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STUDIES ON ANTIBACTERIAL ACTIVITY AND PHYTOCHEMICAL SCREENING OF *FICUS RACEMOSA*

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ABSTRACT

In the present study of antimicrobial activity of *Ficus racemosa* Leaves, Bark, Fruits explored against different pathogens. Antimicrobial component were extracted by solvent extraction where in methanol, ethanol, ethyl acetate, d/w and hot d/w extracts were prepared. The antibacterial nature of extracts was assessed by agar well diffusion methods. Then the methanol, ethanol, ethyl acetate extracts of *Ficus racemosa* leaves and bark were found most effective against pathogens that were used. In leaves Ethanol extract, the highest zone of inhibition was found of 32.5milimeter against *Pseudomonas aeruginosa.* In bark Ethanol and ethyl acetate extract, the highest zone of inhibition was found of 21.5milimeter against *Escherichia. coli* and *Staphylococcus aureus*. Phytochemical screening was carried out according to standard procedures. Alkaloids, Carbohydrates, Phytosterols, Saponins, Phenol, Flavonoids, Proteins and amino acids were found to be present in different extracts prepared by different solvent.

Key words: Bacterial activity, Antibiogram analysis, Agar well diffusion, Solvent extraction,

INTRODUCTION

Medicinal plant represents a rich source of antimicrobial agent. Medicinal plants have been identified and used throughout human history. Plant that having the curing properties called as medicinal plants. Plants are used medicinally in different countries and are a good source of many potent and powerful drugs. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform biological functions and to defence against attack from predators such as insects, fungi, herbivorous mammals **[1]**. The different part used include roots, stem, flower, fruits, twigs exudates and modified plant organs. While some of these raw drugs are collected in smaller quantities by the local communities and folk healers for local uses, many other raw drugs are collected in larger quantities and traded in the market as the raw material for many herbal industries. At least 12,000 such compounds have been isolated so far, a number estimated to be less than 10% of the total **[4]**.

Chemical compounds in plants mediate their effects on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs **[2,3].**

Ficus racemosa linn is studied to be a traditionally used medicinal plant. So it is chosen for determining its Antimicrobial activity.

HERBAL ANTIMICROBIALS

The use of herbal antimicrobials is because of fewer side effects. Natural antimicrobial compound in plants have been found to possess antimicrobial activity. In addition, the antimicrobial property of medicinal plant may differ depending on the forms of added plants such as fresh dried or extracted forms. Search for substance with antimicrobial activity are frequent and medicinal plant have been considered interesting by some researchers since they are frequently used in popular medicines as remedies of many infectious diseases. Medicinal plants have also been considered as healthy source of life for all the peoples **[5,6].**

Ficus racemosa (syn. ***Ficus glomerata*** *Roxb.)* is a species of plant in the Moraceae family. Popularly known as the Cluster Fig Tree, Indian Fig Tree or Goolar (Gular).

This is native to Australia, Malesia, South-East Asia and the Indian Subcontinent. It is unusual in that its figs grow on or close to the tree trunk, termed cauliflory. *Ficus racemosa linn* is an evergreen, moderate to large sized, spreading, lactiferous, deciduous tree, without much prominent aerial roots found throughout greater part of India in moist localities and is often cultivated in villages for its edible fruit.

Plant is propagated using cutting of stem and root suckers. Natural regeneration is very good from seeds dispersed by animals and birds. 4-months old seedlings are transplanted to polythene bags and then planted in field after 1-month **[7,8]**. The tree is medium tall with quite rich green foliage that provides good shade. The leaves are dark green, 7.5-10 cm long, ovate or elliptic. The fruit receptacles are 2-5 cm in diameter, pyriform, in large clusters, arising from main trunk or large branches. The fruits resemble the figs and are green when raw, turning orange, dull reddish or dark crimson on ripening. The seed tiny, innumerable, grain like, the outer surface of the bark consists of easily removable translucent flakes grayish to rusty brown, uniformly hard and non brittle. Bark is grayish green, soft surface and uneven 0.5-1.8 cm thick. On rubbing, white papery flakes come out from the outer surface, inner surface is light brown fracture fibrous, taste mucilaginous without any characteristics odour **[9,10].**

SCIENTIFIC CLASSIFICATION-

Kingdom:	Plantae
(unranked):	Angiosperms
(unranked):	Eudicots
(unranked):	Rosids
Order:	Rosales
Family:	Moraceae
Genus:	Ficus
Species:	*F. racemosa*
Synonyms	*Ficus glomerata Roxb*

All parts of this plant (leaves, fruits, bark and sap of the root) are medicinally important in the traditional system of medicine in India **[11].**

This tree is astringent, anti-diabetic, anti-asthmatic, anti-inflammatory, anti-oxidant, anti-ulcer, anti-pyretic and anti-diarrheal in action. Bark of tree is used to treat infections, swelling and inflammation **[12].**

The astringent nature of the bark has been employed as a mouth wash in spongy gum and also internally in dysentery, menorrhagia and haemoptysis. The leaves powdered and mixed with honey is given in bilious infections. The bark is anti-septic, anti-pyretic and vermicidal, and the decoction of bark is used in the treatment of various skin diseases, ulcers and diabetes. It is also used as a poultice in inflammatory swellings/boils and regarded to be effective in the treatment of piles, dysentry, asthma, gonorrhoea, gleet, menorrhagia, leucorrhea, hemoptysis and urinary diseases **[13].**

METHODOLOGY

Collection of plant materials:

Leaves, fruits, and bark were collected from Vibhuti Khand, Gomti Nagar, Lucknow and from Subhash Nagar, Bareilly. After collection the samples were washed properly and air dried and ground into powdered form **[14]**.

Preparation of solvent extract:

The plant materials were separated and then washed with distilled water. Then these materials were allowed to sun dry. Further powders were prepared by using grinder **[15]**.

The powders were dipped in respective polar and non polar solvents and then incubated for 48 hours at room temperature.

The samples were then filtered and then allowed to evaporate the solvents. After evaporation the remaining residues were dissolved in dimethyl sulphoxide and then preserved for further analysis **[16]**.

Antibiogram analysis:

The samples were screened for their antibacterial activity by using agar well diffusion method. The extracts were loaded in well and then incubated for 24 hours at 37°C. The best extracts were screened on the basis of observation of zone of inhibition **[17]**.

Phytochemical Screening:

The extracts were screened for the identification of presence of secondary metabolites such as flavonoids, terpenoids, steroids, saponins etc **[18, 19, 20]**.

Test for Carbohydrate- Extracts were dissolved individually in distilled water and filtered. The filtrates were used to test for the presence of carbohydrate

a) Via Fehling's test: Equal volume of fehling A (copper sulfate in distilled water) and fehling B (potassium tartarate and sodium hydroxide in distilled water) reagents were mixed along with few ml of extract, boil on water bath, brick red precipitate of cuprous oxide forms, if reducing sugar is present.

2. Test for phenols-

a) Via Ferric chloride test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

3. Test for Alkaloids-

a) Via Wagner's test (Solution of iodine in potassium iodide): Extracts were treated with Wagner's reagent which was prepared as (mix 1.27gm iodine with 2gm potassium iodide and prepared upto 100ml).

4. Test for Protein-

Extract were heated in boiling water bath for 8-10 minutes. When the protein gets coagulated which means protein is present.

5. Test for amino acids-

a) Via ninhydrin test: Extract were treated with ninhydrin reagent and boiled for few minutes, formation of blue colour indicates the presence of amino acids.

6. Test for Flavonoids-

a) Via Alkaline reagent test: Extracts were treated with few drops of sodium hydroxide solution, formation of an intense yellow colour which were turns to colourless on addition of few drops of dilute acid indicate the presence of flavonoid.

7. Detection of phytosterols-

a) Via Salkowski's Test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

8. Test for saponins-

a) Via foam test: Extracts were treated with distilled water and shake it vigorously so that 1cm thick foam is formed. When this foam is still present after 5-8 min, it means saponins are present.

RESULT AND DISCUSSION

Collection of plant:-

Plant parts namely leaves, fruits and bark were collected from Vibhuti Khand Gomtinagar, **Table 1 and Figure 1 (a-c)** below shows the pictures of dried plant parts.

Table 1:- Collection of sample (in gram).

S. No.	Sample (powdered form)	Total weight(in gm)
1.	Leaves	45gm
2.	Fruits	35.334gm
3.	Bark	40gm

Figure 1:- (a) Leaves, (b) Fruits and (c) Bark.

Test pathogens:-

Test pathogens (*Eschrechia coli, Pseudomonas aeruginosa,, Staphylococcus aureus)* were collected from IMTECH, Chandigarh and was subcultured in **MRD LifeSciences, Lucknow**.

Figure 3 below show the picture of culturing of pathogens namely *Pseudomonas aeruginosa, Eschrechia coli, Staphylococcus aureus* in Nutrient Broth and **Figure 2 (A,B,C)** below show the picture of streaking of pathogen namely *Pseudomonas aeruginosa, Eschrechia coli, Staphylococcus aureus* on Nutrient Agar plates.

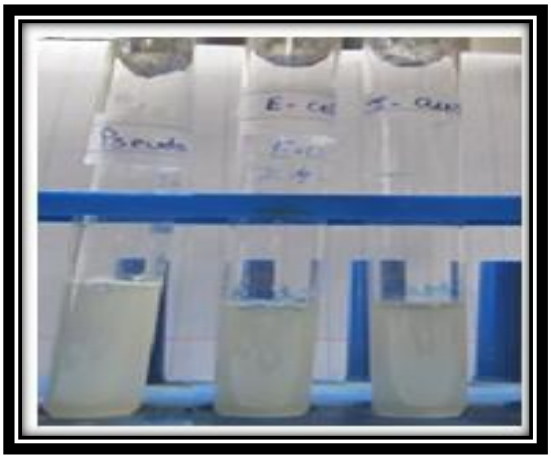

Figure 3:-Different bacterial species (*Pseudomonas aeruginosa, Eschrechia coli, Staphylococcus aureus,*) inoculated in test tubes.

Preparation of solvent extraction:

Extracts were prepared using the solvents such as- ethanol, methanol, ethyl acetate, D/w and hot d/w. **Figure 3** below show the pictures of preparation of solvent extract of Leaves, Bark, Fruit respectively in above mentioned solvents in sterile bottles.

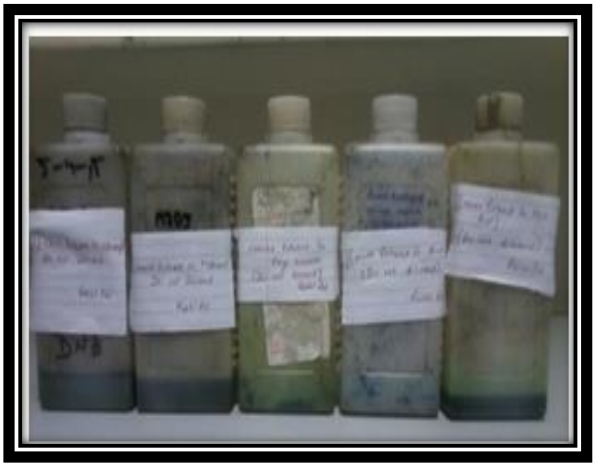

Figure 3:- Leaves extracts in sterile bottles prepared with solvents such as Ethanol, Methanol, Ethyl acetate, D/w, Hot d/w.

Filtrates were collected in the pre weighed bowls below show the picture of collected filtrate of Leaves, fruits and bark respectively in weighed bowls.

Drying of filtrate was done in hot air oven at 50ºC for 24hrs. After drying the secondary metabolites were collected in eppendorf tubes.

ANTIBIOGRAM ANALYSIS

Antibiogram analysis was done to know the antibacterial activity of the plant extracts. Zone of inhibition was marked and calculated in milimeter. Tetracycline was used as positive control and DMSO as negative control. It can be seen from the result below in **Table 5,** in which d/w and hot d/w of leaves extract gave no zone of inhibition against any of the pathogen used.

Table 2:- Antibiogram analysis of leaves extract in D/W and hot d/w

S. No.	Pathogens	ZOI by D/W(mm)	ZOI by hot d/w (mm)	ZOI by tetracycline in(mm)	ZOI by DMSO in (mm)
1	*Escherichia coli*	0	0	26.5	0
2	*Pseudomonas aeruginosa*	0	0	22	0
3	*Staphylococcus aureus*	0	0	24.5	0

Below in **Table 3 and Figure 4 14** In comparison to tetracycline, maximum zone of inhibition was seen in ethanol extract of leaves against *Pseudomonas aeruginosa.* And the lowest zone of inhibition was seen in methanol extract against *Pseudomonas aeruginosa.*

Table 3:- Antibiogram analysis of leaves extract in ethanol and methanol

Pathogens	ZOI by ethanol (mm)	ZOI by methanol (mm)	ZOI by tetracycline in(mm)	ZOI by DMSO in (mm)
Escherichia coli	24.5	29.5mm	26.5	0
Pseudomonas aeruginosa	32.5mm	24mm	22	0
Staphylococcus aureus	26mm	25.5mm	24.5	0

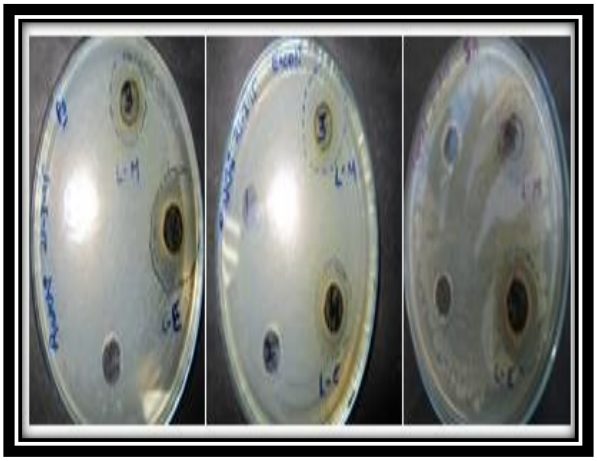

Figure 4- Antibiogram of *ficus racemosa* leaves ethanol, methanol extract against *Pseudomonas aeruginos, Escherichia coli, Staphylococcus aureus*

Below in Table 4, show zone of inhibition of 13mm in leaves ethyl acetate extract which is very less than that of zone shown by tetracycline.

Table 4:- Antibiogram analysis of leaves extract in ethyl acetate.

Pathogens	ZOI by ethyl acetate(mm)	ZOI by tetracycline in(mm)	ZOI by DMSO in (mm)
Escherichia coli	0	26.5	0
Pseudomonas aeruginosa	13	22	0
Staphylococcus	0	24.5	0
aureus			

Below in **Table 5** it can be seen that no zone of inhibition was obtained in d/w and hot d/w of bark extract against any of the used pathogens.

Table 5:- Antibiogram analysis of bark extract in D/W and hot d/w

Pathogens	ZOI by D/W (mm)	ZOI by hot d/w (mm)	ZOI by DMSO in (mm)
Escherichia coli	0	0	0
Pseudomonas aeruginosa	0	0	0
Staphylococcus aureus	0	0	0

Below in **Table 6,** it can be seen that the maximum zone of inhibition was seen in ethanol extract of bark against *Escherichia coli and Staphylococcus aureus.* It is also seen in this that the zone of inhibition made by ethanol extract is less than that of the tetracycline.

Table 7:- Antibiogram analysis of bark extract in ethanol and methanol

Pathogens	ZOI by ethanol (mm)	ZOI by methanol(mm)	ZOI by tetracycline in(mm)	ZOI by DMSO in (mm)
Escherichia coli	21.5	13	26.5	0
Pseudomonas aeruginosa	20	17	22	0
Staphylococcus aureus	21.5	18.5	24.5	0

Below in **Table 9** it can be seen that ethyl acetate in fruit only showed zone against *Staphylococcus aureus.* Hence in comparison from tetracycline zone was less and it can be said that fruit is not very much active against pathogens.

Table 9:- Antibiogram analysis of bark extract in ethyl acetate.

Pathogens	ZOI by ethyl acetate(mm)	ZOI by tetracycline in(mm)	ZOI by DMSO in (mm)
Escherichia coli	0	26.5	0
Pseudomonas aeruginosa	0	22	0
Staphylococcus aureus	21.5	24.5	0

PHYTOCHEMICAL ANALYSIS-

Results were determined on the basis of colour changed by different extracts on addition of different chemicals such as ninhydrin, fehling A and fehling B, Iodine, potassium iodide, HCl, NaOH, Chloroform, sulphuric acid, ferric chloride. Table 8, 9 shows the phytochemical analysis of leaves extract. **Table 10** shows the phytochemical analysis of bark extract.

Table 10:- Alkaloids, Carbohydrates, Phytosterols, Saponins phytochemicals analyzed in Leaves extract.

Leaves extract	**Alkaloids**	**Carbohydrate**	**Phytosterols**	**Saponins**
Methanol	+ve	+ve	-ve	-ve
Ethanol	+ve	-ve	-ve	-ve
Ethyl acetate	-ve	-ve	-ve	+ve
D/w	-ve	+ve	+ve	-ve

NOTE- +VE= POSITIVE AND –VE= NEGATIVE

In Table 11 leaves extract in methanol showed presence of Alkaloids, carbohydrates. And ethanol extract showed presence of alkaloids. Extract of ethyl acetate showed the presence of saponins. Extract of distilled water showed presence of carbohydrates and phytosterols.

Table 11:- Phenols, Flavonoids, Proteins, Amino-acids phytochemicals analyzed in Leaves extract.

Leaves extract	Phenols	Flavonoids	Proteins	Amino acids
Methanol	+ve	-ve	+ve	-ve
Ethanol	+ve	-ve	+ve	-ve
Ethyl acetate	-ve	-ve	+ve	+ve
D/w	-ve	+ve	-ve	-ve

NOTE- +VE= POSITIVE AND –VE= NEGATIVE

In Table 12 leaves extract in methanol and ethanol showed presence of phenols and proteins. Ethyl acetate showed presence of proteins and amino acids. And d/w showed presence of flavonoids.

Table 12:- Alkaloids, Carbohydrates, Phytosterols, Saponins phytochemicals analyzed in Bark extract.

Bark extract	Alkaloids	Carbohydrate	Phytosterols	Saponins
Methanol	+ve	+ve	-ve	-ve
Ethanol	-ve	+ve	-ve	-ve
Ethyl acetate	-ve	-ve	+ve	-ve
D/w	+ve	+ve	+ve	+ve

NOTE- +VE= POSITIVE AND –VE= NEGATIVE

DISCUSSION

Herbal products are best alternative antimicrobial agents demand on plant based therapeutic is increasing in both developing and developed countries. Natural product are safe, easily biodegradable, minimum environmental hazards, have no adverse effects, easily available and more dependable. Plant are the best source of drugs which can be used to treat the disease. Herbal medicines have less or no side effects, allergic problems and very safe to use. Medicinal plants would be the important source of variety of drugs as phytochemicals are more specific, biodegradable and are supposed to have fewer side effects, phytochemical offers unique plateform for structural diversity and biological functionality which is indispensible for drug discovery **[2].**

In Antibiogram analysis of Leaves extract in Methanol gave the positive zone of inhibition of 29.5mm in *Eschrechia coli,* 24mm in *Pseudomonas aeruginosa,* 25.5mm in *Staphylococcus aureus.* In ethanol extract of leaves gave 24.5mm zone of inhibition in *Eschrechia coli,* 32.5mm in *Pseudomonas aeruginosa,* 26mm in *Staphylococcus aureus.* In ethyl acetate extract 13mm zone was found in *Pseudomonas aeruginosa.*

In bark extract, maximum zone of inhibition in Ethanol and Ethyl acetate of bark extract in pathogenic organism i.e *Staphylococcus aureus* and *Eschrechia coli.* Least zone of inhibition was found in methanol extract in pathogenic organism i.e *Eschrechia coli.*

Maximum zone of inhibition in Ethyl acetate of fruit extract in pathogenic organism i.e *Eschrechia coli.* Least zone of inhibition was found in Ethyl acetate in pathogenic organism i.e *Staphylococcus aureus.*

In phytochemical analysis leaves extract in methanol showed presence of Alkaloids, carbohydrates. And ethanol extract showed presence of alkaloids. Extract of ethyl acetate showed the presence of saponins. Extract of distilled water showed presence of carbohydrates and phytosterols. Leaves extract in methanol showed presence of phenols, proteins. Ethanol extract showed presence of phenols and proteins. Ethyl acetate showed presence of proteins and amino acids. And d/w showed presence of flavonoids.

CONCLUSIONS

The multiple benefits of *Ficus racemosa* made it a true miracle of nature. Numerous studies have been conducted on different parts of *Ficus racemosa,* but this plant has not yet developed as a drug by pharmaceutical industries.

From our research we found that *Ficus racemosa* holds various medicinal values and possess anti-bacterial activity. *Ficus racemosa* holds the capacity to inhibit human pathogens such as- *Eschrechia coli, Staphylococcus aureus, Pseudomonas aeruginosa.*

It also showed the presence of phytochemicals. Bark, fruits and leaves of *Ficus racemosa* showed the presence of Alkaloids, carbohydrates, proteins, saponins, phytosterols, phenols, flavonoids and amino-acids. Hence this plant or its extracts can be converted to drugs and subjected to clinical trials for development of new herbal medicines.

In view of the nature of the plant, more research work can be done on humans so that a drug with multifarious effects will be available in the future market.

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