

PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITIES OF *FICUS GLOMERATA* ROXB FRUIT EXTRACTS

KIRANKUMAR SHIVASHARANAPPA, UMESH M K, RAMESH LONDONKAR*

Dept of Biotechnology, Gulbarga University, Gulbarga 585106, Karnataka, India. Email: londonkarramesh53@gmail.com

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ABSTRACT

Objective: The present study designed to evaluate the antibacterial activities of *Ficus glomerata* fruit extracts

Method: Dried fruit powder of *Ficus glomerata* extracted in Soxhlet apparatus using the solvents petroleum ether, chloroform, methanol and water. Preliminary phytochemical and Physico- chemical studies were carried out. The antimicrobial activity of the fruit extract against pathogenic strains evaluated based on the inhibition zone using well diffusion assay, minimum inhibition concentration (MIC) is studied using micro dilution method.

Results: The qualitative phytochemical tests exhibited the presence of common bioactive compounds including alkaloids, terpenoids, flavonoids, saponins and oils as major active constituents. Among the studies conducted against pathogenic microorganisms, the chloroform extract has shown weak activity. Whereas aqueous extract has shown moderate activity and methanolic extract has shown significantly good activity when compared to standard chemotherapeutic agent streptomycin sulphate.

Conclusion: Based on the results we conclude that the *Ficus glomerata* is rich in bioactive principles and exhibits antimicrobial activity against pathogens.

Keywords: *Ficus glomerata* Fruit, Phytochemical analysis, Ash content, Extractive value, MIC.

INTRODUCTION

Ficus glomerata Roxb (Moraceae), commonly known as 'cluster fig', is used widely in Indian folk medicine for the treatment of various diseases [1]. Around 511 species of *Ficus* are present in Asia [2]. One of them is *Ficus glomerata*, which is widely distributed all over India, northern Australia, and other parts of Asia [3]. The fruits of *Ficus* are hollow sphere in shape, its fruit lets have closely lines in the inner wall [4]. Fruits, leaves, bark and latex of the fig tree is used in traditional medicine. The fig tree possesses antibacterial, antifungal, antiseptic and analgesic properties [5]. The *Ficus* genus were analyzed for their constituents and it was found to contain flavonoids, coumarins, alkaloids, steroids, triterpenes and salicylic acid etc [6].

Pathogenic bacteria are the causes of numerous clinical problems across the world. Infectious diseases caused by pathogens are responsible for increased health costs as well as high morbidity and mortality, particularly in developing countries [7]. The increase in the prevalence of multiple drug resistance is necessitated the search for new antimicrobials from alternative or natural sources [8]. One way to prevent the clinical problems arising from infection of pathogens is by using new compounds, which are un-related to the existing synthetic antimicrobial agents [9]. Phytochemicals from medicinal plants showing antimicrobial activities have the potential components of filling this need because their structures are different from those of the more studied microbial sources. Natural products provide clues to synthesize new structural types of antimicrobial and antifungal chemicals that are relatively safe to human [10]. According to the World Health Organization's statement; the traditional healing provides the primary health care needs for a large section (80%) of the population [11]. In India Unani, siddha and Homeopathy prescriptions constitute about 95% of traditional based medicines [12].

Therefore, the present study was formulated in three steps; step I to study physico-chemical and phytochemical profiling of the different extracts of *Ficus glomerata* fruits, Step II includes screening the sensitivity of the pathogenic microbes by agar well diffusion method, step III study includes the calculating of minimum inhibitory concentration using micro dilution method.

MATERIALS AND METHODS

Collection and authentication of plant material

Fresh fruits of *Ficus glomerata* Roxb were collected from Gulbarga University Campus, Gulbarga Karnataka, India, in the month of July,

2012. Plant material was identified and authenticated by the Department of Botany, Gulbarga University, Gulbarga.

Chemicals and Microbial Cultures

All the solvents (analytical grade) used in this experimental work were purchased from Merck, Germany. Standard Antibiotics and Culture media - Mueller-Hinton agar (MHA), Nutrient broth (NB), purchased from Hi-Media Laboratories Mumbai. Standard bacterial cultures of *Escherichia coli* (MTCC 46), *Staphylococcus aureus* (MTCC 96), *Salmonella typhimurium* (MTCC 98), *Enterobacter aerogenes* (MTCC 111) and *Klebsiella pneumoniae* (MTCC 432) were procured from IMTECH, Chandigarh, India.

Preparation of plant extract

Fruits of *Ficus glomerata* plant were shade dried and powdered to mesh. Hot extraction method was employed for extraction of bioactive compounds from fruit powder in soxhlet extraction apparatus using petroleum ether, chloroform, methanol and water as solvents in increasing polarity successively, at the temperature of boiling points of respective solvents and the marc left after methanolic extraction was extracted with boiling distilled water to get aqueous extracts. The concentrated solvent free extract obtained by subjecting the crude extract for slow evaporation in Rota evaporator.

Physico-chemical analysis

The fruit powder was used for analysis of physicochemical studies such as total ash, water-soluble ash and acid insoluble ash values and percentage of extract yielded with respect to different solvents [13].

Preliminary phytochemical screening

All the extracts were subjected to phytochemical tests to find out phytoconstituents present in them [14]. The screened active principles were alkaloids, phenolics, flavonoids, steroids, tannins, Triterpenoids, saponins, carbohydrates, proteins & amino acids, lipids and fats.

Antibacterial assay

Well Diffusion Method

The antibacterial potencies of chloroform, methanol and aqueous extracts were screened using agar-well diffusion method [15]. The

bacterial isolates standardized to 0.5 McFarland in nutrient broth for 18 hr [16]. 100µl of the standardized cell suspension was spreaded on a Mueller-Hinton agar. Wells were punched using a sterile 6 mm cork borer. 50 µl of the crude extract (50 mg/ml) was added into the wells, allowed to stand at room temperature for 1 hr and then incubated at 37°C for 24hrs. The plates were observed for zone of inhibition after incubation period. The effects were compared with standard chemotherapeutic agent streptomycin sulphate (50 mg/ml). 1% Tween 80 was used to dissolve the extracts.

Minimum Inhibitory Concentration

Minimum inhibitory concentration of the extracts was determined in Nutrient broth by micro-dilution method according to the National Committee for Clinical Laboratory Standards [17]. Standardized inoculums (0.1 ml, 10⁶cfu/ml) and the extracts in different concentrations of (25, 12.5, 6.25, 3.12, 1.56 and 0.78 mg/ml) were

taken in test tubes. Test tubes with positive control (Streptomycin), negative control (Methanol) and samples (extracts) incubated at 37°C for 18 h. The lowest concentration that produced no visible bacterial growth compared with the control tubes were regarded as MIC.

RESULTS

Physicochemical analysis

The extractive values of *F. glomerata* different extracts are shown in Fig 1. The Aqueous extract has shown higher amount of yield about 14.50% followed by the Methanol extract 11.30% and chloroform extract 7.10% yield. On the other hand, petroleum ether extract showed lower percentage of yield about 5.20% compared to other solvent extracts. The percentage of acid insoluble ash, water-soluble ash and total ash present in fruits of *Ficus glomerata* is depicted in Fig 2.

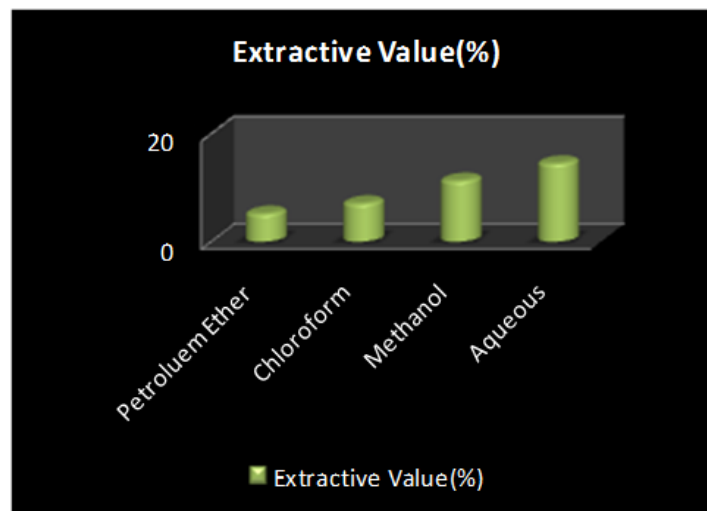


Fig. 1: It shows Extractive value *Ficus glomerata* fruits.

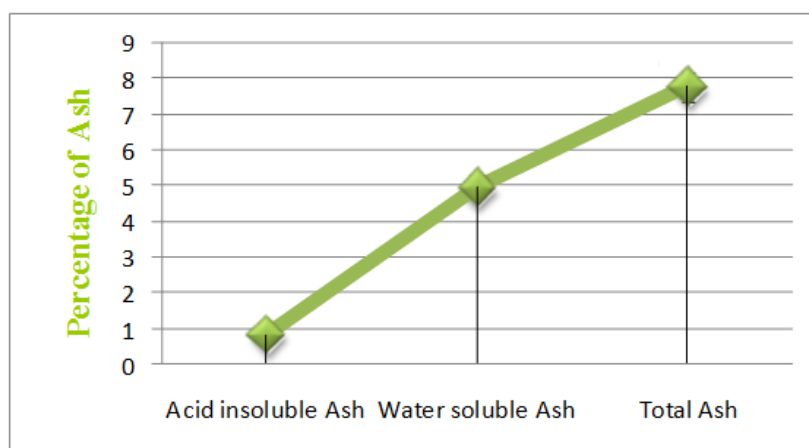


Fig. 2: It shows Ash content analysis of *Ficus glomerata* fruits.

Phytochemical Analysis

Preliminary phytochemical investigation data presented in Table – 1 indicates the presence of considerable amount of secondary metabolites such as alkaloids, saponins, phenolic compounds, tannins, flavonoids, terpinoids and glycosides distributed variably in the plant extracts.

Antimicrobial activity

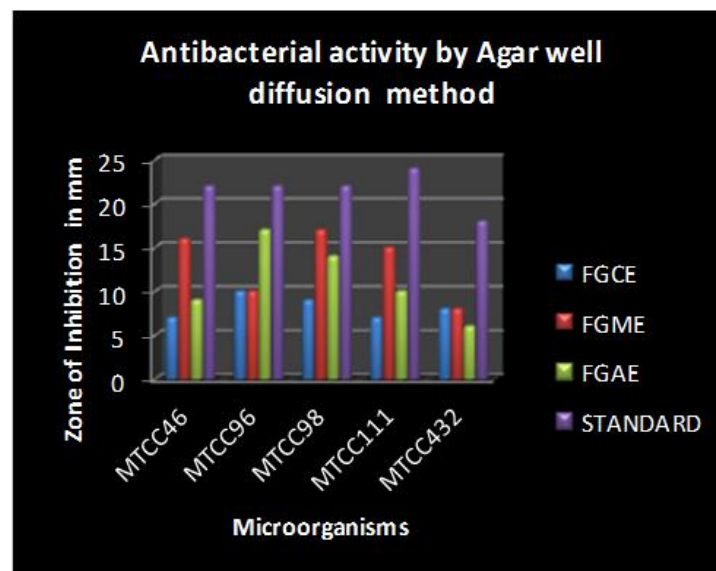
The results of agar well diffusion and minimum inhibitory concentration are shown in Fig 3 and Table 2 respectively, indicates the antibacterial activity against pathogenic organisms exhibited by all three extracts. Comparatively methanolic extract has shown the higher activity followed by aqueous and chloroform extracts.

Table 1: Phytochemical analysis of *Ficus glomerata* crude extracts.

Metabolites	Extracts			
	FGPE	FGCE	FGME	FGAE
Alkaloids	+	+	+	+
Carbohydrates	-	+	+	+
Phenolics	-	-	+	+
Flavonoids	-	-	+	-
Proteins & amino acids	+	+	-	+
Steroids	-	-	+	-
Lipids/fats	+	-	-	-
Saponins	+	-	-	-
Gums & mucilage	+	+	-	-
Triterpenoids	+	-	+	+

(+): Presence of phytoconstituents, (-): Absence of phytoconstituents

(FGPE: *Ficus glomerata* petroleum ether extract, FGCE: *Ficus glomerata* chloroform extract, FGME: *Ficus glomerata* methanol extract, FGAE: *Ficus glomerata* aqueous extract.)

**Fig. 3: It shows Antibacterial activity of *F. glomerata* fruit extracts.**

(MTCC 46: *Escherichia coli*, MTCC 96: *Staphylococcus aureus*, MTCC 98: *Salmonella typhimurium*, MTCC 111: *Enterobacter aerogenes*, MTCC432: *Klebsiella pneumoniae*;) FGCE: *Ficus glomerata* chloroform extract, FGME: *Ficus glomerata* methanol extract, FGAE: *Ficus glomerata* aqueous extract, Standard: Streptomycin sulphate)

Table 2: It shows MIC of *Ficus glomerata* Extracts by micro dilution method.

Organisms	MIC Concentration (mg/ml)		
	FGCE	FGME	FGAE
MTCC 46	6.25	1.56	3.12
MTCC 96	3.12	3.12	6.25
MTCC 98	6.25	1.56	3.12
MTCC 111	3.12	0.78	1.56
MTCC 432	3.12	1.56	0.78

(MTCC 46: *Escherichia coli*, MTCC 96: *Staphylococcus aureus*, MTCC 98: *Salmonella typhimurium*, MTCC 111: *Enterobacter aerogenes*, MTCC432: *Klebsiella pneumoniae*;) FGCE: *Ficus glomerata* chloroform extract, FGME: *Ficus glomerata* methanol extract, FGAE: *Ficus glomerata* aqueous extract, Standard: Streptomycin sulphate)

DISCUSSION

Phytochemical screening of medicinal plants is very important in identifying new source of therapeutically and industrially important compounds. The antimicrobial activities of phenolics and flavonoids have been established in some plants. Flavonoids are becoming the subject of anti-infective research, and some researchers have isolated and identified the structures of flavonoids possessing antifungal, antiviral and antibacterial activity. Previous studies (18,

19 & 20) have reported the presence of alkaloids, carbohydrates, glycosides, proteins and amino acids, phenolic compounds, flavonoids and terpenoids in *Ficus glomerata*. The phytochemicals obtained in this study also correlates with the above report.

In the present study, all the three extracts of *Ficus glomerata* effectively inhibited the tested bacterial strains. The methanol extract has shown antimicrobial activity with zone of inhibition ranging from 12-20 mm, where as aqueous extracts of *Ficus*

glomerata was found to have antimicrobial activity with zone of inhibition ranging from 9-17 mm and the chloroform extract has exhibited least activity among three extracts with zone of inhibition ranging from 9-12mm. The activity of methanol extract was almost equally comparable to the standard chemotherapeutic agent streptomycin sulphate, which ranges from 18-24mm. The inhibitory activity of *Ficus glomerata* extracts against pathogens confirmed the potential use of the plant in the treatment of bacterial diseases. Presence of different chemical compounds in the extract impart significant amount of biological activities of *F. glomerata* thus proving its medicinal value.

CONCLUSION

In the present work, an attempt to assess the status of phytochemical properties in fruits of *Ficus glomerata* for pharmaceutical studies is undertaken. Phytochemical studies reveal that the medicinal plant *Ficus glomerata* appear to be rich in secondary metabolites. The antimicrobial property of fruits of *Ficus glomerata* can be attributed to the presence of high phenolics and flavonoids compounds and their individual or synergistic effect present in the extract. The obtained results of this plant fruit are supportive to carry out further purification, characterization and pharmacological studies of these active principles.

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