SCREENING OF PHYTOCHEMICAL AND ANTIMICROBIAL ACTIVITY OF *BORASSUS FLABELLIFER* L. ROOTS (PALMYRA PALM)

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Abstract

Borassus flabellifer Linn. is one of the medicinally important plants in family Arecaceae. The study was under taken to investigate the phytochemical and antimicrobial activities of *Borassus flabellifer* L. root. The phytochemical analysis of alkaloids, α-amino acid, carbohydrate, glycosides, phenolic compounds, polyphenol, reducing sugars, saponins, starch, terpenoids, tannins, and steroids were present. Antimicrobial activity was analyzed by agar well diffusion method against six strains of microorganism, namely *Escherichia coli*, *Saccharomyces cerevisia*, *Pseudomonous fluorescens*, *Bacillus pumilus*, *Bacillus subtilis*, and *Candida albicans*. Methanol extract of root (12.95-14.68 mm) were active against all test strains. Ethanol and watery extracts (13.23-15.64 mm) against four test strains. Petroleum ether extract did not show antimicrobial activity. Among the various crude extracts, the ethyl acetate extract showed the maximum zone of inhibition was exhibited in *C. albicans* (22.26 mm) compared to tested microorganisms. Therefore, the ethyl acetate extract of the palm root has showed consistently significant inhibitory activity on different tested organisms.

Keywords *B. flabellifer* roots, Phytochemical and Antimicrobial

Introduction

Borassus flabellifer L. is one of the important horticultural crops in many countries. The palm is the family Palmae, or more recently reclassify in Arecaceae, and sub family Coryphoideae with 1200-4000 species. This plant is widely distributed, and cultivated in tropical Asian countries such as Thailand, Bangladesh, India, Myanmar, Sri Lanka, Malaysia, etc.

The different parts of the *B. flabellifer* L. are being used for medicinal properties, such as the various ailments like secondary syphilis, antiperiodic, heart burns, liver, and spleen enlargement (Sandhya *et al.* 2010).

The germinating shoot of the Palmyra palm is either as food, and used for medicinal purposes. The nutritional analysis of the roots has shown 8.54% protein, 23.53% carbohydrates, and 7.29% crude fiber. These roots are found to be high in calories. They are rich in starch, but poor in fats, and proteins (Schneider and wolfing, 2004).

Phytochemical are naturally occurring chemicals produced by plants. They are biological active, and may affect human health. The preliminary phytochemical screening tests may be useful in the detection of the bioactive principles, and subsequently may lead to the drug discovery, and development. Thus, present study was investigated to the phytochemicals, and antimicrobial activities of *Borassus flabellifer* L. roots, and in order to understand their health benefits.

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Materials and Methods

Botanical Studies

Sample collection and preparation

The root samples of *Borassus flabellifer* L. were collected from the Kyauk-pa-daung Township, Mandalay Region. It was peeled and washed with water, minced into small pieces, and dried in the shade for one week then the dried roots were made powder in electric motor grinder. The powdered samples were stored in airtight containers to prevent moisture changes, and contamination.

Chemical Studies

Phytochemical Investigation of *Borassus flabellifer* L. roots

Phytochemical tests of *Borassus flabellifer* L. roots were carried out, to investigate the presence, and absence of phyto-constituents such as alkaloids, α- amino acids, reducing sugar, carbohydrates, flavonoids, glycosides, phenolic compounds, polyphenols, saponins, starch, terpenoids, tannins, and steroids. Phytochemical tests were done according to the methods of (Trease and Evans 1980, Marini-Bettolo, *et al*., 1981, and M-Tin Wa, 1972).

Preparation of crude Extract

Extraction from dried *Borassus flabellifer* L. roots were done using aqueous, ethanol, methanol, ethyl acetate, and petroleum ether as the extraction solvents. 5 g of the powdered sample with 50 ml of solvent in a conical flask, wrapped with aluminum foil, and kept for one week. The sample was shaken twice a day, and filtered using what-man filter paper No. 32. Then, the obtained filtrates were collected, and concentrated using water-bath until a crude viscous extract was obtained.

Antimicrobial activity

Test extracts: Ethanol, Methanol, Ethyl acetate, Petroleum ether, and water.

Medium used in Antimicrobial Activity Test (Ando, 2004)

	(Assay Medium)
Glucose	0.5 g
Yeast extracts	0.2 g
Agar	1.8 g
Distilled water	100ml

Test Organisms used in Antimicrobial Activities (NITE, 2005)

Sr No.	Test Organisms	Infections
1.	*Escherichia coli*	Cholera, diarrhea and vomiting, urinary tract infections
2.	*Bacillus subtilis*	Fever
3.	*Bacillus pumilus*	Wound and burn infection, eye infection
4.	*Saccharomyces cerevisiae*	Food spoilage and fever
5.	*Pseudomonas fluorescens*	Rice disease
6.	*Candida albicans*	Candidiasis, cardiac infection, skin infection

Test for antimicrobial Activity by Agar Well Diffusion Methods (Collins, 1965)

Antimicrobial activity was carried out at the Department of Botany, University of Magway. Sterized cork borer was used to make the wells (8 mm in diameter) in the autoclaved basal antimicrobial test medium. Screening of antimicrobial activity of various crude extracts,

such as ethanol, methanol, ethyl acetate, petroleum ether, and aqueous extract were using by agar well diffusion method.

Assay medium containing glucose (0.5 g), yeast (0.2 g), agar (1.8 g), and distilled water (100 ml) were placed into the sterilized conical flask, and plugged with cotton wool, and then autoclaved at 121 °C for 15 min. After cooling down to 40 °C, 0.5 ml of suspended strain was inoculated to the assay medium with the help of a sterilized disposable pipette near the burner.

About 20 ml of medium was poured into the sterilized petri-dishes and allowed to set the medium. After solidification, the wells (8 mm diameter) were punched in the agar, and 20 µl of extracts were loaded into the wells. The plates were incubated at room temperature 37°C for 24-48 hours. After 24-48 hours of incubation, the antimicrobial activity was assessed by measuring the diameter of the zone of inhibition, and reported on the scale of millimeter.

Results

Scientific Name - *Borassus flabellifer* Linn
English Name - Palmyra palm
Myanmar Name - Htan
Common Name - Toddy palm, Sugar palm
Family - Arecaceae
Part Used - Fruits, Male Inflorescences, and Roots

Outstanding Characters of *Borassus flabellifer* L.

Palmyra palm is a tall dioeciously tree, and single-stemmed evergreen tree up to 30 m high. The plant contains its crown of leaves at the top position.

The leaves are leathery, gray green, palmately fan-shaped, 1-3 m wide, and folded along the midrib, lanceolate, 0.6 -1.2 m along, petiole edges along with hard horny spine scent serrates.

Inflorescence is a spadix. The flowers are unisexual with male spadix branching, and female spadix simple. Male flowers are minute, mixed with scaly bracteoles, sunk in cavities between large overlapping leaves. Female flowers are solitary, scattered on a sparingly branched spadix.

The coconut like fruits are three sided, rounded or more or less oval, 12-15 cm wide, and capped on the greatly enlarged perianth. When the fruit is young, this kernel is hollow, soft as jelly, and translucent like ice, sweetish, and potable. The flowering and fruiting period is December to June.

Figure-1 Habit

Figure-2 Leaves

Figure-3 Mature staminate inflorescence

Figure-4 Pistillate inflorescence

Figure-5 Fruits

Figure-6 Germinating shoots or roots

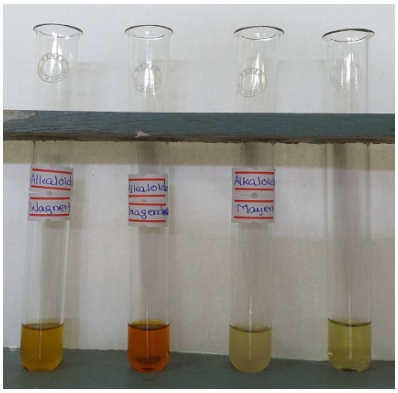
Phytochemical Investigation of Root Sample by Test Tube Methods

The preliminary phytochemical screening of *B. flabellifer* L. roots consisted of alkaloids, α amino acid, carbohydrates, glycosides, phenolic compounds, polyphenols, reducing sugar, saponin, starch, tannins, terpenoids, and steroids. However, flavonoids are not found on it. The results were shown in (Table 1).

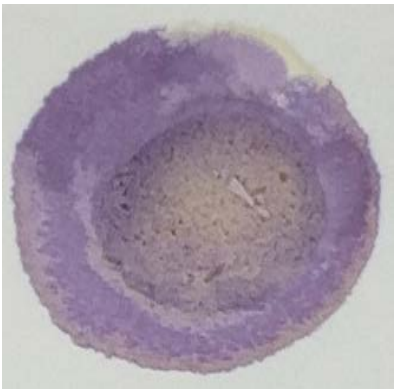
Table 1 Phytochemical constituents of *Borassus flabellifer* L. Root

No.	Tests	Solvent extraction	Test Reagents	Observation	Results
1.	Alkaloids	EtOH	Wagner's reagent	Orange ppt	+
			Dragendorff's reagent	Reddish brown ppt	+
			Mayer's reagent	White ppt	+
2.	α amino acid	H_2O	Ninhydrin reagent	Violet color	+
3.	Carbohydrates	H_2O	10% α-naphthaol & Conc H_2SO_4	Red ring	+
4.	Flavonoids	EtOH	Conc: H_2SO_4, Mg ribbon	-	-
5.	Glycosides	EtOH	10% lead acetate	White ppt	+
6.	Phenolic compounds	H_2O	10% $FeCl_3$	Green	+
7.	Polyphenol	EtOH	1%$FeCl_3$&$K_3Fe(CN)_6$	Greenish blue	+
8.	Reducing Sugar	H_2O	Benedict's solution	Green	+
9.	Saponin	H_2O	Distilled water	Frothing	+
10.	Starch	H_2O	I_2 solution	Deep blue	+
11.	Terpenoids	EtOH	Acetic anhydride, & Conc: H_2SO_4	Reddish brown	+
12.	Tannins	H_2O	10% Lead acetate	White ppt	+
13.	Steroids	H_2O	Acetic anhydride, & Conc: H_2SO_4	Black	+

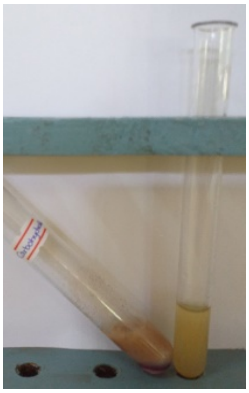
(+) = Presence (-) = Absence (ppt) = Precipitate



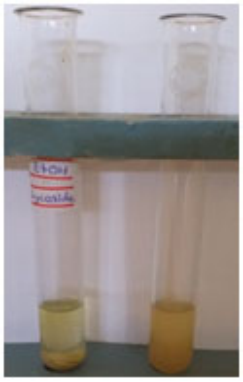
1. Alkaloids



2. α amino acid



3. Carbohydrates



4. Glycosides

5. Phenolic compound

6.Polyphenol

7. Reducing sugar

8. Saponins

9. Starch

10. Terpenoids

11. Tannins

12. Steroids

Figure 7 Phytochemical constituents of *Borassus flabellifer* L. Root

Antimicrobial Activity of Various Crude Extracts of *Borassus flabellifer* L. Root

Screening of antimicrobial activity of root extracts were carried out on different strain of microorganisms by agar well diffusion method. The measureable zone diameter of growth inhibition reflects the degree of antimicrobial activity. The results of the antimicrobial activities were presented in (Table 2).

Table 2 The Antimicrobial Activity of Roots Extract of *Borassus flabellifer* L.

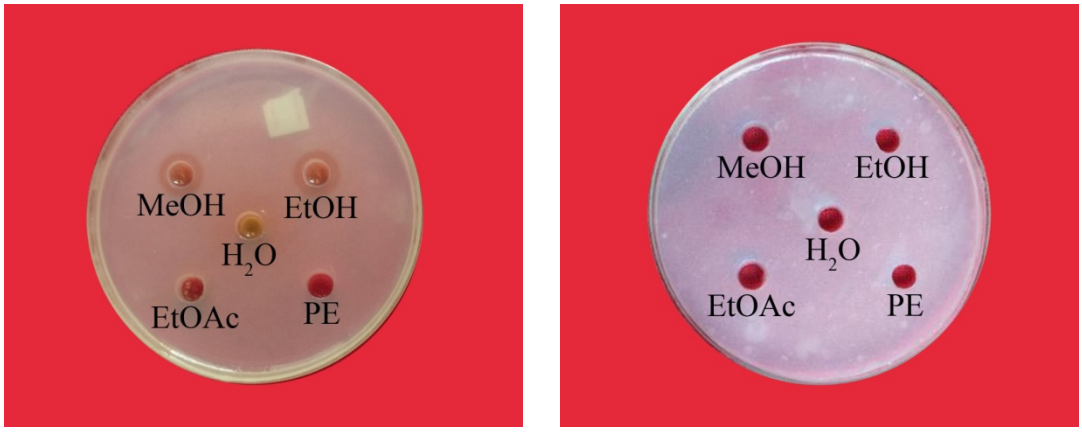
Test microorganisms	Zone of inhibition (mm diameter)				
	Methanol	**Ethanol**	**Water**	**Ethyl acetate**	**Petroleum ether**
Escherichia coli	14.68 (++)	14.75 (++)	14.48 (++)	15.28 (++)	-
Bacillus subtilis	13.12 (+)	14.33 (+)	-	-	-
Bacillus pumilus	14.33 (++)	-	13.37 (+)	-	-
Pseudomonas fluorescens	13.56 (++)	13.24 (+)	14 .28 (+)	-	-
Saccharomyces cerevisiae	12.95 (+)	-	-	-	-
Candida albicans	13.10 (+)	13.23 (+)	15.64 (++)	22 .26 (+++)	-

Agar well - 8mm

10mm - 13mm (+) low activity
14mm - 19mm (++) medium activity
20mm - above (+++) highest activity
(-) No activity

In the present study, the antimicrobial activity of different extracts of *Borassus flabellifer* L. root were tested by agar well diffusion method on six test organisms. Among them, ethyl acetate extract showed the highest activity (22.26 mm) on *Candida albicans* followed by *Escherichia coli* (15.28mm) respectively, and did not show the activity on other four test organisms. The watery extract also showed the medium activity on *Candida albican*s (15.64 mm). Except the petroleum ether extract, all extracts showed the medium activity on *Escherichia coli*. In the methanol extract showed all test organisms (13.10-15.64 mm), and petroleum ether extract did not showed all tested organisms (Table 2 & Fig- 8).

1. *Escherichia coli*



2. *Bacillus subtilis*

3. *Bacillus pumilus*

4. *Pseudomonas flurescens*

5. Sa*cchoromycae cerevisiae*

6. *Candida albicans*

Figure 8 The antimicrobial activity of roots extract of *Borassus flabellifer* L.

Discussion and Conclusion

In this research, the preliminary phytochemical screening of *Borassus flabellifer* L. roots contains alkaloids, α amino acid, carbohydrates, glycosides, phenolic compounds, polyphenols, reducing sugar, saponin, starch, tannins, terpenoids, and steroids. However, flavonoids are not found on it.

In phytochemicals test, alkaloids are one of the largest groups of phytochemicals that have led to the invention of powerful pain killer medications. Also, alkaloids are the most efficient therapeutically significant plant substance (Kam and Liew, 2002).Glycosides are used as antibiotic, anticancer, anti-diabetic, purgative treatment of congestive heart failure, and cardiac arrhythmia.

Tannins are also for antiviral, antimicrobial, and antitumor properties. Steroids are known to be an important cardio tonic activities possess antimicrobial property, and also used in herbal medicines, and cosmetics. Saponins are also important therapeutically as they are shown to have anticancer activity. It is also necessary for activity of cardiac glycosides (Doughari, 2012).

In this research, the screening of antimicrobial activity of various crude extracts such as petroleum ether, methanol, ethanol, ethyl acetate, and water were investigated by employing agar well diffusion method against six species of microorganisms such as *E.coli*, *B. subtilis*, *B. pumalis*, *P. fluroscens*, *S. cerevisia*, and *C. albicans*. The larger the diameter of clear zone the more potent the microbial activity.

In the present study, the Palmyra palm root extracts have showed different degrees of antimicrobial activity ranging from (13.12 mm to 22.26 mm) against studied microorganisms. Ethyl acetate and watery extracts exhibited maximum zone of inhibition against *C. albicans* (22.26 mm & 15.64 mm) respectively. Screening of antimicrobial for inviting us to present research paper activities of these various crude extracts, the ethyl acetate extracts of *Borassus flabellifer* L. root exhibited significant antimicrobial activity.

From this study, it was concluded that *B. flabellifer* L. roots contained many biological active phytochemical and antimicrobial activity. It is recommended that further investigation on vitamins, amino-acids, isolation of active compounds, and efficiencies of this plant are needed for novel herbal medicine.

Acknowledgements

I would like to thank all members of the Myanmar Academy of Art and Science (MAAS) for inviting us to present research paper. I would like to express their profound gratitude to the Department of Higher Education, Ministry of Education in Myanmar, for provision of opportunity to do this research. I would like to express our deepest thanks to Rector Dr. Khin Maung Oo, University of Magway, for allowing to do research paper. I am also grateful thanks to Dr. Sanda Myint, Professor and Head, University of Magway, for her kind permission to use the research facility for the research work.

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