanoids, fixed oil, tannins, gum and mucilage, phytosterols etc.

2.4 Test for carbohydrates

A small quantity of the extract was dissolved separately in 4 ml of distilled water and filtered. The filtrate was subjected to the following testes to detect the presence of Carbohydrate and glycosides.

Molisch test: To 2ml of the extract, add 1ml of α-napthol solution, add concentrated sulphuric acid through the side of the test tube. Purple or reddish violet colour at the junction of the two liquids reveals the presence of carbohydrates.

Fehling test: To 1ml of the extract, add equal quantities of fehling's solution A and B, upon heating formation of a brick red precipitate indicates the presence of sugars

Benedict test: To 5ml of benedict's reagent, add 1ml of extract solution and boil for 2 minutes and cool. Formation of red precipitate shows the presence of sugars.

2.5 Test for glycosides

Legal's test: To the hydrolysate 1 ml of pyridine and few drops of sodium nitroprusside solution were added and then it was made alkaline with sodium hydroxide solution. Appearance of pink color shows the presence of glycosides.

Borntrager's test: To Hydrolysate was treated with chloroform and then the chloroform layer is separated. To this equal quantity of dilute ammonia solution was added. Ammonia layer acquires pink color, showing the presence of glycosides.

2.6 Test for fixed oils and fates

Spot test: Small quantity of extract was pressed between two filter papers. Appearance of oil stain on the paper indicates the presence of fixed oil.

Saponification test: Few drops of 0.5% alcoholic potassium hydroxide were added to a small quantity of various extracts along with a drop of phenolphthalein. The mixture was heated on the water bath for 1-2 hours. Formation of soap pr partial neutralization of alkali indicates the presence of fixed oils and fats.

2.7 Test for proteins and free amino acid

Small quantity of the extract was dissolved in few ml of distilled water and treated with following reagents.

Million's test: Appearance of red color shows the presence of proteins and free amino acids.

Ninhydrin reagent: Appearance of purple color shows the presence of proteins and free amino acids.

Biuret test: Equal volumes of 5% sodium hydroxide solution and 1% copper sulphate solution were added, appearance of pink or purple color shows the presence of proteins and free amino acids.

2.8 Test for saponins

Foam test: The extract was diluted with 20 ml of distilled water and it was agitated in a graduated cylinder for 15 minutes. The formation of 1 cm layer of foam shows the presence of saponins.

Test for phenolic compounds and tannins
Small quantity of the extract was taken in distilled water and test for the presence of phenolic compounds and tannins was carried out with the following reagents.

Dilute ferric chloride solution (5% w/v) -Violet color. **1% solution of gelatin containing 10% sodium chloride-** White precipitate.

10% lead acetate solution-White precipitate.

2.9 Test for phytosterols

Small quantity of the extract was dissolved in 5 ml of chloroform separately. Then this chloroform solution was subjected to the following tests to detect the presence of phytosteroles.

Libermann-Burchard's test: The above prepared chloroform solution was treated with few drops of concentrated sulphuric acid followed by few drops of diluted acetic acid, 3 ml of acetic anhydride. A bluish green color appeared indicates the presence of phytosterols.

Salkowski reaction: To 1 ml of the above prepared chloroform solution, few drops of concentrated sulphuric acid was added. Brown color produced shows the presence of phytosterols.

2.10 Test for Alkaloids

Small quantity of the extract was treated with few drops of diluted hydrochloric acid and filtered. The filtrate was used for the following tests.

Mayer's reagent - cream precipitate
Dragendroff's reagent- Orange brown precipitate
Hager's test - yellow precipitate
Wagner's test- Reddish brown precipitate

2.11 Test for flavonoids

With aqueous naohsolution: Small quantity of the extract was dissolved in aqueous sodium hydroxide. Appearance of yellow colour indicates the presence of flavonoids.

With conc. Sulphuric acid: To a small portion of extract, concentrated sulphuric acid was added. Yellow orange color was obtained shows the presence of flavonoids.

3. Results

Preliminary phytochemical screening of petroleum ether, ethyl acetate, chloroform and methanolic extracts of seeds of *Mucuna Utilis* revealed the presence of different kind of phytochemical components that are summarized in table 1

Table 1: Preliminary phytochemical screening of petroleum ether, ethyl acetate, chloroform and methanolic extracts of seeds of Mucuna Utilis

Sr. No.	Phytochemical test	Extracts			
		Petroleum ether	Ethyl acetate	Chloroform	Methanol
1.	**Carbohydrates:**				
	I) Molisch' test	-	+	-	+
	II) Fehling test	-	+	-	+
	III)Benedict test	-	+	-	+
2.	**Glycosides:**				
	Legal's test	-	+	-	+
	Borntrager's test	-	+	-	+
3.	**Fixed oils and fats:**				
	Spot test	+	+	+	+
	Saponification test	-	+	-	+
4.	**Proteins and amino acids:**	-	+	-	+
	Mellon's test	-	+	-	+
	Ninhydrin reagents				
	Iii) Biuret's test	-	+	-	+
5.	**Saponins:**				
	Foam test	+	+	+	+
6.	**Phenolic compounds and tannins:**				
	I) Dilute ferric chloride solution	-	+	-	+
	II) 1% solution of gelatin containing 10% sodium chloride	-	+	-	+
	III) 10% lead acetate solution	-	+	-	+
7.	**Phytosterols:**				
	I) Libermann-Burchard's test	-	+	-	+
	II) Salkowski reaction	-	+	-	+
8.	**Alkaloids:**				
	I) Mayer's reagent	+	+	-	+
	II) Dragendroff's	+	+	-	+
	reagent	-	+	+	+
	III) Hager's test	+	+	-	+
	IV) Wagner's test				
9.	**Test for flavonoids:**				
	I) With aqueous naoh solution	-	+	-	+
	II) With conc.Sulphuric acid	+	-	+	+

4. Discussion

These results obtained in the present paper are in good consonance with the earlier reports of *Mucuna Utilis*. Carbohydrates, proteins & amino acid, glycosides, fixed oils & fats, saponins, tannins, phytosterols, alkaloids and flavonoids were present in ethyl acetate & methanolic extracts of seeds of *Mucuna Utilis*. The medicinal values of the seeds may be related to their constituent phytochemical.

5. Conclusion

In conclusion, the findings of the present paper suggest that carbohydrates, proteins & amino acid, glycosides, fixed oils & fats, saponins, tannins, phytosterols, alkaloids and flavonoids were present in ethyl acetate & methanolic extracts of seeds of *Mucuna Utilis*.

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