

RESEARCH ARTICLE

EVALUATION OF PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF *FICUS AURICULATA* LOUR. (MORACEAE) – AN TRADITIONAL MEDICINAL PLANTRekka Raja^{1,*}, Nirubama Kumar² and Moorthy Duraisamy¹¹Department of Botany, Kongunadu Arts and Science College, Coimbatore - 641029, Tamil Nadu, India.²Department of Biochemistry, Kongunadu Arts and Science College, Coimbatore - 641029, Tamil Nadu, India.

ABSTRACT

Ficus auriculata is a huge tropical, deciduous and evergreen tree is cultivated in India for its edible fruits and also this plant fruits have been used for the treatment of diabetes, asthma, male and female infertility by Malayali tribals in Yercaud hills. The main aim of this study was to screening of phytochemical properties of the fruit of this plant and also evaluated their potency. The investigation of phytochemical has been done by chemical tests and using some chemical reagents and it showed the presence of various classes of compounds such as carbohydrates, glycosides, phenolic compounds and Tannins, alkaloids, proteins and amino acids, flavonoids, saponins, terpenoids, phytosterols and fixed oils and fat and absence of Anthraquinones. This study summarizes the information concerning the bioactive constituents present in methanol fruit extract which may be responsible for various therapeutically effects. Phenol content of the fruit extract was 1.03mg/g dr.wt, flavonoids content of the fruit extract was 0.64mg/g dr. wt., and alkaloids content was 0.15 mg/g dr.wt. The antibacterial activity of the extracts was established by disc diffusion method and the extract showed a clear zone of inhibition against *Proteus vulgaris*, *Staphylococcus epidermidies*, *E. coli*, *Klebsiella pneumonia*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium* and *Pseudomonas aeruginosa*. The methanolic fruit extract of the *F. auriculata* showed a wide range of activity against all the bacterial studied. The zone of inhibition increased with the increase in concentration. Highest activity was seen in *Mycoplasma genitalium* and *Staphylococcus epidermidies* in a concentration of 60 µg. the results provide justification for the use of *F. auriculata* in folk medicine to treat various infectious disease.

Keywords: *Ficus auriculata*, methanol fruit extract, bioactive constituents, antibacterial activity.

1. INTRODUCTION

Plants have been the most important source of medicines by human for the treatment of various diseases for more than 60 thousand years ago. As of record about 20,000 plant species are used for medicinal purposes across the globe and around 70 % of them are from Indian subcontinent [1]. In recent times, focus on medicinal plant research has increased all over the world and large body evidence has collected to shown enormous potential of medicinal plants used in various traditional systems. Medicinal potential of these plants lies in bioactive phytochemical constituents such as alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins, phenolic compounds to act as an defense system against diseases or more accurately, to protect against diseases [2].

Ficus auriculata is a huge tropical, deciduous and evergreen tree is cultivated in India for its edible fruits. Various parts of this plant such as bark, root, leaves, fruit seed and latex are frequently used for the treatment of various illnesses, particularly this plant fruit have been used for the treatment of diabetes, asthma, male and female infertility by Malayali tribals in Yercaud hills [3]. *Ficus* species are rich source of polyphenolic compounds, flavonoids which are responsible for strong antioxidant properties that help in prevention and therapy of various oxidative stress related diseases such as neurodegeneration and hepatic diseases [4,5].

The present study is aimed at preliminary phytochemical screening of the methanolic fruit extract of *Ficus auriculata* and evaluation of the same for potential antibacterial activity. It efficacy as

antibacterial activity will open new avenues to scrutinize rich natural resources for further analysis in order to develop the potential of herbal medicine. Such screening and scientific validation may provide the basics for developing novel antibacterial agents without possible side effect. These can be expected to be used on a large scale as their cost and availability will pose no problem and there will be no limitation factor as in case of synthetic drugs.

3. MATERIALS AND METHODS

2.1. Collection and authentication of plant material

The plant materials were collected from Yercaud hills of Salem district, Tamil Nadu. The plant specimens were verified with Botanical Survey of India (BSI), Coimbatore, Tamil Nadu, India (BSI/SRC/5/23/2016/Tech/832).

2.2. Preparation of plant powder

The fruits were washed thoroughly with tap water, shade dried, homogenized to fine powder and stored in air tight bottles for further studies.

2.3. Preparation of Extraction

The dried fruit powder of *F. auriculata* was subjected to methanolic extraction in the ratio of methanol : water as 80:20, adopting Soxhlet method. The extract was concentrated under few reduced pressure to yield semisolid mass which was dried in a desiccators and stored properly for further study.

2.4. Preliminary phytochemical screening

2.4.1. Quality analysis

The quality analysis was carried out on the methanolic extracts of fruit of *Ficus auriculata* to determine the presences of various phytochemical constituents as per the standard protocol [6].

2.4.2. Quantitative estimation of chemical constituency

The total alkaloids content is estimated by the method of Anonymous [7]. The total phenolic content is tested by using Folin-Ciocalteu reagent by the method of Sidduraj and Becker [8]. The total flavonoids content is determined using the procedure described by Jia *et al.* [9].

2.5. Antibacterial Assay of methanolic fruit extract of *Ficus auriculata* Lour.

The antibacterial potential of methanolic fruit extract of *F. auriculata* Lour. was estimated by disc diffusion method. The disc diffusion is a simple

and reliable test to find out the effect of a particular substance on a specific bacterium.

2.5.1. Source of Microbial Strains

The strains of common pathogenic microorganisms were used in this study such as *Proteus vulgaris*, *Staphylococcus epidermidies*, *E. coli*, *Klebsiella phemoniae*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium* and *Pseudomonosa aeruginosa*. All the bacterial cultures were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India. The young bacterial broth cultures were prepared before the screening procedure.

2.5.2. Preparation of Muller Agar Media:

38g of Muller Hinton agar was dissolved in 1000ml of glass water. The pH was adjusted to 7 and autoclaved for 30 minutes in 15lb pressure.

2.5.3. Preparation of Culture Plates

20ml of sterile Muller Hinton agar medium was poured into petriplates under sterile condition and kept in laminar air flow chamber for solidification. After solidification the plates were dried for 30minutes in an oven to remove excess of moisture from the surface.

2.5.4. Preparation of Inoculums

Nutrient agar	- 1gm
Bacteriological peptone	- 0.5gm
Sodium chloride	- 0.25gm
Distilled water	-100ml

The above components were dissolved one by one in 100ml of glass distilled water and the pH was adjusted to 7. 10ml of medium was poured into test tube and the mouth of the tube was covered with sterile cotton. The test tubes were autoclaved for 30minutes in 15lb pressure. After autoclaving the test tubes were cooled in laminar air flow chamber and selected microorganisms were inoculated into the medium separately. The tubes were incubated overnight in 37°C and used for inoculation.

2.5.5. Inoculation

The test microorganisms were inoculated in nutrient agar medium by spread plate method. About 10 µl (10⁶ cells/ml) of nutrient broth of overnight bacterial cultural was spread evenly on the solidification medium. Sterile cotton swabs were dipped separately into inoculums of organisms and swabbed inside the wall of the tubes. The agar surface of the plates was streaked in three directions by turning the plates to 60° angle between each

streaking. The lid of the petriplates was on and kept at room temperature for 5-10 minutes to get confluent growth for accurate results.

2.5.6. Preparation and Application of Disc

Sterile discs (Hi Media) of 6mm were used to load the plant extract. Various concentration of extract such as 30, 40, 50, 60 mg were dissolved in Dimethyl Sulfoxide (DMSO) and loaded in the discs. The standard antibiotic generation was used as a control due to its broad spectrum of activity against various organisms.

The impregnated discs were incubated at 37°C for an hour. The dried discs were placed over the surface of swabbed medium with equal distance to avoid the overlapping of the zones of inhibition. The discs were gently pressed on the surface of the medium and they were placed at least 25mm away from the edge.

2.5.7. Incubation

The plates were incubated at 37°C for 16-18 hours in an incubator.

2.5.8. Measurement of Zone Inhibition:

The diameter of the zone of inhibition was measured in mm at the end of incubation period of 18 hours and recorded. Each experiment was done in triplicate.

2.5.9. Determination of Activity Index (AI):

The activity index of the crude plant extract was calculated by comparing the mean value of the extracts with the mean value of zone of inhibition of standard antibiotic, using the following formula,

$$\text{Activity index (AI)} = \frac{\text{Zone of inhibition of extract.}}{\text{Zone of inhibition of Standard antibiotic drug.}}$$

3. RESULTS AND DISCUSSION

3.1. Preliminary phytochemical screening of Methanolic fruit extract of *Ficus auriculata* Lour.

The preliminary phytochemical screening revealed the presence of carbohydrates, glycosides, phenolic compounds and Tannins, alkaloids, proteins and amino acids, flavanoids, saponins, terpenoids, phytosterols and fixed oils and fat. The detailed results of the analysis are given in Table 1. Ritu Mishra and Ashok Kumar Tiwari [10] reported that the preliminary phytochemical screening of extracts of *Ficus racemosa* Linn observed the presence of Carbohydrate, tannin, protein, resin and saponin whereas Shivani *et al.*, [11] reported that

alkaloids, tannin, flavonoids, carbohydrates, terpenoids are present in leaves; flavonoids, carbohydrates, terpenoids in root and alkaloids, tannin, flavonoids are present in bark of *Ficus retusa* Linn. However alkaloids, glycosides, flavonoids, saponins, carbohydrates, phenolic compounds and tannin and steroids are found to be present in *Morus alba* as identified by Shikha Srivastava *et al.* [12]. While, Jasreet [13] investigated that alkaloids, carbohydrate, tannins, flavonoids, saponin, glycosides, steroids and triterpenoids are present in preliminary phytochemical investigation of *Ficus pumila* leaves. As in other plant of Moraceae *F. auriculata* contains alkaloids, flavonoids and phenolic compounds that may enhance the medicinal property as reported in other plants observe by Ajayi *et al.* [14]. Carbohydrates, alkaloids, saponins, resins, phenols, protein and aminoacids present in hexane, chloroform and methanol bark extract of *F. auriculata* [15] and a similar finding was also observed in our present study. The medicinal value of the plant depends on the phytochemicals such as alkaloids, flavonoids, phenolic and other nutrients like amino acids and protein [16]. Based on the earlier reports and the present study, we propose that the active principle in *F. auriculata* because of having these phytoconstituents.

3.2. Quantitative phytochemical analysis

The phytochemicals present in the plant plays an important role in biological studies. The quantities analyzed by phytochemical analysis in *Ficus auriculata* Lour was given in table 2. Phenol content of the fruit extract was 1.03mg/g dr.wt, flavonoids content of the fruit extract was 0.64mg/g dr.wt, and alkaloids content was 0.15 mg/g dr.wt. The estimation of phytochemicals revealed that the quantities of secondary metabolites like phenol (1.03 mg/g dr. wt), flavonoids (0.64 mg/g dr.wt) and glycosides (0.61 mg/g dr.wt) were higher proportion. These results expose that the plant has quite a number of chemical constituents, which may be responsible for many pharmacological actions.

3.3. Antibacterial Assay of methanolic fruit extract of *Ficus auriculata* Lour.

The antibacterial activity of the extracts was established by disc diffusion method. The methanolic fruit extract of *Ficus auriculata* were active against seven different bacteria. Four concentrations of the extract were used (30, 40, 50 and 60 µl). The methanolic fruit extract showed a

Table 1. Qualitative phytochemical analysis of methanol extracts of *F. auriculata* Lour.

S. No	Constituents	Test	Colour	Reaction
1	Carbohydrates	Molisch's test	Violet	++
		Felhing's test	Brick red	+++
		Benedict's test	Red	+++
		Selivanoff's test	Cherry red	+++
2	Glycosides	Legal's test	Blood red	+++
		Keller-Killiani test	Blue	+++
		Conc. H ₂ SO ₄	Red	-
		Borntrayer's test	Pink	++
3	Phenolic compound and Tannins	Ferric chloride test	Violet precipitate	+++
		Lead acetate test	White precipitate	+++
		Elagic acid test	Niger brown	++
4	Alkaloids	Mayer's test	White precipitate	-
		Dragendroff's test	Reddish brown precipitate	+++
		Wanger's test	Reddish brown precipitate	+
		Hager's test	Yellow precipitate	+
5	Proteins and free amino acids	Million's test	White	+++
		Biuret test	Violet	+++
		Nihydrén reagent test	Violet	+++
		Xanthoproteic test	Orange	++
6	Flavanoids	Ferric chloride test	Blackish red	+++
		Conc. H ₂ SO ₄	Yellow	+
		Alkaline reagent test	Yellow	+++
		Fluorescence test	Fluorescence green	+++
7	Saponins	Foam test	Foam	+
8	Terpenoids and Steroids	-	Green bluish	+++
9	Phlotannins	-	Red precipitate	+++
10	Phytosterols	Salkowasi test	Red brown	++
11	Fixed oils and fats	Filter paper test		-
		Saponification test	Formation of soap	+++
12	Anthraquinones	-	Pink to rose	-

Table 2. Quantities analysis of fruit powder of *F. auriculata* Lour.

S. No	Name of the phytochemical content	Quantity mg/g (Dry weight)
1	Phenol	1.03
2	Flavonoids	0.64
3	Alkaloids	0.15

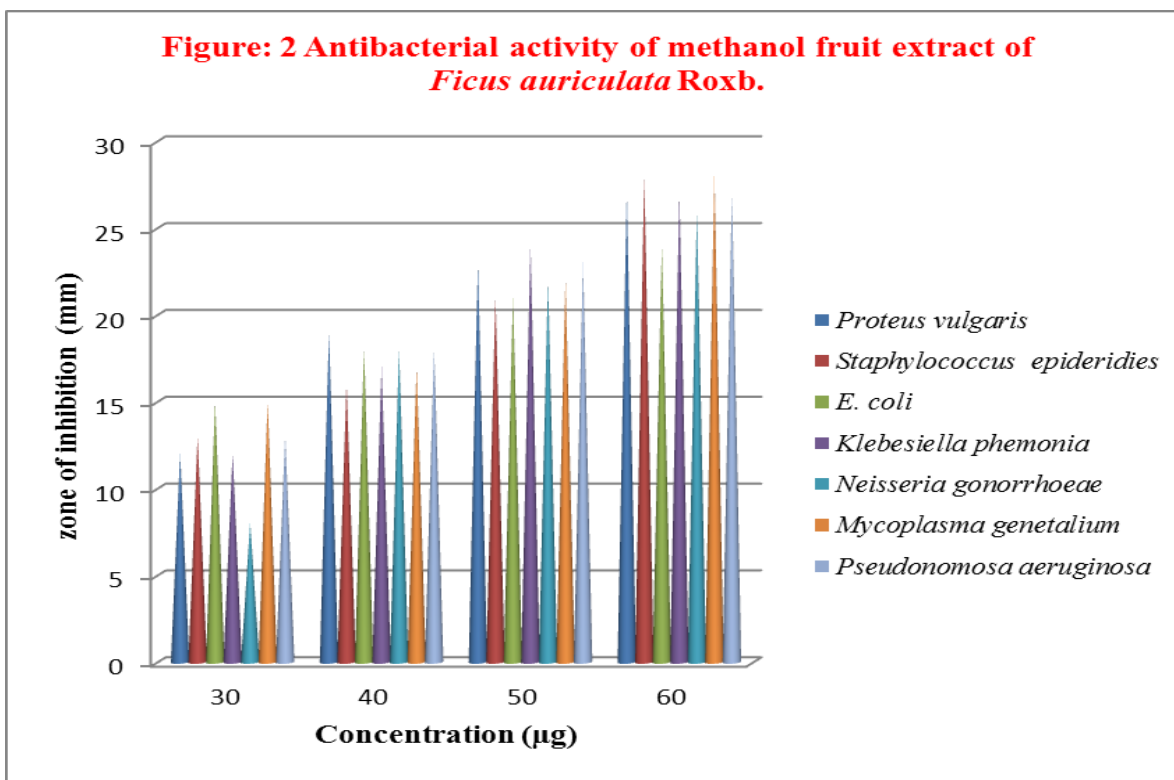
Table 3. Antibacterial activity of methanol fruit extract of *Ficus auriculata* Lour.

S. No	Organism Name	Control (Amoxicillin)	Concentration(μ g)			
			30	40	50	60
Zone of Inhibition (mm)						
1.	<i>Proteus vulgaris</i> (MTCC No: 1771)	17	12	19	23	27
2.	<i>Staphylococcus epidermidies</i> (MTCC No: 435)	16	13	16	21	28
3.	<i>E.coli</i> (MTCC No: 443)	17	15	18	21	24
4.	<i>Klebsiella phemonia</i> (MTCC No: 109)	21	12	17	24	27
5.	<i>Neisseria gonorrhoeae</i> (MTCC No: 19424)	17	08	18	22	26
6.	<i>Mycoplasma genitalium</i> (MTCC No: 2288)	16	15	17	22	28
7.	<i>Pseudonomosa aeruginosa</i> (MTCC.No: 2488)	14	13	18	23	27



Figure 1. *Ficus auriculata* Lour - Fruit

Figure: 2 Antibacterial activity of methanol fruit extract of *Ficus auriculata* Roxb.



clear zone of inhibition against *P. vulgaris*, *S. epidermidies*, *E. coli*, *K. phemonia*, *N. gonorrhoeae*, *M. genetalium* and *P. aeruginosa*. The methanolic fruit extract of the *F. auriculata* showed a wide range of activity against all the bacteria studied. Methanolic fruit extract showed significant antibacterial activity as compared to standard antibiotics (amoxicillin). The zone of inhibition increased with the increase in concentration as stated in table 3; figure 2). Among the various microorganisms, the methanolic fruit extract of *F. auriculata* was more active against *Mycoplasma genetalium* (28 mm) and *Staphylococcus epidermidies* (28 mm) in concentration 60 µg and lowest effect in *E. coli* (24 mm) in concentration 60µg.

There is an influence of certain microbial infection on male infertility. Several investigators have reported difference types of microorganisms in seminal fluid, Oligospermia and azospermia are most common causes of male infertility which has been reported due to bacterial infections [17]. Based on this information, the above microorganisms were selected for this study and

also Ali Hussein Al-Marzoqi *et al.* [18] identified *P. vulgaris*, *S. epidermidies*, *E. coli*, *K. pneumonia*, *N. gonorrhoeae*, *M. genetalium* and *P. aeruginosa* in seminal fluid and associated with male infertility. Antibacterial assay revealed, the methanolic fruit extract of *F. auriculata* having capacity to control these bacterial infections. The antibacterial property was claimed to be conferred by phytochemicals present in the plant. Tannins and flavonoids have been reported to inhibit the growth of many fungi, yeast, bacteria and viruses [19], alkaloids widely well known to have anti diabetic and antimicrobial activity [20], terpenoids, steroids and saponins may also responsible for the antibacterial activity [21]. Methanol fruit extract of *F. auriculata* showed the presence of these compounds in preliminary phytochemical screening.

4. CONCLUSION

The present study concludes with the pharmacological standards of the *F. auriculata* Lour which helps in the identification and quality assessment of these plants. The pharmacological study established the antibacterial efficacy of the

extracts. The phytochemical studies on the *F. auriculata* revealed the presence of some phenol, flavonoids and other terpenoids in these extracts may be responsible for the pharmacological activity of the extract. The findings of phytochemical and pharmacological studies support the traditional knowledge of Malayali traditional healers and also support folkloric usage of the *F. auriculata* Roxb plant. These findings will take the drug research to the next level in upcoming years.

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