INVESTIGATION ON SOME PHYTOCHEMICAL CONSTITUENTS AND BIOACTIVITIES OF SEED EXTRACTS OF *Mucuna Pruriens* L.(KHWE-LEYA)

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Abstract

The research focused on the investigation of some phytochemical constituents of *Mucuna pruriens* (L.) seed and some of its biological activities. The sample was collected from Ingapu Township, Ayeyawady Region and it was identified at Department of Botany, Hinthada University. Preliminary phytochemical tests have revealed the presence of alkaloids, carbohydrates, flavonoids, α-amino acids, starch, organic acids, phenolic compounds, saponins, glycosides, reducing sugars, steroids and terpenoids in the sample according to test tube methods and TLC profile. The seed sample was found to contain 14.39 % of moisture, 3.43 % ash, 23.22 % of protein, 8.98 % of dietary fiber, 1.68 % of crude fat, 48.30 % of carbohydrate, and 302 kcal /100 g of energy value based on dried sample. The seed sample was found to have relatively highest content of K and P whereas minor components of S, Ca, Fe, Zn, Cu and Mn according to EDXRF analysis. The *in vitro* antimicrobial activities of PE, EtOAc, $CHCl_3$, 95 % EtOH and H_2O extracts from *M. pruriens*seed were screened by agar well diffusion method on six species of microorganisms, namely *Bacillus pumilus*, *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. All extracts of seed sample were observed to possess antimicrobial activity. Among the tested crude extracts, EtOH extract was found to possess the most potent antimicrobial activity (inhibition zone diameter ranged between 14~16 mm). *In vitro* antioxidant activities of 95 % EtOH and watery extracts from *M. pruriens* seed were assessed by DPPH radical scavenging activity assay. The antioxidant activity of watery extract (IC_{50}= 3.62 µg/mL) was found to be higher than ethanol extract (IC_{50}= 4.49 µg/mL). From the results of phytochemical constituents, antioxidant and antimicrobial activities of the *M. pruriens* seed observed in the present study, the seed could be applied as the local health remedy for the local indigenous communities of our country.

Keywords: *Mucuna pruriens* (L.) (Khwe-leya), phytoconstituents, TLC profile, antimicrobial activity, antioxidant activity

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Introduction

Traditional Medicine and Medicinal Plants

Pharmacognosy is the first step in deciding the status of a plant organ as a crude medicine, hence the current study was done. The present study comprises macroscopy, microscopy, histochemistry, physicochemical parameters, fluorescence analysis and preliminary phytochemistry. The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health, has been widely observed.

Traditional medicine is the sum total of the knowledge, skill and practices based on the theories, beliefs, and experiences, indigenous to different cultures, whether explicable or not, used in maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness. It refers to health practices, approaches, knowledge and beliefs incorporating plants, animal and mineral based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination to treat, diagnose and prevent illnesses or maintain well-being.

The diseases as described and defined in Modern medicine were in existence globally since long times and were named differently in various civilizations. Ayurveda, considered as the oldest "Science of Life" describes various diseases and its treatment with herbs, minerals, and parts of animals. The Ayurveda describes various neurological diseases such as kampavat (Parkinson's disease), apasmar (epilepsy), Unmad (schizophrenia), smrutinash (dementia), avsaada (depression), manas mandata (mental retardation), etc. Kampavata, (meaning tremors caused by excess of vata), is characterized in Ayurveda by tremors, rigidity, akinesia, dyskinesia, loss of olfaction, uncontrollable body movements, difficulty in step initiation, difficulty in maintenance of posture, etc. *Mucuna pruriens* is the most commonly used herb in treatment of Parkinson's disease, either alone or with other herbs.

The incidence of Parkinson's disease (PD) is very high in aged population and levodopa is still the gold standard in management of PD. Prolonged use of levodopa leads to dyskinesias, toxicity, and diminishing efficacy (Cenci, 2007). Despite of several advancements in drug development, the control over progression of neurological damage is inadequate. Before the

introduction of modern medicine, every civilization had it own traditional system of medicine. Several plants, minerals, and biochemical substances were used in almost every traditional system of medicine. The diseases, as defined by the modern system of medicine, were described along with their symptoms in the traditional system of medicine also.

Houghton and Howes (2005) and have reviewed plants having neuroprotective activity in rat model of PD. The Ayurvedic physicians treat PD disease using seeds of *Mucuna pruriens* and some other medicinal plants such as *Celastrus paniculatus, Withania somnifera* and *Tinospora cordifolia, Nardostachys jatamansi*, etc. depending on the symptoms. There are pharmacological studies supporting therapeutic practice of using the above mentioned plants.

Mucuna pruriens L.(Khwe-Leya)

It is mainly distributed in Asia, Africa, Pacific Islands and the United States. *M. pruriens* has been of keen interest in phytochemical and Ayurvedic research due to its excellent medicinal values. Medicinal herbs are moving from fringe to main stream use with a great number of people speaking remedies and health approaches free from side effects caused by synthetic chemicals. *M. pruriens* is highly regarded as an universal panacea in the ayurvedic medicine. It is one of the universal plant having medicinal activities.

Botanical aspects of *Mucuna pruriens*

Family - Fabaceae

Botanical Name - *Mucuna pruriens*

Myanmar Name - Khwe-Leya

English name - Velvet beans, Cowharge, Cowitch, Lacuna bean, Lyon bean

Parts used - Seed

Chemical constituents of *Mucuna pruriens*

The seeds of *M. pruriens* (Figure 1) in addition to the levodopa also contained protein, lipid, dietary fiber and carbohydrates, minerals such as sodium, potassium, calcium, magnesium, iron, zinc, copper, manganese, and phosphorus. The seeds also contain phenolics, tannins and phytic acid. The fatty acids found in the seeds were palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid and behenic acid. The seeds also contained niacin and ascorbic acid. The amino acids found in seeds were glutamic acid, aspartic acid, serine, threonine, proline, alanine, glycine, valine, cystine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine, tryptophan, and arginine. Sridhar and Bhat (2007) have reported a number of value-added phytochemicals of *Mucuna* seeds of medicinal importance (e.g. alkaloids, alkylamines, arachidic acid, beta-carboline, harmine, bufotenin, dopamine, flavones, galactose, gallic acid, genistein, glutathione, hydroxygenistein, 5-hydroxytryptamine, N,N-dimethyltryptamine (DMT), 5-methoxy-dimethyltriptamine(5-MeO-DMT), 6-methoxyharman, mucunadine, mucunain, mucunine, myristic acid, nicotine, prurienidine, prurienine, riboflavin, saponins, serotonin, stizolamine, trypsin, tryptamine, vernolic acid (Figure 2). Mucunadine, prurienine and pruricininine are the additional alkaloids isolated from seed extracts (Mehta and Majumdar, 1994).

Medicinal uses of *Mucuna pruriens*

The root is used as a blood purifier, diuretic, emmenagogue, for asthma, cholera, dropsy, delirium, elephantiasis, fevers, gout, kidney stones rheumatism, to relieve dysmenorrhea, in catarrh and dropsy. The leaf is used as an aphrodisiac, diuretic, nerve tonic, uterine stimulant, for scorpion stings and in dysentery. The infusion of the pods is also good for dropsy. The seed cure night dreams and impotency and to promote fertility, for sexual debility, seminal weakness and spermatorrhea, as an aphrodisiac to increase seminal fluid and manly vigour, antivenin, diarrhea, diabetes.

(a)

(b)

Figure 1 Photographs of (a) *Mucuna pruriens* seeds in pod (b) seeds

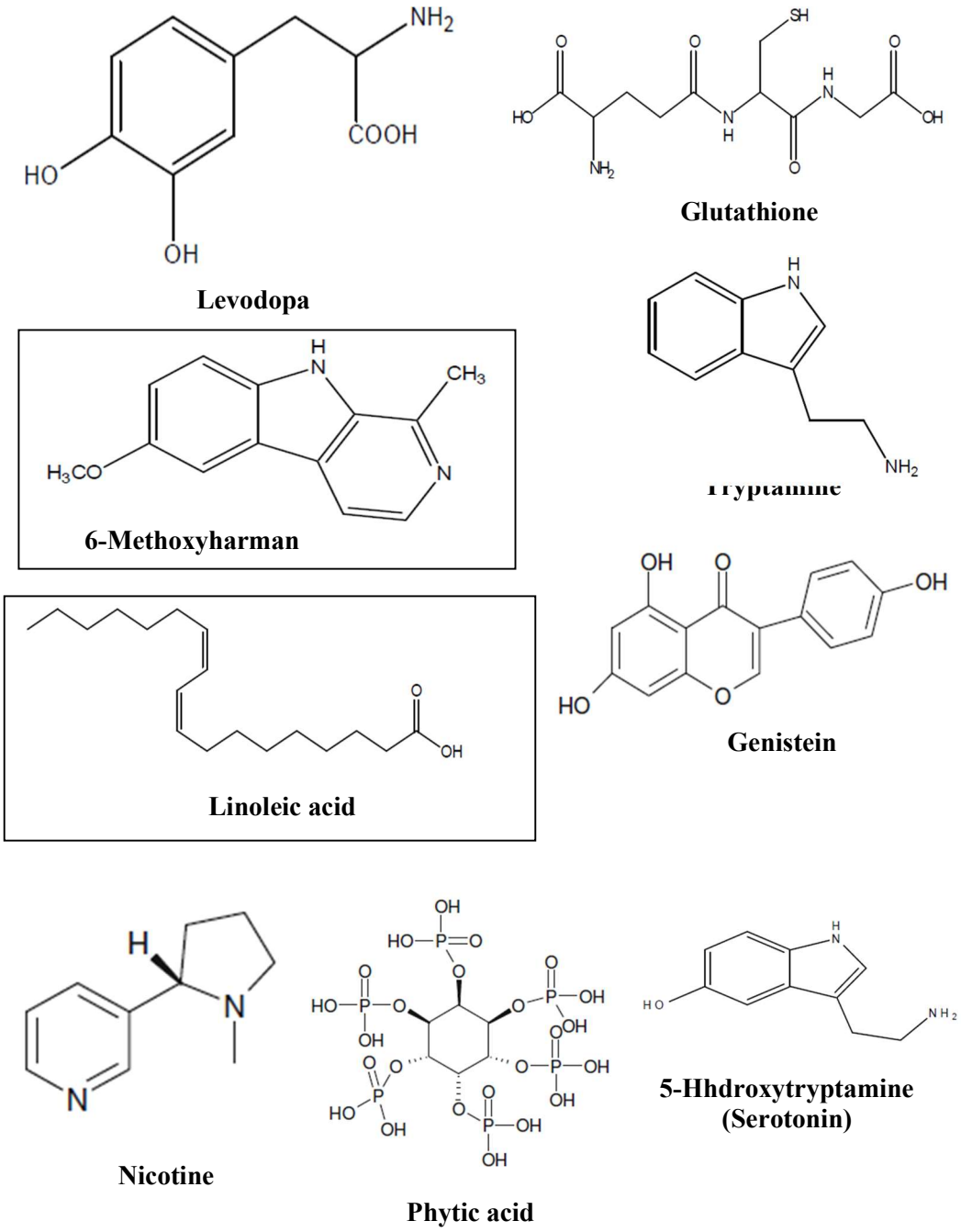

Figure 2 Structures of some chemical constituents of *Mucuna pruriens* seed

The main aim of this study was to investigate some phytochemical constituents from the seed of *Mucuna pruriens* L. (Khwe-Leya) and to study some of its biological activities such as antimicrobial activity and antioxidant activity.

Materials and Methods

Sample Collection

The seed sample of *Mucuna pruriens* (L.) (Khwe-leya) was collected from Ingapu Township, Ayeyawady Region in October, 2016. After being collected, the scientific name of the sample was identified by authorized botanists at Botany Department, Hinthada University.

Sample Preparation

The fresh sample was cleaned by washing with water and air-dried. The dried sample was ground using grinding machine. And then this powdered sample was kept in the sealed air-tight container to prevent moisture changes and other contamination. It was then used without further purification or refining.

Qualitative Screening of the Phytochemicals

In order to classify the types of organic constituents present in seed samples, preliminary phytochemical tests on samples were carried out according to the series of test tube methods.

Elemental Analysis of the Seed of *Mucuna pruriens* L. by Energy Dispersive X-ray Fluorescence (EDXRF) Spectrometry

Relative abundances of elements in the seed were determined by Energy Dispersive X-Ray Fluorescence (EDXRF) Spectrometer.

Determination of Some Nutrient Values of the Samples

Determinations of some nutritional values present in seed samples such as moisture, ash, fiber, protein, fat and carbohydrate contents were carried out by Association of Official Analytical Chemists method (AOAC,1990).

Separation of some Organic Constituents from Crude Extracts of *Mucuna pruriens* L. (Khweleya) Seed

Thin layer chromatographic examinations on methanol, ethyl acetate and petroleum ether extracts were performed by using silica gel GF_{254} precoated plate and a variety of solvent systems. TLC plates used in the laboratory were purchased as 20 cm x 20 cm sheets. Each large sheet cut into plate was measured. Using a pencil, a line was drawn across the plate at the 0.5 cm mark. About 1 mg of petroleum or ethyl acetate or methanol extracts was dissolved in 1 mL of a respective solvent. The tip of the capillary tubes was dipped into the solution and then gently touches the end of it into the proper location on the TLC plate.

The developing container for TLC was designed as chamber, a beaker with a watch glass on the top. Each solvent (various ratio of petroleum ether or ethyl acetate or MeOH) was poured into the chamber to a depth of just less than 0.5 cm. The beaker was covered with a watch glass, swirled gently, and it was allowed to stand for 5 minutes.

The prepared TLC plate was placed in the developing beaker; the beaker was covered with the watch glass. The solvent was raised up the TLC plate by capillary action. The plate was allowed to develop until the solvent was about half a centimeter below the top of the plate. The plate was removed from the beaker and immediately marked the solvent front with a pencil. The plate was allowed to dry. After developing the chromatograms, these were viewed with the various spraying agents such as Liebermann Burchard reagent, 5 % H_2SO_4 solution, $AlCl_3$ solution, Dragendorff's reagent and 5 % $FeCl_3$ solution to develop colour and classify the seed constituents .

Screening of Antioxidant Activity of Crude Extracts from *M. pruriens* Seed by DPPH Assay

DPPH (1,1-diphenyl-2-picryl-hydrazyl) 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.

In this experiment, the antioxidant activity was studied on 95% ethanol extract and watery extract from selected seed sample by DPPH free radical scavenging assay.

Antimicrobial Activity Screening by Agar Well Diffusion Method

The antimicrobial activities of different crude extracts such as pet ether, ethyl acetate, chloroform, 95% ethanol and water extracts were determined against six microorganisms such as *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans* and *E. coli* species by employing agar well diffusion method at Pharmaceutical Research Department, Ministry of Industry, Yangon, Myanmar.

Results and Discussion

The phytochemical investigation was carried out according to the test tube method. In Table 1, α-amino acids, alkaloids, carbohydrate, flavonoids, glycosides, phenolic compounds, reducing sugars, starch, saponins, steroids, terpenoids, oganic acids were found to be present in *M. pruriens* seed.

Table 1 Results of Phytochemical Investigation on *M. pruriens* Seed

Sr.No.	Tests	Extract	Test Reagents	Observation	Remark
1.	α-amino acids	H_2O	Ninhydrin reagent	pink colour	+
2.	Alkaloids	1% HCl	Mayer's reagent	white ppt	+
			Dragendorff's reagent	orange ppt	+
				brown ppt	+
			Wagner's reagent	yellow ppt	+
			Sodium picrate		
3.	Cyanogenic glycosides	H_2O	Sodium picrate solution	no brick red	-
4.	Carbohydrate	H_2O	10% α-naphthol & H_2SO_4	red ring	+
5.	Flavonoids	EtOH	Mg ribbon & conc.HCl	pink colour	+
6.	Glycosides	EtOH	10% lead acetate	white ppt	+
7.	Phenolic	EtOH	1% $[K_3Fe(CN)_6]$ &	Deep blue	+

Sr.No.	Tests	Extract	Test Reagents	Observation	Remark
	compounds		5% $FeCl_3$	colour	
8.	Reducing sugars	H_2O	Benedict's solution	brick-red ppt	+
9.	Starch	H_2O	Iodine solution	Blue colour	+
10.	Saponins	H_2O	Distilled water	frothing	+
11.	Steroids	Toluene	Acetic anhydride & conc. H_2SO_4	green colour	+
12.	Tannins	H_2O	Gelatin & 1% $FeCl_3$	no white ppt	-
13.	Terpenoids	$CHCl_3$	Acetic ahydride & conc. H_2SO_4	pink colour	+
14.	Organic acids	H_2O	Bromocresol green	blue colour	+

(+) presence ; (-) absence

The relative abundances of elements present in *M. pruriens* seed were determined by EDXRF spectrometer. In Table 2 K was found to be the most abundant element followed by P,S,Ca,Fe,Zn,Cu,Mn.

Table 2 Relative Abundances of Some Elements in *M. pruriens* Seed (By EDXRF)

No.	Elements	Relative Abundance (%)
1.	K	1.294
2.	P	0.353
3.	S	0.236
4.	Ca	0.102
5.	Fe	0.011
6.	Zn	0.04
7.	Cu	0.002
8.	Mn	0.002

The nutrient values such as moisture, ash, proteins, fiber, fat and carbohydrate contents were determined and by AOAC method and energy value was found and seed sample. The results are described in Table 3.

Table 3 Some Nutritional Values of *M. pruriens* (Khwe-Leya) Seed

No.	Parameters	Contents (%)
1.	Carbohydrate	48.30
2.	Crude Protein	23.22
3.	Moistures	14.39
4.	Crude Fiber	8.98
5.	Ash	3.43
6.	Crude Fat	1.68
7.	Energy value	302 (kcal/100g)

The antioxidant activity of the seed sample was determined by DPPH assay. The results obtained are shown in Table 4 and illustrated with Figures 3 and 4. From these observations the activity of aqueous extract (IC_{50} = 3.62 μg/mL) was more potent than ethanol extract (IC_{50} = 4.49 μg/mL)

Table 4 % Oxidative Inhibition and IC_{50} Values of 95 % EtOH and Aqueous Extracts of *M. pruriens* Seed and Standard Ascorbic Acid

Extracts	% Inhibition (mean ±SD) in different concentrations (μg/mL)						IC_{50} (μg/mL)
	0.625	1.25	2.5	5	10	20	
Ethanol	13.98 ± 2.35	17.06 ±1.34	41.00 ±0.84	53.08 ±0.84	57.35 ±0.84	61.61 ±1.01	4.49
Aqueous	14.95 ±1.15	26.63 ±2.31	43.21 ±0.96	57.34 ±2.69	61.96 ±1.54	64.13 ±1.15	3.62
Ascorbic acid	14.04 ±2.09	54.83 ±2.48	72.44 ±3.83	81.13 ±1.47	87.40 ±2.23	91.21 ±0.48	1.17

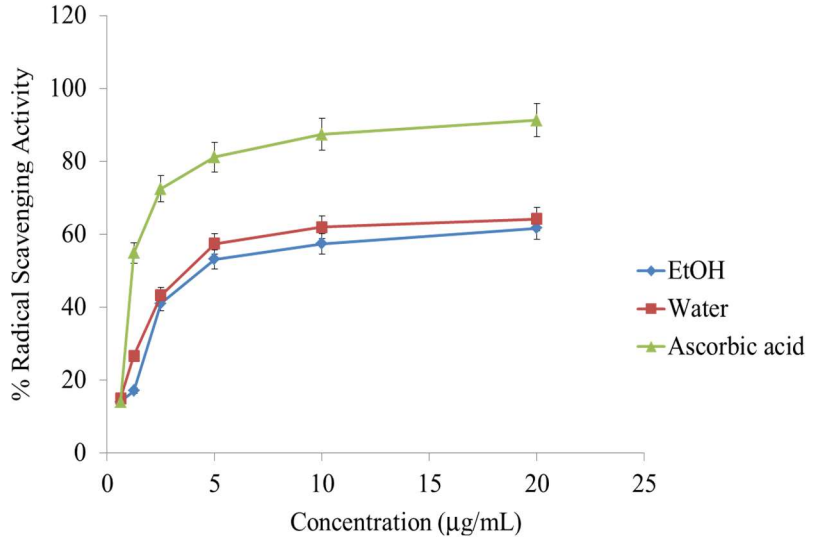

Figure 3 Plot of % oxidative inhibition Vs concentrations (µg/mL) of ethanol and aqueous crude extracts of *M. pruriens* seed in comparison with ascorbic acid

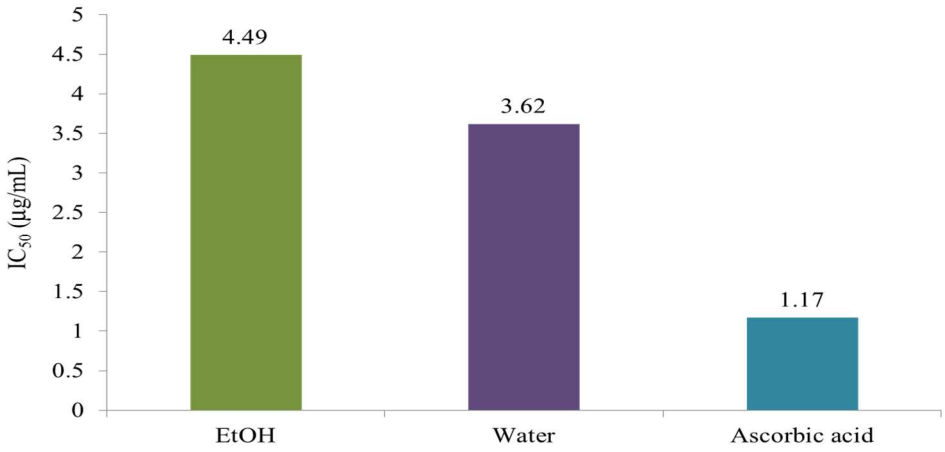

Figure 4 A bar graph of IC_{50} values of 95 % EtOH and aqueous extracts of *M. pruriens* seed in comparison with ascorbic acid

In vitro antimicrobial activity of various crude extracts such as PE, EtOAc, EtOH and H_2O extracts was investigated by employing agar well diffusion method against *Bacillus pumilus, Bacillus subtilis, Candida albicans, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* microorganisms. The inhibition zone diameter (ID) showed the degree of the antimicrobial activity. The larger the inhibition zone diameters, the higher the antimicrobial activity. Inhibition zone diameters of crude extracts of *M. pruriens* seed against six microorganisms are shown in Table 5 and Figure 6.

Table 5 Inhibition Zone Diameters of Crude Extracts of *M. pruriens* seed against Six Microorganisms

Microorganisms	**Types of Microorganisms**	**Inhibition Zone Diameters (mm)**				
		PE	**$CHCl_3$**	**EtOAc**	**EtOH**	**H_2O**
Bacillus pumilus	Gram (+) ve	-	13	13	15	-
Bacillus subtilis	Gram (+) ve	12	13	12	15	-
Candida albicans	Fungi	13	15	13	14	11
Escherichia coli	Gram (–) ve	12	14	13	16	-
Pseudomonas aeruginosa	Gram (–) ve	-	11	13	14	15
Staphylococcus aureus	Gram (+) ve	12	14	13	15	-

Agar well diameter = 10 mm

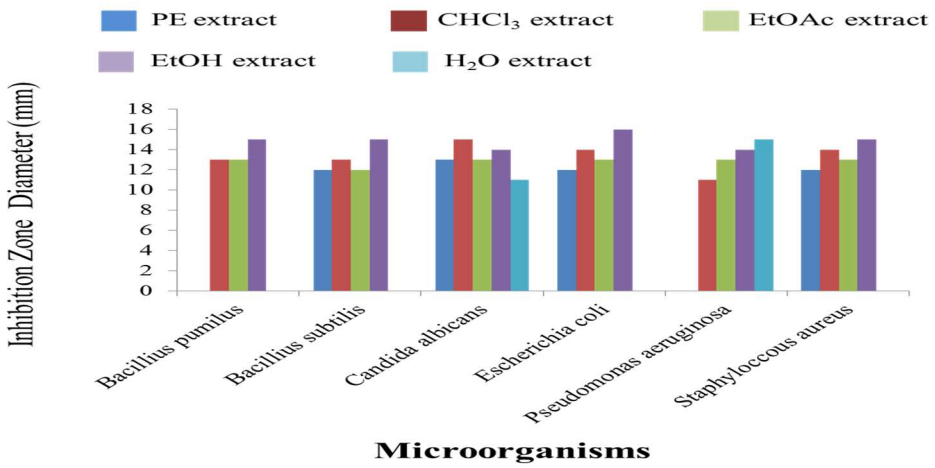

Figure 6 Comparison of inhibition zone diameters for various crude extracts against six microorganisms

Conclusion

From the overall assessment of the present work, the following inferences could be deduced.

(i) The preliminary phytochemical tests on *M. pruriens* (Khwe-leya) seed revealed the presence of α-amino acids, alkaloids, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugars, starch, saponins, steroids, terpenoids and organic acids but the absence of cyanogenic glycosides and tannins.

(ii) From EDXRF spectrum, K, P, S, Ca, Fe, Zn, Cu and Mn were found in the seed of *M. pruriens* (Khwe-leya).

(iii) The nutritional values for *M. pruriens* (Khwe-Leya) seed were found to be 14.39 % of moisture, 1.68 % of crude fat, 8.98 % of dietary fiber, 23.22 % of protein and 3.43 % ash. 48.30 % of carbohydrate was observed to be present in the seed sample and thus *M. pruriens* seed can be utilized as good source of carbohydrate and protein.

(iv) According to the antioxidant activity screening of two crude extracts such as ethanol and aqueous extracts from *M. pruriens* seed using DPPH assay, the order of antioxidant activity was as aqueous extract (IC_{50}= 3.62 μg/mL) > ethanol extract (IC_{50}= 4.49 μg/mL). From these observations, the radical scavenging activity of aqueous extract of *M. pruriens* seed was found to be more effective than that of ethanol extract.

(v) Screening of antimicrobial activity of various crude extracts showed that $CHCl_3$, EtOAc, and EtOH extracts of *M. pruriens* seed responded antimicrobial activity against all tested microorganisms ranging the inhibition zone diameter 11-16 mm. Among these, EtOH extract of *M. pruriens* seed possessed more potent activity, exhibiting the inhibition zone diameters ranging 14-16 mm against all six tested organisms. PE, H_2O extracts of *M. pruriens* seed showed inactive against *Bacillus pumilus.* All extracts except PE extract exhibited inhibition zone within the range between 11-15 mm against *Pseudomonas aeruginosa*. H_2O extract showed activity against *Candida albicans* and *Pseudomonas aeruginosa*.

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