

A Study on the Antimicrobial Activities of *Ziziphus mauritiana* Lamk. Seeds Kernels

Htay Htay Aung¹

Abstract

Ziziphus mauritiana Lamk. is belonging to the family Rhamnaceae. It is one of the edible wild plants and some are cultivated in central Myanmar. The seeds kernels (cotyledons) of *Ziziphus mauritiana* Lamk. are used in traditional medicine and exported to other countries for medicine. In this research, the kernels of Zee thi were purchased from local market, Nyaung Oo Township, Mandalay Region, Myanmar. The powdered of the kernels of Zee thi were also used to preliminary phytochemical test and antimicrobial activities. The presence of alkaloids, glycosides, phenolic compounds, flavonoids, steroids, terpenoids, tannins, saponins, α -amino acid, proteins, reducing sugars, starch and carbohydrates were found in the examination. The acetone, chloroform, ethyl acetate, ethanol, methanol, pet-ether and distilled water extracts from the kernels of Zee thi were tested with six different types of microorganisms and their activities were recorded. The ethyl acetate extracts of the kernels of Zee thi responds high activities on different pathogenic microorganisms. These results showed that the kernels of Zee thi are endowed with many active chemical constituents and antimicrobial activities that can be found in medicinal uses.

Key words: phytochemical constituents, antimicrobial activities

Introduction

Plants are part of life. Without them nobody can live. When a plant has antibiotic properties, it is called medicinal plants. About 80% of the world population use traditional medicines, which are predominantly based on plant materials (WHO; 1993).

Ziziphus mauritiana Lamk., is commonly known as Indian Jujube in English and Zee thi in Myanmar. It is a most common fruit tree found in rural area of Central Myanmar around, Mandalay, Magway and Sagaing Region. *Ziziphus mauritiana* Lamk. is a spiny, evergreen shrub or small tree up to 15 m high, with trunk 40 cm or more in diameter; spreading crown; stipular spines and many drooping branches. Fruits are eaten fresh, dried and made into confestionery or drinks can be made from the juice in Myanmar. Moreover, the seeds kernels (cotyledons) of *Ziziphus mauritiana* Lamk. are used in traditional medicine and export to other countries.

In literature, many studies reported that *Ziziphus mauritiana* Lamk. have some medical benefits such as antioxidant, anti-microbial, anti-diarrheal, anti-diabetic, hepato-protective and anti-cancer. Phytochemical screening of the extract revealed the presence of triterpenoids, reducing sugars, sugars, alkaloids, phenolics, catechins, flavonoids, saponins, tannins and amino acids. (Website-1).

Plant extracts have therapeutic effect with or without chemical modification for various infectious diseases cause by bacterial activities and other disease-causing organisms. Plants used for the treatment of diseases is as old as mankind, medicinal plants are an important source of potentially useful structures for the development of new chemotherapeutic agents(Website-2).

In this paper the physicochemical analysis, phytochemical constituents and antimicrobial activity of the seeds kernels (cotyledons) of *Ziziphus mauritiana* Lamk. were studies. So, this study is to contribute the knowledge on the medicinal uses plants and to give the better health care for the people.

¹ Dr, Lecturer, Department of Botany, Yenanchaung Degree College

Materials and Methods

The specimens were collected from Bagan Nyaung Oo Townships during Flowering and Fruiting period from September to November. Fresh specimens of the vegetative and reproductive parts were used for identification in the Department of Botany, Yenanchaung Degree College with the help of literatures of key to the family (Hooker, 1885; Backer, 1965; Dassanayake, 1987; Hundley and chit Ko Ko, 1987 and Kress *et al*, 2003).

The dried fruits together with the seeds are pounded using pestle and motor, and are separated to the kernels. The seeds kernels (cotyledons) of *Ziziphus mauritiana* Lamk. were dried in room temperature for two weeks. When it is complete, dried samples were pulverized for powder by grinding machine. And then, physicochemical test, phytochemical investigation and antimicrobial activities were conducted at the Department of Botany, Yangon university.



Figure. 1. Dry fruits of *Ziziphus mauritiana* Lamk.



Figure. 2. Seed-coat (hull) of *Ziziphus mauritiana* Lamk.

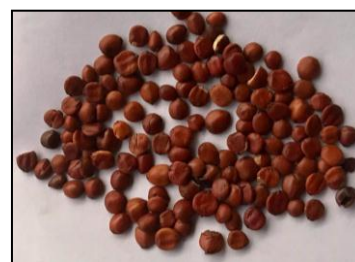


Figure. 3. Seeds kernels of *Ziziphus mauritiana* Lamk.

Physicochemical Test

The physicochemical properties of the seeds kernels (cotyledons) of *Ziziphus mauritiana* Lamk. were determined according to “The British Pharmacopoeia” (1968) as follows:

Petroleum ether soluble matter

Fifty gm of powdered were soaked with 300 ml of pet-ether in a closed flask for 72 hrs and kept over three nights. The mixture was filtered rapidly taking precautions against loss of alcohol and the filtrate was evaporated in a weighed petridish on a boiling water bath, until it was completely dried. The evaporated residue together with the petridish was weighed. The procedure was repeated until a constant weight was obtained. The difference in weights gave the pet-ether soluble extractive value. The above procedure was repeated with 300 ml of chloroform, ethyl acetate, acetone, methanol, ethanol, distilled water soluble matter instead of pet-ether. Then the respective soluble extractive values were calculated.

Preliminary Phytochemical Investigation

For preliminary phytochemical investigation, the air-dried powders of the seeds kernels were used. Tests for alkaloids, glycosides, phenolic compounds, flavonoids, steroids, terpenoids, tannins, saponins, α -amino acid, proteins, reducing sugars, starch and carbohydrates were investigated by using various solvents. These results were carried out according to the British Pharmacopoeia, 1968; Marrini Bettalo *et al.*, 1981; Central Council for Research in Unani Medicine, 1987 and Trease and Evans, 2002.

Antimicrobial Activity Test

The different solvent extracts were tested against six pathogenic microorganisms by using paper disc diffusion method. The antimicrobial activity of the extracts was measured for the diameter zone of inhibition by using paper disc diffusion method (Cruickshank, 1975) at Central Research and Development Center (CRDC).

Paper Disc Diffusion Assay

Isolated bacterial strains grown on nutrient agar were inoculated into 50 ml conical flasks containing 10 ml of sterile growth medium. Then, they were incubated at 30°C for 72 hours on a reciprocal shaker at 200 rpm.

Test organisms were *Aspergillus flavous*, *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Pseudomonas fluorescens* and *Xanthomonas oryzae*. 0.3 ml of test organisms was added to assay medium, then poured into plates. After solidification, paper discs impregnated with broth samples were applied on the test plates and these plates were incubated for 24-36 hours at 30°C. After for 24-36 hours, clear zones (inhibitory zones) surrounding the test discs indicate the presence of bioactive compounds which inhibit the growth of test organisms.

Assay medium (SY) for test organisms

Agar	2.0 g
Sucrose	1.0 g
Yeast extract	0.3 g
NaCl	0.1 g
Distilled water	100 ml
pH	7.0

Results

Morphological characters

Scientific name	-	<i>Ziziphus mauritiana</i> Lamk.
Family name	-	Rhamnaceae
English name	-	Indian jujube
Local name	-	Zee thi
Flowering period	-	September to November
Part uses	-	Fruits, leaves, stem, bark, seed

Medium-sized tree, bushy shrub to tree, ever green or semi-deciduous, erect or wide-spreading, drooping thorny branches, zigzag branchlets; the bark deep, longitudinal furrows, greyish brown. Leaves, alternate, ovate or oblong elliptic with rounded apex, with 3 depressed longitudinal veins at the base; petiolate, long; spinous stipules, paired, one curved and one straight or both curved. Inflorescences fasciculate cyme shortly pedunculate. Flower, tiny, bisexual, actinomorphic, yellowish white, faintly fragrant. Sepal 5, free acute, pubescent. Petal 5, free. Stamens 5, free, alternate to the petals; filament filiform, anther dithecous, basifixed. Ovary semisuperior, bilocular with 2 ovules in each locule on axile placentae; style terminal; stigma 2-cleft, disk fleshy 10 ribbed. Fruit drupe, glabrous, globoid to ovoid, greenish, yellowish or reddish. Seed pyrene, kernels brown (Figure 4).



Habit



Zigzag branchlet



Inflorescence



Flower

Figure. 4. Morphological characters of *Ziziphus mauritiana* Lamk.

Physicochemical properties of the seeds kernels of *Ziziphus mauritiana* Lamk.

The different extractive value of the various solvents in the seeds kernels (cotyledons) of *Ziziphus mauritiana* Lamk. were quantitatively presented in Table 1. Acetone (20%) and ethyl acetate (25%) and ethanol (20%) were more significantly soluble than other solvents pet-ether, chloroform, methanol and distilled water in seeds kernels.

Table 1. Physicochemical Properties of the seeds kernels

No	Physicochemical characters	Quality (%)
		Seeds Kernels
1	pet-ether	10
2	CHCL ₃	15
3	MeoH	5
4	Acetone	20
5	EtoAc	25
6	EtoH	20
7	H ₂ O (D.W)	5

Preliminary Phytochemical Investigation of the kernels of *Ziziphus mauritiana* Lamk.

The results of these tests confirmed the seeds kernels of *Ziziphus mauritiana* Lamk. showed the presence of alkaloids, glycosides, phenolic compounds, flavonoids, steroids, terpenoids, tannins, saponins, α -amino acid, proteins, reducing sugars, starch and carbohydrates. The results were shown in Figures (5) and Table (2)



Figure. 5. Preliminary Phytochemical tests of *Ziziphus mauritiana* Lamk. Seeds Kernels

Table (2) Preliminary Phytochemical Investigation of the kernels

No.	Tests	Extracts	Test Reagents	Observations	Results
1	Alkaloids	Methanol	1% HCl+Mayer's reagent	White ppt.	+
		Methanol	1% HCl+Wagner's reagent	Brown or reddish brown ppt.	+
		Methanol	1% HCl+Hager's reagent	Yellow ppt.	+
2	Glycosides	Methanol	1ml H ₂ O+NaOH	Yellow green color	+
3	Phenolic compounds	Methanol	2ml H ₂ O+10% FeCl ₃	Blackish/green color	+
4	Flavonoids	Methanol	Mg coil + HCl (dil)	Pink/reddish brown color	+
5	Steroids/ Terpenoids	Methanol	CHCl ₃ + H ₂ SO ₄ (conc.)	Green/reddish brown color	+
6	Tannins	water	5% FeCl ₃ + H ₂ SO ₄ (dil)	Yellow brown ppt./reddish brown ppt.	+
7	Saponin	water	Shaken with 2ml H ₂ O	Foaming	+
8	α Amino acids	water	Ninhydrin reagent	Pink spot	+
9	Proteins	water	Million's reagent (heated)	White ppt. turned red when heated	+
10	Reducing sugar	water	1 ml H ₂ O and mixture equal part fehling's A and B	Brick red ppt.	+
11	Starch	water	Iodine	Blue black/brown ppt.	+
12	Carbohydrates	water	1 ml benedict's reagent and boil for few minute	Brick red ppt./red ppt.	+

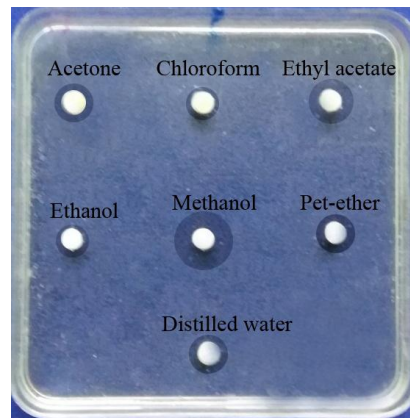
(+) present (-) absent

Antimicrobial Activities on the seeds kernels of *Ziziphus mauritiana* Lamk.

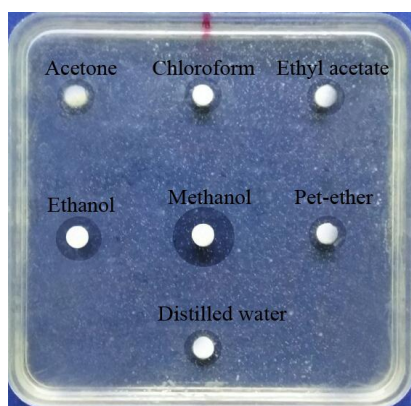
Six microorganisms namely *Aspergillus flavous*, *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Pseudomonas fluorescens* and *Xanthomonas oryzae* were subjected to test microbial activity. In this experiment, the extractives using acetone, chloroform, ethyl acetate, ethanol, methanol, pet-ether and distilled water exhibits showed effectiveness. Among then, the ethyl acetate extracts of the kernels of Zee thi responds high activities on different pathogenic microorganisms as shown in Figures 6 and Table 3.



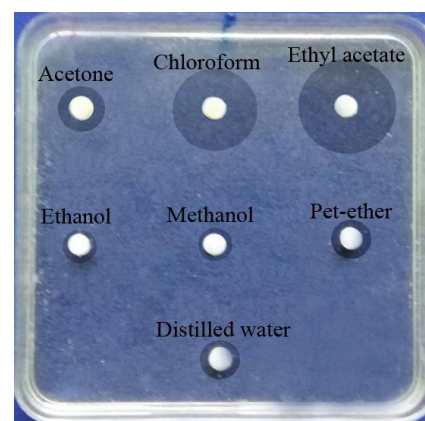
a. *Aspergillus flavous*



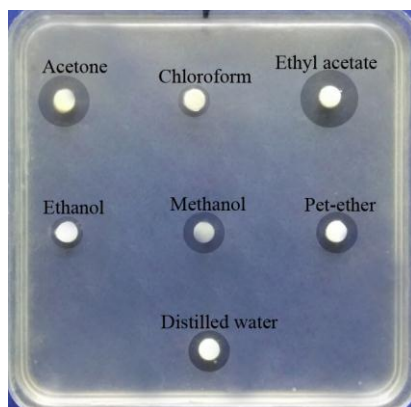
b. *Bacillus subtilis*



c. *Candida albicans*



d. *Escherichia coli*



e. *Pseudomonas fluorescens*



f. *Xanthomonas oryzae*

Figure 6. Antimicrobial activities of *Ziziphus muritiana* Lamk. Seeds kernels

Table 3. Antimicrobial activities of the kernels of *Ziziphus mauritiana* Lamk.

No.	Test Organisms	1	2	3	4	5	6	7
a	<i>Aspergillus flavous</i>	14 mm	14 mm	16 mm	12 mm	10 mm	8 mm	8 mm
b	<i>Bacillus subtilis</i>	10 mm	8 mm	12 mm	8 mm	14 mm	10 mm	10 mm
c	<i>Candida albicans</i>	8 mm	8 mm	8 mm	10 mm	14 mm	8 mm	8 mm
d	<i>Escherichia coli</i>	10 mm	14 mm	16 mm	8 mm	8 mm	8 mm	8 mm
e	<i>Pseudomonas fluorescens</i>	12 mm	8 mm	14 mm	8 mm	10 mm	10 mm	10 mm
f	<i>Xanthomonas oryzae</i>	10 mm	8 mm	14 mm	12 mm	8 mm	8 mm	10 mm

Paper disc size = 6 mm

Solvent Extracts

1. Acetone
2. Chloroform
3. Ethyl acetate
4. Ethanol
5. Methanol
6. Pet-ether
7. Distilled water

Discussion and Conclusion

In this research paper, morphological characters, physicochemical properties and antimicrobial activities of the seeds kernels of *Ziziphus mauritiana* Lamk. were carried out. The fruits of *Ziziphus mauritiana* Lamk. (Indian jujube) are one of the best edible wild plants and some are cultivated. It provides food, drink, fuel and wood as a source of income from its products. The health-promoting properties of Indian jujubes are increasingly being recognized.

The pericarp of Indian jujube is consumed either fresh or dried while its seeds are usually discarded as waste. The dried fruits together with the seeds are pounded using pestle and mortar, and are separated to the kernels and the seed-coat (hull) can be used for fuel, but the kernels in the hard endocarp are very useful for traditional medicine and it is one of the export products.

In physicochemical properties, the seeds kernels of *Ziziphus mauritiana* Lamk. showed that acetone and ethyl acetate and ethanol were more significantly soluble than other solvents pet-ether, chloroform, methanol and distilled water in seeds kernels.

The phytochemical constituents, alkaloids, glycosides, phenolic compounds, flavonoids, steroids, terpenoids, tannins, saponins, α -amino acid, proteins, reducing sugars, starch and carbohydrates were found in the examination.

In the antimicrobial activities of various seven extracts (acetone, chloroform, ethyl acetate, ethanol, methanol, pet-ether and distilled water) were carried by using paper disc diffusion assay with test six organisms (*Aspergillus flavous*, *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Pseudomonas fluorescens* and *Xanthomonas oryzae*). Ethyl acetate extracts exhibited greater activity against *Aspergillus flavous*, *Escherichia coli*, *Pseudomonas fluorescens* and *Xanthomonas oryzae* (16mm and 14mm). Overall study noticeably illustrates *Aspergillus flavous*

with relatively higher susceptibility against all the extracts and lowest *Candida albicans* as presented in Table 3.

Ziziphus mauritiana Lamk. endocarp extractions indicated the presence of triterpenoids, reducing sugars, sugars, alkaloids, phenolics, catachins, flavonoids, saponins, tannins and amino acids. They found high amount of fructose, glucose, potassium, phosphorus, calcium manganese and vitamin C including soluble and insoluble dietary fiber (Website-1).

Ziziphus mauritiana Lamk. seeds kernels possesses terrific medicinal. Phytochemically alkaloids, glycosides and flavonoids, terpenoids and others are present in the kernels. Each ingredient has unique and multifactorial properties and the importance of such constituents in health products. In this research it becomes more understandable about the medicinal values of the seeds kernels of *Ziziphus mauritiana* Lamk.. It is suggested that the seeds kernels of *Ziziphus mauritiana* Lamk. are very valuable for the people and medicinal uses in Myanmar. For the future research, the bioactivity of this plant for antioxidant activity, antifungal activity and anticancer activity should be investigated.

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