Investigation of Some Chemical Constituents and Antimicrobial Activity of *Mucunapruriens* (L.) DC. (Khwele-ya) Seeds

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Abstract

The present research deals with the studies on some chemical constituents and antimicrobial activity of *Mucunapruriens* (L.) DC. (Khwele-ya) seeds. The preliminary phytochemical investigation of *M. pruriens* was carried out by Test Tube method. From the investigation, alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, organic acids, phenolic compounds, reducing sugars, saponins, steroids, tannins and terpenoids were observed in the sample. Semiquantitative elemental analysis of *M. pruriens* seeds was carried out by EDXRF method. From the results, potassium, phosphorus, sulphur, calcium, iron, zinc, manganese, copper, rubidium and strontium were observed in the sample. Determination of nutritional values has also carried out by AOAC method resulting moisture (3.94 %), ash (3.89 %), protein (24.21 %), fiber (1.91 %), fat (4.65 %), carbohydrate(33.83 %) and energy values (274.01 kcal/100g) in the sample. The antimicrobial activity of *M. pruriens* was screened by agar well diffusion method. From the screening, the six microorganisms such as *B. subtilis*, *S. aureus*,*P. aeruginosa*, *B. pumilus*, *C. albicans*and *E. coli*. From the results, EtOAc extract exhibited higher antimicrobial activity than other extracts in the sample. According to the experimental results, *M. pruriens* may be used in medicinal formulation for human health.

Keywords: Antimicrobial activity, EDXRF, *M. pruriens*, Nutritional values, Phytochemical investigation

Indroduction

Description of *M. pruriens*

*Mucunaprurines*L. (DC), the family Fabaceace is commonly known as Velvet bean. It is distributed in Africa, West Indies, tropical America, the Pacific Islands and the United Statae. Velvet bean is an annual twinning, climbing shrub with long vines that can reach over 15 m in length and 3-18 m in height. It can grow in well-drained soil, sandy soils and also in the soils with great acidity range but will not able to survive in very saturated soils. The shapes of terminal leaflets are rhomboid oval and smaller, while the lateral ones are vary unequal sided with their lower halves much broader. Flowers are white to dark purple in color and hang in long clusters. The pods are, 2 to 3 inch in length and half an inch broad. The pods produced in clusters which contain seeds known as *mucuna* beans. The seeds pods are covered with reddish-orange hairs that are not easily detached and can cause intense irritation to the skin when they come in contact to the body. The seed are 15-20 mm long and 7-15 mm broad and 4 to 6.5 cm thick reniform or ovoid in shape. The seed coat is hard, thick and glossy occasionally mottled (Verma*et al.,* 2014).TheVelvet bean exhibits wide array of phytoconstituentsphysiological and pharmacological activities. *M. prurines*have pharmacological activities like anti-parkinson's, hypoglycemic,

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antioxidant, antivenom, aphrodisiac, antimicrobial, analgesic and antipyretic (Kavitha*et al*., 2014)

Botanical aspects of *Mucunapruriens*

Family : Fabaceae

Genus : *Mucuna*

Species : *M. pruriens*

Botanical name : *Mucunapruriens*(L.) DC.

Myanmar name :Khwele-ya

Part used : Seeds

Figure 1.*M. pruriens* fruits and seeds

Materials and Methods

Collection and Preparation of Plant Samples

*M. pruriens*seeds were collected from ChaungOo Township, Sagaing Region, Myanmar. The plants wereidentified and authenticated at the Department of Botany, Yangon University. The preliminary phytochemical investigation of *M. pruriens* was carried out by Test Tube method (Tin-Wa, 1972). Shimadzu EDX-8000 spectrometer can analyze the elements from Na to U under vacuum condition. In this research work relative abundance of elements present in *M. pruriens*seeds was determined by EDXRF spectrometer.The nutritional values such as moisture, ash, crude protein, crude fiber, crude fat, carbohydrate contents and energy value of *M. pruriens*seedsweredetermined according to AOAC method at Department of Research and Innovation Analysis Department (DRI), The Government of the Republic of the Union of Myanmar Ministry of Education (GRUMME), Yangon, Myanmar (A.O.A.C, 2000).Antimicrobial activities of different crude extracts of the *M. pruriens* seeds were screened *in vitro* by agar well diffusion method on nutrient agar medium

(Perez *et al*., 1990). This experimentwas carried out at Pharmaceutical Research Department, Insein, Yangon, Myanmar.

Results and Discussion

Preliminary Phytochemical Screening in*M. pruriensSeeds*

In order to detect the various constituents present in *M. pruriens* seeds, the seeds extracts were subjected to the qualitative test analysis using standard methods. Test reagents, observations and inferences for the analyses are summarized in Table 1.

Table 1.Results of Preliminary Phytochemical Investigation of sample *M. pruriens* Seeds

No	Types of compounds	Extract	Reagent	Observation	Remark
1	Alkaloids	1% HCl	Dragendorff'sMayer's Wagner's reagent	Orange pptWhite pptReddish brown ppt	+ + +
2	α–amino acids	H_2O	Ninhydrin	Purple sport	+
3	Carbohydrates	H_2O	10% α-napthol, conc: H_2SO_4	Red ring	+
4	Cyanogenic glycosides	MeOH	Conc: H_2SO_4 and sodium picrate	No change	-
5	Flavonoids	EtOH	Mg turning,conc: HCl	Pink solution	+
6	Glycosides	H_2O	10% lead acetate	White ppt	+
7	Organic acids	MeOH	Bromo-cresol green	Deep blue colour	+
8	Phenolic compounds	H_2O	5% $FeCl_3$ solution	Green solution	+
9	Reducing sugar	Dil H_2SO_4	Benedict's solution	Brick red ppt	+
10	Saponins	H_2O	D.W	Forthing	+
11	Steroids	PE	Acetic anhydride and H_2SO_4	Green solution	+
12	Tannins	H_2O	2% gelatin solution	White ppt	+
13	Terpenoids	$CHCl_3$	Acetic anhydride and H_2SO_4	Pink solution	+

(+) present, (-) absence

Semi-quantitative Elemental Analysis of *M. pruriens*Seeds

As shown in Table 2 and Figure 2 and 3, it can be seen that organic compounds are predominant in the samples, and other element, K is also present in reasonable composition but P, S and Ca were present in medium amount and Fe, Zn, Mn, Cu, Sr and Rb were present in very little amounts based on the relative abundance of elements.

Table 2.Relative Abundance of Elements of *M. pruriens* Seeds byED-XRF

No.	Elements	Relative Abundance (%)
1	Potassium (K)	1.413
2	Phosphorus (P)	0.461
3	Sulphur (S)	0.298
4	Calcium (Ca)	0.107
5	Iron (Fe)	0.007
6	Zinc (Zn)	0.003
7	Manganese (Mn)	0.002
8	Copper (Cu)	0.002
9	Strontium (Sr)	0.001
10	Rubidium (Rb)	0.001
11	COH	97.706

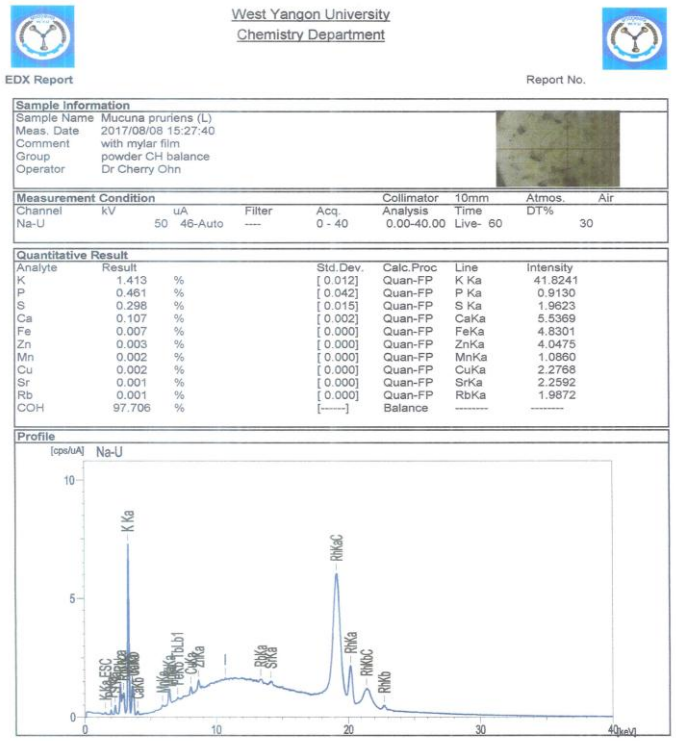

Figure 2.ED-XRF spectrum of *M. pruriens* seeds

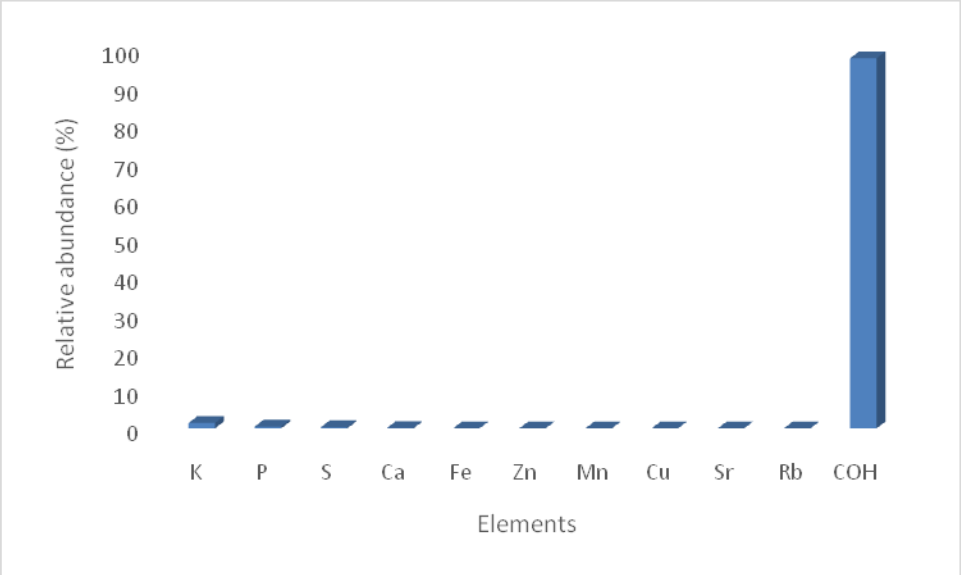

Figure 3. Relative abundance (%) of elements in the *M. pruriens* Seeds by using ED-XRF methods

Nutritional Values of Khwele-ya Seeds

The nutritional values of the seed of *M. pruriens*such as moisture, ash, crude protein, crude fiber, crude fat, carbohydrates and energy values were determined by using standard methods for food analysis (AOAC, 2000) and the nutritional composition of the samples are described in Table 3 and Figure 4. These analyses revealed some interesting findings. *M. pruriens* seedswere found to contain higher carbohydrate content but lower fiber content.

Table 3. Nutritional Compositions of*M. pruriens* Seeds

Parameter	Nutritional Composition
Moisture (%)	3.94
Ash (%)Crude protein (%)	3.89
Crude fiber (%)	24.21
Crude fat (%)	1.91
Carbohydrate (%)	4.65
Energy value (kcal/100g)	33.82
	274.01

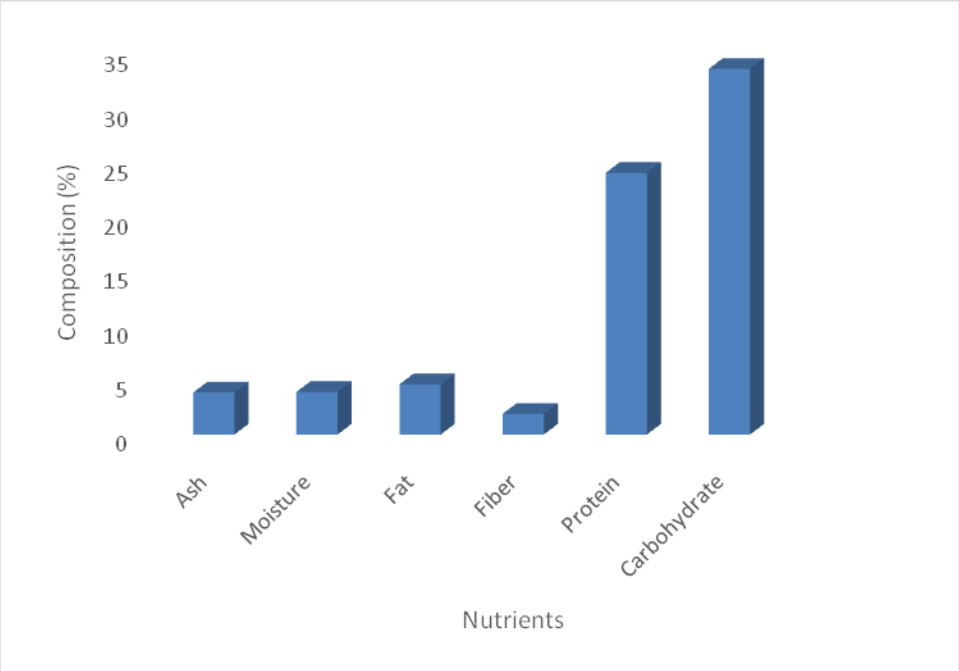

Figure 4. Nutritional values in *M. pruriens*seeds

Antimicrobial Activity

Antimicrobial activity was studied by agar well diffusion method according to Perez *et al*., 1990. *In vitro* antimicrobial screening of both samples extracts was carried out at Pharmaceutical Research Department, Insein, Yangon, Myanmar. The antimicrobial activity was assessed by agar well diffusion method which is equally suited to the screening of antibiotics or the products of plant evaluation and is highly effective for rapidly growing microorganisms and the activities of the test extracts are expressed by measuring the zones (mm) of inhibition. Generally the more susceptible the organism, the bigger is the zone of inhibition.

Petroleum ether, ethyl acetate, ethanol, methanol and aqueous extracts of the *M. prurines* seedswere used to determine the antimicrobial activity against five bacterial strains such as *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus* and *Escherichia coli*, and one fungal strain: *Candida albicans*.

The observed antimicrobial activity of*M. pruriens* seeds was expressed as the zone diameters of inhibition shown in Tables 6. The photographs of agar plates showing the inhibition zones are illustrated in Figures 5. Among the extracts, EtOAc extract of *M. pruriens*seeds (12 ~ 27 mm) was observed to possess higher antimicrobial activitythan other extracts.

Table 6. Inhibition Zone Diameters of *M. pruriens* Seed Extracts

croorganisms	Inhibition zone diameters (mm) of different extracts			
	PE extract	tOAc xtract	tOH xtract	H_2O tract
B.subtilis	11 (+)	12 (+)	2 (+)	l (+)
S.aureus	13 (+)	12 (+)	1 (+)	l (+)
.aeruginosa	11 (+)	27 (+++)	-	-
B.pumilus	12(+)	11 (+)	1 (+)	l (+)
C.albicans	12 (+)	17 (++)	1 (+)	l (+)
E.coli	12 (+)	15 (++)	2 (+)	l (+)

Agar well (diameter)-(10 mm), 10 mm - 14 mm (+),15 mm - 19 mm (++), 20 mm - above (+++)

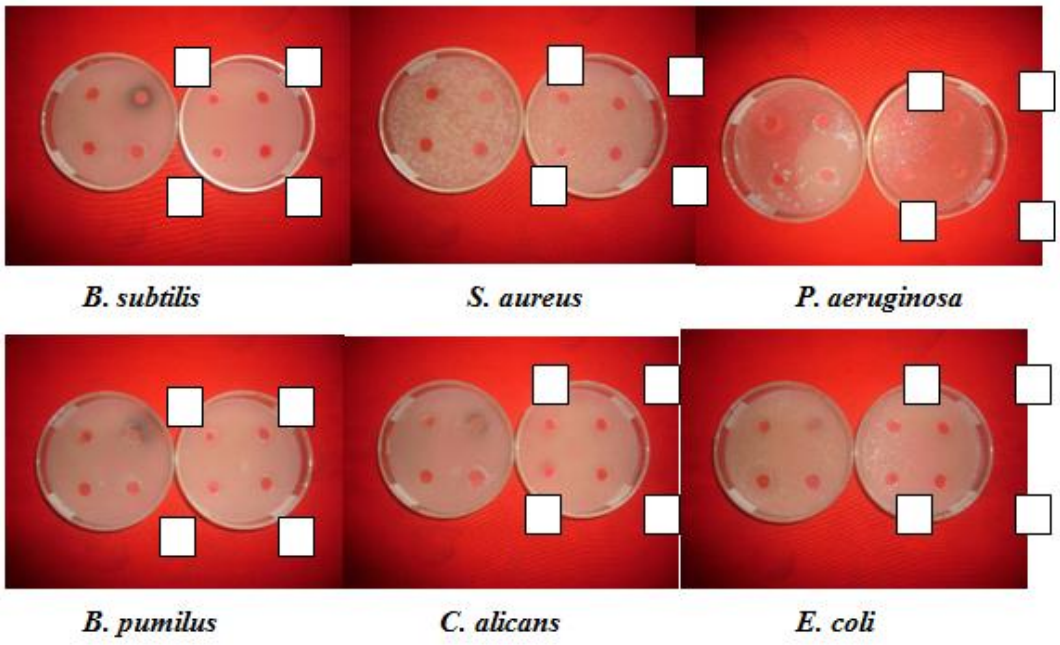

Figure 5. Agar wells indicating inhibition zones of various extracts from the *M. pruriens* seeds

1 = PE extract 2 = EtOAC extract 3= EtOH extract 4 = H_2O

Conclusions

From the overall assessments of the present work concerning with the investigation of some chemical constituents and antimicrobial activity in *M. pruriens* seed, the following inferences can be deduced. The preliminary phytochemical tests have revealed that the presence of alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, organic acids, phenolic compounds, reducing sugars, saponins, steriods, tannins and terpenoids in the examined samples. According to the phytochemical investigation, the *M. pruriens* seeds may be used for the medicinal formulation. From the analysis of element by ED-XRF spectroscopic method, it was found that relative abundance of potassium (1.413 %), phosphorus (0.461 %), sulphur (0.298 %), calcium (0.107 %), iron (0.007 %), zinc (0.003%), manganese (0.002 %), copper (0.002 %), Strontium (0.001 %), rubidium (0.001 %), and organic compounds (97.706 %). The elemental analysis by ED-XRF indicated that *M. pruriens* seed sample had the highest potassium and trace amount of phoshorus, sulphur, calcium, iron, zin, manganese, copper, Strontium and rubidium. As the high concentration of potassium, the M. *pruriens* may be continued to test for the use in hypertension.*In vitro* antimicrobial activity of the different crude extracts were screened by using agar well diffusion method against *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albicans* and *Escherichia Coli* species. All the crude extracts of the sample (except ethanol and water extracts of *Pseudomonas aeruginosa*)exhibited antimicrobial activity against the organisms tested. Among the extracts, EtOAc extract of *M. pruriens* seeds (12 ~ 27 mm) was observed to possess higher antimicrobial activitythan other extracts.*M. pruriens* seeds may be used in the medicinal formulation of human health.

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