Phytochemical Investigation and Antimicrobial Activities of Leaves of *Carica papaya* L.

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***Abstract*— *Carica papaya* L. belongs to the family Caricaceae. It is known as papaya in English and Thinbaw in Myanmar. Morphological features of *Carica papaya* L. were studied in the present investigation. Samples for this study were collected from the Loikaw University Campus during July to November in 2016. Phytochemical screening of *Carica papaya* L. revealed the presence of many therapeutically important classes of phytoconstituents such as alkaloid, glycoside, reducing sugar, carbohydrate, phenolic compound, tannin, steroid, terpenoid, flavonoid and neutral compound. Moreover, the leaves samples of *Carica papaya* L. were extracted with various solvents: pet-ether, acetone, chloroform, methanol, ethanol, ethyl acetate and water. The antimicrobial activities of the extracts were tested by the well diffusion method. In this case, the ethyl acetate extracts had the highest activity against *Pseudomonas aeruginosa* and showed the narrow spectrum of the bacterial activity. The present work aimed to generate information regarding relevant pharmacological and phytochemical data needed for the proper identification of medicinal plants.**

***Keywords*— Carica papaya L., leaves, phytoconstituents, antimicrobial activities, ethyl acetate extract.**

I. INTRODUCTION

The plant materials and their products are being applied to treat various diseases for a period of several times. They have been denoted as natural resources in the environments of human beings [10]. Their parts and exudates constitute major portion of drugs used in traditional herbal systems of medicine. The drugs efficiently used in these systems greatly depend on the use of proper and genuine raw materials. In order to assure safety, quality and subsequent efficacy of the medicinal plants and herbal products has now become a major and key issue [15].

Carica papaya L. belonging to family Caricaceae is commonly known as papaya in English, Thinbaw in Myanmar [6]. The papaya is a large, tree-like plant, with an unbranched stem growing from 7 to 20 ft. tall, leaves are spirally arranged to the top of the trunk [13]. *Carica papaya* L. and several species of Caricaceae have been used as remedy against various diseases. It is a neutraceutical plant and has a wide range of pharmacological activities. It is a powerhouse of nutrients and is available throughout the year [9]. Moreover, papaya leaves possess antibacterial activity which might prevent the multiplication of wound infection-causing bacteria. The antibacterial properties of papaya leaves were conducted against some wound-infection causing pathogens and justified plant-based compounds to replace synthetic ones [2].

Medicinal plants are defined as a plant containing substances useful for therapeutic purposes or precursors for the manufacturing of drugs useful for disease therapy. Since medicinal plants do not nearly save people from feeling pain but permit them to emerge unscathed, they deserve investigation. The local use of natural plants as primary health remedies is quite common in Asia, Latin America, and Africa [2].

In the present study, phytochemical investigation and antimicrobial activities of *Carica papaya* L. were conducted. This information helped to identify medicinal plants and apply these plants as nutraceuticals and medicines: antibiotics.

II. MATERIALS AND METHODS

A. Collection and Identification of Carica papaya L.

In this study, the plant specimens were collected from Loikaw University Campus during July to November in 2016. The morphological characters of the specimens were studied, measured and recorded. The plants were identified with the help of available literatures: Flora of China by [5]; Flora of Ceylon by [3].

B. Preparation of Powdered Samples

The samples were thoroughly washed with water and air-dried at room temperature for about two to three weeks. The dry samples were pulverized by grinding machine into powder and kept in air-tight container for further study.

C. Preliminary Phytochemical Examination of Leaves of Carica papaya L.

Phytochemical examination was conducted according to [16], [12].

(1.) Test for alkaloids

The leaves extracts were stirred with 2 ml of dilute hydrochloric acid separately and filtrate was tested with the following reagents;

1. Dragendroff's test: Dissolve a few mg of alcoholic or aqueous extract of the drug in 5ml of distilled water, add 2 M hydrochloric acid until an acid reaction

occurs, then add 1 ml of Dragendroff's reagent. An orange or orange-red ppt. is produced immediately [17].

2. 2. Mayer's test: Add a few drops of Mayer's reagent to 1 ml of acidic aqueous extract of the drug. A white or pale yellow ppt. is formed [17].
3. Wagner's test: Acidity 1 ml of alcoholic extracts of the drug with 1.5% V/V of hydrochloric acid and adds a few drops of sodium picrate solution reagent. A yellow of brown ppt. is formed [17].

(2.) Test for α-amino acids

Each extract 2 ml of the leaves was added with two drops of ninhydrin reagent. The formation of violet color appears due to the presence of α-amino acid [8].

(3.) Test for carbohydrates

Each extract 2 ml of the leaves was introduced into a test tube and a few drops of 10% α-napthol were added and shaken. The test tube was then inclined at an angle of 45˚ and concentrated sulphuric acid was added slowly along the side of the tube. A red ring or violet ring was formed between the two layers, showing the presence of carbohydrate [17].

(4.) Test for flavonoids

In a test tube containing 0.5 ml of alcoholic extract of the drug, add 5 – 10 drops of dilute hydrochloric acid followed by a small piece of zinc or magnesium. Boil the solution for a few minutes. In the presence of flavonoids, a pink, reddish pink or brown color is produced [17].

(5.) Test for glycosides

Dissolve a small amount of alcoholic extract of the drug in 1 ml of water, and add sodium hydroxide solution. A yellow color indicates the presence of glycosides [17].

(6.) Test for cyanogenic glycoside

Two grams of powered sample were mixed with distilled water and a few drops of concentrated sulphuric acid were added. The sodium picrate paper was inserted with the cork at the top of the test tube. Then, the resulting mixture was gently heated by means of a spirit burner. If the sodium picrate paper turns pink, it indicates the presence of cyanogenic glycoside.

(7.) Test for phenolic compound

Each extract 2 ml of the leaves was added 2 drops of 4 % ferric chloride solution in a test tube separately. If formation of green or blue color which may indicate that the presence of phenolic compound [17].

(8.) Test for reducing sugar

Each extract 2 ml of the leaves was added a few drops of Benedict's solution in a test tube separately solution appear green, yellow or red ppt., which indicated that the presence of reducing sugar [14].

(9.) Test for saponin

Each extract 2 ml of the leaves was added a few drops of distilled water. Then the mixture was vigorously shaken for a few minutes. Observation was made to see if foaming took place, indicating the presence of saponin [8].

(10.) Test for steroid and terpenoid

Each extract 2 ml of the leaves was added two drops of concentrate H_2SO_4. This formation of blue or green color shows the presence of steroid and formation of deep red color, greenish color or blue color indicating the presence of terpenoid [17].

(11.) Test for tannin

Each extract 2 ml of the leaves was added with a few drops of 1 % ferric chloride solution in a test tube separately. If yellowish brown ppt. or blue green color was resulted indicating that the presence of tannins [17].

(12.) Test for acid or base or neutral compound

Two grams of powered sample was boiled with distilled water for about 30 minutes and filtered. A few drops of bromocresol green were added. If no change in color was found, it indicates a neutral compound. If yellow color was found, it indicates acid compound. If blue color was found, it indicates base compound.

D. Antimicrobial Screening of Leaves of Carica papaya L.

The antimicrobial activities of leaves of *Carica papaya* L. were conducted at Pharmaceutical Research Department, Yangon.

(1.) Solvent and Extraction

Solvents: Pet-ether, Chloroform, Methanol, Acetone, Ethyl acetate, Ethanol and water were used for the extraction of leaves of *Carica papaya* L. Five grams of dried leaf powder were soaked in 50 ml of different solvents for a week. The extracting solvents were filtered and the filtrates were concentrated by using water-bath.

(2.) Test Microorganisms

The test microorganisms: *Bacillus subtilis* (N.C.T.C – 8236), *Staphylococcus aureus* (N.C.P.C – 6371), *Pseudomonas aeruginosa* (6749), *Bacillus pumilus* (N.C.I.B – 8982*), Candida albicans* and *Escherichia coli* (N.C.I.B – 8134) were used for the determination of antimicrobial activity.

(3.) Preparation of plates for antimicrobial activity

The antimicrobial activities were performed by agar-well diffusion method. Nutrient agar was prepared according to the method described by [4]. Nutrient agar was boiled and 20 – 25 ml of the medium was poured into a test tube. They were plugged with cotton wool and autoclaved at 121˚C for 15 minutes. Then they were cool down to 60˚C and poured into sterilized petridishes. Then, 0.1 ml of spore suspension was

added into the dishes and the agar was allowed to set for 30 minutes.

(4.) Agar well diffusion method

In agar well diffusion method, a cork borner was used to make a small well (10 mm) in the nutrient agar. After that 0.15 ml of crude extract was introduced into the agar well and the plates were incubated at 37 ˚C for 24 hrs. The inhibition zone appeared around the agar well indicating the presence of antimicrobial activity.

III. RESULTS AND DISCUSSION

A. In Taxonomic Description

Scientific Name : *Carica papaya* L.

Synonyms : *Papaya carica* Gaertner.

Family : Caricaceae

Local name : Thinbaw

Common name : Papaya

Flowering period : Throughout the year.

B. Preliminary Phytochemical Investigation of the Leaves of Carica papaya L.

The various extracts of the plant showed the presence of alkaloid, glycoside, reducing sugar, carbohydrate, phenolic compound, tannin, steroid, terpenoid, flavonoid and neutral compound (Table 1).

Table 1:- Result of Preliminary Phytochemical Investigation of the Leaves of *Carica papaya* L.

No.	Chemical constituent	Extract	Reagent used	Observation	Results
1.	Alkaloid	1% HCl	1. Mayer's reagent 2. Dragendroff's reagent 3. Sodium picrate solution	white ppt. orange ppt. yellow ppt.	+ + +
2.	Glycoside	H_2O	10% lead acetate solution	white ppt.	+
3.	Reducing sugar	H_2O	Fehling solution	reddish ppt.	+
4.	Saponin	H_2O	Distilled water	No frothing	-
5.	Carbohydrate	H_2O	10% α-napthol, Conc. H_2SO_4 acid	pink ring	+
6.	Cyanogenic glycoside	H_2O	Conc. H_2SO_4 acid, sodium picrate paper	No change in color	-
7.	Phenolic compound	H_2O	$FeCl_3$ solution	deep brown ppt.	+
8.	Tannin	H_2O	1% gelatin solution	white ppt.	+
9.	Steroid	Pet – Ether	Acetic anhydride, Conc. H_2SO_4 acid	pale green	+
10.	Terpenoid	EtOH, $CHCl_3$	Conc. H_2SO_4 acid	reddish brown	+
11.	Flavonoid	MeOH	Conc. HCl acid, Mg turning	pink color	+
12.	α-amino acid	H_2O	Ninhydrin solution	No change in color	-
13.	Acid/Base/Neutral Compound	H_2O	Bromocresol green	green color	**Neutral compound**

C. Antimicrobial Activity of Different Solvent Extracts of Leaves of Carica papaya L.

The antimicrobial activity assays were conducted by using agar well diffusion method. The leaves samples of *Carica papaya* L. were extracted with various solvents: pet-ether, acetone, chloroform, methanol, ethanol, ethyl acetate and water. The antimicrobial activities of the extracts were tested by six test microorganisms. In this case, the ethyl acetate extracts had the highest activity against *Pseudomonas aeruginosa*. The ethanol extracts showed the moderate activity against *Pseudomonas aeruginosa*. The other extracts showed the least activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Bacillus pumilus*, *Candida albicans* and *Escherichia coli*(Table2).

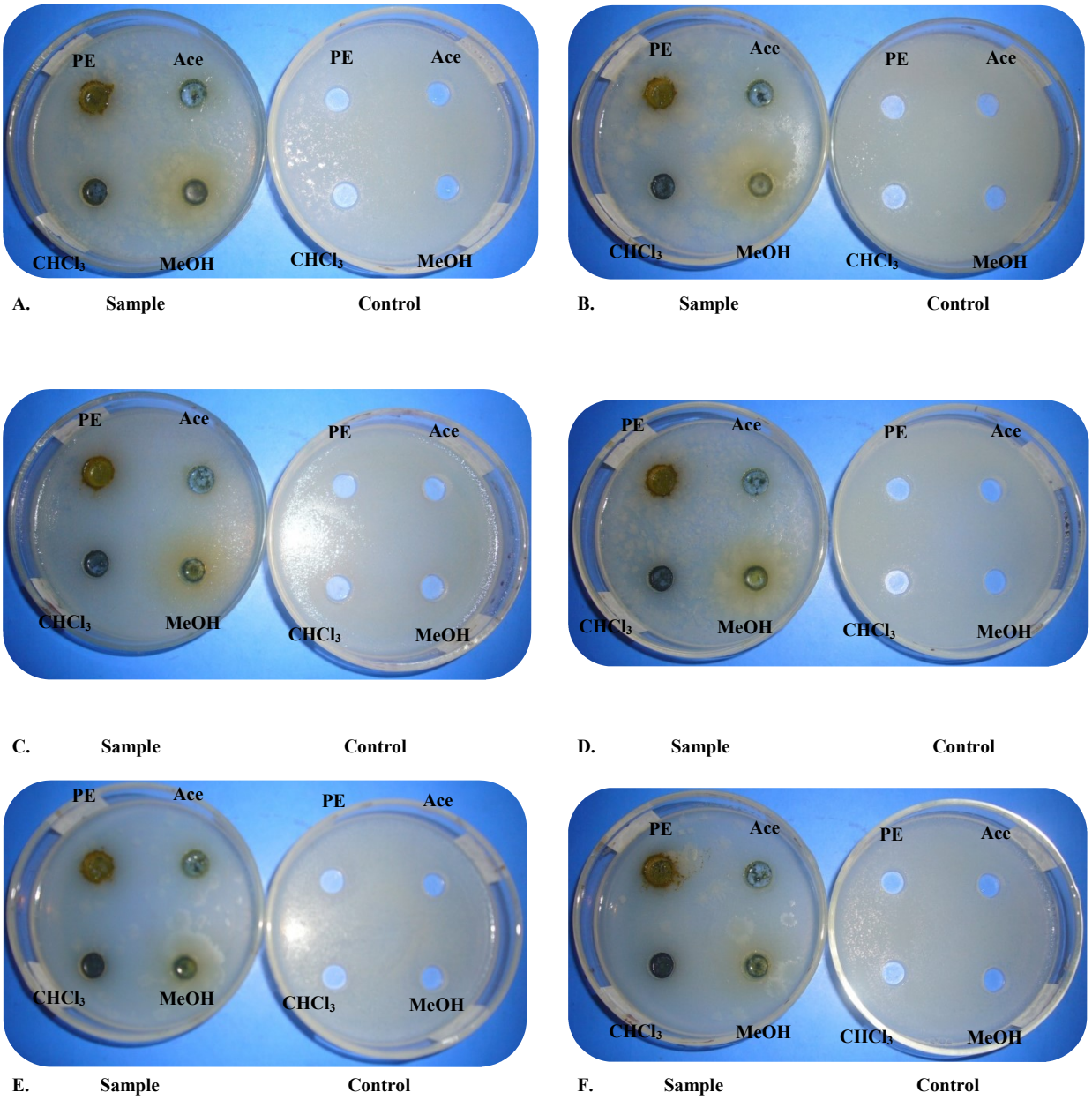

Fig. 1:– Antimicrobial Activity of Different Solvent Extracts of Leaves of *Carica papaya* L.

(A.) *Bacillus subtilis*

(B.) *Staphylococcus aureus*

(C.) *Pseudomonas aeruginosa*

(D.) *Bacillus pumilus*

(E.) *Candida albicans*

(F.) *Escherichia coli*

Different Solvents:
PE – Pet – ether
Ace – Acetone
$CHCl_3$ – Chloroform
MeOH – Methanol

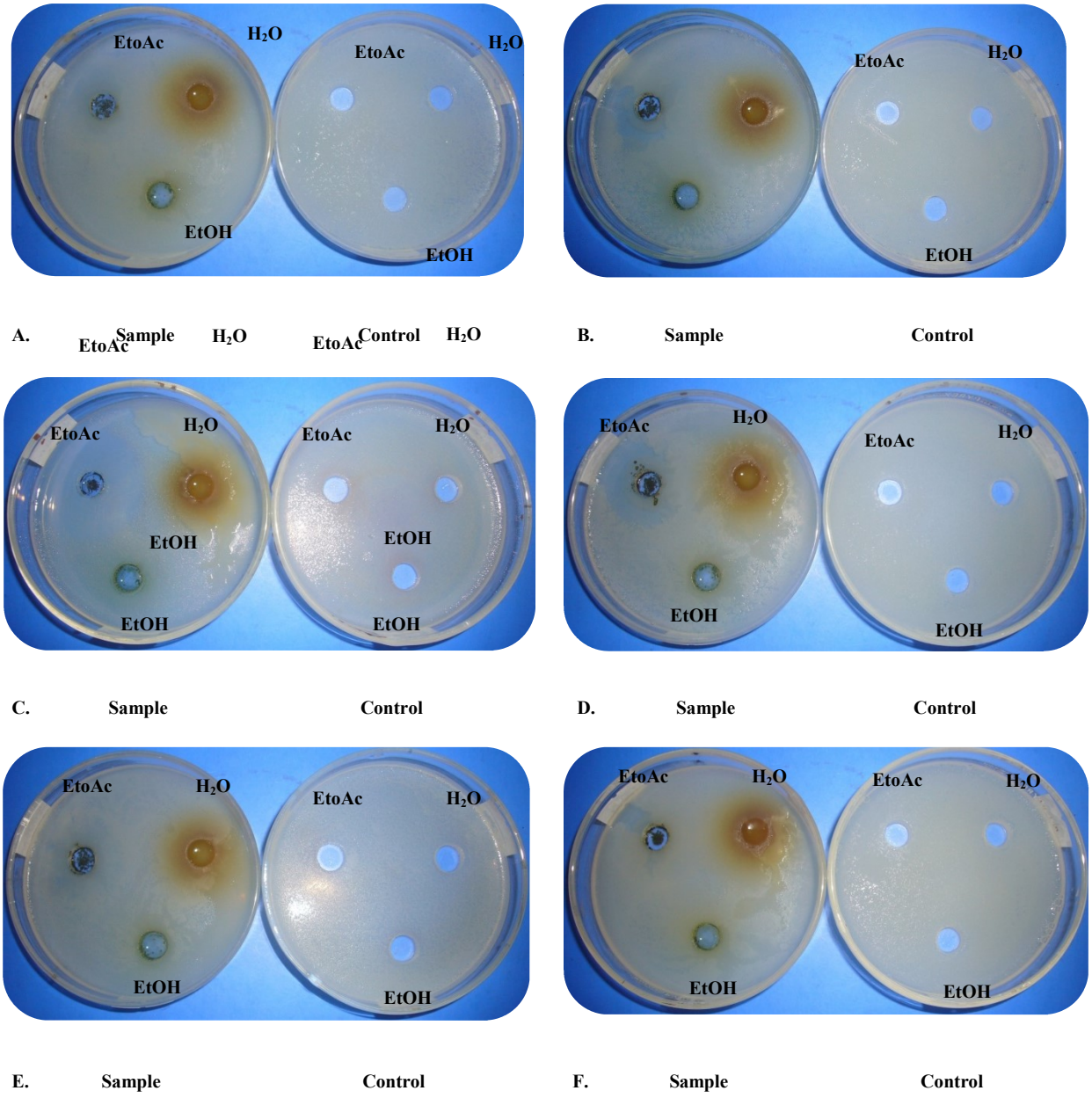

Fig. 2: – B. Antimicrobial Activity of Different Solvent Extracts of Leaves of *Carica papaya* L.

(A.) *Bacillus subtilis*

(B.) *Staphylococcus aureus*

(C.) *Pseudomonas aeruginosa*

(D.) *Bacillus pumilus*

(E.) *Candida albicans*

(F.) *Escherichia coli*

Different Solvents:
EtoAc – Ethyl acetate
H_2O – Aqueous
EtOH – Ethanol

Table 2:- Antimicrobial Activity of Six Extracts of *Carica papaya* Leaf on Six Pathogenic Microorganisms (well diffusion method)

		Test Organisms					
Samples	**Solvent**	***B. subtilis***	***S. aureus***	***P. aeruginosa***	***B. pumilus***	***C. albicans***	***E. coli***
Carica papaya L.	pet-ether	12 mm (+)	12 mm (+)	11 mm (+)	12 m.m (+)	11 mm (+)	11 mm (+)
	$CHCl_3$	14 mm (+)	12 mm (+)	12 mm (+)	13 mm (+)	14 mm (+)	14 mm (+)
	MeOH	12 mm (+)	12 mm (+)	12 mm (+)	12 mm (+)	14 mm (+)	14 mm (+)
	Acetone	14 mm (+)	13 mm (+)	12 mm (+)	13 mm (+)	14 mm (+)	14 mm (+)
	EtoAc	11 mm (+)	12 mm (+)	40 mm (+++)	-	-	-
	EtOH	13 mm (+)	13 mm (+)	16 mm (++)	13 mm (+)	12 mm (+)	12 mm (+)
	H_2O	11 mm (+)	13 mm (+)	14 mm (+)	11 mm (+)	12 mm (+)	12 mm (+)

Agar well – 10 mm

10 mm – 14 mm (+)

15 mm – 19 mm (++)

20 mm & above (+++)

Test Organisms

(1.) ***Bacillus subtilis*** **(N.C.T.C – 8236)**
(2.) ***Staphylococcus aureus*** **(N.C.P.C – 6371)**
(3.) ***Pseudomonas aeruginosa*** **(6749)**
(4.) ***Bacillus pumilus*** **(N.C.I.B – 8982)**
(5.) Candida albicans
(6.) ***Escherichia coli*** **(N.C.I.B – 8134)**

In the present investigation, the morphological characters of *Carica papaya* L. were in agreement with those described by Ho, Ting – naung & Robert Ornduff, (1995); & Dassanayake, (1996). Phytochemical examination was carried according to British Pharmacopoeia, (1968) and Trease and Evens, (1978), (Table. 1).

Peter J.K. *et al*., (2014) discussed that the papaya leaves were made into tea as a treatment of malaria. The leaves of papaya plant contained chemical compound of karpain which killed microorganisms that often interfered with the digestive function. Antimicrobials of plant origin were effective in the treatment of infectious diseases. He observed that aqueous extracts of papaya leaves had antibacterial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella typhi*. The chloroform extracts did not show any inhibition against the bacterial pathogens. In the present study, all extracts of the leaves showed the antibacterial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*.

Taemchuay D. *et al*., (2009) reported that the ethanol extracts of the leaves of papaya had more potential antibacterial activity against *Staphylococcus aureus* than the water extracts. Anibijuwon I.I. & Udeze A.O. (2009) showed that the leaves of papaya had the phytoconstituents such as alkaloids, tannins, saponins, glycosides and phenols. The leaf extracts of papaya were active against gram negative and gram positive bacteria and fungi, indicating that a broad spectrum of activity. This was significant because the developing therapeutic substances must be active against multidrug-resistant organisms.

In the present study, it has been reported that the extracts of the leaves of *Carica papaya* L. was found to be the least activity against gram positive bacteria: *Bacillus subtilis*, *Staphylococcus aureus*, *Bacillus pumilus* and gram negative bacteria: *Escherichia coli* and fungi: *Candida albicans*. The ethanol extracts of the leaves of *Carica papaya* L. that showed the moderate activity and the ethyl acetate extracts of the leaves of *Carica papaya* L. showed the highest activity against gram negative bacteria: *Pseudomonas aeruginosa* and no activity against: *Bacillus pumilus*, *Candida albicans*, and *Escherichia coli* (Table. 2); (Fig. 1 & 2). It can be noted that the leaves of *Carica papaya* L. contained various phytochemical constituents that were active against pathogenic microorganisms. Thus, they were able to be utilized for nutraceuticals as well as modern and traditional medicines.

IV. CONCLUSION

It can be concluded that the present work was conducted to obtain some pharmacognostical standards for *Carica*

papaya L. The ethyl acetate extract of leaves of *Carica papaya* L. against *Pseudomonas aeruginosa* showed the narrow spectrum indicating the presence of effective phytoconstituents. The above studies provided information dealing with their identification and chemical constituents may be useful for pharmacognostical study and standardization for the medicinal plants. The potential of leaves of *Carica papaya* L. in the discovery of novel compounds with activity against microorganisms has been realized, and hence open exciting avenues in the field of biomedical research.

ACKNOWLEDGMENT

We would like to acknowledge with gratitude to Dr Nwe Nwe Yin, the Acting Rector of Panglong University for her permission and administrative support to publish this research journal. We would like to acknowledge to all the staff, Department of Botany, Loikaw University for their invaluable helps, suggestions and comments necessary in this work. We also thank the Department of Higher Education for their financial support. Our sincere thanks are due to our colleagues at Pharmaceutical Research Department, Yangon, for their invaluable guidance, advice, suggestion and comments.

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