HISTOLOGICAL CHARACTERS, NUTRITIONAL VALUES OF FRUITS FROM *ZANTHOXYLUM ARMATUM* DC. AND ITS ANTIMICROBIAL ACTIVITY

Yin Yin Khaing[1], Myat Myat Moe[2]

Abstract

Zanthoxylum armatum DC. is an aromatic medicinal plant belonging to the family Rutaceae has been undertaken. They were collected from Loikaw Township, Kayah State during from September to November 2019. The collected plants were identified, classified, and studied by the literature. The morphological characters of vegetative and reproductive parts and microscopical characters of the leaves and fruits were conducted to ascertain their correct identification. The plant was small tree with thorny branches. Leaves were distinctly trifoliate with wing leaf-stalk. Inflorescence were terminal, axillary and bracts presented. Petals were absent. For microscopical studies, lamina, midrib, petiole and fruits were examined by preparing free hand sections. The upper surface of the lamina was polygonal in shaped and lower surface was wavy. Stomata present only on lower surface were numerous and anomocytic type. In transverse section of lamina, calcium oxalate crystals were presented in palisade layer. The preliminary phytochemical tests were examined from the powdered fruits. Alkaloid, carbohydrate, flavonoid, glycoside and phenolic compound, saponin and tannin were present in this examination. The nutrient contents of powdered fruits were analyzed protein, fat, fiber and carbohydrate by using David Pearson and Kieldaha method. Antimicrobial activities of various crude extracts of fruits were carried out by using paper disc diffusion method with six test organisms. Acetone and ethyl acetate extracts indicated more effective against on Candida albicans than other tests organisms. Medicinal plants contained numerous biologically active compounds which are used for helpful in improving the human health.

Kewwords : *Zanthoxylum armatum*, thorny, anomocytic, *Candida albicans*

Introduction

Nature has been a source of medicinal agent for thousands of years. Herbal medicine represents one of the most important fields of traditional medicine all over the world. Different extracts from traditional medicinal plants have been tested to identify the source of therapeutic outcome. Developing countries still depend mainly on medicinal herbs due to their cheaper cost and their intervention in the treatment of various infectious diseases with lesser side effects (Butkhup *et al*, 2011).

Medicinal plants, which form the backbone of traditional medicine, have in the last few decades been the subject for very intense pharmacological studies; this has been brought about by the acknowledgment of the value of medicinal plants as potential sources of new compounds of therapeutic value and as sources of lead compounds in drug development. In developing countries, it is estimated that about 80% of the population rely on traditional medicine for their primary health care (Taylor and Attaur, 1994).

Zanthoxylum armatum DC. is an aromatic tree or shrub with winged petiolate prickly imparipinnate compound leaves. Flowers are small and polygamous. Male flowers have 6 to 8 stamens with rudimentary ovary. Female flowers have 1-2 carpels. Fruit is drupe, splitting into two when ripe. Seeds are rounded and shining black. The plant grows in shady or semi shady habitat at altitude from about 800m up to 1500m. In many tropical countries, rural people traditionally harvest of leafy vegetables and fruits from wild because of its taste, cultural uses, as food supplements or to tide over food shortage (Kebu and Fassil, 2006).

[1] Lecturer, Department of Botany, Dagon University
[2] Professor and Head, Department of Botany, Dagon University

A family of about 140 genera and 1200 species, widely distributed in temperate and tropical regions of northern and southern hemispheres but most numerous in South Africa and Australia. The Rutaceae are distinguished by the presence of translucent pellucid dots in the foliage, the lobed ovary evaluated on a disc, and the outer stamens usually opposite the petals (Lawrance, 1964). The fruit of several species is used to make the spice Sichuan pepper.

Zanthoxylum armatum DC is small tree mostly with a strong pungent and aromatic smell. The seeds and barks are used in fever, dyspepsia and cholera. The fruits and thorn are used as remedy for toothache, also as stomachic and carminative. Various parts of *Zanthoxylum armatum* DC. are used in the preparation of tooth powder and medicinal purposes. It is an evergreen shrub or small tree with stem and branches, sharp prickles. It's also found throughout China, Japan, Pakistan, Nepal, and Malaysia. Its seeds and barks are used for the treatment of various diseases such as fever, cholera, heartburn or indigestion. Fruits and seeds of this plant are used in fever, dyspepsia and skin diseases. Leaves and barks were reported for hepatoprotective activity, anticancer and antidiabetic activity The nutritional values and mineral contents of these fruits were richer than that of the commercial fruits and could be used for nutritional purpose (http://florajournal.com).

A family consisting mainly of trees or shrubs and a very small proportion of herbs all of which flourishing in the tropics and sub-tropics. The ground tissues of both leaf and axis is nearly always characterized by the presence of secretory cavities appear to the naked eyes as transparent dots in the leaf. The usually abundant crystals of calcium oxalate may be either solitary or clustered or a mixture of both of these types (Melcheft & Chalk, 1950).

Bioactive constituents such as alkaloids, sterol, saponins, tannins, phenols and flavonoids were quantitatively evaluated in leaves, barks and fruits of *Zanthoxylum armatum* DC. The results bring out the presence of bioactive constituents in the fruits contained alkaloids, sterols, saponins, phenol and flavonoids (http://academicjournals.org).

Immature shoots are used as toothbrush and useful for curing gum diseases. Its fruits and seeds are edible and used as potherb species. Fruit is utilized for toothache, dyspepsia, as carminative and for stomachache. The seeds are applied as condiment and seasoning agent (Arshad and Ahmad, 2004).

Zanthoxylum armatum DC. (Rutaceace), also known as toothache tree residents an important the history of Indian system of medicines. It is used as carminative, stomachic and anthelmintic and in the intervention of toothache. It contains volatile oil with active constituents such as limonene and lagan. Its fruits branches and thorns are in general used as carminative, stomachic, and remedy for toothache. In India, different parts of the plants are used in Ayurvedic practices for the intervention of skin diseases, abdominal pain, anorexia, and ataxia. This review focuses on the detailed phytochemical composition and medicinal uses along with pharmacological activity of different parts (nopr.niscair.res.in/handle).

Aspergillus flavus is found through the world as saprophyte in soils and causes disease on many important agriculture crops. In mammals, pathogen can cause liver cancer through consumption of contaminated feed through invasive growth.

Bacillus species are bacteria used as a soil inoculate in agriculture and horticulture. *Candida albicans* is a genus of yeast that can cause fungal infection in human and other animals (Cruickshank *etal*.,1968).

Escherichia coli are a gram-negative bacteria and motile by peri trichous flagella or non-motile. The genus of *E. coli* occurs in the human intestinal tracts (Ronald, 1988).

Pseudomonas fluorescens (an antibiotic) can be produced, which has been found to be utile in treating of skin, ear, and eye disorders. *Pseudomonas fluorescens* is a common Gram-negative, rod-shaped bacterium.

Xanthomonas oryzae are slow-growing, mucoid and straw-colored to yellow in colour and those are fairly slow-growing, usually pale-yellow, round,

smooth, domed and mucoid. Both bacteria are Gram-negative rods, capsulated and motile with a polar flagellum. The bacterium gets in the leaf through stomata or wounds. Spread within a crop occurs by mechanical contact and in rain and irrigation water; under favorable warm wet conditions, rapid and terrible disease development can occur (Reddy,1984).

In this paper, morphological and microscopial characters of fresh specimens, preliminary phytochemcial tests, nutritional values and antimicrobial activity of the dried powdered fruits of *Zanthoxylum armatum* DC., were carried out. The aim of the present was studied to identify the plant of *Zanthoxylum armatum* DC., to examine the microscopical characters of leaves and fruits, nutritional values and to study the phytochemical analysis and antimicrobial activities of the fruits.

Materials and Methods

Botanical Studies

The specimens of *Zanthoxylum armatum* DC. were collected from Loikaw Township, Kayah State during from June to November 2019. After collection, the specimens were identified with the help of available literatures Hooker (1885), Kirtikar and Basu (1935), Dutta (1979), Dassanayake (1999). Both the vegetative and reproductive parts of the fresh specimens were used for the morphological and microscopical characters studies.

The samples were washed and dried at room temperature and then crushed into powder to study the powdered characteristic. For microscopical studies, leaves and fruits were examined by preparing free hand sections from the fresh specimens, according to the methods by Esau (1965), Metcalfe and Chalk (1950), Trease and Evans (1978), Pandey (2004) and Tandon (2011). Chloral hydrate solution was used as a clearing reagent. The presence of calcium oxalate crystals was tested by 80% sulphuric acid.

Chemical Studies

Preliminary Phytochemical Examination

Preliminary phytochemical study the fruits of *Zanthoxylum armatum* DC. has been conducted with test reagent in Department of Botany, Dagon University. The experiment was carried out to determine the presence or absence of alkaloids, amino acid, carbohydrate, flavonoid, glycoside, phenolic compound, reducing sugar, saponin, starch and tannin according to the methods described by using Vogel (1956), Marini-Bettalo (1981).

Nutritional values of Powdered fruits of *Zanthoxylum armatum* DC.

Nutritional values

The experimental process for the nutritional contents in powdered fruits of *Zanthoxylum armatum* DC. was carried out at the Small Scale Industries Department, Ministry of Agriculture, Livestock and Irrigiation, North Okkalapa Township, Yangon Region. According to the experiments carbohydrate, protein, fiber, fat and moisture were analyzed by using the method of David Pearson (1976) and Kjeldahl (1883).

Antimicrobial Activities

Microorganisms were divided into four main types, bacteria, fungi, virus and protozoa. The most microorganisms were free living and performed useful activity that benefits animals and plants life. Bacteria are pathogenic microorganisms with a relatively simple and primitive form of cellular organization and are smaller than those of protozoa and fungi (Cruickshank, 1968).

Various crude extracts of powdered fruits such as acetone, ethyl acetate, ethanol, methanol, pet-ether and distilled water extracts were used for antimicrobial activities. Screening of antimicrobial activity was done by paper disc diffusion assay according to Madigan and Martinko, 2005 at microbiology lab Department of Botany, University of Yangon. The six test organisms were utilized for antimicrobial activity. The assay medium (agar 2.0 g, sucrose 1.0g, pH 7.0) was utilized for these test organisms. Test organisms (0.3ml) was added to 1000 ml assay medium, then poured into plates. After solidification, about 0.2 ml of crude extract was impregnated on to the paper disc with the size of 6mm diameter on the test agar plate and these plates were incubated for 24-36 hours at 30C. After 24-36 hours, clear zones surrounding the test discs were measured.

Table 1 Type of microorganisms, their respective code numbers and diseases

No.	Test organisms	Source	Diseases
1.	*Aspergillus flavus*	-	Bronchitis
2.	*Bacillus subtilis*	JAP-0225215	Pathogenic group, anthrax in animals
3.	*Candida albicans*	IFO-1060	Skin infection, veginal candidiasis alimentart tract infection
4.	*Escherichia coli*	ACTT-25922	Cholera, diarrhea and vomiting urinary tract infection
5.	*Pseudomonas fluorescens*	-	Bacteria for leaf blight
6.	*Xanthomonas oryzae*	-	Bacteria for leaf blight

Results

Botanical Studies

Morphological characters of *Zanthoxylum armatum* DC.

Scientific name : *Zanthoxylum armatum DC.*
Myanmar name : Mak-kha
English name : Winged Prickly Ash
Family : Rutiaceae
Flowering and fruiting period : August- December
Distribution : Wildly grows in cold region Myanmar.
Part used : Fruits

Tree; stems woody with thorny branches. Leaves were distinctly trifoliate with the wing leaf-stalk. Leaflets were stalk less. Inflorescence racemose. Petals were absent

Microscopical Characters of leaves of *Zanthoxylum armatum* DC.

Lamina

In surface view, epidermal cells of both surfaces were parenchymatous cells, thin-walled and compactly arranged. Anticlinal walls of the lower surface were wavier than the upper ones. Stomata were absent on upper surface and abundant in lower surface. They were anomocytic type.

Stomata were elliptic in shape with very small pores; guard cells were reniform shape with chloroplast.

In transverse section, the lamina was dorsiventral and smooth cuticle present on both surfaces. The upper epidermal cell was one layer on both sides, rectangular in shape and the lower epidermal cells were barrel shaped. The mesophyll composed of palisade and spongy parenchyma. The palisade mesophyll was made up of two layers of vertically elongated cylindrical cells, which were closely packed with one another compactly arranged. The spongy mesophyll consisted of 3-4 layers of cells, irregular to isodiametric shape and loosely arranged. Calcium oxalated crystals were presented in palisade layer.

Midrib

In surface view, the epidermal cells were parenchymatous and compactly arranged and rectangular in shaped. In transverse section, convex at both sides covered with thin cuticle. Both epidermal cells were rounded shaped. The lower epidermal cells were similar to those the upper epidermal cells. Below the epidermis, the cortex was differentiated into collenchyma and thin-walled parenchyma cells. The angular types of collenchymatous cells were 1-2 layers in thickness towards the upper surface and 2-3 layers in thickness towards the lower surface. The parenchyma cells were 3 to 4 layers in thickness above the vascular bundle and 5 to 6 layers in thickness below the vascular bundle. They were thin- walled and irregularly rounded or oval in shape. Intercellular spaces were numerous druses crystals of calcium oxalate were present in both parenchymatous cells. The vascular bundle was rounded or oval in outline, collateral and closed type.

Petiole

In surface view, the epidermal cells were parenchymatous, thin-walled and mostly rectangular in shape and elongated along the length of the petiole. In transverse section, the petiole was oval in outline. The cuticle layer was thick. The epidermal cells were rounded- shaped. Hairs consist of thick or thin-walled multicellular of trichomes were present. The cortex was made up of collenchymatous and parenchymatous tissues. The collenchymatous type tissues consisted of 4 to 5 layers in thickness. The parenchymatous tissues composed of 8 to 9 layers in thickness. The parenchyma cells were oval to isodiametric in shape. Intercellular spaces were numerous among the tissue. Druses of calcium oxalate crystals were present in the cells. The vascular bundles were more or less rounded in outline and embedded in the parenchymatous tissues. Vascular bundles were arranged in shaped, collateral and surrounded by a bundle sheath. The xylem was present in the abaxial side and the phloem was present in the adaxial sides.

Microscopical characters of fruits of *Zanthoxylum armatum* DC.

Fruits

In surface view, epidermal cells of fruit surface were parenchymatous cell. In transverse section, covering with cuticle was presented. The fruit wall showed three well differentiated layers. The epicarp composed of one layer of selerenchymatous cells. Epidermal cells were compactly elongated. The mesocarp was made up of parenchyma tissue about 5 to 6 layers. Oil cavity were presented in mesocarp. The endocarp was found below the mesocarp and consists of 4 to 5 layers of parenchymatous cells.

Figure 1 Habit

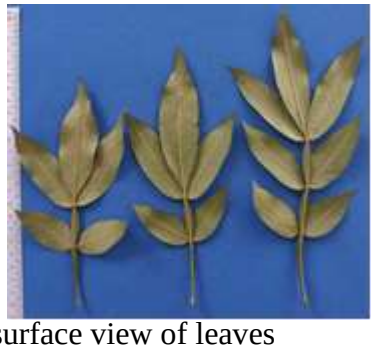


Figure 2 Upper and lower surface view of leaves

Figure 3 Inflorescence

Figure 4 Flowers

Figure 5 L.S of Flower

Figure 6 T.S of Ovary

Figure 7 Fruits

Figure 8 Seeds

Morphological characters of *Zanthoxylum armatum* DC

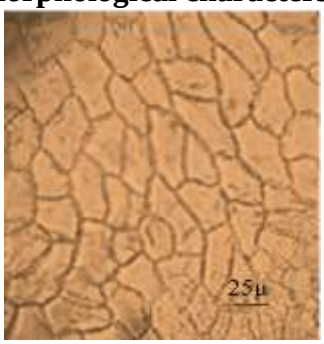


Figure 9 Upper surface view of lamina

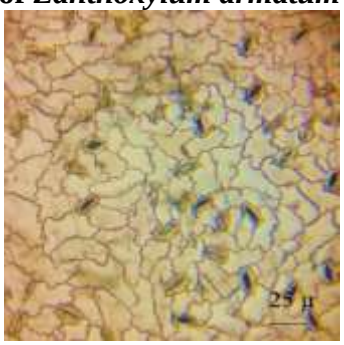


Figure 10 Lower surface view of lamina

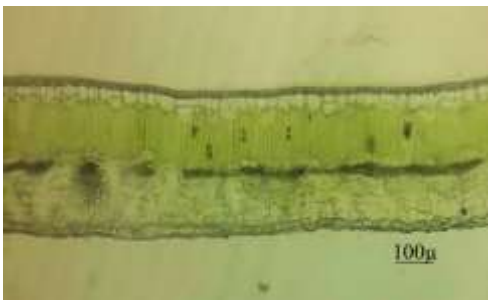


Figure 11 T.S of lamina

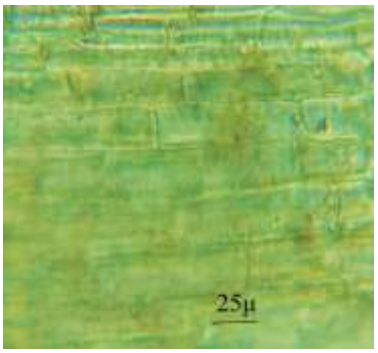


Figure 12 Surface view of midrib

Figure 13T.S of midrib

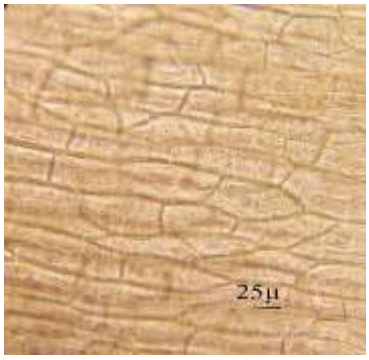


Figure 14 Surface view of petiole

Figure 15 T.S of petiole

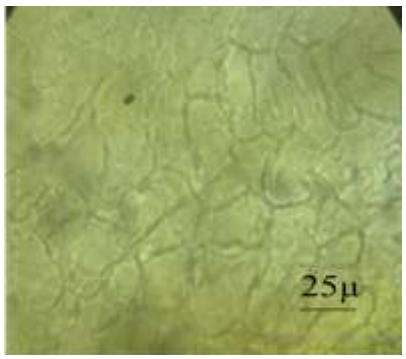


Figure 16 Surface view of fruit

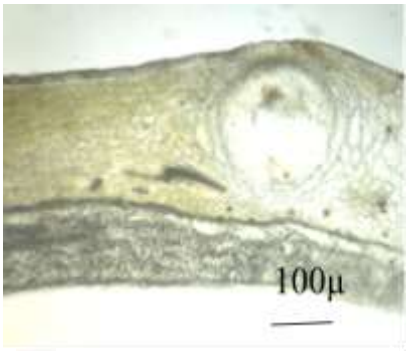
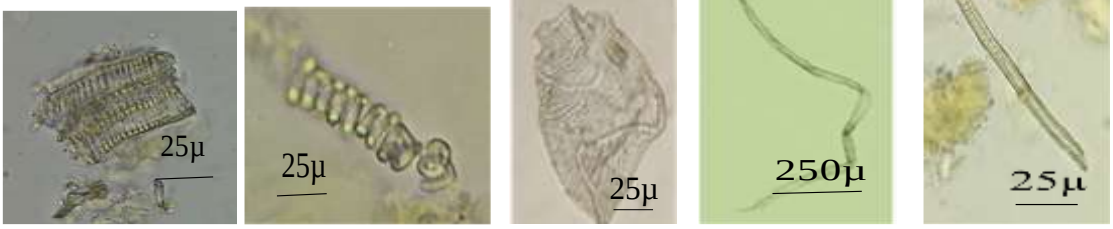


Figure 17 T.S of fruit

Microscopical characters of *Zanthoxylum armatum* DC.



Spiral Vessels Pitted Vessel Fiber Tracheid

Figure 18 Diagnostic Characters of Powdered fruits of *Zanthoxylum armatum* DC.

Chemical Studies

Preliminary Phytochemical Tests

The phytochemical investigation of the fruits *Zanthoxylum armatum* DC. indicated the presence of alkaloids, carbohydrate, glycosides, phenolic compound, saponin, tannins and flavonoid. α -amino acid, starch and reducing sugar were absent in fruits. The results of phytochemical examination were shown in Table (2).

Table 2 Preliminary phytochemical test of powdered fruits from *Zanthoxylum armatum* DC.

No	Test	Extract	Test Reagents	Observation	Result
1	Alkaloid	H_2O, 1%HCL	(1) Mayer's Reagent (2) Wagner's Regent (3) Dragendroff's reagent	White ppt Brown ppt Brown ppt	+ + +
2	α-amino acid	H_2O	Ninhydrin solution	Two layer	-
3	Carbohydrate	H_2O	10% α-naphthol+conc-H_2SO_4	Pink ring, ppt	+
4	Starch	H_2O	I_2solution	Brown color	-
5	Reducing sugar	H_2O	Benedict's solution	Reddish brown	-
6	Glycoside	H_2O	10%lead acetate solution	Pale brown ppt	+
7	Phenolic compound	H_2O	Ferric chloride	Black ppt	+
8	Saponin	H_2O	Distilled water	Frothing	+
9	Tannin	H_2O	Ferric chloride	Dark brown ppt	+
10	Flavonoid	H_2O	Mg turning, Conc HCL	Pale brown ppt	+

(+) = present (-) = absent

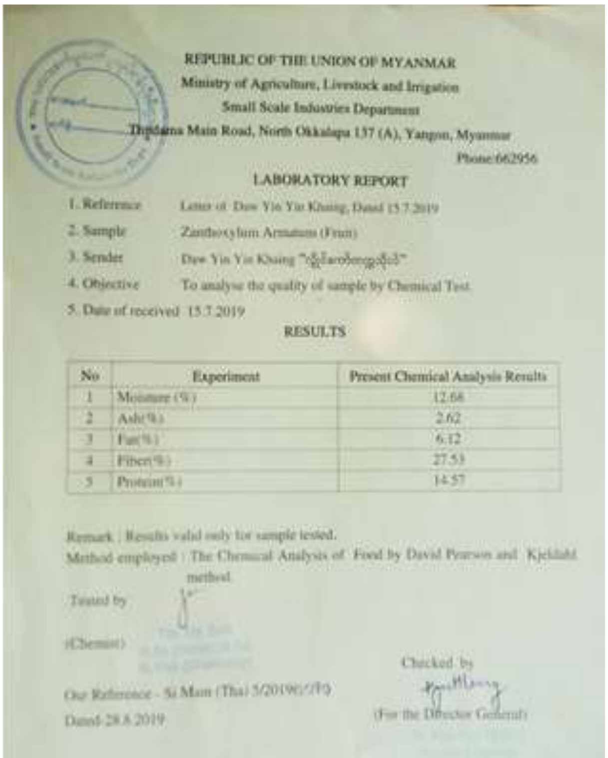
Nutritional values of Powdered fruits of *Zanthoxylum armatum* DC.

Nutritional values

The experimental work for the nutritional contents in powdered fruits of *Zanthoxylum armatum* DC. was carried out. One of the purposes of this research was to study the nutritive values of food content such as carbohydrate, fat, protein, fiber and protein of fruits have been analyzed. The results were shown in Figure (19) and Table (3).

Table 3 Determination of nutritional values from Powdered fruits of *Zanthoxylum armatum* DC.

No	Type of nutrients	Percentage %
1.	Protein	14.57
2.	Moisture	12.68
3.	Ash	2.62
4.	Fat	6.12
5.	Fiber	27.53
6.	Carbohydrate	36.48



REPUBLIC OF THE UNION OF MYANMAR
Ministry of Agriculture, Livestock and Irrigation
Small Scale Industries Department
Thuddama Main Road, North Okkalapa 137 (A), Yangon, Myanmar
Phone 662956

LABORATORY REPORT

1. Reference Letter of Daw Yin Yin Khaing, Dated 15.7.2019
2. Sample Zanthoxylum Armatum (Fruit)
3. Sender Daw Yin Yin Khaing "[illegible]"
4. Objective To analyse the quality of sample by Chemical Test
5. Date of received 15.7.2019

RESULTS

No	Experiment	Present Chemical Analysis Results
1	Moisture (%)	12.68
2	Ash(%)	2.62
3	Fat(%)	6.12
4	Fiber(%)	27.53
5	Protein(%)	14.57

Remark : Results valid only for sample tested.
Method employed : The Chemical Analysis of Food by David Pearson and Kjeldahl method

Tested by

(Chemist)

Checked by

Our Reference - Si Man (Tha) 5/2019([illegible])
Dated 28.8.2019

(For the Director General)

Figure 19 Nutritional composition of powdered fruits of *Zanthoxylum armatum* DC.

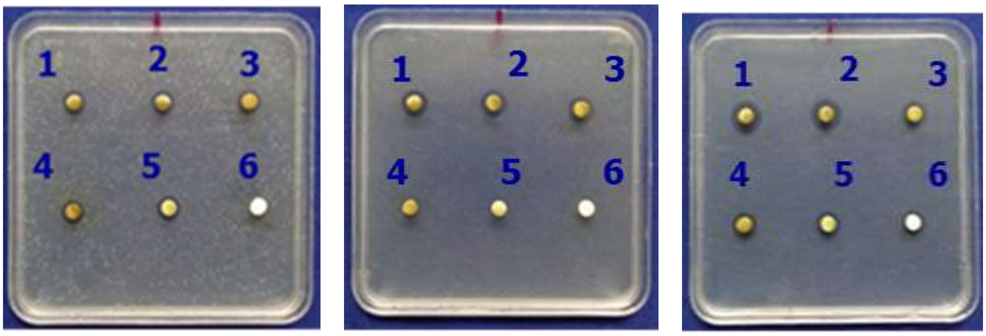
Antimicrobial activities of various fruits extracts

In the results, various crude extracts of powdered fruits *Zanthoxylum armatum* DC. showed the effective on five strains of microorganism. The acetone and ethanol extracts of the fruits were showed the highest activity, especially sensitive against on *Candida albicans*. The results were shown in Figures (20) and Table (4).

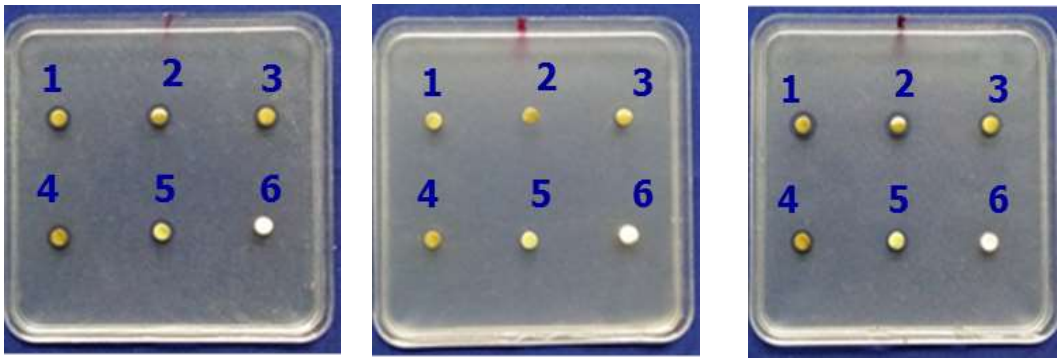
Table 4 Antimicrobial activity from various fruits extracts of *Zanthoxylum armatum* DC.

Extracts	Test Organisms					
	Aspergillus flavous	***Bacillus subtitis***	***Candida albicans***	***Escherichia coli***	***Pseudomonas fluorescens***	***Xanthomonas oryzae***
PE	8 mm	-	10mm	8 mm	-	8 mm
Acetone	8 mm	10 mm	12 mm	8 mm	-	10 mm
EtoAc	8 mm	10 mm	12 mm	8 mm	-	10 mm
EtOH	8 mm	10 mm	10 mm	8 mm	-	8 mm
MeOH	8 mm	-	10 mm	8 mm	-	8 mm
H_2O	-	-	8 mm	-	-	-

Paper disc size – 6 mm



Aspergillus flavous *Bacillus subtitis* *Candida albicans*



Escherichia coli *Pseudomonas fluorescens* *Xanthomonas oryzae*

1. Acetone, 2. EtoAc, 3. EtOH, 4. MeOH, 5. PE, 6. H_2O

Figure 20 Antimicrobial activity of various extracts from fruits of *Zanthoxylum armatum* DC.

Discussion and conclusion

In this paper, the morphological and microscopical studies of leaves, fruits, nutritional values and antimicrobial activity of *Zanthoxylum armatum* DC. have been undertaken.

In morphological study, the plant of *Zanthoxylum armatum* DC. is tree; stems woody with thorny branches. Leaves are alternate, palmate distinctly trifoliate with the wing leaf-stalk. Inflorescence racemose, bracteate, ebracteolate, petals were absent and female flowers have 1-2 ovary, 1- loculed, the ovule solitary and basal, stigma capitates. Fruits are small, ovoid. These characters are in agreement with those mentioned by Hooker (1885), Kirtikar and Basu (1935), Dutta (1979), Dassanayake (1999).

In microscopical study, stomata are abundant on the lower surface. They are anomocytic type. In transverse section of the lamina, palisade cells are present and cluster small of crystals are found in the palisade cells. In transverse section of petiole, the vascular bundles are arranged as a curved line. These characters are in agreement with those stated by Esau (1965), Metcalfe and Chalk (1950), Trease and Evans (1978), Pandey (2004) and Tandon (2011).

In phytochemical test on powdered fruits of *Zanthoxylum armatum* DC. has shown the presence of alkaloids, carbohydrate, glycosides, phenolic compound, saponin, tannins and flavonoid. These factors are described by (http://academicjournals.org).

The nutritional analysis of powdered fruits, protein (14.57%), fat (6.12%), fiber (27.53%) and carbohydrate (36.48%) are obtained from the powdered fruits. These data showed that the fruits are rich sources of nutritional values for human diet. Carbohydrates are body's source for plants and animals that depend on the plants for food. Fiber found in plant cells, cannot be digested but play an essential role in human health. Protein is a major constituent of almost every cell in the body. These factors are mentioned by Passmore (1974).

The antimicrobial activities of various fruits extracts of *Zanthoxylum armatum* DC. fruits such as acetone, ethyl acetate, ethanol, methanol, pet-ether and distilled water extracts were determined by paper disc diffusion method with six different microorganisms. Crude extracts of fruits were indicated that the antimicrobial activities showed against five microorganisms except *Pseudomonas fluorescens* The acetone and ethyl acetate crude extract of fruits showed the highest activity and especially sensitive against on *Candida albicans*.

In conclusion, the species of *Zanthoxylum* include many chemical constituents which are used for medicinal purposes. Hence, the family Rutaceae are not only the medicinal plants but also the economic ones. Therefore, it is sincerely hoped that this present study can be beneficial for the future research.

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References

Arshad M, Ahmad M (2004). Medico-Botanical Investigation of Medicinally Important Plants from Galliyat Areas, NWFP (Pakistan). Ethnobotanical, 2004:6.

Butkhup, L., S., Samppito. (2011). "In Vitro Free Radical Scavenging and Antimicrobial Activity of Some Selected ThaiMedicinal Plants". *Res. J. Med. Plant,* 5, 254-265

Cruickshank, R., J.P Guguid and R.H.A, Swain, (1968). Medicinal Microbiology 11th ed. The English Language Book Society and F, and S. Livingstone Ltd., London

Dassanayake, M.D.and W.D.Clayton. (1999). A Revised Handbook to the Flora of Cyelon, (Vol.XIII), Amerind Publishing Co.Pvt. Lid., New Delhi.

David Pearson. (1976). The Chemical Analysis of Food. Churchill Livingsstone, New York.

Dutta, A.C. (1979). Botany for degree students. (5th ed.)., New Delhi: Oxford University Press.

Esau, K. (1965). Plant anatomy. New York: John Wiley & Sons, Inc.

Hooker, J.D. (1885). Flora of British India, (Vol.IV), L.Reeve and Co., 5, Henrietta Street, Covent Garden, London.

Kebu, B. and K. Fassil, (2006). Ethnobotanical study of wild edible plants in Derashe and Kucha Districts. South Ethiopia. J. Ethnobiol. Ethnomed., 2: 53.

Kirtikar, K.B and B.D. Basu. (1935). Indian Medicinal Plants, (vol.III). Lalit Mohan Basu, M.B.49, leader Road, Allahabad, India.

Kjeldahl, J. (1883). New Method for the Determination of Nitrogen. Chem-News.

Lawrance, G.H.M. (1964). Taxonomy of Vascular Plants. The Macmillan Company, New York.

Marini - Bettalo G.B., M.nicoletti and M. Patamia. (1981). Plant Screening by chemical and Chromatographic procedure under field condition. Journal of Chromatography, 31,14-17.

Metcalfe C.R and L. Chalk. (1950). Amatomy of the Dicotyledons Leaves, Stemand Wood in Relation to Taxonomy with Notes on Economic Uses, (Vol.II), Oxford.

Pandey, S.N. (2004). Plant Anatomy and Embryology, Vikas Publishing House PVT LTD 576 Masjid Road, Jangpura, New Delli.

Passmore, R.N. and M.N.Rao. (1974). Handbook on Human Nutritional Requirement FAO Food and Nutrition.Series No.4, FAO, Rome.

Reddy, P.R. (1984) Kresek phase of bacterial blight of rice. Oryza 21, 179-187.

Ronald, M.A. (1988). *Microbiology fundamentals and applications*. (2nd ed.). USA: Macmillan Publishing Company.

Tandon Neeraj. (2011). Quality Standards of India Medicinal Plants, (Vol. 9), Medicinal Plants Unit India Council of Medicinal Research, New Delhi.

Taylor, W.C. and R. Attaur, (1994). "Constituents of some Asian Medicinal Plants". *Pure Appl. Chem.*,66: 2375-2378

Trease, G. E. and W. C. Evans. (1978). Pharmacognosy. (11th ed.). London: Casselk & Collier Macmillan Publishers Ltd.

Vogel, A. I. (1956). A text book of Practical Organic Chemistry. Longmans Green& Co., Ltd., London.

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