

Phytocontent Screening of *Mucuna* Seeds and Exploit in Opposition to Pathogenic Microbes

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ABSTRACT

Plants and plant-based medicaments are the basis of many of the modern pharmaceuticals which we use today for our diverse ailments. The aim of the study was to find out the bio active chemical constituents and to evaluate the antimicrobial activity of the ethanolic extract and methanolic extract of herbal plant *Mucuna pruriens* and *Mucuna bracteata*. A qualitative phytocontent analysis was performed for the detection of alkaloids, glycosides, terpenoids, steroids, flavonoids, tannins, reducing sugar etc. All the plant extracts were subjected to individual microbiological tests to ascertain their antimicrobial activity against four pathogenic microorganisms: *Salmonella typhi*, *Escherichia coli*, *Shigella dysenteriae* and *Bacillus subtilis*. The antimicrobial activity of the extracts was determined by measuring the diameter of zone of inhibition (ZI) exhibited by the extracts. The extract of *Mucuna pruriens* was more effective against *Escherichia coli* (ZI = 2.8 cm) and less effective *Bacillus subtilis* (ZI= 2.1 cm). The extract of *Mucuna bracteata* was more effective against *E. coli* (ZI= 2.4 cm) and less against *Salmonella typhi* (ZI= 1.8 cm)

Key Words: Phytocontent, ethanolic extract, pathogenic microorganisms, antimicrobial activity, zone of inhibition.

INTRODUCTION

The seeds of *Mucuna* spp. are large, weighing 99 to 190 mg each and are black in colour with a hard seed coat. In view of hard seed coat, the seeds did not germinate under ambient conditions. According to world health organization (WHO 1976), more than 80% of the world's population relies on traditional medicines for their primary health care needs. The medicinal value of plants lies in some chemical substances that produce a definite physiologic action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds (Duke 1995). The phytochemical research based on ethno-pharmacological information is generally considered an effective approach in the discovery of new anti-infective agents from higher plants (Duraipandiyan, et. al. 2006). Knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information may be of value in disclosing new sources of such economic materials as tannins, oils, gums, precursors for the synthesis of complex chemical substances. (Sofowora, 1993 Fasola, 2000). In addition, the knowledge of the chemical constituents of plants would further be valuable in discovering the actual value of folkloric remedies (Mojab, et. al. 2003). Chemically constituents may be therapeutically active or inactive. The ones which are active are called active constituents and the inactive ones are called inert chemical constituents (Iyengar, 1995). The chemical constituents may be used for the various purposes such as against pathogenic bacteria.

MATERIAL AND METHODS

Sample Selection

For the present study, the plant material *Mucuna pruriens* and *Mucuna bracteata* were selected because of the *Mucuna* seeds have high content of alkaloids, glycosoids, terpenoids, saponins, tannins and reducing sugars.

Sample Collection and Preparation of Extract

Mucuna seeds were collected from CIMAP Lucknow, and powdered. The powdered crude drug was macerated with 80% ethanol. However, methanol was also used as solvent for the extraction of *Mucuna pruriens* and *Mucuna bracteata*. The solvent was then evaporated at a constant temperature of 72°C until a

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very concentrated extract was obtained. Identification tests for the various chemicals were carried out to test the presence of various chemical constituents.

Identification Tests

The tests were done to find the presence of the active chemical constituents such as alkaloids, anthraquinones, flavonoids, glycosides, terpenoids and steroids, reducing sugar and tannin etc. by the following procedure:

a. Alkaloids

Alkaloids are basic nitrogenous compounds with definite physiological and pharmacological activity. Alkaloid solution produces white yellowish precipitate when a few drops of Mayer's reagents are added (Siddiqui and Ali, 1997). Most alkaloids are precipitated from neutral or slightly acidic solution by Mayer's reagent (Evans, 2002). The alcoholic extract was evaporated to dryness and the residue was heated on a boiling water bath with 2% hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Mayer's reagent. The samples were then observed for the presence of turbidity or yellow precipitation.

b. Glycosides

Glycosides are compounds which upon hydrolysis give rise to one or more sugars (glycones) and a compound which is not a sugar (aglycone or genine). To the solution of the extract in glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid are added, and observed for a reddish brown coloration at the junction of two layers and the bluish green color in the upper layer (Siddiqui and Ali, 1997).

c. Terpenoids and steroids

Four milligrams of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet color was observed for terpenoid and green bluish color for steroids (Siddiqui and Ali, 1997).

d. Flavonoids

Four milliliters of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red color was observed for flavonoids and orange color for flavones (Siddiqui and Ali, 1997).

e. Tannins

To 0.5 ml of extract solution 1 ml of water and 1-2 drops of ferric chloride solution was added. Blue color was observed for gallic tannins and green black for catecholic tannins (Iyengar, 1995).

f. Anthraquinones

About 0.5 g of the extracts was boiled with 10% HCl for few minutes in a water bath. It was filtered and allowed to cool. Equal volume of CHCl_3 was added to the filtrate. Few drops of 10% NH_3 were added to the mixture and heat. Formation of rose-pink colour, indicates the presence of anthraquinones (Siddiqui and Ali, 1997).

g. Saponins

About 0.2 g of the extract was shaken with 5ml of distilled water and then heated to boil. Frothing (appearance of creamy mass of small bubbles) shows the presence of saponins (Iyengar, 1995).

h. Phlobatanins

The extract (0.5 g) was dissolved in distilled water and filtered. The filtrate was boiled with 2% HCl solution. Red precipitate shows the presence of phlobatanins.

i. Reducing Sugar

To 0.5 ml of extract solution, 1 ml of water and 5-8 drops of Fehling's solution was added at hot and observed for brick red precipitate (Iyengar, 1995).

Antimicrobial Activity Test

All the plant extracts were subjected to individual microbiological tests to ascertain their antimicrobial activity against four species of microorganisms: *Escherichia coli*, *Salmonella typhi*, *Sigella dysenteriae* and

Bacillus subtilis. The antimicrobial activity of the extracts was determined by measuring the diameter of zone of inhibition (ZI) exhibited by the extracts. The tests were carried out by cup plate method and the diameter of the borer used was 6 millimeter (Herborne 1973). The antibiotic Streptomycin was used as control to judge against the data obtained. The microorganisms used were the isolates of a local hospital of Dehradun.

RESULTS AND DISCUSSION

The phytochemical constituents of the *Mucuna* plants shown in Table 1. From the phytocontent screening, alkaloids, glycosides, terpenoids, saponins, tannins and reducing sugars were found in both *Mucuna* plants shown in fig. 2, 3a, 3b, 3c, 3d, 3e, and 3f. Steroids, flavonoids, anthraquinones, phlobatanins and saponins were not detected in methanolic extract of *Mucuna* seeds. But flavonoids were present in ethanolic extract of *Mucuna bracteata* and steroids detected in ethanolic extract of *Mucuna pruriens*. Though the one percent extracts of all the plants showed some degree of antimicrobial activity, it was significant in *Mucuna pruriens* (ZI= 2.8 cm). The extract of *Mucuna bracteata* was effective against *Escherichia coli* (ZI = 2.4 cm). The zone of inhibition against *E. coli* was 2.8 cm, 2.4 cm and 3.1 cm, against *Sigella dysenteriae* 2.4 cm, 2.3 cm, 3.4 cm, against *Salmonella typhi* was 2.6 cm, 1.8 cm and 3.2 cm and against *Bacillus subtilis* 2.1 cm, 2.6 cm and 3.0 cm, extract of *Mucuna pruriens*, *Mucuna bracteata* and antibiotic Streptomycin respectively. Zone of inhibition of the individual plant extracts are shown in table 2 and depicted in graph 1.

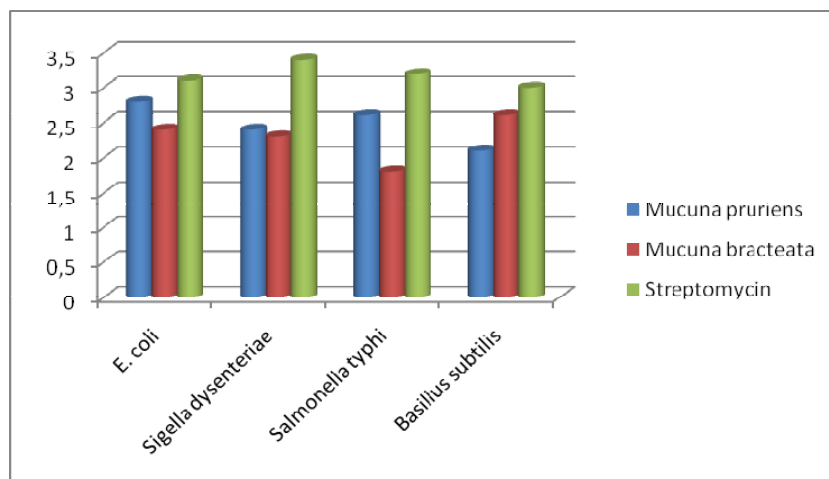
Table 1. Phytochemical content of the plant (seeds) extracts

Chemical Components	Methanolic Extract of <i>Mucuna pruriens</i>	Methanolic Extract of <i>Mucuna bracteata</i>	Ethanolic Extract of <i>Mucuna pruriens</i>	Ethanolic Extract of <i>Mucuna bracteata</i>
Alkaloids	+	+	+	+
Glycosides	+	+	+	+
Terpenoids	+	+	+	+
Steroids	-	-	+	-
Flavonoids	-	-	-	+
Tannins	+	+	+	+
Anthraquinones	-	-	-	-
Saponins	-	-	+	+
Phlobatanins	-	-	-	-
Reducing sugars	+	+	+	+

Key: + = detected
- = not detected

Table 2. Zone of inhibition of individual plant (seeds) extracts

Bacteria	Plants	1% extract solution
<i>Escherichia coli</i>	<i>Mucuna pruriens</i>	2.8 cm
	<i>Mucuna bracteata</i>	2.4 cm
	Streptomycin (0.5%)	3.1 cm
<i>Shigella dysenteriae</i>	<i>Mucuna pruriens</i>	2.4 cm
	<i>Mucuna bracteata</i>	2.3 cm
	Streptomycin (0.5%)	3.4 cm
<i>Salmonella typhi</i>	<i>Mucuna pruriens</i>	2.6 cm
	<i>Mucuna bracteata</i>	1.8 cm
	Streptomycin (0.5%)	3.2 cm
<i>Bacillus subtilis</i>	<i>Mucuna pruriens</i>	2.1 cm
	<i>Mucuna bracteata</i>	2.6 cm
	Streptomycin (0.5%)	3.0 cm



Graph 1: Microbial Activity of Mucuna Seeds against Pathogenic Micobes



Figure 1 a: Seeds of *Mucuna bracteata*



Figure 1 b: Seeds of *Mucuna pruriens*



Figure 2: Various Phytocontent Test for Mucuna Seeds



Figure 3 a: Test of Alkaloids



Figure 3 b: Glycosides Test



Figure 3 c: Terpenoids Test.



Figure 3 d: Tannins Test.



Figure 3 e: Saponins Test

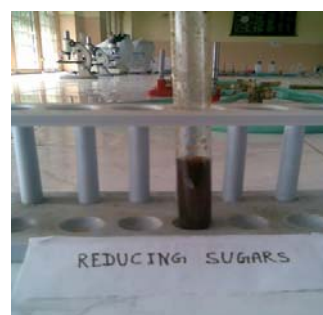


Figure 3 f: Reducing Sugars Test



Figure 4 a: ZI for *Bacillus subtilis*



Figure 4 b: ZI for *Salmonella typhi*



Figure 4 c: ZI for *E. coli*



Figure 4 d: ZI for *Shigella dysenteriae*

CONCLUSIONS

The ethanolic and methanolic extracts of the considered plant contained many bioactive chemical constituents including alkaloids, glycosides, terpenoids, saponins, tannins and reducing sugars. The extract of *Mucuna pruriens* was more effective against *Escherichia coli* (ZI = 2.8 cm) and less effective against *Bacillus subtilis* (ZI= 2.1 cm). The extract of *Mucuna bracteata* was more effective against *E. coli* (ZI= 2.4 cm) and less against *Salmonella typhi* (ZI= 1.8 cm). The present study can be used in future for the formulation of the active chemical ingredients present in the *Mucuna* seeds and be used as natural drug against a variety of pathogenic microorganisms at very little expenditure.

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