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Antibacterial potential and phytochemical analysis of *Flacourtia indica* (Burm.f.) Merr. root extract against human pathogens

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ABSTRACT

Objective: To evaluate antibacterial potential and phytochemical analysis of *Flacourtia indica* (*F. indica*) root extract. **Methods:** Phytochemical screening of the root extract was done to determine the phytochemicals in the methanol extract. The study included the determination of antibacterial activity by agar well diffusion assay, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of methanolic root extract. The antibacterial activity of the root extract was tested against selected human pathogenic bacteria. Further, the methanolic extract of *F. indica* was screened for the range of phytochemicals by thin layer chromatography and bioactive compound was identified using GC-MS. **Results:** The extract was effective on tested pathogenic bacteria and MIC values ranged between 50-200mg/ml. While the phytochemical screening of the root extract revealed the presence of flavonoids, saponins, alkaloids, tannins, terpenoids, glycosides and phenolic compounds. **Conclusion:** These findings indicate that the root extract of *F. indica* possesses pharmacological activity and potential to develop natural products based pharmaceutical drugs. The result of this study justifies the use of the plant in folk medicine.

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Introduction:

Plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well being. In India, plants are in use as medicine since Vedic period. A major part of the total population in developing countries still uses traditional folk medicine obtained from plant resources. The use of traditional medicines holds a great promise as an easily available source and as effective medicinal agents to cure a wide range of ailments among the people particularly in tropical developing countries like India. Plant extracts or bioactive herbal compounds have been evaluated for their biological activities. Phytochemicals are non-nutritive plant chemicals that have protective or having disease preventive properties. India is a country rich in indigenous herbal resources which grow on their varied topography and under changing agro-climatic conditions permitting the growth of almost 20,000 plant species of which about 2,500 are having medicinal value. Therefore, globally India would be place for new drugs to manage various challenging diseases because of its rich biodiversity of medicinal plants^[1].

The present study was undertaken to analyze the antibacterial and phytochemical compounds of the *Flacourtiaindica*. *Flacourti aindica* (Burm.f.)Merr.Synonymous to *Flacourtia ramontchi* L. Herit. belong to the family Flacourtiaceae. It is commonly known as 'Baichi' or 'Katai' is an indigeneous medicinal plant axillary thorn and often with tufts of branched thorns on the stem has been widely distributed in Bangladesh and India. The botanical name is of particular historical and geographical interest^[2]. This plant has been reported as an effective remedy for the treatment of a variety of diseases. Fruits are used as appetizing and digestive, in jaundice and enlarged spleen^[3]. Barks are used for the treatment of intermittent fever. Roots are used on nephritic colic and gum in cholera. The leaves range from 11mm to 9 mm and are useful in pruritis and scabies. Previous phytochemical investigation on this plant resulted in the isolation of β -sitosterol (a well-known phytosterol), β -sitosterol- β -D-glucopyranoside, ramontoside, butyrolactonelignan disaccharide^[4] and flacourtin^[5, 20]. Further the presence of coumarin such as scoparone and aesculetin^[6] was also been reported. Though this plant is being used in the traditional medical practice by the indigenous physicians in Bangladesh and India, but its ethnomedical values have not been evaluated through scientific screening significantly. Further, some traditional population of coastal region of India, use the root extract for anti-pyrogenic activities.

Flacourtia indica (Flacourtiaceae) is the most useful traditional medicinal plant in India. It is now considered as a valuable source of unique natural products for development of medicines and targeting against various diseases. Although different parts (leaves, bark, stem, fruits, root and even whole plant) of the *Flacourtia indica* have been demonstrated for several pharmacological activities^[17], the potential of root extract of this plant is yet to be resolved; therefore, potential of the root extract was evaluated for pharmaceutical and antibacterial activity.

Materials and Methods

Flacourtia indica

Flacourtia indica roots in this study were collected during October, 2009 from natural habitat (Hawajae, Udupi district, Karnataka, India). Roots from the plant were washed using running tap water followed by sterilized distilled water, shade dried and then powdered with the help of sterilized pestle and mortar and the powder was further subjected for different extraction protocols.

Aqueous extraction

10 g of the powder was boiled in 400 ml distilled water till one-fourth, evaporated, filtered and centrifuged. The supernatant was again filtered (Whatman filter No.1) and stored in fresh sterile bottles at 4°C until further use.

Organic solvent extraction

Preparation of cold extracts

10 g of *Flacourtia indica* powder was mixed with 100 ml organic solvent (viz., methanol, acetone and hexane) incubated for 24 h on rotary shaker/150 rpm. Mixing solution was filtered, (Whatman filter No.1), evaporated under reduced pressure with rotatory vacuum evaporator to yield the pure extract. Stock solution of crude extracts for each type of organic solvent was prepared by mixing well with appropriate amount of dried extracts with 20% dimethyl sulphoxide (DMSO) to obtain a final concentration of 100 mg/ml and was used for evaluation of antibacterial activities.

Preparation of hot extracts (Soxhlet extraction)

About twenty five gram of the *Flacourtia indica* powder was extracted with methanol by hot extraction process (soxhlet) for 72h. After completion of the extraction the solvent was recovered by distillation and concentrated under vacuum and resulted semisolid mass was vacuum dried using rotary flash evaporator to yield a solid residue. After complete evaporation, the solvent extract was weighed and preserved at 4°C in airtight bottles until further use. 100 mg of methanol solvent residue was dissolved in 1 ml of 20% DMSO and was used as the test extracts for antibacterial activity assay.

Microbial cultures

The bacterial cultures for the present study were kindly supplied by the Dept. of Microbiology, SS Institute of Medical College, Davangere, India. These included both Gram-positive and Gram negative bacterial strains, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella sp.*, *Shigella dysenteriae*, *Pseudomonas sp.*, *Vibrio cholerae ogawa*, *Vibrio cholerae enaba*, and *Proteus sp.* The bacterial cultures were grown in Nutrient Agar (NA) plates at 37°C. Then the stock cultures were maintained.

Antibacterial activity by agar well diffusion assay

Both aqueous and organic extracts of *F. indica* root extract were subjected for antibacterial activities by *in vitro* agar well diffusion method. Autoclaved Nutrient agar was plated and seeded with the 100µl with the inoculums concentration (10^6 CFU/ml) and spread on the solid agar plates, and 6mm diameter was made on the agar media using sterile cork borer. 100µl of plant root extract was filled in the respective wells and the plates were incubated at 37°C for 18hr. DMSO was used as negative control and tetracycline antibiotic as positive control (40µl/ml). The diameter of zone of inhibition was measured in mm.

Determination of minimum inhibitory concentration (MIC)

In order to determine the MIC values, the methanol root extract was dissolved in 20% DMSO and different concentrations such as 25, 50, 100, and 200 mg/ml were prepared. Three test tubes containing media (C_M), media plus sample as antimicrobial control (tube containing extract and the growth medium without inoculums C_{MS}) and media plus inoculums as organism control (the tube containing the growth medium and the inoculums C_{MI}) were also maintained. A 16h culture was diluted in 10ml of sterile distilled water with reference to the 0.5McFarland standards to achieve inocula of approximately 10^6 colony forming units. Series of nutrient broth tubes were prepared and 100µl of each concentration of plant extract was added. Then tubes were inoculated with 10 µl of bacterial suspension per ml of nutrient broth, homogenized and incubated at 37°C for 24h. One ml of the sample was added to C_{MS} and mixed well. 10µl of inoculums was added to C_{MI} to observe the growth of the organisms in the media. C_M containing media was used to check the sterility of the solution. The lowest concentration of the extracts which inhibited microbial growth was recorded as the MIC [9, 10].

Results were recorded in terms of turbidity. The test was performed in triplicates and the mean of three readings were recorded

For the determination of minimum bactericidal concentration (MBC), samples were taken from the nutrient broth tubes that showed no visible growth after 24 h incubation and sub cultured onto freshly prepared sterile nutrient agar medium. The least concentration that did not produce growth after 24 h was regarded as the MBC and results were tabulated.

Qualitative phytochemical screening of the *F. indica* root extract

Phyto-chemical investigation of the root extract was carried out to confirm the presence of secondary metabolites for alkaloids, carbohydrates, proteins and amino acids, steroids, phenolic compounds and tannins, and flavonoids by using standard protocols^[11-14] with modifications.

Flavonoids: : 0.5g of root extract was dissolved in 5ml of ethanol and treated with few drops of conc. HCl and 0.5% of magnesium turning and observed for the formation of pink color.

Saponins: For 0.5g of the extract with 5ml of distilled water. The development of stable persistent froth upon vigorously shaking was recorded.

Tannins: About 0.5g of sample was taken in a test tube and boiled with 20ml distilled water and then filtered. 0.1% of ferric chloride was added and mixed well and allowed to stand for few min and observed for the formation of brownish green or blue black colour.

Terpenoids: To 0.5g of the extract, 2ml of chloroform was added and then 3ml of conc. H₂SO₄ was added to form a layer and observed for the formation of reddish brown colour at the interface.

Phenols: 0.5g of the extract was mixed with water, and 2ml of ferric chloride was added and observed for the formation of green or blue color.

Alkaloids: For 2ml of the extract, Mayer's reagent was added and observed for the formation of dark-orange red precipitate.

Steroids: 2ml of acetic anhydride was added to 0.5g of extract and 2ml of H₂SO₄ was added along the sides of the test tube and the formation of reddish brown colour at the interface was observed.

Pholabatannins: Few drops of 1% HCl was added to the extract in a boiling tube and then allowed to stand and then observed for the development of red precipitate.

Anthraquinone (Borntragers test): About 0.5g of the extract was taken into test tube and 5ml of chloroform was added and shaken for 5min. The extract was filtered and the filtrate was shaken with equal volume of 10% ammonia solution. A pink violet or red color in an ammonical layer was observed for the presence of anthraquinones.

Cardiac Glycosides (Salkowski test): 2ml of the extract was dissolved with 2 ml of chloroform and Conc. H₂SO₄ was added and cooled. Formation of brownish green indicated the presence of cardiac glycosides.

Coumerin: To the test sample, 10% of sodium hydroxide and chloroform was added. Formation of yellow precipitate indicated the presence of coumerin.

Proteins: To the test solution, Biuret Reagent is added. The blue reagent turned to violet in the presence of proteins.

Reducing sugars: 0.5ml of sample was added with equal volumes of Fehling's solution A and B and heated in boiling water bath for 5min and observed for the formation of brick red colored precipitate.

Thin Layer Chromatography (TLC) of *F. indica* root extract

To evaluate the total phytochemical present in the methanol root extract of *F. indica* was subjected to TLC.

Preparation of the plates

The stationary phase (adsorbent) used for TLC was Silica gel-G. The precoated plates were heated in an oven at 110°C for 30min for activation. 5µl of the test sample was applied in the form of narrow bands of 5mm length at the distance of 5cm apart using 20 µl capillary tubes.

Development solvent system

Ethyl acetate: Methanol: Water and n-Butanol: Acetic acid: Water (80:20:20) solvent systems were used in the present study. When the solvent front has reached three quarters of the length of the plate, the plate was removed from the developing chamber and the position of the solvent front was immediately marked^[15]. After development of plates, the TLC plates were taken out, air-dried and visualized in visible light, UV-light (254-nm and 366nm) and Iodine chamber and numbers of spots were noted and Rf value of each spot was determined.

Gas-chromatography and mass spectra (GC-MS) analysis

The extracts was subjected to GC-MS (QP 2010, IISc, Bangalore, India) equipped with a VF-5ms fused silica capillary column of 30m length, 0.25mm diameter and 0.25µm film thickness. For GC-MS detection, an electron ionization system with ionization energy of 70eV was used. Helium as a carrier gas as at constant flow rate of 1.51ml/min, with injector and mass transfer line temperature was set at 200°C and 240°C respectively. The oven temperature was programmed from 70°C to 220°C at 10°C /min. and held isothermal for 1 min and finally raised to 300°C at 10°C /min. 2µl of respective diluted sample was manually injected in the split less mode, with split ratio of 1:40 and with mass scan of 50-600 amu. Total running time of GC-MS was 35 min. The relative percentage of the root extract constituents was expressed as percentage with peak area normalization. To identify the compounds in the extracts a comparison of their retention indices and mass spectra fragmentation pattern with those stored on the computer library of NIST-MS type version-2, 2005 and with those reported in the literature was used^[16].

Statistical Analysis:

Diameter of zone of inhibition (excluding well diameter) resulted from replicates were expressed as mean ± standard deviation (SD). The data obtained were subjected to one way ANOVA test. P value < 0.001 was considered as significant and mean values were compared by using Least Significant Difference (LSD) test using Microsoft excel software.

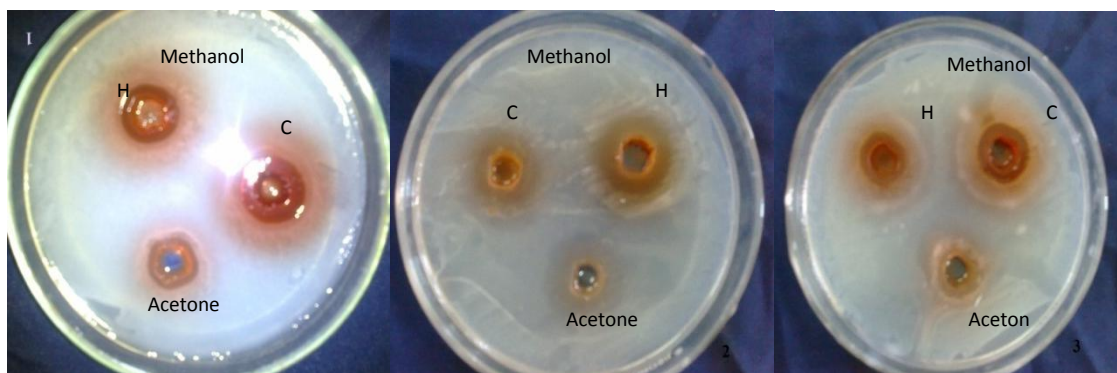
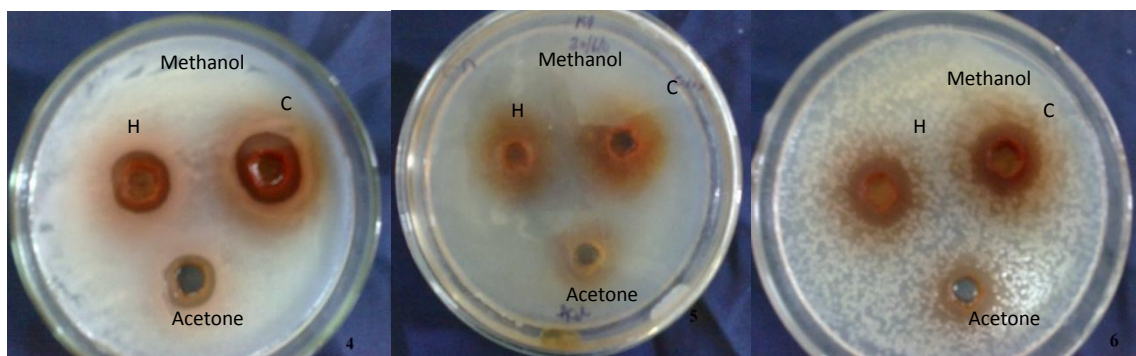
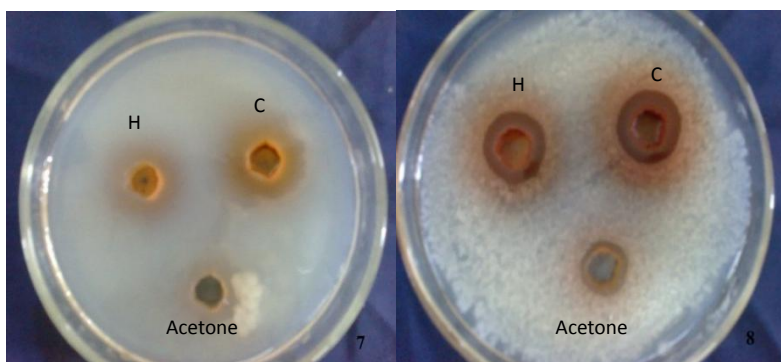
Results

Antibacterial assay

The antibacterial activity of root extracts of *F. indica* in methanol (hot and cold extract), acetone, hexane and aqueous solution along with standard antibiotic tetracycline against human pathogenic bacteria was screened by agar well diffusion method (Fig 1-8).

Of the eight test bacteria, *Pseudomonas* sp (Fig-2) and *Shigella dysenteriae* (Fig-6) were found to be sensitive to methanol root extract producing wide inhibition zone of 12±0.2 and 12 ±0.5 respectively. Further, *Shigella dysenteriae* (12 ±0.5), *V. cholerae* ssp.*Inaba* (10 ±0.5) and *V. cholerae* ssp. *Ogava* (11 ±0.5) exhibited greater sensitivity to cold methanol when compared to antibiotic tetracycline which is producing a inhibition zone of 10±0.6, 9±0.4 and 10±0.3 respectively (table-1). *Klebsiella* sp was most resistant to all organic solvent extracts producing less inhibition zone when compared to antibiotic. All tested bacteria were also sensitive to acetone and hexane extract except *Escherichia coli* which was resistant to hexane extract. Aqueous extract did not show any antibacterial activity towards any tested bacteria. Negative control, DMSO doesn't show any

activity. Therefore extract prepared in organic solvents consistently produced better antibacterial activity when compared to aqueous extract. Furthermore, extract prepared in hot and cold methanol were found to be highly inhibitory (inhibition zone ranging from 0.6 to 12 mm) followed by those prepared in hexane and acetone (Table-1 and 2).

Fig.1: *Escherichia coli*Fig.2: *Pseudomonas sp*Fig.3: *Staphylococcus sp*Fig.4: *Proteus sp*Fig.5: *Klebsiella sp*Fig.6: *Shigella dysenteriae*Fig.7: *V. cholerae* ssp. *Inaba* Fig.7: *V. cholerae* ssp. *Ogava*

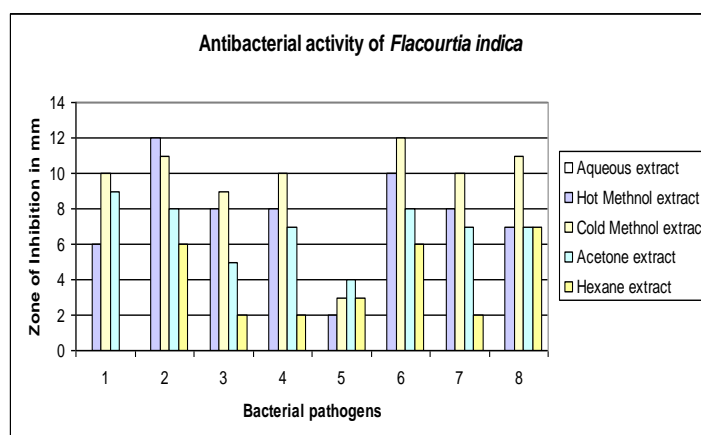
(H=hot methanol extract, C=cold methanol extract)

Fig.1-7: Antibacterial assay of clinically important bacteria against root extract of *Flacourtia indica*

Table 1. Antibacterial activity (zone of inhibition) of aqueous and organic root extracts of *F. indica* compared with commercial antibiotic (tetracycline).

| Sr. No | Microorganisms | *Zone of Inhibition(mm) | | | | | Tetracycline (40µg/ml) |
|--------|------------------------------|-------------------------|---------------------------------------|-------------------------------------|---------------------------|--------------------------|---------------------------|
| | | Aqueous | Hot (H) methonol (100 mg/ml) | Cold (C) methonol (100 mg/ml) | Acetone (100 mg/ml) | Hexane (100m g/ml) | |
| 1 | <i>Escherichia coli</i> | NA | 6.0±0.5 | 10±0.3 | 9.0±0.3 | ND | 19±0.4 |
| 2 | <i>Pseudomonas sp</i> | NA | 12±0.2 | 11±0.2 | 8.0±0.4 | 6.0±0.3 | 15±0.2 |
| 3 | <i>Staphylococcus aureus</i> | NA | 8.0±0.6 | 9.0±0.5 | 5.0±0.4 | 2.0±0.2 | 15±0.3 |
| 4 | <i>Proteus sp</i> | NA | 8.0±0.5 | 10±0.6 | 7.0±0.2 | 2.0±0.0 | 14±0.2 |
| 5 | <i>Klebsiella sp</i> | NA | 2.0±0.1 | 3.0±0.4 | 4.0±0.3 | 3.0±0.5 | 20±0.7 |
| 6 | <i>Shigella dysenteriae</i> | NA | 10±0.4 | 12±0.5 | 8.0±0.6 | 6.0±0.3 | 10±0.6 |
| 7 | <i>V. cholera ssp. Inaba</i> | NA | 8.0±0.6 | 10±0.5 | 7.0±0.3 | 2.0±0.0 | 9.0±0.4 |
| 8 | <i>V. cholera ssp. Ogawa</i> | NA | 7.0±0.2 | 11±0.5 | 7.0±0.6 | 7.0±0.4 | 10±0.3 |

*The values are mean inhibition zone (mm) ± S.D of average of triplicates. **ND** =not detected, **NA** =No activity:

Table 2 Antibacterial activity of aqueous and organic extracts of *Flacourtia indica* against some bacterial pathogens

Bacterial pathogens: 1-*Escherichia coli*, 2-*Pseudomonas sp*, 3-*S. aureus*, 4- *Proteus sp*, 5-*Klebsiella sp*, 6- *S. dysenteriae*, 7- *V. cholerae inaba*, 8- *V. cholerae ogawa*

Minimum inhibitory concentration (MIC)

The positive methanol root extract for antibacterial activity was further tested to determine Minimum inhibitory concentration (MIC) at different concentrations viz, 25, 50, 100, and 200 mg/ml (Table-3). The tested extract at various concentrations showed significant variation in MIC values depending upon the test bacteria. In the present study, among tested bacterial pathogens the methanol fraction of the root extract of *F. indica* showed the MIC value of 50 mg/ml against *Escherichia coli*, *Proteus sp* and *Vibrio cholerae* ssp *ogawa*. For *Staphylococcus aureus*, and *Pseudomonas sp* MIC value of 100mg/ml was found to be effective. While, *Shigella dysenteriae* was found to be inhibited at the concentration of 200mg/ml. Among eight bacterial pathogens tested, *Klebsiella sp.* and *Vibrio cholerae enaba* were found to be highly resistant and showed turbidity even at 200mg/ml.

The test isolates which did not show any turbidity in MIC was chosen for Minimum Bactericidal Concentration (MBC) study (*Escherichia coli*, *Staphylococcus aureus*, *Proteus sp*, *Pseudomonas sp*, *Shigella sp*, and *Vibrio cholerae spp ogawa*). 100mg/ml was the least bactericidal concentration for *Pseudomonas sp.* and *Vibrio cholerae spp ogawa* showed no growth on nutrient agar media while, *Escherichia coli*, *Staphylococcus aureus*, *Proteus sp*, and *Shigella dysenteriae* showed growth at the concentrations of 50, 100, and 200 mg/ml (Table-4) and were found to be bacteriostatic.

Table 3. Minimum Inhibitory Concentration (MIC) of Methanol extracts of *F. indica* root against different bacterial strains

| Organisms | Positive control (C _{MS}) | MIC(mg/ml) Concentrations in mg/ml | | | | Negative control (C _{MI}) | Media (C _M) |
|--|--|---------------------------------------|-----|-----|-----|--|----------------------------|
| | | 25 | 50 | 100 | 200 | | |
| <i>Escherichia coli</i> | - | ++ | - | - | - | +++ | - |
| <i>Staphylococcus aureus</i> | - | +++ | +++ | - | - | +++ | - |
| <i>Proteus sp.</i> | - | ++ | - | - | - | +++ | - |
| <i>Pseudomonas sp.</i> | - | ++ | ++ | - | - | +++ | - |
| <i>Shigella dysenteriae.</i> | - | ++ | ++ | - | - | +++ | - |
| <i>Vibrio cholarae</i> ssp <i>ogawa</i> | - | +++ | +++ | +++ | +++ | +++ | - |
| <i>Vibrio cholarae</i> ssp <i>enaba</i> | - | +++ | +++ | +++ | - | +++ | - |
| | - | ++ | - | - | - | +++ | - |
| | | +++ | +++ | +++ | +++ | +++ | |

* = Mean of 3 determinations. - = No Turbidity (Inhibition), + = Slightly Turbid, ++ = Moderately Turbid, +++ = Very Turbid (No Inhibition). (C_M) Media, (C_{MS}) Media and sample, (C_{MI}) Media and inoculums

Table-4. Minimum Bactericidal Concentration (MBC) of Methanol extracts of *F. indica* root against selected bacterial strains

| *Minimum Bactericidal Concentration(MBC) | | | |
|---|-----------------|------------------|------------------|
| Organisms | 50 mg/ml | 100 mg/ml | 200 mg/ml |
| <i>Escherichia coli</i> | + | + | + |
| <i>Staphylococcus aureus</i> | + | + | + |
| <i>Proteus sp</i> | + | + | + |
| <i>Pseudomonas sp</i> | ND | - | - |
| <i>Shigella dysenteriae</i> | ND | ND | + |
| <i>Vibrio cholerae ogava</i> | ND | - | - |

* = Mean of 3 determinations. ND= Not Determined since there was no inhibitory activity.

- = absence of growth, + = Presence of Growth

Phytochemical screening

Qualitative phytochemical analysis

Phytochemical analysis of methanolic root extract of *F. indica* indicated high *in vitro* potential for antibacterial activity to varying degrees against all bacterial pathogens tested. The results of Phytochemical tests indicated the presence of tannins, saponins, alkaloids, phenolic compounds, glycosides, terpenoids, steroids and flavonoids in the methanol root extract of *F. indica* (Table-5).

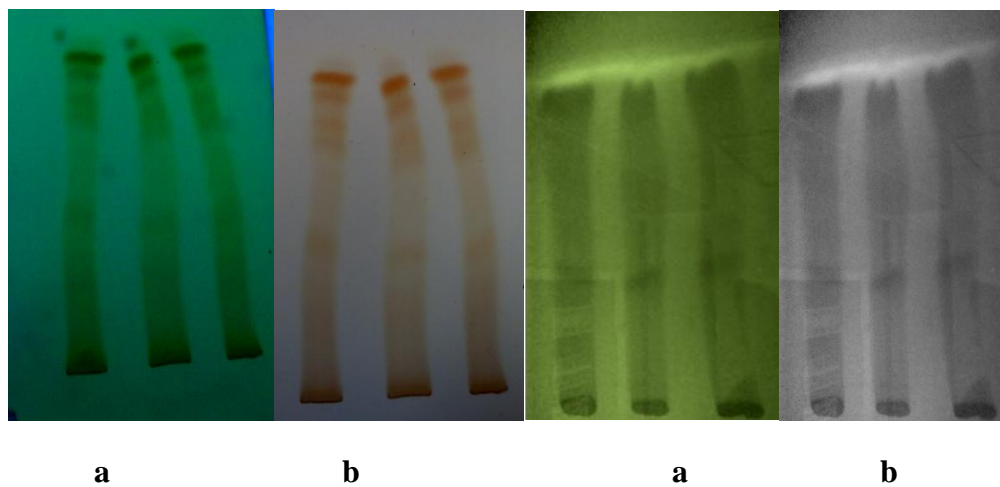
Thin Layer Chromatography (TLC):

Thin layer chromatographic technique is a useful analytical tool for the isolation and identification of organic compounds. The data of the quantitative separation of secondary metabolites from root extract of *F. indica* by TLC indicated (Table-6). Various phytochemicals with different R_f values in different solvent system. Compound showing high R_f value in the solvent system have low polarity and with less R_f value have high polarity. In the present experiment, TLC profiling of the plant extract in two different solvent systems indicated the presence of different groups of phytochemicals in the plants. In the solvent system of ethyl acetate: methanol: water, there are six bands were observed with the R_f values of 0.20 (band-1), 0.30 (band-2), 0.56 (band-3), 0.61 (band-4), 0.68 (band-5) and 0.82 (band-6). In the solvent system of n-Butenol: Acetic acid: Water, two bands were observed with R_f values of 0.57(band-1) and 0.79(band-2). Ethyl acetate: methanol: water solvent system particularly useful for the separation of polar compounds such as glycosides, anthroglycosides, arbutin, alkaloids, cardiac glycosides, bitter principles, flavonoids, and saponins (Fig.8).

Table-5: Qualitative phytochemical analysis of Methanol extracts of *F. indica* root.

| Sl.no | Phytoconstituents | Result |
|-------|--------------------|--------|
| 1 | Flavonoids | + |
| 2 | Saponins | + |
| 3 | Tannins | + |
| 4 | Terpenoids | + |
| 5 | Phenols | + |
| 6 | Alkaloids | + |
| 7 | Steroids | + |
| 8 | Pholabatannins | + |
| 9 | Anthroquinones | + |
| 10 | Cardiac glycosides | + |
| 11 | Coumerins | + |
| 12 | Reducing sugars | + |

+ = Presence of constituent, - = absence of constituent



Solvent system -*Ethyl acetate: Methanol: Water* Solvent system-*n-Butanol:Aceticacid:Water*

Fig8: TLC Profile of the methanol extract of *Flacourtia indica* root.a). Under UV-light, b). Under Visible light

Table-6: TLC profile of *Flacourtia indica* root extract* Average R_f value (R_f = Retention factor)

| Solvent System | No. Bands | * R_f values |
|--------------------------------|-----------|----------------|
| Ethyl acetate: Methanol: Water | Band-1 | 0.20 |
| | Band-2 | 0.30 |
| | Band-3 | 0.56 |
| | Band-4 | 0.61 |
| | Band-5 | 0.68 |
| n-Butanol:Aceticacid:Water | Band-6 | 0.82 |
| | Band-1 | 0.57 |
| | Band-2 | 0.79 |

GC-MS analysis

The identification of bioactive compounds in the root extract of *Flacourtia indica* was based on the peak area, retention time and molecular formula. By GC-MS compound identified was 4-Benzoyl-3-methoxyisocoumarin with Molecular formula: $C_{17}H_{12}O_4$ and Molecular weight: 280 with retention time (7.69) (Fig.9).

Discussion

The indiscriminate use of antibiotics has developed many resistant micro-organisms creating immense clinical problems in the treatment of infectious diseases such as those caused by multi drug resistant *S. aureus*, *E. coli*, *Salmonella* sp, *Shigella* sp, *Pseudomonas* sp etc. Therefore, there is a need to develop alternative antimicrobial agents for the treatment of these infectious diseases. A non-antibiotic approach to the treatment and prevention of these infections includes the application of the potential of higher plants as source of new antimicrobial agents in modern medicine. There are many reports available on the antimicrobial, antiviral, antifungal properties of plant^[9]. These findings are useful in designing of new strategies for the development of new therapeutic agents. There are many investigations carried on the aerial parts (leaves) of *Flacourtia indica* by various workers however, not many studies are available on the antimicrobial and pharmacological potential of *Flacourtia indica* roots.

This plant has been reported as an effective remedy for the treatment of a various diseases. The roots are sweet, refrigerant, depurative, alexipharmic and diuretic. They are useful in vitiated conditions of pitta and

vataaphthae, poisonous bites, skin diseases, pruritus, erysipelas, strangury, nephropathy and psychopathy and are used on nephritic colic and anti-pyrogenic, fruits are used as appetizing and digestive in jaundice and enlarged spleen, barks are used for the treatment of intermittent fever, gum in cholera and leaves are useful in pruritis and scabies [3, 17, 20].

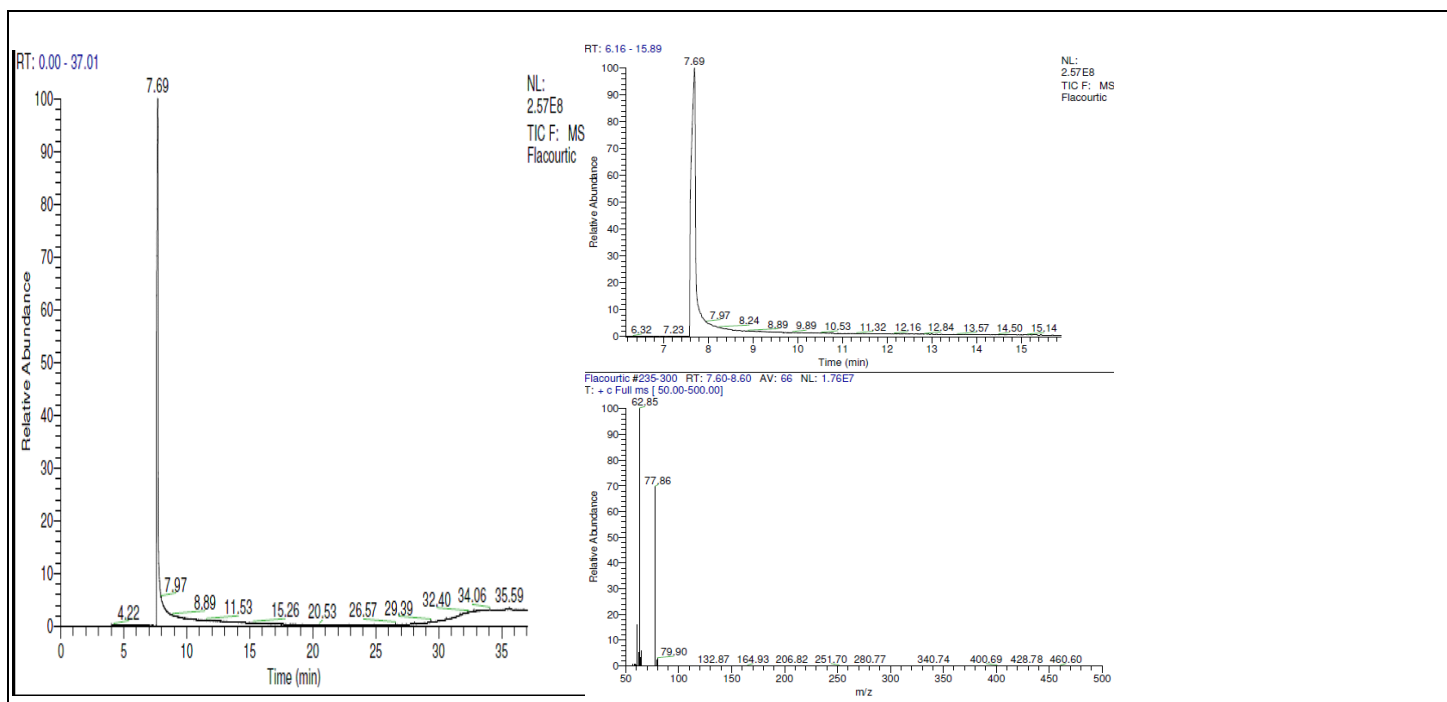


Fig.9: GC-MS chromatogram of methanol root extract of *Flacourtia indica*. Peak with retention time of 7.69 was identified as 4-Benzyl-3-methoxyisocoumarin and as the major phytochemical of the plant while other peaks were of the various phytochemicals present.

The present study had been undertaken to evaluate the potential of the root extract of *F. indica* as a source for antimicrobial and phytochemical compounds. The results of this study revealed that methanolic root extract of *F. indica* exhibited *in vitro* antibacterial activity to varying degrees against all tested microorganisms compared to acetone, hexane and aqueous extract. The results obtained from agar diffusion assay indicated that both Gram positive and Gram negative bacteria were effectively inhibited by the root extract of *F. indica*. Our results also showed that some of the pathogenic bacteria selected have a high range of MIC values. This could be due to the nature and level of antimicrobial compounds present in the root extract and their mode of action as reported in this study.

Furthermore, this result is congruent with the work of Hajra and their group [10], who evaluated the antibacterial activity of *Cassia fistula* and *Flacourtia indica* leaves on both Gram-positive and Gram-negative bacteria at varying concentrations. The organic solvents (methanol, acetone and ethanolic) extracts of *F. indica* leaves showed significant antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Klebsiella pneumoniae*, and *Salmonella typhimurium* [10].

In another study, acetone extract of *Flacourtia inermis* fruits exhibited significant antifungal activities against opportunistic human pathogens such as *A. flavus*, *A. niger*, *A. fumigatus*, *M. ramosissimus* and *Chrysosporium* species. The fruit extract showed highest activity against *A. fumigatus* (47mm), least was shown by *A. niger* (30mm) [19]. Methanolic extract of *F. indica* leaves also showed antifungal activity against *R. stolonifer* (13.2±0.27) [10].

Previous phytochemical investigation on this plant resulted in the isolation of β -sitosterol, β -sitosterol- β -D-glucopyranoside, ramontoside, butyrolactonelignan disaccharide, flacourtin, coumarin such as scoparone and aesculetin²⁻⁴^[20]. Tannins inhibit the cell protein synthesis by the formation of irreversible complexes with proline-rich proteins^[10, 17]. The preliminary phytochemical investigation on the leaves extract of *Flacourtia ramonchi* revealed that the presence of secondary metabolites like triterpenoids and lignans^[5]. However, this qualitative analysis alone may not ascertain the pharmacological action of the plant.

Several studies have also been reported the presence of flavonoids, polyphenols, pyrocatechol, homaloside-D and poliothryoside^[14, 15]. So far, no phytochemicals investigations reports on root extract of *F. indica*. In the present study, an attempt was made to reveal secondary metabolites in the root extract of *F. indica* by TLC. The TLC profiling of the root extract in ethyl acetate: methanol: water as well as n-butenol: acetic: water gave an impressive result that directing towards the presence of number of phytochemicals. Better separations achieved in the ethyl acetate: methanol: water solvent (6 bands) system when compared to n-butenol: acetic: water (2 bands). Various phytochemicals gave different R_f values. This variation in R_f values of the phytochemicals provided a very important clue in understanding of their polarity and also helps in selection of appropriate solvent system for separation of pure compounds by column chromatography. This may suggest that of the four solvents used for extraction in this study, methanol extracts demonstrated the highest relative antibacterial activity; advocating that, methanol solvent might have had higher solubility for antimicrobial phytoconstituents. Further GC-MS analysis on the root extract of this plant resulted in the identification of phytochemical compound, 4-Benzyol-3-methoxyisocoumarin.

Previous study on *Flacourtia ramonchi* suggested the leaves of the plant were useful in treating inflammation and infectious diseases. Further, the report suggested that ethanolic extract of *F. indica* leaves exhibited anti-asthmatic activity^[8]. In another study, aqueous extract of leaves of *F. indica* possesses potent free radical scavenging and antioxidant activity as well as hepatoprotective activity and protected liver against CCl_4 and methotrexate induced oxidative stress^[6, 20, 21]. It was concluded that apart from the folklore use of *F. indica* as antioxidant agents, the ethanolic extract of leaves of the plant also possess anti-asthmatic activity^[8]. Our findings suggested that *Flacourtia indica* root extract possessed antibacterial activity due the presence of various phytoconstituents which may be contributing to the antimicrobial activity.

Conclusion:

The results of this study revealed a correlation between traditional therapeutic use and the *in vitro* antibacterial activity. The phytochemical studies confirmed the traditional uses of the *Flacourtia indica* as antimicrobial, antibacterial and anti-inflammatory agent. Most of therapeutic effects explained due to the presence of glycosides, tannins, sugar, flacourtin, β -sitosterol, β -sitosterol- β -D-glucopyranoside, ramontoside, butyrolactonelignan disaccharide, coumarin such as scoparone and aesculetin etc. The plants ability to exhibit antibacterial activity in this study may be attributed to the presence of these phytochemical constituents which are known to be biologically active. The extract of the plant could therefore be useful in the treatment of many bacterial infections. Their quantification of the individual phytoconstituents as well as pharmacological profile based on *in vitro*, *in vivo* studies and on clinical trials needs to be investigated.

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