# Phytochemical Screening, Antimicrobial Activities, Antioxidant Activities and Isolation of Organic Compounds from *Ficus glomerata* Roxb.

## <sup>1</sup>Thidar Khaing, <sup>2</sup>Yinn Kay Khaing, <sup>3</sup>Khin Htay Win

Lecturer Department of Chemistry Mandalay University, Mandalay Myanmar

*Abstract*: One Myanmar indigenous medicinal plant, *Ficus glomerata*, locally known as Ye-Thapan, belonging to the family Moraceae was selected for evaluation of preliminary phytochemical screening, antimicrobial activities, antioxidant activities, elemental composition and isolation of pure compounds. The preliminary phytochemical screening was performed using a standard procedures. Preliminary phytochemical analysis revealed that alkaloid, flavonoid, glycoside, steroid, polyphenol, phenolic compound, reducing sugar and saponin were present in the fleshy part of *Ficus glomerata*. The antimicrobial activities of various extracts were tested against six different microorganisms using the agar well diffusion method in vitro assay. The antioxidant activities of ethanol extract was determined by using 1, 1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging assay. The elemental composition of sample was investigated by Energy Dispersive X-rays Fluorescence (EDXRF) spectral data. Moreover, pure compounds were isolated and purified from ethyl acetate by using thin layer and coloum chromatographic techniques. The prominent functional groups in pure compounds were assigned by Fourier Transform Infrared (FT-IR) spectral data.

Keywords: phytochemical, antimicrobial, antioxidant, EDXRF, FT-IR

## I. INTRODUCTION

The demands of herbal medicines are increasing because their potent pharmacological activity and economical values have been proving to be beneficial for the people [2]. The leaves of this plant are rich in flavonoids, triterpenoids (basically lanosterol), alkaloids and tannins. A new triterpene namely gluanol acetate and racemosic acid were isolated from the same part [10].

Bergenin (flavonoid) was the major component of the stem bark, which was isolated using both the hot and cold water extraction methods. In addition, kaempferol and coumarin were also extracted in a purified form by using the hot water extraction method. The other major components obtained from the stem bark were glycosides, sterols ( $\beta$ -ditosterol, stigmasterol,  $\alpha$ -amyrin acetate, lupeol and lupeol acetate) and tannins (ellagic acid) [1].

The trank bark is rich in various types of sterols like  $\beta$ -sitosterol, lupenol and stigmasterol [10]. Gluanol acetate is the major component of fruits. The other components are glucanol, tiglic acid, taraxasterol, lupeol acetate, friedelin and hydrocarbons. The latex contains various types of steroids such as euphol, isoeuphorbol,  $\beta$ -sitasterol, 4-deoxyphorbol, cycloartenol and cyclloeuphordenol.

From ancient time all of the parts of this plant have been used for their medicinal value. The fruits of this plant are active against leprosy, blood disorders, burns, dry cough, urinary tract infection [6]. The bark has multiple action and is helpful in gynecological disorders. The roots are popularly used for the treatment of hydrophobia [10].

Today, the plant find application in pharmaceutical, cosmetic, agriculture and food industry [3].

## **Botanical Description**

Family Name	-	Moraceae
<b>Botanical Name</b>	-	Ficus glomerata
Hindi Name	-	Fular, Goolar
Local Name	-	Ye-thapan
English Name	-	Cluster fig
Part Used	-	Fleshy Part of Fruit



Figure (1) The fruits of Ye-thapan

#### **II. MATERIALS AND METHODS** Sample collection

The fruits of *Ficus glomerata* were collected from Kyaukse Township, Mandalay Region. The fleshy part of fruits were chopped into small pieces and air dried at room temperature for used throughout the experiment.

## Preliminary Phytochemical Constituents of the Fleshy Part of Ficus glomerata

Phytochemical investigation on the extracts of sample was carried out according to standard procedures and the presence of chemical constituents were identified and each of test was expressed as negative (-) or positive (+) [5].

### Antimicrobial Activities of Crude Extracts of Ficus glomerata

The antimicrobial activities of crude extract sample of the fleshy part of fruit were examined by using agar well diffusion method on six selected microorganisms, at Central Research and Development Centre (CRDC), Insein, Yangon [8], [12].

### Antioxidant Activity of Crude Extracts of Ficus glomerata

The antioxidant activity of the fleshy part of fruit of sensitive plants was done by using DPPH assay method at Department of Chemistry, University of Mandalay. In this experiment, the antioxidant activity of the bark of sensitive plants was done by using DPPH assay method at Department of Chemistry, University of Mandalay. In this experiment, 1, 1-diphenyl-2-picryl hydrazyl (DPPH) powder was used as stable free radical. Ascorbic acid was used as standard antioxidant. Ethanol (Analar grade) was also used as solvent. The absorbance was determined at 517 nm wavelength [11], [7].

### Mineral Contents of Fleshy Part of Ficus glomerata

The elemental composition of fleshy part of **fruits** of *Ficus glomerata* were examined by the Energy Dispersive X-ray Fluorescence (EDXRF) spectrophotometer at Department of Chemistry, Monywa University (SPECTRO XEPOS EDXRF Spectrometer, Germany).

## Extraction and Isolation of Pure Organic Compounds from freshly Part of Ficus glomerata

The sample 600 g was percolated with 95% ethanol 2100 mL for about two months and then filtered and the filtrate was concentrated. The residue was re-extracted with 300 mL of ethyl acetate (EtOAc) and checked by TLC. The EtOAc extract (3.29 g) was separated by column chromatography using silica gel and eluent as n-hexane and ethyl acetate. The pure compound I (pale yellow neddle crystral form) and compound II (yellow oil form) were obtained. The R<sub>f</sub>value of pure compound I is 0.53 (n-hexane:ethylacetate 2:3 v/v) and pure compound II is 0.41(n-hexane: ethylacetate 1:1 v/v).

## Study on FT-IR Spectrum of Pure Compounds

The Fourier Transform Infrared spectrum of compound I and II were measured at Department of Chemistry, University of Mandalay. The FT-IR spectrum informs the prominent functional groups containing the compounds. The infrared spectrum of compound I and compound II were described in Figure.

#### **III. RESULTS AND DISCUSSION**

#### Preliminary Phytochemical Test for Freshly Part of Ficus racemes

Phytochemical test were carried out to detect the presence of organic constituents in the fleshy part of *Ficus glomerata*. The result were tabulted in Table (1).

No	Test	Solvent extract	Test reagent	Observation	Remark
1.	Alkaloid	1%HCL	Dragendorff's Reagent	Pale orange ppt	+
			Wagner's Reagent	Reddish Brown ppt	+
2.	Flavonoid	95% Ethanol	Conc: HCl, Mg turning	Yellow	+
3.	Glycoside	D/W	10% lead acetate	White ppt	+
4.	Steroid	95% ethanol	CHCl <sub>3</sub> , acetic anhydride Conc: H <sub>2</sub>	Green colour	+
			SO <sub>4</sub>		
5.	Tannin	D/W	10% FeCl <sub>3</sub> , dil H <sub>2</sub> SO <sub>4</sub>	-	
6.	Terpene	Pet - ether	Acetic anhydride, CHCl <sub>3</sub> , conc:		
			HC1		
7.	Polyphenol	95% ethanol	1% K <sub>3</sub> [Fe (CN) <sub>3</sub> ]	Greenish blue ppt	+
8.	Phenolic	D/W	10% FeCl <sub>3</sub>	Brown colour	+
9.	Reducing Sugar	D/W	Benedict's Solution	Brick red	+
10.	Saponin	D/W	Shaken	Forth	+

## Table (1) Phytochemical Tests of the Fleshy Part of Ficus glomerata

(+) = Presence, (-) = absence

D/W = distilled water

According to these results, the fleshy part of *Ficus glomerata* glomerata extract consists of alkaloid, flavonoid, glycoside, steroid, , polyphenol, phenolic compound, reducing sugar and saponnin respectively.

## Determination of Antimicronial Activities of the Fleshy Part of Ficus glomerata

The antimicrobial activities of crube sample of the fleshy part of *Ficus glomerata* were examined by using agar well diffusion method at Development Center of Pharmaceutial and food technology, Insein, Yangon. The results are shown in Table (2). Table (2) Antimicrobial Activities of Fleshy Part of *Ficus glomerata* 

1

	Inhibition Zone						
Samples	Solvent	Ι	II	III	IV	V	VI
	n- hexane	+	_	_	+	+	_
Ficus glomerata freshly part of fruit	t EtOAc	+	+	+	+	+	+
	EtOH	+	+	+	+	+	+
Agar well – 10mm Organ	isms						
$10 \text{mm} \sim 14 \text{mm} (+)$ (I) Be	icillus Sublilis						

10mm ~ 14mm (+)	(1) Bacillus Sublilis
15mm ~ 19mm (++)	(II) Staphyolococcus aureus
20mm above (+++)	(III) Pseudomonas aerginosa
	(IV) Bascillus pumilus
	(V) Candida albican
	(VI) E - coli

According to this table (2) n-henane crude extract responds low activities on three tested organism such as *Staphyolococcus aureus*, *Pseudomonas aerginosa* and E - coli. Moreover, ethyl acetate and ethanol extracts show low activities on all tested organisms such as *Bacillus Sublilis*, *Staphyolococcus aureus*, *Pseudomonas aerginosa*, *Bascillus pumilus*, *Candida albican*, and E - coli respectively.

## Determination of Antioxidant Activity of the Standard Ascorbic Acid

Table (3) % Inhibition of Various Concentration of Standard Ascorbic Acid

Sample Concentration(µg/mL)	Mean Absorbance	Mean% inhibition	IC <sub>50</sub> (µg/mL)
200	0.136	79.01	
160	0.157	75.77	12 72
120	0.176	72.83	12.72
80	0.198	69.44	
60	0.218	66.35	]
20	0.392	39.50	

IC50 value was calculated by using linear regressive equation





### **Determination of Antioxidant Activity of Fleshy Part of** *Ficus racemasa* Table (4) % Inhibition of Various Concentration of Sample

Sample concentration (µg/mL)	Mean Absorbance	Mean inhibition	IC <sub>50</sub> (mg/mL)
DPPH alone	0.645		
20	0.188	70.85	]
16	0.201	68.83	7.05
12	0.292	54.72	1
8	0.301	53.33	1
4	0.358	44.19	1

 $IC_{50}$  value was calculated by using linear regressive equation.



Figure (3) Plot of % Inhibition V<sub>3</sub> Concentration of Fleshy part of Ficus glomerata

From the table, the antioxidant activity of the plant of fleshy part of *Ficus glomerata* was determined in DPPH free radical scavenging assay. In DPPH screening assay the IC<sub>50</sub> value of the fleshy part of *Ficus glomerata* was found to be 7.05 mg/ mL. It was very much higher than that of standard ascorbic acid ( $I_{50} = 12.72 \ \mu g/ mL$ ). So the sample extract has lower antioxidant activity than standard ascorbic acid.

### Mineral Contents of Fleshy Part of Ficus glomerata

The mineral contents of fleshy part of Ficus glomerata were determined and the result are show in Table (5).

Table (5) Mineral Contents of Part of Ficus rocemosa

No	Symbol	Element	Amount of Concentration
1.	К	Potassium	1.100%
2.	Ca	Calcium	0.502%
3.	Р	Phosphorous	0.129%
4.	S	Sulfur	0.092%
5.	Fe	Iron	0.012%
6.	Cu	Copper	0.001%
7.	Ti	Titanium	-0.010%
8.	Zn	Zinc	0.001%
9.	Mn	Manganese	0.001%
10.	Rb	Rubidium	0.001%
11	Sr	Strontium	0.001%

From the above data, potassium is the highest amount in the sample.



Figure (4) EDXRF Analysis of Fleshy Part of Ficus glomerata

## Thin- Layer Chromatography of Pure Compound I and II

The pure compound I and II were checked by TLC (using n hexane : ethyl acetate (2:3, v/v) and (1:1/v/v) iodine developer. the  $R_F$  values of these two pare compound were determined. [4]

Compound I				Co	mpound II
Solvent system	=	n-hexane :EtOAc (2:3, v/v)	Solvent system	=	n-hexane : EtOAc (1:1,v/v)
Developer	=	Uv and Iodine	Developer	=	UV and Iodine
Adsorbent R <sub>F</sub> value of	=	Silica-gelplate	Adsorbent	=	Silica-gel plate R <sub>F</sub> value of
Compound I	=	0.53	Compound II	=	0.41
	0			¢	

## Table (6) FT – IR Assignments of Isolated Compound (I)

No	Frequencies (cm <sup>-1</sup> )	Assignment (Functional group)		
1	3015	C-H stretching vibration of sp <sup>2</sup> hydrocarbon		
2	2854.36, 2924.60	Asymmetric and symmetric C-H stretching vibration of sp <sup>3</sup> hydrocarbon		
3	1736.05	C= O stretching vibration of carbonyl group		
4	1640.37	C = C ring skeletal stretching vibration of alkanic benzene ring		
5	1366.34, 1379.23	C – H bending vibration of gem-di methyl group		
6	1241.74	C–O stretching vibration of ester group		
7	1026.66	C–C–O stretching vibration of ether group		
8	980.27	C-H Out of plane bending vibration of trans or E alkenic		



According to FT-IR spectrum, the compound I showed the presence of C –H stretching vibration of  $sp^2$  hydrocarbon,  $sp^3$  hydrocarbon, carbonyl group, C –C ring skeletal stretching vibration of alkanic benzene ring, C–H bending vibration of gem-di methyl group, C-O stretching vibration of ester group C- C-O stretching vibration of ester group and trans or E alkenic.

Table (7)	FT - 1	<b>IR</b> Assignments	of Isolated	Compound (	(III)
			01 1001000	compound (	·/

No	Frequencies (cm <sup>-1</sup> )	Assignment (Functional group)
1	3355.94	OH stretching vibration of alcohol group
2	2853.52,2924.34	Asymmetric and symmetric vibration of sp <sup>3</sup> hydrocarbon
3	1712.31	C = O Stretching vibration of carbonyl group
4	605.35, 516.92	C =C stretching vibration of alkanic group
5	1287.94	C–O stretching vibration of ether group
6	1192.82	C–O–C stretching vibration of ether group
7	942.03	C-H Out of plane bending vibration of trans or E alkenic



According to FT-IR spectrum, the compound II showed the presence of OH functional group,  $sp^3$  hydrocarbon, carbonyl group, C = C stretching vibration of alkanic group, C- O stretching vibration of ether group, C-O-C stretching vibration of ether group and trans or E alkene.

### **IV. CONCLUSION**

In this research work, the crude extract of fleshy part of *Ficus glomerata* gave positive tests for alkaloid, flavonoid, glycoside, steroid, polyphenol, phenolic, reducing sugar and saponin respectively.

According to antimicrobial tests, n-henane extract responds low activities on three tested organism such as *Staphyolococcus aureus, Pseudomonas aerginosa* and *E. coli*. Moreover, ethyl acetate and ethanol extracts show low activities on all tested organisms such as *Bacillus Sublilis, Staphyolococcus aureus, Pseudomonas aerginosa, Bascillus pumilus, Candida albican, and E – coli* respectively.

The antioxidant activity of fleshy part of *Ficus glomerata* was determined in DPPH free radical scavenging assay. In DPPH screening assay the IC<sub>50</sub> value of the fleshy part of *Ficus glomerata* was found to be 7.05 mg/ mL. It was very much higher than that of standard ascorbic acid (I<sub>50</sub> = 12.72  $\mu$ g/ mL). So the sample extract has lower antioxidant activity than standard ascorbic acid.

The elemental composition of fleshy part of *Ficus racemes* were determined by using EDXRF method. The elements are potassium, calcium, phosphorus, sulfur, iron, copper, titanium, zinc, manganese, rubidium and strontium respectively. The highest value of potassium and calcium were observed.

The yield percent of Compound I and Compound II were observed as 0.0039% and 0.027%. The  $R_f$  values of compound I and II are 0.53 and 0.41. The prominent functional groups in pure compound I and compound II were assigned by FT-IR spectrum. According to FT-IR spectrum, the compound I showed the presence of C –H stretching vibration of sp<sup>2</sup> hydrocarbon, sp<sup>3</sup> hydrocarbon, carbonyl group, C –C ring skeletal stretching vibration of alkanic benzene ring, C–H bending vibration of gem-di methyl group, C-O stretching vibration of ester group C- C-O stretching vibration of ester group and trans or E alkenic. The compound II showed the presence of OH functional group, sp<sup>3</sup> hydrocarbon, carbonyl group, C = C stretching vibration of alkanic group, C-O-C stretching vibration of ether group and trans or E alkene.

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