

## INVESTIGATION OF *CURCUMA PETIOLATA* ROXB. RHIZOMES FOR THE PRESENCE OF CURCUMIN AND ITS ANTIMICROBIAL ACTIVITY

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### Abstract

The plant *Curcuma petiolata* Roxb., Myanmar name “Marlar” belongs to the family Zingiberaceae. The plants are widely distributed in Myanmar. It was collected from Dawei University Campus, Tanintharyi Region from July to October (2018). In this study, phytochemical properties, curcumin percentage and antimicrobial activity of *Curcuma petiolata* Roxb. rhizomes were investigated. Phytochemical tests of *Curcuma petiolata* Roxb. showed the presence of alkaloid,  $\alpha$ -amino acid, carbohydrate, flavonoid, glycoside, phenolic compound, protein, reducing sugar, saponin, starch, steroid, tannin and terpenoid. The percentage of curcumin content from the rhizomes of *Curcuma petiolata* Roxb. was done at Kayin State Medicinal Plants Resource Center by using HPLC. The yield percentage of curcumin content was 4.3%. Antimicrobial activity of rhizomes of *Curcuma petiolata* Roxb. was carried out at Botany Department, University of Yangon by using different solvent extracts (petroleum ether, chloroform, ethyl acetate, acetone, ethanol, methanol and water). Antimicrobial activity was also investigated on six microorganisms such as *Aspergillus flavus*, *Bacillus subtilis*, *Candida albicans*, *Echerichia coli*, *Pseudomonas fluorescens* and *Xanthomonas oryzae*. The extracts of *Curcuma petiolata* Roxb. rhizomes indicated antimicrobial activity against *Aspergillus flavus*, *Bacillus subtilis*, *Candida albicans*, *Echerichia coli*, *Pseudomonas fluorescens* and *Xanthomonas oryzae*. Among them, chloroform extract showed the most significant antimicrobial activity against *Aspergillus flavus* and acetone and aqueous extracts on *Echerichia coli*. Aqueous extract showed the most prominent antimicrobial activity against *Bacillus subtilis* and *Pseudomonas fluorescens*. Only methanolic extract showed antimicrobial activity against *Candida albicans*. Ethyl acetate extract showed the most powerful antimicrobial activity against *Xanthomonas oryzae*.

**Keyword:** *Curcuma petiolata* Roxb., curcumin percentage and antimicrobial activity.

### Introduction

The plant *Curcuma petiolata* Roxb. belongs to the family Zingiberaceae. This family consists of 50 genera and 1300 species (Heywood, *et al.*, 2007 and Trease and Evans, 2009). The member of this family distributed in South and South East Asia, some species in America and subtropical and warm-temperate Asia (Te-lin and Larsen, 2000).

In Myanmar, this family consists of about 18 genera and 125 species (Hundley and Chit ko ko, 1961). According to the Kress, *et al.*, (2003), genus *Curcuma* contains 24 species of Myanmar are listed in Zingiberaceae. Some members of the Zingiberaceae yield dyes, spices, perfumes, and medicines. Various species are cultivated for their showy flowers (Te-lin and Larsen, 2000 and Heywood, *et al.*, 2007).

The genus *Curcuma* is one of the largest genera in the Zingiberaceae, with about 80 species, and distributed throughout tropical Asia from India to South China, Southeast Asia, Papua New Guinea and Northern Australia. They are grown in wide range of altitudes from 100 –1300m on limestone hills. Generally, most *Curcuma* grows well in loose and sandy soil in shaded areas (Sirirugsa, *et al.*, 2007).

The rhizomes of these species are used in traditional medicines (Perry, 1980). *Curcuma* species possess antioxidant activity and the pharmacological effects. Bioactive components such

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as curcuminoids are responsible for anti-oxidative and anti-inflammatory properties, wound healing, hypoglycemia and antimicrobial activities (Beghel, *et al.*, 2013).

*Curcuma petiolata* Roxb. is one of *Curcuma* species which widely cultivated as an ornamental plant and has long been used as a folk botanical in Asia (Perry, 1980). The *Curcuma petiolata* Roxb. rhizomes extract contain high amount of curcumins with potent DPPH radical scavenging, ferrous reducing power and inhibition of lipid peroxidation activities (Thakam, *et al.*, 2012). Curcumin has a wide range of biological functions, especially the anticancer activity including bladder cancer (Gao, *et al.*, 2012), pancreatic cancer (Plengsuriyakarn, *et al.*, 2012), prostate cancer (Zhou, *et al.*, 2014). Curcumin has also been found to greatly inhibit the metastasis of breast cancer cells. Previous reports have revealed that curcumin can inhibit cell proliferation of chronic granulocytic leukemia (CGL), glioblastoma, and oesophageal cancer through inducing autophagy (Chen, *et al.*, 2014). In this paper, phytochemical test, curcumin percentage and antimicrobial activity of *Curcuma petiolata* Roxb. rhizomes were carried out.

The aim and objectives are to determine the preliminary phytochemical tests and to examine the curcumin percentage and to examine the antimicrobial activities from the different solvent extracts by using on six types of microorganisms.

## **Materials and Methods**

### **Collection, Drying and Pulverization**

The plant *Curcuma petiolata* Roxb. was collected from Dawei University Campus, Tanintharyi Region from July to October (2018). The sample of *Curcuma petiolata* Roxb. rhizomes were thoroughly washed and cut into small pieces and air-dried in room temperature for several days. When constant weight was obtained, the dried samples were pulverized by grinding machine to get powder and stored in airtight containers to prevent from moisture and air-borne contamination.

### **Phytochemical investigation of *Curcuma petiolata* Roxb. rhizomes**

In this investigation, the powdered *Curcuma petiolata* Roxb. rhizomes were tested to find out the presence or absence of chemical constituents such as alkaloid,  $\alpha$ -amino acid, carbohydrate, flavonoid, glycoside, phenolic compound, protein, reducing sugar, saponin, starch, steroid, tannin and terpenoid compounds. Preliminary phytochemical tests were carried out at the Hpa-an University according to the methods of Marini Bettolo, *et al.*, (1981), Central Council for Research in Unani Medicine (1987) and Sasikala and Sundaraganapathy (2017).

### **Determination of total curcumin content**

The percentage of curcumin content from the rhizomes of *Curcuma petiolata* Roxb. were done at Kayin State Medicinal Plants Resource Center. The curcumin content of extract was determined by using HPLC model-L 7300.

### **Antimicrobial activities of different solvent extracts from *Curcuma petiolata* Roxb. rhizomes**

Antimicrobial activities of different solvent extracts of *Curcuma petiolata* Roxb. rhizomes were tested on six pathogenic microorganisms by using paper disc diffusion method at the Department of botany, University of Yangon.

### Preparation of crude extracts

The powdered of *Curcuma petiolata* Roxb. rhizomes were extracted with various solvents such as petroleum-ether, chloroform, ethyl-acetate, acetone, ethanol, methanol and water. The filtrates were evaporated by using water bath.

### Preparation of sample for testing antimicrobial activity

Screening of Antimicrobial activity of crude extracts had been done by paper disc diffusion method. Paper disc having six millimeter diameter were utilized for antimicrobial test. Assay medium was prepared according to the method described by Cruickshank (1975). Assay medium was boiled and 20- 25 ml of the medium was poured into each conical flask, plugged with cotton wool and autoclaved at 121°C for 15 minutes. Then the conical flasks were cooled down to 40- 45°C and each of 0.1- 0.2 ml of test organisms were also added into the flask and then, poured into sterilized petridishes. After solidification, paper disc impregnated with sample were applied on the agar plates and incubated at 37°C for 24 hours. Then the diameter of inhibitory zone was measured with the help of a transparent ruler.

## Results

### Phytochemical investigation of *Curcuma petiolata* Roxb. rhizomes

Preliminary phytochemical tests indicated the presence of alkaloid,  $\alpha$ -amino acids, carbohydrate, flavonoid, glycoside, phenolic compound, protein, reducing sugar, saponin, starch, steroid, tannin and terpenoid of *Curcuma petiolata* Roxb. rhizomes. The experimental results were shown in Table (1).

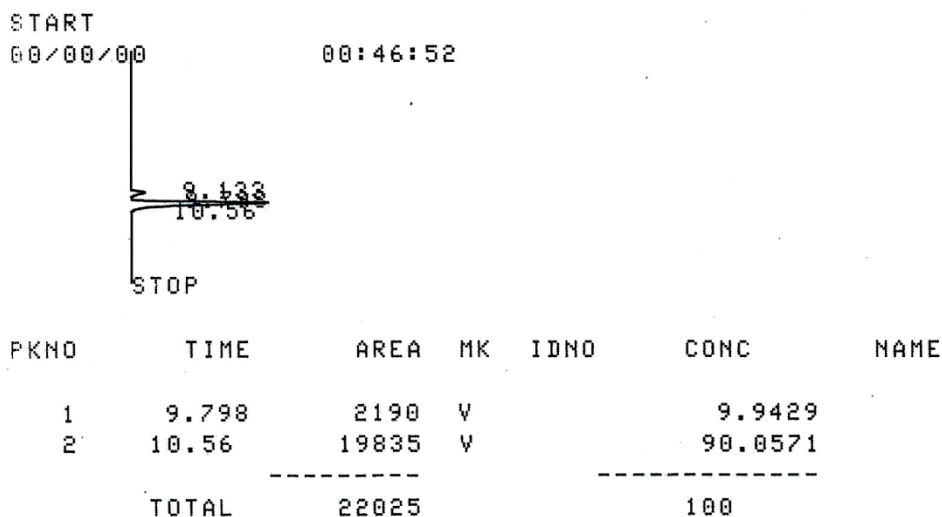
**Table 1** Phytochemical test of *Curcuma petiolata* Roxb. rhizomes

No	Test	Extract	Test reagents	Observation	Results
1.	Alkaloid	EtOH	1. Dragendorff's reagent 2. Mayer's reagent 3. Wagner's reagent 4. Hager's reagent	Orange brown ppt White ppt Reddish brown ppt Yellow ppt	+ + + +
2.	$\alpha$ -amino acids	H <sub>2</sub> O	Ninhydrin reagent	Pink spot	+
3.	Carbohydrate	H <sub>2</sub> O	Benedict's solution	Brick red ppt	+
4.	Flavonoid	EtOH	HCl / Mg	Pink color	+
5.	Glycoside	EtOH	H <sub>2</sub> O + NaOH	Yellow color	+
6.	Phenolic compound	EtOH	H <sub>2</sub> O + 10% FeCl <sub>3</sub>	Green color	+
7.	Protein	H <sub>2</sub> O	Millon's reagent	White ppt turns red on heating	+
8.	Reducing sugar	H <sub>2</sub> O	Fehling's solution A and B	Brick red ppt	+
9.	Saponin	H <sub>2</sub> O	H <sub>2</sub> O	Frothing	+
10.	Starch	H <sub>2</sub> O	Iodine solution	Blue black	+
11.	Steroid	EtOH	CHCl <sub>3</sub> + conc:H <sub>2</sub> SO <sub>4</sub>	Green color	+
12.	Tannin	H <sub>2</sub> O	5% FeCl <sub>3</sub> + H <sub>2</sub> SO <sub>4</sub>	Yellow brown ppt	+
13.	Terpenoid	EtOH	CHCl <sub>3</sub> + conc:H <sub>2</sub> SO <sub>4</sub>	Pink color	+

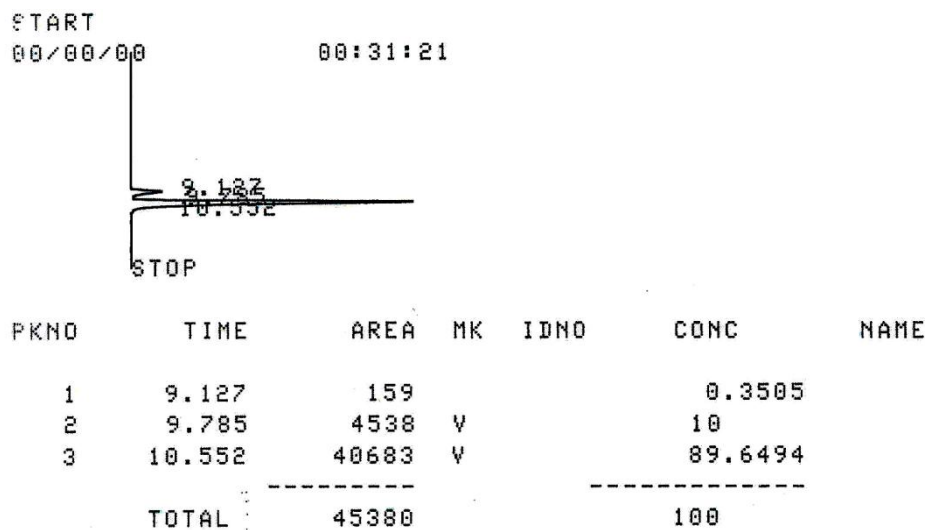
(+) = Present

### Determination of total curcumin content

The curcumin content of extract was determined by using HPLC model-L 7300. The yield percentage of total curcumin was 4.3 %. The experimental results were shown in Figure (1 to 4).



**Figure 1** HPLC chromatogram of curcumin standard 25 ppm



**Figure 2** HPLC chromatogram of curcumin standard 50 ppm

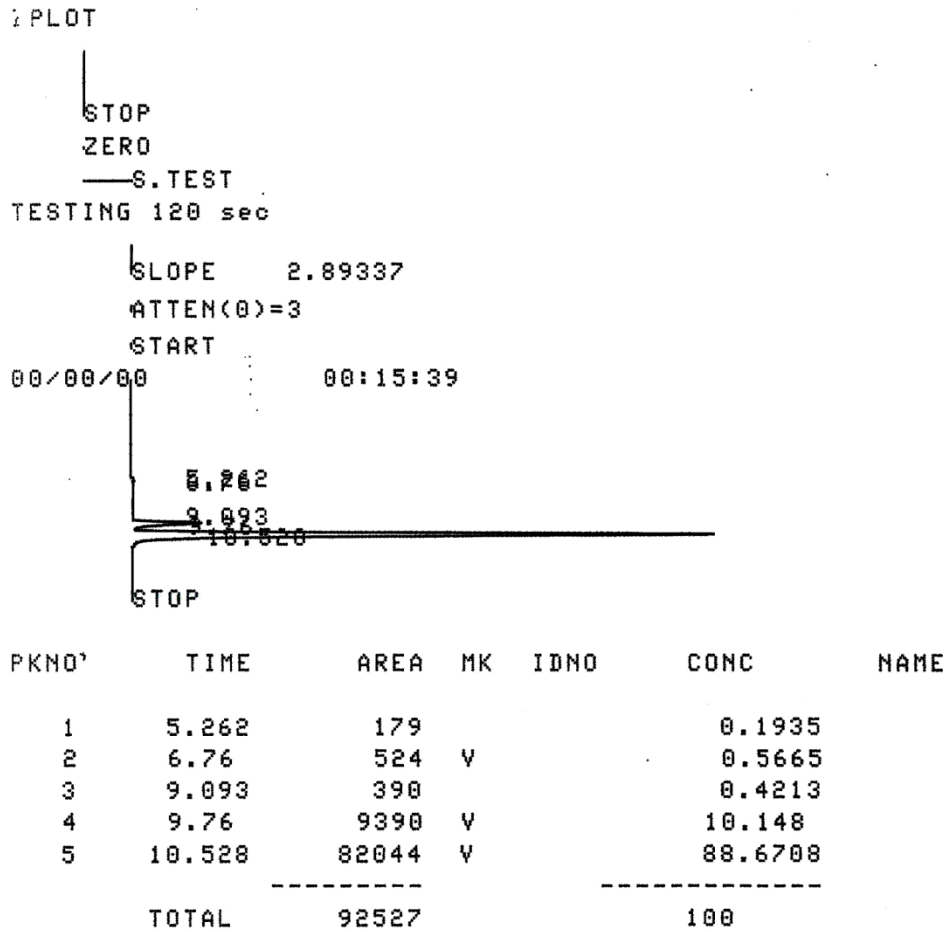


Figure 3 HPLC chromatogram of curcumin standard 100 ppm

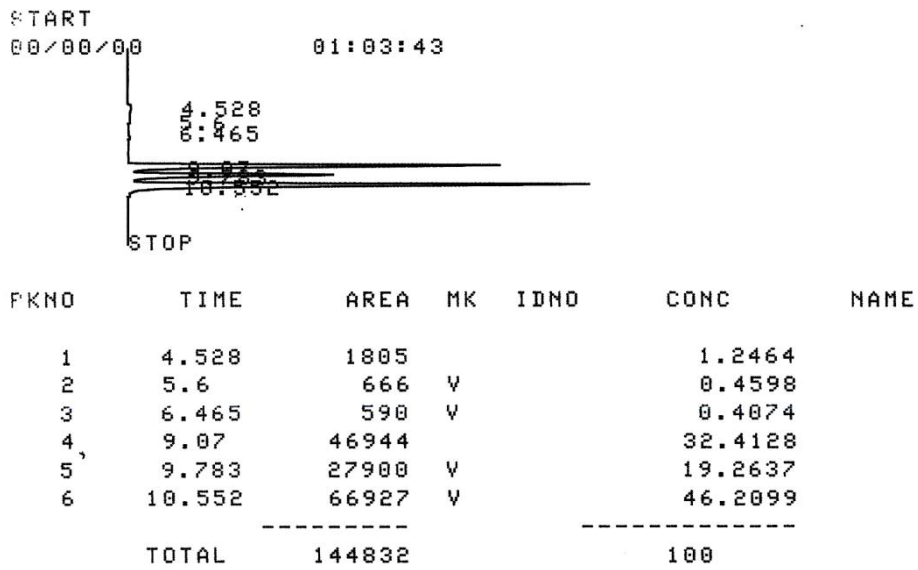


Figure 4 HPLC chromatogram of curcumin from rhizomes extract of *Curcuma petiolata* Roxb.

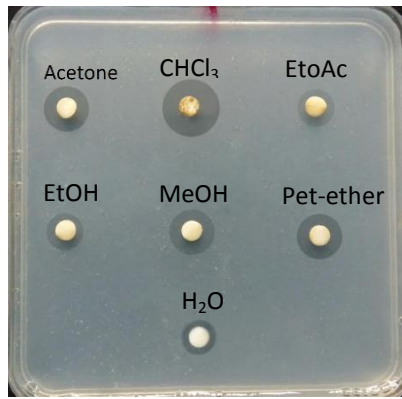
### Antimicrobial activities of different solvent extracts of *Curcuma petiolata* Roxb. rhizomes by using paper disc diffusion method

The powdered of *Curcuma petiolata* Roxb. rhizomes were extracted with petroleum ether, chloroform, acetone, ethyl-acetate, ethanol, methanol and distilled water. The different extracts were tested on six pathogenic microorganisms by using paper disc diffusion method. According to this experiment, all extracts showed antimicrobial activity on *Aspergillus flavus* and *Echerichia coli*. Among them, chloroform extracts showed most significant antimicrobial activity against *Aspergillus flavus* (16mm) and acetone and aqueous extracts on *Echerichia coli* (14mm). Chloroform, ethyl acetate, acetone and aqueous extracts showed antimicrobial activity against *Bacillus subtilis*. Among them, aqueous extract showed the most significant antimicrobial activity against *Bacillus subtilis* (14mm). Petroleum ether, ethanol and methanol extracts did not show on *Bacillus subtilis*. Only methanol extracts showed antimicrobial activity against *Candida albicans* (14mm). Petroleum ether, chloroform, ethyl acetate, acetone ethanol and aqueous extracts did not show on *Candida albicans*. Chloroform, ethanol and aqueous showed antimicrobial activity on *Pseudomonas fluorescens*. Among them, aqueous extract showed the most significant antimicrobial activity against *Pseudomonas fluorescens* (16mm). Petroleum ether, ethyl acetate, acetone and methanol extracts did not show on *Pseudomonas fluorescens*. Chloroform and ethyl acetate extracts showed antimicrobial activity on *Xanthomonas oryzae*. Among them, ethyl acetate extract showed antimicrobial activity on *Xanthomonas oryzae* (18mm). Petroleum ether, acetone, ethanol, methanol and aqueous extracts did not show on *Xanthomonas oryzae*. The results were shown in Table (2) and Figure (5).

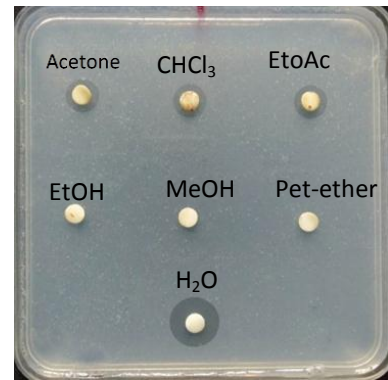
**Table 2 Antimicrobial activities of different solvent extracts from *Curcuma petiolata* Roxb. rhizomes against (6) tested organism**

No	Solvents	<i>A. flavus</i>	<i>B. subtilis</i>	<i>C. albicans</i>	<i>E. coli</i>	<i>P.fluorescen</i>	<i>X. oryzae</i>
1.	Acetone	14 mm	10 mm	-	14mm	-	-
2.	Chloroform	16mm	10 mm	-	12mm	10mm	12 mm
3.	Ethyl acetate	10 mm	10mm	-	10 mm	-	18 mm
4.	Ethanol	10mm	-	-	10 mm	12mm	-
5.	Methanol	14 mm	-	14mm	10mm	-	-
6.	Pet- ether	12mm	-	-	10 mm	-	-
7.	Aqueous	10mm	14 mm	-	14 mm	16 mm	-

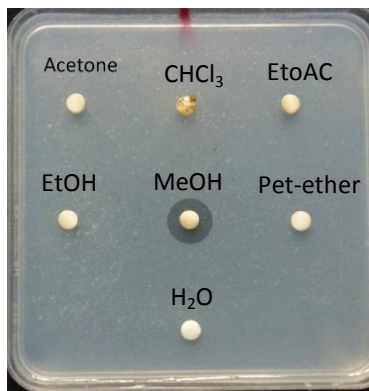
Paper disc size = 6 mm



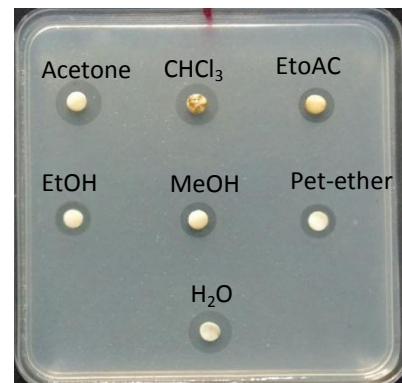
*Aspergillus flavus*



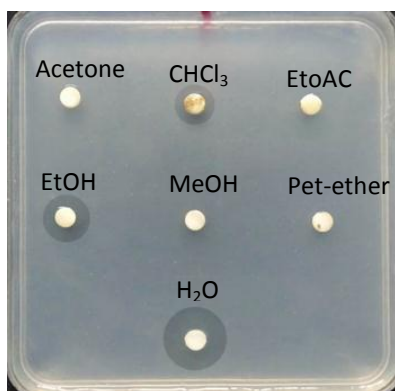
*Bacillus subtilis*



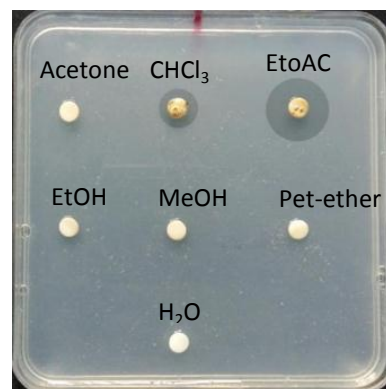
*Candida albicans*



*Escherichia coli*



*Pseudomonas fluorescens*



*Xanthomonas oryzae*

**Figure 5** Antimicrobial activities of *Curcuma petiolata* Roxb. rhizomes

### Discussion

In this investigation, phytochemical test, curcumin percentage and antimicrobial activity of *Curcuma petiolata* Roxb. rhizomes were carried out.

Khin Tar yar Myint, *et al.*, (2018) stated that preliminary phytochemical test of *Curcuma petiolata* Roxb. rhizomes showed the presence of alkaloids,  $\alpha$ -amino acid, carbohydrate, flavonoids, phenolic compound, reducing sugar, steroid and terpenoid.

In this research, the powdered sample of *Curcuma petiolata* Roxb. rhizomes contained alkaloid,  $\alpha$ - amino acid, carbohydrate, flavonoid, glycoside, phenolic compound, protein, reducing sugar, saponin, starch, steroid, tannin and terpenoid.

Thakam, *et al.*, (2012) reported that the curcumin content of rhizomes extract of *Curcuma petiolata* Roxb. was determined by high performance liquid chromatography. The yield percentage of total curcumins content per weight of plant was 13.34 %.

In this research, the curcumin content of *Curcuma petiolata* Roxb. rhizomes extract was determined by using HPLC. The powdered sample of *Curcuma petiolata* Roxb. rhizomes contained 4.3% of curcumin.

The different extracts of *Curcuma petiolata* Roxb. rhizome were tested on six pathogenic microorganisms by using paper disc diffusion method. According to this experiment, all extracts showed antimicrobial activity on *Aspergillus flavus* and *Echerichia coli*. Among them, chloroform extracts showed most significant antimicrobial activity against *Aspergillus flavus* (16mm) and acetone and aqueous extract on *Echerichia coli* (14mm). Chloroform, ethyl acetate, acetone and aqueous extracts showed antimicrobial activity against *Bacillus subtilis*. Among them, aqueous extract showed the most significant antimicrobial activity against *Bacillus subtilis* (14mm). Only methanol extracts showed antimicrobial activity against *Candida albicans* (14mm). Chloroform, ethanol and aqueous extracts showed antimicrobial activity on *Pseudomonas fluorescens*. Among them, aqueous extract showed the most significant antimicrobial activity against *Pseudomonas fluorescens* (16mm). Chloroform and ethyl acetate extracts showed antimicrobial activity on *Xanthomonas oryzae*. Among them, ethyl acetate extracts showed antimicrobial activity on *Xanthomonas oryzae* (18mm).

### Conclusion

The plant *Curcuma petiolata* Roxb. belongs to family Zingiberaceae. *Curcuma petiolata* Roxb. rhizomes contains alkaloid,  $\alpha$ -amino acid, flavonoid, glycoside, phenolic compound, protein, reducing sugar, saponin, steroid, tannin and terpenoid.

The yield percentage of total curcumin content *Curcuma petiolata* Roxb. rhizomes was 4.3 %. Curcumin has a wide range of biological functions, especially the anticancer activity including bladder cancer, glioblastoma, and esophageal cancer, pancreatic cancer prostate cancer. Therefore, *Curcuma petiolata* Roxb. rhizomes possess anticancer activity.

The extracts of *Curcuma petiolata* Roxb. rhizomes indicated antimicrobial activity against *Aspergillus flavus*, *Bacillus subtilis*, *Candida albicans*, *Echerichia coli*, *Pseudomonas fluorescens* and *Xanthomonas oryzae*. Among them, chloroform extracts showed most significant antimicrobial activity against *Aspergillus flavus* and acetone and aqueous extracts on *Echerichia coli*. Aqueous extract showed the most significant antimicrobial activity against *Bacillus subtilis* and *Pseudomonas fluorescens*. Only methanol extracts showed antimicrobial activity against *Candida albicans*. Ethyl acetate extracts showed the most powerful antimicrobial activity against *Xanthomonas oryzae*.

Therefore, extracts of *Curcuma petiolata* Roxb. rhizomes is effective in protecting against bronchitis caused by *Aspergillus flavus*, alimentary tract infection, cardiac infection, sores and inflammation by *Candida albicans*, diarrhoea, dysentery by *Echerichia coli*. Fever, nausea and vomiting and rapid heart rate in human and leaf blight caused by *Pseudomonas fluorescens*. Extracts of *Curcuma petiolata* Roxb. rhizomes can prevent rice blight caused by *Xanthomonas oryzae*. So, *Curcuma petiolata* Roxb. rhizomes is effective on protection of diseases which caused by microorganisms.



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