

## Investigation on Phytochemical Constituents and Antimicrobial Activity of Leaves of *Cayratia trifolia* (Linn.) Domin

Khin Khin Shwe<sup>1</sup>, Cho Cho Win<sup>2</sup>

### Abstract

The present research focused on the investigation of phytochemical constituents and the antimicrobial activity from leaves of *Cayratia trifolia* (Linn.) Domin. The preliminary phytochemical investigation of crude extracts revealed the presence of alkaloids, flavonoids, and glycosides, phenolic compounds, reducing sugars, saponins, steroids, tannins and terpenoids.  $\alpha$ -amino acids, carbohydrates and starch were not found in this sample. According to quantitative elemental analysis, five elements such as Na (10.08 ppm), Ca (8.16 ppm), K (6.45 ppm), Mg (4.29 ppm) and Fe (0.29 ppm) can be detected by AAS method. In the antimicrobial screening, the different crude extracts such as pet-ether, ethyl acetate, ethanol and watery extracts from leaves sample was evaluated on microorganisms such as *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albican* and *Escherichia coli* by employing agar well diffusion method. Ethyl acetate extract showed the highest activities against all microorganisms with the zone of inhibition ranging above 20mm except *Escherichia coli*. The ethanol extract possesses mild activities against all six strains with the ranging from 15-19mm. The watery extract showed the activity on *Pseudomonas aeruginosa* only with the zone of inhibition diameter of 12mm. Pet-ether extracts did not show any antimicrobial activity.

**Keywords:** *Cayratia trifolia* (Linn.) Domin, phytochemical constituents, antimicrobial activity, crude extracts, agar well diffusion method

### Introduction

Medicinal plants are very ancient and true natural medicines which are useful for the treatment of different diseases. They can be used directly or in extracted forms for the management of various ailments due to the presence of various secondary metabolites. The use of plants in the traditional remedy of many other cultures has been widely documented. These plant-based systems continue to play a significant role in health care and it has been projected by the World Health Organization that around 80% of the world's inhabitants rely mainly on traditional medicines for their primary health care, although plant products also play a main role in the health care systems of the remaining 20% of the population mostly residing in developed countries (Sowmyaet al., 2016). Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases. Plants with potent bioactive principles are regarded as components of phytomedicine. Plant based natural constituents were derived from any part of the plant like leaves, bark, roots, fruits, seeds, etc. Many plants contain a variety of phytopharmaceuticals, which have found very important applications in the fields of agriculture, human and veterinary medicine (Sowmyaet al., 2015).

The three-leaf *Cayratia trifolia* (L.) Domin, Family Vitaceae, commonly known as fox grape in English and locally known in the Philippines askalit-*kalit*, is a weak herbaceous climber in thickets and open forests at low altitudes. It is widely distributed in tropical and subtropical Asia, Africa, India, Australia and Pacific islands where its wide-ranging medicinal values are well-documented both in folk medicine and pharmacological (Ragasaet al., 2014). It has been reported to contain a huge amount of bioactive compounds such as yellow waxy oil, steroids, terpenoids, flavonoids and tannins. Stem, leaves, and roots are reported to possess

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<sup>1</sup> Dr., Lecturer, Department of Chemistry, Dagon University

<sup>2</sup> Dr., Professor and Head, Department of Chemistry, Dagon University

hydrocyanic acid and delphinidin. Several flavonoids such as cyanidins are reported in the leaves. Infusion of seeds along with extract of tubers is traditionally given orally to diabetic patients to check sugar level of blood. The whole plant is used in diuretics, tumors, neuralgia and splenopathy (Singh *et al.*, 2012). The paste of tubers is applied on the affected part in the treatment of snake bite. It is reported to possess antiviral, antibacterial, antiprotozoal, hypoglycaemic, anticancer and diuretic activity, etc. Leaf decoction or the juice of the freshleaves is used to cure scurvy in the Philippines, to prevent head itch and dandruff in Java, to relieve inflammation and high fever in Thailand and Peninsular Malaysia while the young leaves are eaten as vegetable in Moluccas. The root is used as an antidote against snake bite, while the stem is reportedly with aphrodisiac property (Sowmya *et al.*, 2016).



**Figure.1** *Cayratia trifolia* (L.) Domin (Man-thone-kwa)

### Materials and Methods

The leaves of *Cayratia trifolia* (L.) Domin were collected from Maha Aung Myae Township, Mandalay Region. Firstly, phytochemical investigation of the leaves sample was carried out by Test Tube method. Then, quantitative elemental analysis of the fruit sample was performed by AAS method. Antimicrobial activity of the different crude extracts was screened by agar well diffusion method against *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, and *Escherichia coli* species in Pharmaceutical Research Department (PRD).

### Results and Discussion

#### (a) Preliminary Phytochemical Results of Leaves of *Cayratia trifolia* (Linn.) Domin

Preliminary phytochemical investigation was performed to examine the different types of chemical constituents present in the powdered sample of *C. trifolia*. The results are shown in Table 1. It was observed that these tests show the presence alkaloids, flavonoids, glycosides, phenolic compounds, reducing sugars, saponins steroids, tannins and terpenoids.  $\alpha$ -amino acids, carbohydrates and starch were not found in the leaves sample.

**Table.1 Results of Phytoconstituents in Leaves of *Cayratia trifolia* (L.) Domin**

No.	Tests		Observation	Remark
			Dragendroff's reagent	Orange ppt. +
1	Alkaloids	1% HCl	Mayer's reagent	White ppt. +
			Wagner's reagent	Brown ppt. +
2	$\alpha$ -Amino acids	H <sub>2</sub> O	Ninhydrin reagent	No purple spot -
3	Carbohydrates	H <sub>2</sub> O	10% $\alpha$ -Naphthol, conc : H <sub>2</sub> SO <sub>4</sub>	No red ring -
4	Flavonoids	EtOH	Mg ribbon, conc: HCl	Pink colour +
5	Glycosides	H <sub>2</sub> O	10% lead acetate solution	White ppt. +
6	Phenolic compounds	EtOH	1% FeCl <sub>3</sub> sol., 1% K <sub>3</sub> Fe(CN) <sub>6</sub>	deep blue solution +
7	Reducing sugars	H <sub>2</sub> O	Benedict's solution	Brick-red ppt. +
8	Saponins	H <sub>2</sub> O	Distilled water	Frothing +
9	Starch	H <sub>2</sub> O	1% Iodine	No blackish blue colouration -
10	Steroids	PE	Acetic anhydride, Conc. H <sub>2</sub> SO <sub>4</sub>	Green colour +
11	Tannins	H <sub>2</sub> O	1% Gelatin, 10% NaCl	White ppt. +
12	Terpenoids	CHCl <sub>3</sub>	Acetic anhydride, Conc. H <sub>2</sub> SO <sub>4</sub>	Pink colour +

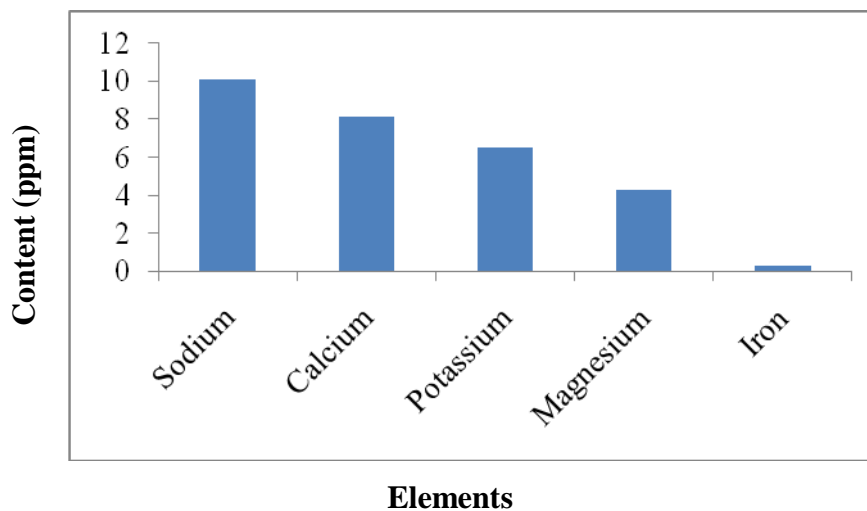
(+) = presence, (-) = absence, (ppt.) = precipitate

### (b) Quantitative Elemental Results of Leaves of *Cayratia trifolia* (Linn.) Domin

Quantitative elemental analysis of Leaves of *C. trifolia* sample was determined by AAS method. According to the elemental analysis AAS data, Sodium was found as the larger amount than others. Sodium helps the body keep fluids in a normal balance and plays a key role in normal nerves and muscle function. Calcium is the role of the teeth and bone to be strong for human and potassium is a very important mineral for the proper function of all cells, tissues and organs in the human body. Potassium is crucial to heart function, plays a key role in skeletal and smooth muscle contraction. Magnesium and iron elements are essential for the metabolism and the growth of human body.

**Table.2 Elemental Results of Leaves Sample**

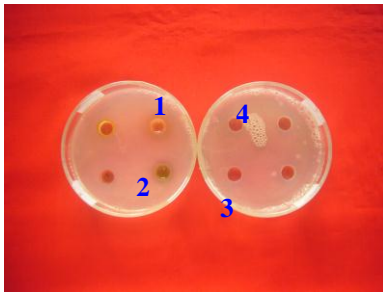
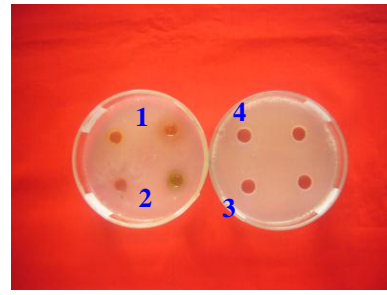
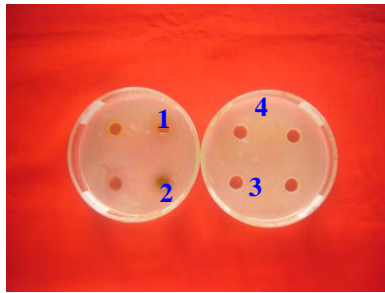
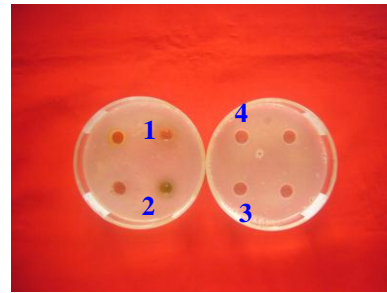
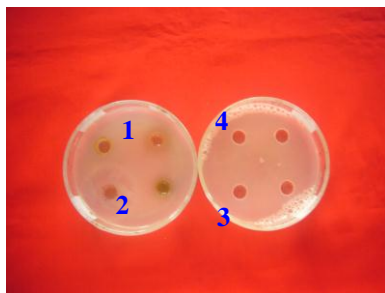
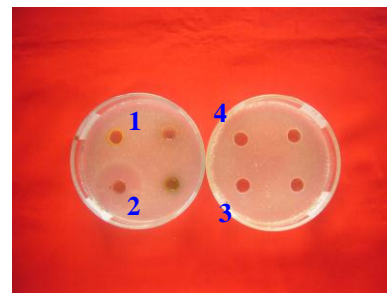
No.	Elements	Concentration (ppm)
1	Na	10.08
2	Ca	8.16
3	K	6.49
4	Mg	4.29
5	Fe	0.29

**Figure.2 Histogram of elemental results data of leaves sample**

### **Antimicrobial Activity of Leaves of *Cayratia trifolia* (Linn.) Domin**

Screening of antimicrobial activity of crude extracts has been done by agar well diffusion method. In the present work, antimicrobial activity of four extracts (PE, EtOAc, EtOH and H<sub>2</sub>O) obtained from leaves of *C. trifolia* sample was determined against on six different strains of microorganisms which include *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albicans* and *E.coli* species. The measurable zone diameter, including the agar well diameter shows the degree of antimicrobial activity.

From the screening, the ethanol and ethyl acetate extracts were effective against all six strains although ethyl acetate extract was observed to possess the highest activities against all microorganisms except *E.coli*. The watery extract showed inhibitory effect against *Pseudomonasaeruginosa* only and other microorganisms were not inactive and also observed that the PE extract of *Cayratia trifolia* Linn. did not showed any antimicrobial activity.

*Bacillus subtilis**Staphylococcus aureus**Pseudomonas aeruginosa**Bacillus pumilus**Candida albicans**Escherichia coli***Anti-clockwise**

1 – PE extract

2 – EtOAc extract

3 – EtOH extract

4 – H<sub>2</sub>O extract

**Figure.3 Antimicrobial activity of various extracts in Leaves of  
*Cayratia trifolia* (L.) Domin**

**Table.3 Inhibition Zone Diameters of Crude Extracts of Leaves of *Cayratia trifolia* (L.) Domin**

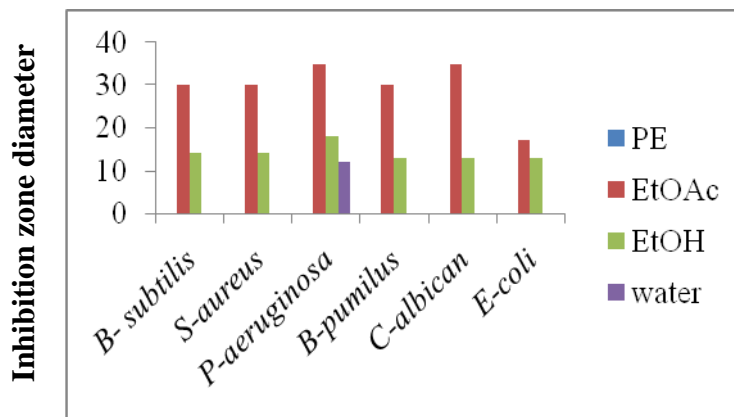
Microorganisms	Inhibition Zone Diameter (mm)			
	PE	EtOAc	EtOH	H <sub>2</sub> O
<i>Bacillus subtilis</i>	-	30	14	-
<i>Staphylococcus aureus</i>	-	30	14	-
<i>Pseudomonas aeruginosa</i>	-	35	18	12
<i>Bacillus pumilus</i>	-	30	13	-
<i>Candida albicans</i>	-	35	13	-
<i>Escherichia coli</i>	-	17	13	-

Agar well - 10mm

10 mm ~ 14 mm      Mild activity

15 mm ~ 19 mm      Medium activity

20 mm above      Highest activity



#### Microorganisms

**Figure 4** Histogram of inhibition zone diameters of different extracts of leaves of *Cayratia trifolia* (L.) Domin

#### Conclusion

From the present work preliminary phytochemical investigation was performed to examine the different types of chemical constituents present in the powdered sample of *C. trifolia*. It observed that these tests showed the presence alkaloids, flavonoids, glycosides, phenolic compounds, reducing sugars, saponins steroids, tannins and terpenoids.  $\alpha$ -amino acids, carbohydrates and starch were not found in the sample.

In addition, the fruit sample contains Na (10.08 ppm), Ca (8.16 ppm), K (6.49 ppm), Mg (4.29 ppm), Fe (0.29 ppm) by AAS analysis. According to the elemental analysis AAS data, Na was found as the larger amount than others. Ca, K and Fe elements are essential for the metabolism and the growth of human body. It is a very important mineral for the proper function of all cells, tissues and organs in the human body.

The antimicrobial activity of crude extracts: petroleum ether, ethyl acetate, ethanol and watery extract of the leaves of *C. trifolia* sample were screened against on six microorganisms by using agar well diffusion method. On antimicrobial screening of the leaves of *C. trifolia*, the various crude extracts were examined on six microorganisms such as *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albicans* and *E. coli* species. From the screening, the ethyl acetate extracts were showed the highest activities against all of the tested organisms *Bacillus subtilis*,



*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus* and *Candida albicans* with the zone of inhibition ranging from above 20 mm. Ethanol extract possessed mild activity against on *Bacillus subtilis*, *Staphylococcus aureus*, *Bacillus pumilus*, *Candida albicans* and *E. coli* with the ranging of inhibition zone from 10-14 mm but medium activity on *Pseudomonas aeruginosa* with the inhibition diameter of 18 mm. The watery extract showed mild activity against on *Pseudomonas aeruginosa* only with the inhibition diameter of 12 mm. The pet ether extract of leaves of *C. trifolia* did not show any antimicrobial activity.

On the basis of above scientific data, leaves of *Cayratia trifolia* (Linn.) Domin (Manthone-kwa) seems to be promoting as the natural products uses and may be utilized for diseases caused by bacteria.

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