PHYTOCHEMICAL, PHYSICOCHEMICAL AND ANTIMICROBIAL ACTIVITIES ON FLOWERS OF *CAREYA ARBOREA* ROXB.

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Abstract

Careya arborea Roxb. belongs to the family Lecythidaceae is found in many places of the world. It is also known as Bumbwe in Myanmar and Wild guava in English. The plants were collected from West Yangon University campus during the flowering period of May - November, 2019. The flowers are traditionally used to treat cough and cold, fever and tonic. In this study, the plant is deciduous trees, alternate and broadly obovate simple leaves, terminal spike with yellowish-white flowers, stamens with three whorls, inferior cup shaped ovary and large berry fruit with persistent style and calyx. In phytochemical screening, the presence of alkaloid, glycoside, saponin, phenolic compound, flavonoid, carbohydrate, steroid, terpenoid, tannin and reducing sugar were detected in flowers. However, cyanogenic glycoside and amino acid were absent. In the physicochemical properties, the powdered flowers are the most soluble in aqueous extract. The elemental analysis of powdered sample was determined by using Energy Dispersive X-ray Fluorescence Spectrophotometer. It was observed that the potassium was principal element. Antimicrobial activities of plant extracts are tested with six types of microorganisms by using agar well diffusion method. Ethanol extract of flowers exhibited effective against on *Pseudomonas aeruginosa*.

Keywords: Antimicrobial Study

Introduction

Careya arborea Roxb. is have been used for timber and ornamental plants and several parts of these species use as medicines. This plant is planted in gardens and roadsides for its large conspicuous leaves and showy flowers and fruits (Kumar *et al*., 2010). These species occur from Afghanistan through India to Ceylon and east to Thailand (Dassanayake, 1981). Range of these plants is Myanmar to the Malay Peninsula (Perry, 1980). In Myanmar, these plants occur in Western Thayetmyo, Katha, Bhamo, Mogok and Northern Tanintharyi of moist teak forest. It is also found in Pyinma forest on the plains and alluvial flats bordering the Bago Yoma forests (Kress *et al*., 2003).

Ecologically, the plant prefers a well-drained, sandy or even rocky soil and requires a sunny position. The tree is highly fire resistant and coppies well (Dassanayake, 1981). The vernacular names of this plant are Ka Li Yu Rui in Chinese, Kradonbok in Thailand, Kumbhi in Hindi and Katabhis in Sanskrit (Prabhakaran *et al*., 2014). The flowers of *Careya arborea* Roxb. have triterpenoid, steroid and tannin (Shantha *et al*., 1987).

The flowers and fruits are also used for relieving cold and cough (Ambardar and Aeri, 2013). Paste of flower of *Careya arborea* Roxb. prepared by macerating in ghee is taken orally in empty stomach to treat infertility (Mahishi *et al*., 2005). Aerial parts of this plant also exhibit antioxidant activity, antimicrobial activity against the bacteria like *Bacillus subtilis*, *Staphylococus aureus*, *Pseudamonas aeruginosa*, *Bacillus pumilus*, *Escherichia coli* and *Candida albican* (Navya and Anitha, 2018).

Therefore, the plant was chosen for this study to inform the medicinal value. To achieve this aim, the objectives were to detect the phytochemical tests for the presence or absence of

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chemical constituents in the flowers and to test the antimicrobial activities of the crude extracts by using agar-well diffusion method.

Materials and Methods

1. Morphological study of *Careya arborea* Roxb.

The plants materials were collected from West Yangon University campus, during the flowering period. The collected specimens were photographed to record the data. The plants were classified and identified by using Hooker (1879) and Dassanayake (1981).

The flowers were washed with water and then cut into small pieces and air dried at room temperature for three weeks. When constant weight was obtained, the dried samples were pulverized by grinding machine and stored in air tight bottles for further use.

2. Chemical study of *Careya arborea* Roxb.

Preliminary phytochemical investigation on flower of *Careya arborea* Roxb.

In this investigation, the powdered flowers of *Careya arborea* Roxb. were tested to find out the presence or absence of chemical constituents such as alkaloids, glycoside, saponin, cyanogenic glycoside, phenolic compounds, flavonoid, carbohydrate, steroids, terpenoid, tannins, amino acid and reducing sugars. Preliminary phytochemical tests of flowers were carried out at the Chemistry Department,West Yangon University according to the methods of British Pharmacopoeia (1968) and Central Council for Research in Unani Medicine (1987). The results were shown in Table (1).

Physicochemical analysis on flower of *Careya arborea* Roxb.

Physicochemical properties which include moisture content, total ash, acid insoluble ash, water soluble ash and solubility of nonpolar and polar solvents such as pet-ether, chloroform, ethyl-acetate, acetone, ethanol, methanol and water soluble matter contents of *Careya arborea* Roxb. flowers powered were carried out by the methods of British Pharmacopoeia (1968). The results were shown in Table (2).

Elemental analysis on flowers of *Careya arborea* Roxb. by using Energy Dispersive X-ray Fluorescence Spectrophotometer (EDXRF)

Element analysis was performed by EDXRF (Energy Dispersive X-ray Fluorescence Spectrophotometer) at Chemistry Department, West Yangon University. The EDX-700 spectrophotometer SHIMADZU Co.Ltd., Japan is used for determination of elements. The results were shown in Table (3) and Figure (10).

3. Antimicrobial screening of different solvent extracts from flowers of *Careya arborea* Roxb.

Extraction

The powdered samples of flowers (five gram) were soaked in petroleum ether (60-80˚ C), chloroform, methanol, acetone, ethyl acetate, 95% ethanol and distilled water for about 3 weeks and then filtered. (British Pharmacopoeia 1968). The solvents were then evaporated by using water bath to obtain a paste.

Test Organisms

The different solvent extracts were tested against six tested microorganisms by using agar well diffusion method. The six microorganisms were *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Escherichia coli and Candida albicans* obtained from **Central Results Development Centre**. (N.C.T.C-8236), (N.C.P.C-6371), (6749), (N.C.I.B-8982), (N.C.I.B – 8134)

The extracts of antimicrobial activity were measured from the diameter zone of inhibition. The results were shown in Table (4) and Figure (11-16).

Procedure

Nutrient agar was prepared according to the method of Cruickshank, 1975. Nutrient agar was boiled and 20 - 25 ml of the medium was poured into a conical flask and plugged with cotton wool and autoclaved at 121° C for 15 minutes. Then the tubes were cooled down to 30 - 35° C and poured into sterilized petridishes and 0.1 - 0.2 ml of test organisms were also added into the dishes. The agar was allowed to set for 2 - 3 hours. After that, 10 mm plate agar-well made with the help of sterilized agar well cutter. About 0.2 ml of sample was introduced into the agar-well and incubated at 37° C for 24 - 48 hours. The inhibition zone appeared around the agar well, indicated that the presence of antibacterial activity. This antimicrobial activity test was conducted in CRDC (Central Research Development Centre).

Results

1. Morphological study of *Careya arborea* Roxb Pl. Coromandel 3(1): 14, t. 218 (1811).

Deciduous trees, stem erect, cylindrical, pubescent; bark greyish-brown cracked and flakeing in thin stripes. Leaves alternate, crowded towards the end of the branches, simple, lamina broadly abovate, the base tapering cunneate, the margin serrate, the tips rounded with the pointed, dull-green glabrous, on the both surfaces, petiolate; the petioles cylindrical, glabrous, exstipulate. Inflorescences terminal spike, cymes and wide, sub pedunculate. Flower yellowish-white, bractcate, the bracts 3, unequal, linear- to lanceolate, green, glabrous, ebracteolate, sessile, bisexual, actinomorphic, 4-merous, epigynous. Calyx (4) synsepalous, campanulate, the lobe oval, green, imbricate, glabrous. Corolla 4-5, free petals, oblong, elliptic, light yellowish, glabrous, very fragile and soon falling. Stamens numerous twice as long as the petals in 3 whorls; outer whorl longest, without anther, reddish-white; middle whorl medium, fertile; the innermost whorl short without anther, all united at the base into a thick fleshy ring, the filaments, slender, the anther dark-brown in age, dithecous, oblongoid, basifixed, longitudinal dehiscence. Ovary inferior, cup shaped disc at the top, ovoid, tetra-carpellary, syncarpous, axile placentation, 4 - α ovules in each locule in T.S; the style long, slender long, glabrous; the stigma capitate. Fruit berry globose, bright-green pericarp in young, brownish in mature, fibrous crowned with the sepals and style, with fleshy pulp; seed, many, white, oblong embedded in fleshy pulp of the fruits.

Figure 1 Natural Habit

Figure 2 Inflorescence

Figure 3 Flower as seen

Figure 4 Stamens

Figure 5 Calyx & Style

Figure 6 L.S of flower

Figure 7 T.S of ovary

Figure 8 Fruit

Figure 9 Seeds

2. Chemical study of *Careya arborea* Roxb

Table 1 Preliminary Phytochemical Investigation on Flowers of *Careya arborea* Roxb.

No	Chemical Constituents	Extract	Test Reagent	Observation	Results
1	Alkaloid	1%HCL	1 Mayer's reagent 2 Dragendorff's reagent 3 Wagner reagent	White ppts Orange ppts Yellow ppts	Present
2	Glycoside	H_2O	10% Lead acetate solution	White ppts	Present
3	Saponin glycoside	H_2O	Distilled water	Frothing	Present
4	Cyanogenic glycoside	H_2O	Conc H_2SO_4 acid+Sodium Picrate solution	No Colour Change	Absent
5	Phenolic Compound	H_2O	$FeCl_3$ Solution	Brown colour	Present
6	Flavonoid	MEOH	Mg turing + Conc HCl	Pink Colour	Present
7	Carbohydrate	H_2O	10% α naphathol+ Conc H_2SO_4	Pink Ring	Present
8	Steroid	PE	Acetic Anhydride+Conc H_2SO_4 acid	Pink Colour	Present
9	Terpenoid	EtOH $CHCl_3$	Conc H_2SO_4acid	Reddish brown	Present
10	Tannin	$CHCl_3$	10% Gelatin solution	White ppts	Present
11	Amino acid	H_2O	Ninhydrin	No change in colour	Absent
12	Acid/Base/Nutral	H_2O	Bromocresol green	Green	Nutral
13	Reducing Sugar	H_2O	1. Benedicts Solution 2. Fehling Solution	Red ppts	Present

Table 2 Physicochemical Analysis on Flowers of *Careya arborea* Roxb.

No	Physics chemical properties	Yield percent (%)
1	Moisture content	6.65%
2	Total ash	1.03%
3	Acid insoluble ash	5.10%
4	Water soluble ash	28.47%
5	Ethanol soluble matter content	5.75%
6	Methanol soluble matter content	6.42%
7	Petroleum ether soluble matter content	2.07%
8	Ethyl acetate soluble matter content	2.41%
9	Chloroform soluble matter content	2.43%
10	Acetone soluble matter content	0.68%
11	Aqueous soluble matter content	14.17%

Table 3 Elemental Analysis on Flowers of *Careya arborea* Roxb. by using EDXRF

Elements	**Average %**
Potassium (K)	1.986
Sodium (Na)	0.206
Calcium (Ca)	0.121
Iron (Fe)	0.095
Titanian (Ti)	0.014
Manganese (Mn)	0.005
Zince (Zn)	0.002
Rubidium (Rb)	0.002
Chromium (Cr)	0.001
Copper (Cu)	0.001
Strontium (Sr)	0.001
Zirconium (Zr)	0.000
COH balance	97.566

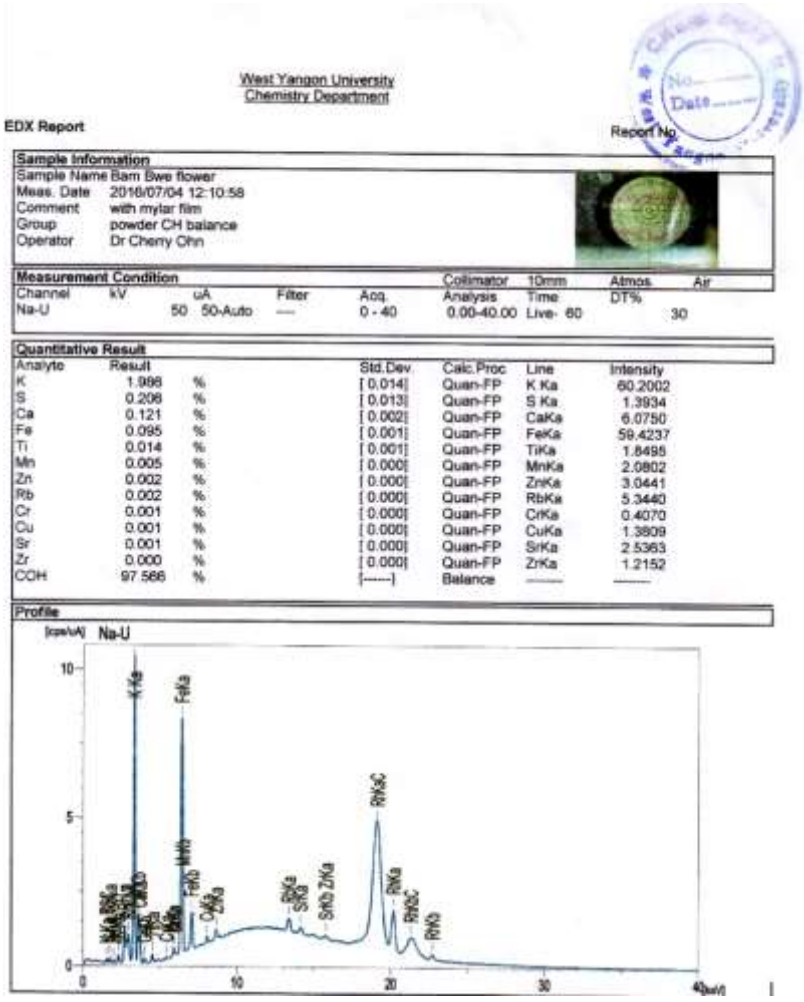

Figure 10 Elemental analysis on flowers of *Careya arborea* Roxb. by using Energy Dispersive X-ray Fluorescence Spectrophotometer (EDXRF)

3. Antimicrobial screening of different solvent extracts from flowers of *Careya arborea* Roxb.

Table 4 Antimicrobial Activity from the Different Solvent Extracts on Flowers of *Careya arborea* Roxb.

Test Organisms	Solvents							
	Control	Pet-ether	Chloroform	Methanol	Acetone	Ethyl acetate	Ethanol	Aqueous
Bacillus subtilis	-	-	-	20 mm (+++)	14 mm (+)	13 mm (+)	16 mm (++)	14 mm (+)
Staphylococcus aureus	-	-	-	16 mm (++)	14 mm (+)	12 mm (+)	21 mm (+++)	14 mm (+)
Pseudomonas aeruginosa	-	-	-	16 mm (++)	13 mm (+)	-	24 mm (+++)	13 mm (+)
Bacillus pumilus	-	12 ml (+)	12 mm (++)	18 mm (++)	17 mm (++)	11 mm (+)	18 mm (++)	15 mm (++)
Candida albicans	-	11 ml (+)	12 mm (++)	18 mm (++)	16 mm (++)	12 mm (+)	19 mm (++)	17 mm (++)
Escherichia coli	-	11 ml (+)	12 mm (++)	17 mm (++)	15 mm (++)	12 mm (+)	20 mm (+++)	15 mm (++)

Agar well – 10 mm

10 mm ~ 14 mm (+)

15 mm ~ 19 mm (++)

20 mm above (+++)

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) *E-coli* (N.C.I.B-8134)

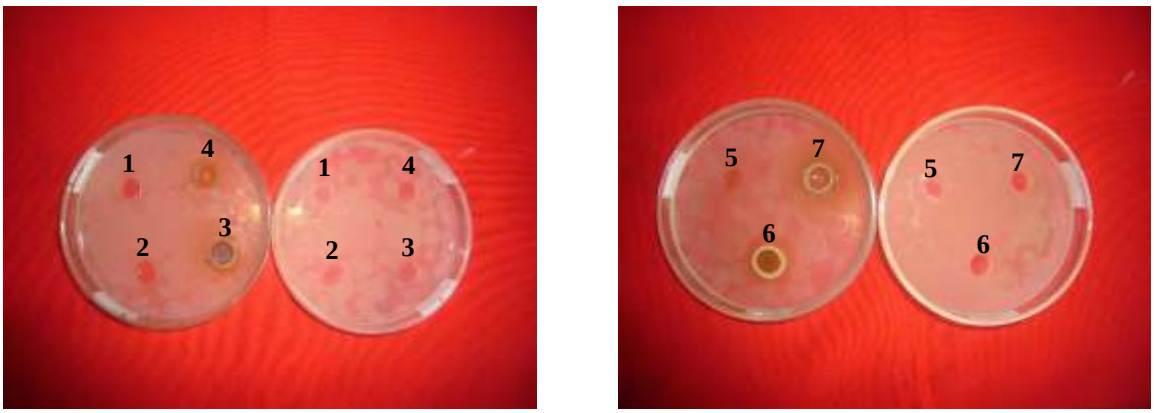

Figure 11 Antibacterial activity of the flowers extracts on *Bacillus subtilis*

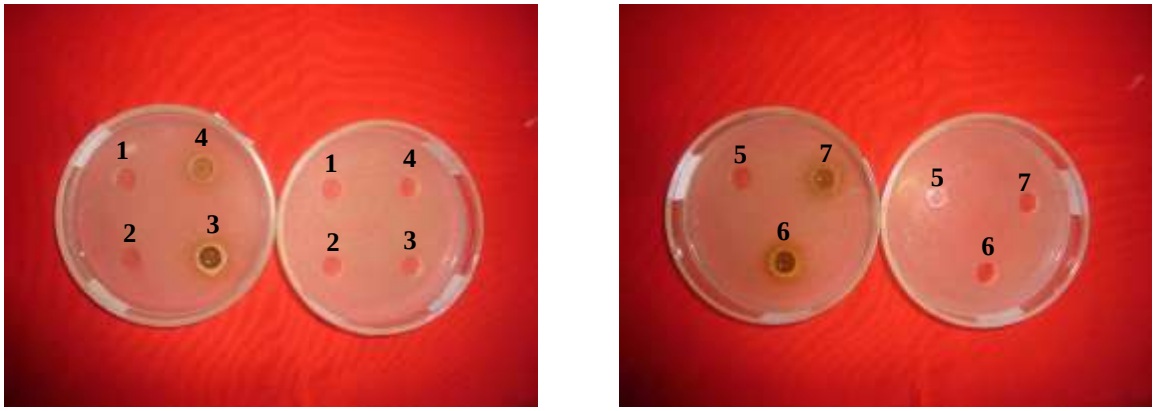

Figure 12 Antibacterial activity of the flowers extracts on *Staphylococcus aureus*

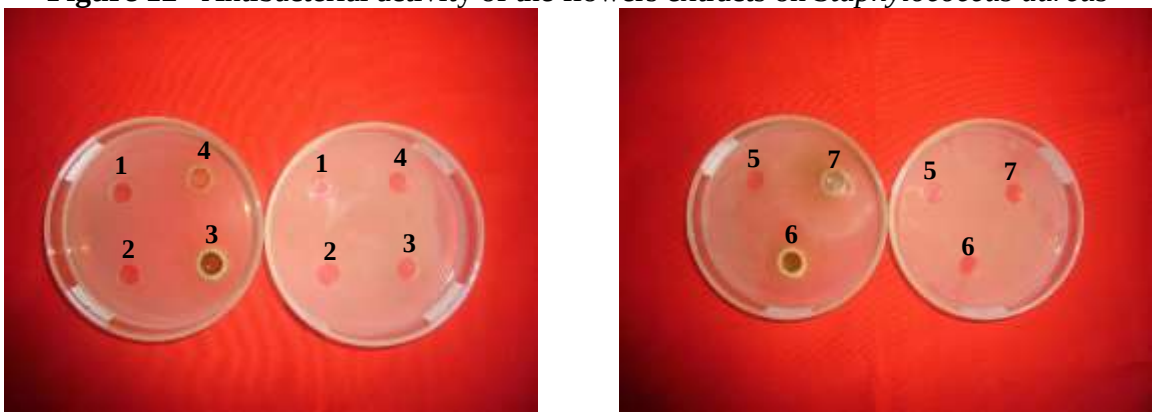

Figure 13 Antibacterial activity of the flowers extracts on *Pseudomonas aeruginosa*

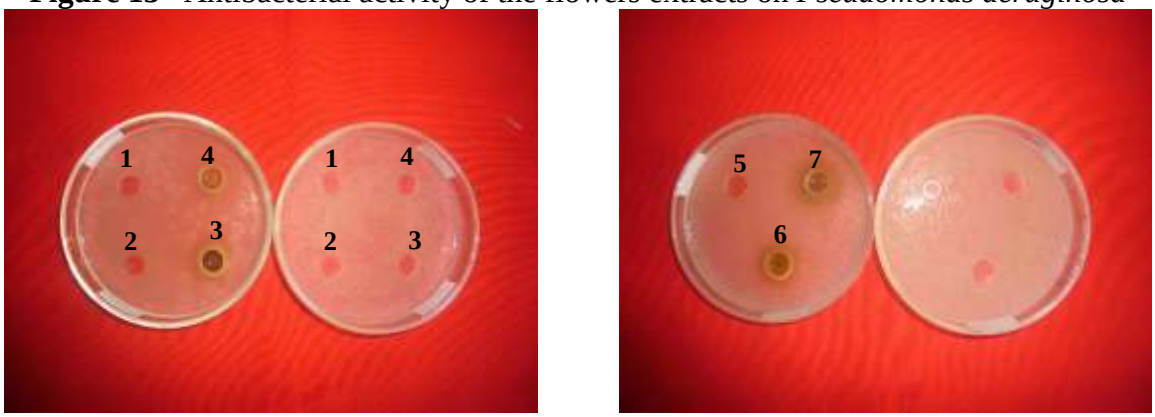

Figure 14 Antibacterial activity of the flowers extracts on *Bacillus pumilus*

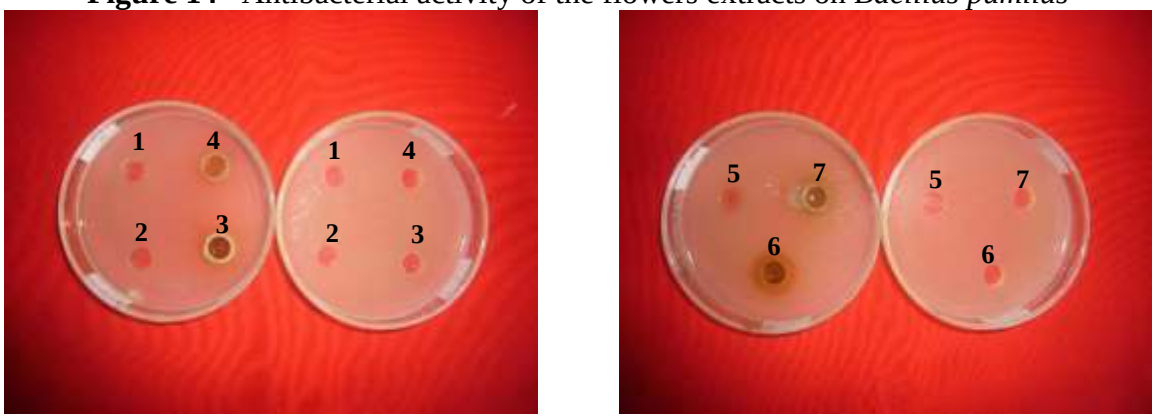

Figure 15 Antifungal activity of the flowers extracts on *Candida albicans*

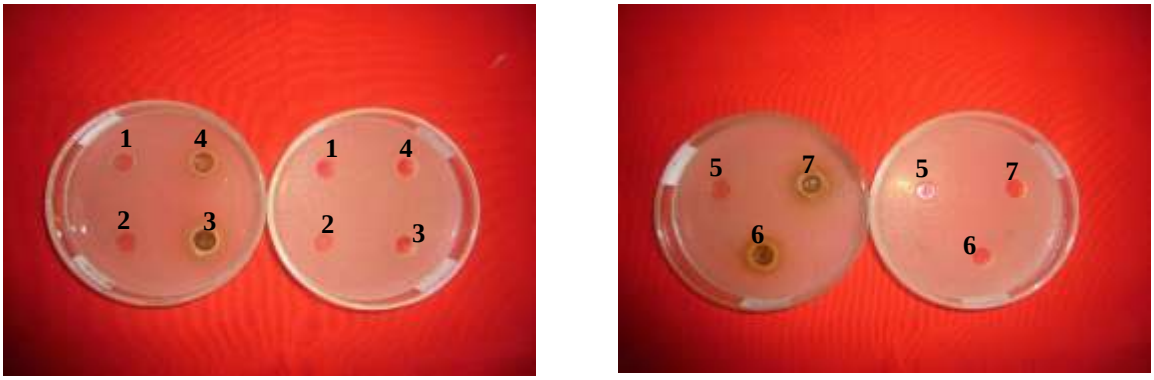

Figure 16 Antibacterial activity of the flowers extracts on *Escherichia coli*

1= pet-ether 2 = chloroform 3 = methanol 4 = acetone 5 = ethyl acetate 6 = ethanol 7 = aqueous

Discussion and Conclusion

In the morphological study, *Careya arborea* Roxb. are deciduous trees with alternate leaves, crowded to wards the end of branches; large and yellowish white flowers in crowded erect spikes; campanulate tube of calyx and oblong elliptic petals; numerous stamens in 3 whorls and connate at the base; inferior ovary with 4 – numerous ovulesin each locule in T.S; style long and simple; large and globose fruit with crowned calyx and numerous seeds immersed in the fleshy pulp. These characters are in agreement with those mentioned by Hooker (1879) and Dassanayake (1981) and Khaliq (2016).

Phytochemical analysis of air dried flowers of *Careya arborea* Roxb. showed the presence of many secondary metabolites of phytoconstituents like alkaloid, glycoside, saponin, phenolic compound, flavonoid, carbohydrate, steroid, terpenoid, tannin and reducing sugar. Cyanogenic glycoside and amino acid are absent. These characters were similar to those of Kapoor *et al*., (1972), Atal (1978) and Shantha (1987).

The results of the physicochemical investigation, the most soluble in aqueous soluble was 14.17%. It was higher than those of other solvents and the least soluble in acetone was 0.68%. Elemental analysis was to determine the amount of elements in *Careya arborea* Roxb. flowers. Potassium is found as principal element.

The antimicrobial activities were tested with six types of microorganisms. Ethanolic extracts of flowers against *Pseudomonas aeruginosa* and showed higher activity as reported early by Ambardar and Aeri (2013).

Plants or plant products use as the therapeutic agents in treating various ailments by virtue of their phytoconstituents (Chalia *et al*., 2009). At present, most of the people including Myanmar have to rely on the herbal medicines as the remedies for various illness. Since ancient times plants have served producer beings as a nutral source of treatments and therapies, among them medicinal herbs have gained attention because of its wide use and less side effects. Although uses of herbal medicines reduce side effects need to assurance of the safety, quality and efficacy of medicinal for plants and herbal products.

In this work, the flowers of the *Careya arborea* Roxb. showed antimicrobial activities. Phytochemical screening of the extracts revealed the presence of phyto-compound such as triterperoids, steroids, flavonoids and tannins as major phyto-constituents with known antimicrobial agents. These phyto-constituents may be responsible for the antimicrobial activity of *Careya arborea* Roxb. (Mahadev and Shailaja, 2015). Therefore, this research will be highlighting the flowers of *Careya arborea* Roxb. for the medicinal uses in order to know the scientifically knowledge for the local people.

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