Investigation on the Phytochemical Constituents, Antimicrobial Activities and Antioxidant Activities in the Seeds of *Mucuna pruriens* (L.) DC

Nyunt Nyunt Than[1], Suu Suu Win[2], Kathy Myint Thu[3]

Abstract

In this research work one Myanmar indigenous medicinal plant, ***Mucuna pruriens*** (L.) DC. was selected for chemical analysis. Firstly, the preliminary phytochemical screening was performed by using test tube method. According to the phytochemical tests, the seeds of ***Mucuna pruriens*** consist of alkaloids, flavonoids, glycosides, reducing sugars, tannins, saponins, terpenes, steroids and phenolic compounds respectively. Moreover, antimicrobial activities of various solvent extracts such as petroleum ether, chloroform, methanol, ethyl acetate and ethanol of seeds of ***Mucuna pruriens*** were tested by using agar well diffusion method on six selected organisms, ***Bacillus pumilus, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans*** and ***Escherichia coli***. Ethyl acetate extract of seeds of ***Mucuna pruriens*** respond high activity on ***Pseudomonas aeruginosa***. In addition the antioxidant activity of water and ethanol extract of seeds of ***Mucuna pruriens*** were determined by DPPH radical scavenging assay methods. The IC_{50} value for water and ethanol extract were 40.30 μg/mL and 44.86 μg/mL respectively. Therefore, antioxidant activity of water extract of velvet seeds is higher than ethanol extract.

Keywords **:** ***Mucuna pruriens*** (L.) DC., phytochemical, antimicrobial activity, antioxidant activity, DPPH

Introduction

Human beings have relied on natural products as a resource of drug for thousands of years. Herbal drugs constitute only those traditional medicines, which primarily use medicinal plant preparation for therapy. According to world health organization, traditional medicine is the synthesis of therapeutic experience of the generation of indigenous systems of medicine. Since ancient times, various parts of plants, such as root, stem, bark, seed and leaf, have been used for the treatment and prevention of many ailment and diseases and have shown a tremendous resource for the development of new drugs. In addition, the knowledge of the chemical constituents of plants would further be valuable in discovering the actual value of folkloric remedies.

Mucuna pruriens, commonly known as velvet bean, is one of the potential tropical legume having good nutrition qualities and medicinal properties. Velvet plant is typically found in tropical regions and used for various purposes in traditional medicine in several countries. The main aim of this research is to study some phytochemical constituents present in the seeds of ***Mucuna pruriens*** and to investigate the antimicrobial and antioxidant activities from the seeds of ***Mucuna Pruriens*** (L.) DC.

In the present study, we have attempted to evaluate some phytochemical constituents of velvet seeds with their antimicrobial activities and antioxidant activities in the hope of ensuring this data to be used for variety of pharmaceutical industries.

[1] Lecturer, Dr, Department of Chemistry, Sagaing University of Education
[2] Associate Professor, Dr, Department of Chemistry, Sagaing University of Education
[3] Professor and Head, Dr, Department of Chemistry, Sagaing University of Education

Botanical Aspects of *Mucuna pruriens* (L.) DC.

Figure 1 . Seeds and flowers of *Mucuna pruriens* (L.) DC.

Family Name	:	*Fabaceae*
Botanical Name	:	***Mucuna pruriens*** (L.) DC.
Local Name	:	Khwelaype, Khwelayya
Common Name	:	Velvet bean, Cowitch, Cowhage
Part utilized	:	Seeds

Description

Velvet beans are annual, herbaceous, vigorous climbing vines, which can reach 18m in length when grown on supports and even on the ground can attain a length of 5.5 m but 2-3 m is normal. Flowers are the inflorescence is axillary and the flowers, usually 5-30, are showy, and purple, red or greenish-yellow in colour. Pods are 9-14 cm long, hard, curved, slightly ridged and covered with soft black, white or grey hairs which give them a velvety appearance. The seeds are shiny black or brown, ovoid and 12 mm long.

Distribution

Mucuna pruriens (L.) DC. is widely distributed in the wood lands of tropical areas especially South East Asia largely found in Bangladesh, India, Sri lanka, Malaysia. It is also found in Asia, America and Africa.

Cultivation

Mucuna pruriens is cultivated in any types of soil and environment in rainy seasons. It is successfully grown in acidic soil (pH < 5-3) humid area with annual rainfall > 400 mm and annual temperature 19-27 °C.

Medicinal uses of *Mucuna pruriens* (L.) DC.

Several medicinal properties have been attributed to ***Mucuna pruriens*** **(L.) DC.** Roots and seeds are the main parts of velvet plant for therapeutic potentials and have been used by traditional medicinal practitioners, as remedying diabetes, gout, rheumatic disorders and tuberculosis. Decoction of root is for blood purifier, diuretics. Roots are bitter, emollient, stimulant, aphrodisiac, purgative, febrifuge and tonic. It is widely used as a treatment for Parkinson's disease. Seeds have several functions like to treat impotence, worms, nerve disorders and antidepressant activity. Velvet seeds are also used in traditional medicine to prevent the toxic effects of snake bites, which are mainly triggered by potent toxins such as neurotoxins, cardiotoxins, cytoxins, phospholipase and proteases.

Materials and Methods

Plant Materials

The velvet seeds were collected from Salin Township, Magway Region in March, 2017. The botanical name was identified as ***Mucuna pruriens*** (L.) DC. by authorized botanist from Botany Department. The seed samples were sun-dried for four hours and were removed husks. The raw materials were then ground and dried powdered samples were stored in an air-tight bottle.

Phytochemical Screening on Seeds of *Mucuna pruriens* by using Test Tube Method

Phytochemical investigation was carried out on powdered, dried sample of velvet seeds with a view to determining the presence or absence of alkaloids, flavonoids, terpenes, steroids, reducing sugars, saponins, phenolic compounds, glycosides and tannins respectively (Harbone, J.B., 1984).

Antimicrobial Actiyity of Seeds of *Mucuna pruriens* by Agar Well Diffusion Method

Antimicrobial activities of various crude extracts such as petroleum ether, chloroform, methanol, ethyl acetate and ethanol extracts of sample were studied by agar well diffusion method in CRDC (Central Research and Development Centre), Insein, Yangon. The applying organisms are *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans* and *Escherichia coli.* The extent of antimicrobial activities of crude extracts was measured by inhibition zone diameters.

Screening of Antioxidant Activity of Isolated Compounds by DPPH assay

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay was chosen to assess the antioxidant activity of seed materials. This assay has been widely used to evaluate the free radical scavenging effectiveness of various flavonoids and polyphenols in food system (Leea, et. al., 2002).

Results and Discussion

Phytochemical Investigation of the Seeds of *Mucuna pruriens* (L.) DC.

After preparation of the plant sample, the determination for the types of compounds present in the sample was made by using phytochemical test tube method. The results were shown in Table 1. According to the phytochemical tests, the seeds of ***Mucuna pruriens*** consist of alkaloids, flavonoids, terpenes, steroids, reducing sugars, saponins, phenolic compounds, glycosides and tannins groups receptively.

Table1. Result of phytochemical test on the seeds of ***Mucuna pruriens* (L.) DC.**

.	Tests	Solvent Extract	Test Reagents	Observation	Remark
1	Alkaloids	1% HCl	Dragendorff's reagent	Orange ppt	+
			Wagner's reagent	Brown ppt	+
2	Flavonoids	95%EtOH	Mg turning, conc:HC1	pink color solution	+
3	Terpenes	95%EtOH	Acetic anhydride, $CHC1_3$,conc:H_2SO_4	Reddish brown color solution	+
4	Steroids	95%EtOH	Acetic anhydride, conc: H_2SO_4	green color solution	+
5	Tannins	95%EtOH	10% $FeCl_3$,dil H_2SO_4	Brown color solution	+
6	Reducing Sugars	Distilled water	Benedict's solution	Brick red ppt	+
7	Saponins	Distilled water	Distilled water	perment frothing	+

8	Phenolic compounds	Distilled water	10% $FeC1_3$	greenish blue color solution	+
9	Glycosidse	Distilled water	10% lead acetate	white ppt	+

(+) = Presence (-) = Absence ppt = Precipitate

Antimicrobial activities of the Seeds of *Mucuna pruriens* (L.) DC.

Antimicrobial activities of various crude extracts such as petroleum ether, chloroform, ethyl acetate and ethanol extracts were studied by agar well diffusion method. The extent of antimicrobial activities of crude extract was measured by the diameter of inhibition zones as shown in Table 2 and Figure 3.

Table 2. Results of Antimicrobial activities of the **Seeds of *Mucuna pruriens* (L.) DC.**

Inhibition zone diameters of various solvent extracts against microorganisms

Sample	Solvent	I	II	III	IV	V	VI
***Mucuna pruriens* (Seeds)**	Petroleum ether	-	-	-	-	-	-
	Chloroform	14 mm (+)	13 mm (+)	14 mm (+)	12 mm (+)	13 mm (+)	14 mm (+)
	Methanol	13 mm (+)	13mm (+)	18 mm (++)	14 mm (+)	13 mm (+)	14 mm (+)
	Ethyl acetate	18 mm (++)	17 mm (++)	28 mm (+++)	17mm (++)	17 mm (++)	18 mm (++)
	Ethanol	16 mm (++)	14 mm (+)	19 mm (++)	18 mm (++)	15 mm (++)	17 mm (++)

Agar-well ~ 10mm Organisms

10mm~ 14mm (+) Low Activity

I = *Bacillus subtilis* (N.C.I.B-8326)

15mm~ 19mm (++) Medium Activity II = *Staphylococcus aureus* (N.C.P.C-6371)

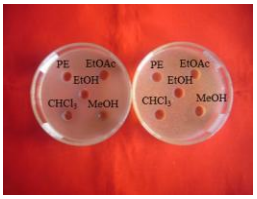
20mm above (+++) High Activity III = *Pseudomonasus aeruginosa* (6749)

(-) No Activity IV = *Bacillus pumilus* (N.C.I.B-8982)

V = *Candida albicans*

VI = *Escherichia coli*(N.C.I.B-8134)

The lengths of the diameters show the degree of antimicrobial activity. The larger the inhibition zone diameter, the higher the antimicrobial activity.According to this table, ethyl acetate extract of velvet seeds gives the highest activity on ***Pseudomonus aeruginosa*** and medium activities on the remaining five selected organisms such as ***Bacillus subtilis, Staphylococcus aureus, Bacillus pumilus,* C*andida albicans*** and ***E.coli***. Exception of ***Staphylococcus aureus***, ethanol extract of velvet seeds responds medium activity on other five selected organisms, ***Bacillus subtilis, Psedomonus aeruginosa, Bacillus pumilus,* C*andida albicans*** and ***E.coli*** respectively. Methanol extract shows medium activities on ***Psedomonus aeruginosa*** and chloroform extract responds low activity on all six selected organisms. Petroleum ether extract showed no the antimicrobial activity. Therefore, the velvet seeds were found to be more potent antimicrobial activities in ethanol and ethyl acetate extracts.



Bacillus subtilis

Staphylococcus aureus

Pseudomonas aeruginosa

Bacillus pumilus

Candida albicans

Escherichia coli

Figure 3. Inhibition Zone of Various Extracts of the Seeds of *Mucuna pruriens*

Determination of Antioxidant Activities of Seeds of *Mucuna pruriens* (L.) DC.

Antioxidant activities of water and ethanol extracts have been investigated by DPPH assay at 517 nm using UV-7504 spectrophotometer. On the basis of absorbance values, % RSA (Radical Scavenging Activity) of different concentration was calculated and the result obtained is described in Figure 4.

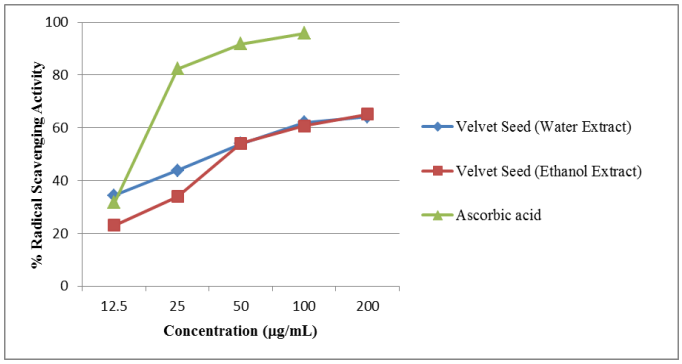


Figure 4. Radical Scavenging Activity of different concentrations of crude extracts of Velvet Seeds

From the result, 50 % inhibition concentration (IC_{50}) for water and ethanol extracts was determined by linear regressive excel program and the results were described in Figure 4 and 5. According to Table 3, the IC_{50} values of water and ethanol extract were found to be 40.30 µg/mL and 44.86 µg/mL respectively. The lower the value of IC_{50} indicates the higher the free radical scavenging activity. The IC_{50} value of the standard vitamin C is 17.76 µg/mL. Therefore, antioxidant activity of water and ethanol extracts of sample is lower than the standard vitamin C.

Table 3. %RSA and IC_{50} Values of Water and Ethanol Extracts of Velvet Seeds

Tested Sample	% RSA ± SD in different concentration (µg/mL)					IC_{50} (µg/mL)
	12.5	25	50	100	200	
Mucuna pruriens (H_2O extract)	34.29 ± 0.64	43.81 ± 1.71	53.93 ± 2.35	61.94 ± 1.71	64.20 ± 0.64	40.30
Mucuna pruriens (EtOH extract)	23.07 ± 1.37	33.98 ± 2.34	54.15 ± 7.42	60.78 ± 1.56	65.20 ± 0.39	44.86
Ascorbic acid	31.59 ± 1.73	82.40 ± 4.56	91.76 ± 3.24	95.76 ± 1.51		17.76

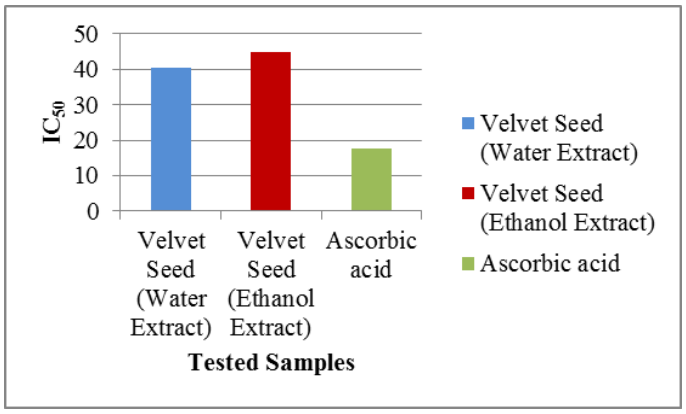


Figure 5. Bar graph for the comparison of IC_{50} values of tested samples

Conclusion

From the phytochemical screening, the seeds of ***Mucuna pruriens* (L.) DC.** contained alkaloids, flavonoids, terpenes, steroids, reducing sugars, saponins, phenolic compounds, glycosides and tannins respectively. In addition, antimicrobial activities of this sample were examined by agar well diffusion methods using six microorganisms. According to antimicrobial activity tests, ethyl acetate extract of the sample showed the highest activity on ***Pseudomonas aeruginosa*** and medium activity on the remaining five selected organisms. Hence, ethyl acetate extract of seeds of ***Mucuna pruriens*** should be studied for more detailed chemical analysis.

Furthermore, from the DPPH radical scavenging assay, IC_{50} values of watery and ethanol extract of velvet seeds gave 40.30µg/mL and 44.86µg/mL respectively. Therefore, watery extract was found to be more potent antioxidant activity than ethanol extract. However, from the results of

present investigation, further studies are recommended to isolate the active components of ***Mucuna pruriens*** seeds for their several medicinal properties.

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