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Exploring Phytoconstituents of Chloroform and Ethanolic Extract of *F. Indica* and their Antibacterial Potency

Sangeeta Parida[1], Suman Jagatee[2], Saktikanta Rath[3], Chandi Charan Rath[4]

[1,2]Post Graduate Department of Life Science, Rama Devi Women's University, Vidya Vihar, Bhubaneswar-751022, Odisha, India.
[3]Associate Professor, Post Graduate Department of Life Science, Rama Devi Women's University, Vidya Vihar, Bhubaneswar-751022, Odisha, India.
[4]Prof.Chandi Charan Rath, Post Graduate Department of Life Science, Rama Devi Women's University, Vidya Vihar, Bhubaneswar-751022, Odisha, India.

Abstract
Natural plant products have found a place in traditional systems of healing across the globe since ages. Recently, they have only been applied as isolated and described molecules for modern drug discovery and development. The current study aimed at studying the antibacterial properties as well as phytochemicals of *Flacourtia indica.* Alkaloids, flavonoids, glycosides, phenols, steroids, tannins, and terpenoids were found to be the main phytoactive compounds, on phytochemical screening. For the purpose of quantifying Gallic acid and Quercetin in the chloroform and ethanolic extract of *F. indica*, a modest, trustworthy, and predictable high-performance thin-layer chromatography (HPTLC) technique was developed. Densitometry analysis was executed out in absorption mode at 254 and 366 nm, with a scanning speed of 100 mm/s and a 6 × 0.45 nm micro-slit dimension. Presence of Alcohol phenols, Aliphatic amines Alkenes, Aromatics, Nitro compounds, Alcohol, Alkyl halides, Carboxylic acid, Esters, Ethers, Primary, Secondary amines were confirmed through FTIR analysis, with peaks at 3326.85 cm^{-1}, 2974.87cm^{-1}, 2853.37cm^{-1}, 1462.49cm^{-1}. Antibacterial activity of the extract was observed against both Gram positive and Gram negative bacteria such as *Staphylococcus aureus, Streptococcus pneumoniae, Corynaebacterium kroppenstedtii, Pseudomonas aeruginosa, Vibrio cholerae* and *Escherichia coli* by Agar Cup Well method. Minimum Inhibitory Concentration of the extract was observed in Serial dilution method using 96 well microtiter plate using Resazurin dye. Potent antibacterial activity against both Gram positive and Gram negative bacteria was shown by *F. indica* which creates opportunities to go for pharmaceutical research activities on the plant which has been used as traditional medicine since long time.

Keywords: *Flacourtia indica*, HPTLC finger printing, FTIR, Resazurin

1. Introduction

Healing with medicinal plants is as old as mankind itself. Traditional medical practitioners commonly

employ medicinal plants in their regular practise to alleviate many different kinds of ailments (Sofowora *et al*., 2013). *Flacourtia indica* or *Flacourtia ramontchi* (synonym) is commonly known as "BainchaKoli" (in Odia), Governer's plum or Indian plum. *F. indica* is a small shrub known for its edible fruits and medicinal properties. It belongs to the Flacourtiaceae family and is distributed throughout tropical regions of East Africa and Asia. Its therapeutic potentials there by provide a basis for future research. Every component of the plant, including the leaves, bark, stem, fruit, root system, and even the entire plant, has been demonstrated to have a variety of pharmacological properties. Antibacterial potency and phytochemical characterisation of *Flacourtia indica* (Burm.f.) Merr. root extract against human pathogens was reported by Eramma *et al.,* (2013) which is due to the presence of butyrolactonelignan disaccharide, coumarin such as scoparone and aesculeti, glycosides, flacourtin, tannins, sugar, β-sitosterol, β-sitosterol-β-D-glucopyranoside, ramontoside. The plant also possess antibacterial (Chingwaru *et al.,* 2020), antifungal (Eramma *et al.,* 2021), antioxidant (Saxena *et al.,*2010), anti-malarial (Kaou *et al.,* 2010 and Sashidhara *et al.,* 2013), anti-diabetic (Makuttan *et al.,* 2023), analgesic, anti-inflammatory and antipyretic effects (Islam *et al*. (2022), anti-asthmatic (Tyagi *et al*.,2011), hepatoprotective (Akter *et al*.,2020), antioxidant and anticancerous (Bashera *et al*., 2024) benefits.

Methanolic and Aqueous leave Extract of *Flacourtia indica* Merr possess significant antioxidant property because of alkaloids, flavonoids, glycosides, phenolic compounds, saponins, steroids, tannins and terpenoids (Saxena *et al.,* (2010). The analgesic, anti-inflammatory and antipyretic properties of the ethanolic extract of *Flacourtia indica* was studied by Islam *et al*., (2022). Root extract of *Flacourtia indica* has shown presence of 41 distinct secondary metabolites including Heneicosane, Squalene, Cholesterol, tetradecamethyl,2,4 Ditertbutylphenol, Cycloheptasiloxane hexadecamethyl, Cyclononasiloxane octadecamethyl and n-Hexadecanoic acid. All these compounds found to have antibacterial, antifungal, antioxidant, anti-cancer and anti-proliferative properties as a probable source of various pharmacological actions (Eramma *et al.,* 2023). Along with antioxidant activity, the *Flacourtia indica* extracts have anti-proliferative potential against a lung cancer cell line for the first time as investigated by Bashera *et al.,* (2024). Numerous ailments have been effectively treated with this plant. While most of the parts of *Flacourtia indica* (leaves, bark, stem, fruits, root, and even the entire plant) have been shown to have various pharmacological properties, the potential of the stem/leaf extract of the plant remains unexplored. The phytochemical screening of the medicinal plants followed by chromatographic methods provided basic information on secondary metabolites. A detailed literature review on *F. indica* under investigation has shown that, so far, possible phytoconstituents of the chloroform and ethanolic extract of stem/leaf extract of the plant through phytochemical screening, TLC, HPTLC, FTIR methods, their quantification and their possible antibacterial activities against test bacterial strain through ACPM, MIC, MBC through serial dilution method yet to be explored.

2. Experimental

2.1 Plant material and sample preparation

Fresh samples of *Flacourtia indica* were collected from Regional Plant Resource Centre (RPRC), Bhubaneswar, identified and authenticated by Dr P.C Panda, Principal scientist, RPRC. The fresh stem/leaf of plant were fully rinsed with tap water and subsequently with distilled water, shade dried for 15 to 20 days, chopped into small pieces, made to coarse powder with the help of electric grinder and were put in storage in air tight bottles until further analysis. Different solvents (polar and non-polar) like water, methanol, ethanol, benzene, n-hexane, chloroform were used to get the extract from the coarse powder of

the sample by using Soxhlet apparatus for 72 hrs at room temperature. The liquid extract was filtered (Whatman filter paper No. 1), subsequently concentrated by rotary vacuum evaporator (BUCHI TYPE,CEMC/RS/01) and preserved at 4° C in sealed bottle until further analysis.

2.2 Qualitative phytochemical screening

The presence of Phenol, Flavonoids, Tanin, Alkaloids, Coumarin, Terpenoids, Steroids and other phytoconstituents were screened to know their presence in the plant extract following the protocol of Harbone, (1988).

2.3 Quantification of secondary metabolites

2.3.1 Phenolics Estimation

The quantity of total phenolics present in the extract was evaluated by the method of Khaabadi *et al*., (2011). The calibration curve alongside Gallic acid as the standard solution was used for estimating the phenol concentration. The unit of measurement is milligrams of Gallic acid equivalents per milligram of dried matter.

2.3.2 Flavonoids Estimation

To determine the amount of flavonoid of a sample, the method of Queitter, 2000 was followed. By taking Rutin as the standard, the calibration curve was used to determine the amount of flavonoid, which was subsequently expressed as mg of Rutin equivalents/g of dried sample.

2.3.3Tannins Estimation

The amount of tannin present in the sample was studied following the method of Marinova (2005).

2.4 Microbial culture

The microbial cultures used for the experiment were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh. Gram +ve bacteria like *Staphylococcus aureus* (MTCC 8785), *Streptococcus pneumoniae* (MTCC 655), *Corynebacterium kroppenstedtii* SPAB2 and Gram -ve bacteria including *Pseudomonas aeruginosa (*MTCC 7837), *Vibrio cholerae* (MTCC 3900), *Escherichia coli* (MTCC 1687) were used for the study.

2.4.1 Antibacterial activity by agar cup method (ACPM)

Agar cup plate method (ACPM) described by Eramma *et al*., (2013) was carried out for antibacterial activity study of the plant extract of *F. indica*. Ciprofloxacin was taken as standard antibiotic. Zone of Inhibition (ZOI) was measured by an antibiotic Zone scale following an overnight incubation.

2.4.2 Minimum Inhibitory Concentration (MIC)

The study aimed to determine the minimum antimicrobial potential of plant extracts that inhibit human pathogen growth using a modified tube dilution method (Rath *et al*., 1999). The minimum amount of extract inhibited was determined by the concentration at which the broth medium showed no bacterial growth. Ten test tubes were filled with sterile nutritional broth, and the extract was transferred to each tube, creating a uniform emulsion. Ciprofloxacin was used as a control, and 0.2 ml of the inoculum was introduced to each tube. The MIC was determined by preventing turbidity or no bacterial growth in the broth medium. The study also used a two-fold dilution approach to find the minimum inhibitory concentration with different concentration range of several extracts against test microorganisms.

Minimum Bactericidal Concentration (MBC) of the plant extract.

The bacteriostatic (merely inhibiting the growth of pathogens by the extract) or bactericidal (killing of

pathogens by the extract) activity were studied by sub-culturing one loop of the sample from MIC tubes on to the nutrient agar plates and incubated at 37°C overnight. The minimum concentrations which exhibit no growth of microbes was the MBC of that extract.

2.4.3 Antimicrobial susceptibility testing (AST)

In order to evaluate the antibacterial qualities, serial microdilution method (Jorgensen and Ferraro, 2009) was adopted where resazurin dye, serves as a redox indicator. As per O'Brien *et al*. (2000), when resazurin dye was added to the well, the live bacterial cells convert the non-fluorescent resazurin dye (blue in colour) into the fluorescent resorufin (pink in colour).

In brief, sterile Nutrient broth (NB) 100µl amount were dispensed separately into all wells of a sterile microtiter plate including the control well. 100µl of the plant extract was transferred to the first well using a sterile pipette and mixed for a homogenous emulsion. From the 1stwell, 100µl was taken and added to the 2ndwell, then to the 3rdwell and so on to the last tube and finally 100µl of emulsion was discarded. 20µl of test microorganism (over-night fresh culture) was added to each well using a Pasteur pipette. Ciprofloxacin was served as control and incubated for 18hrs at 37°C. After incubation 100 µl of Resazurin (0.015%) was added to each well, and then incubated again for further 2–4 hours to observe any colour change. The MIC for the test extract and bacterial strain was computed as the average of three readings. Pink colour represents viability, while blue colour represents growth inhibition. To find the minimum bactericidal concentration (MBC), the contents of wells having concentrations greater than the MIC value were immediately plated. The MBC value was computed when there was no colony growth in the immediately plated contents of the wells. The test was repeated twice with three replicates per assay.

2.5 Thin Layer Chromatography

Thin Layer Chromatography of the plant extract with the following solvents (Methanol, Ethanol, Chloroform, Benzene, n-Hexane) were processed for analysis of the phytoconstituents of *Flacourtia indica.* TLC plate of size 12x24 cm with 3mm thickness was prepared by uniformly pouring the slurry of silica gel and sterile water (1: 3). The plates were air dried and activated by drying at 110ºC in hot air oven for one hour. For separation of the metabolites, chamber was saturated with the solvent system i.e. Ethyl acetate and Benzene (1: 5) for one hour. Using micropipette, about 10µl of the extracts was loaded gradually over the plate and the process was repeated several times and air dried. Gallic acid served as standard. The plate was then placed in the saturated chamber for separation of components and after separation it was removed from the chamber and air dried at room temperature. To locate the spot the plate was sprayed with Folin Ciocalteau (FCR) reagent.

2.6 HPTLC

2.6.1 Preparation of standard solutions

Sigma-Aldrich (St. Louis, MO, USA) provided the standard gallic acid (CAS Number 149-91-7; ≥ 97.5%) and quercetin (CAS Number 117-39-5; ≥ 95%). Gallic acid and quercetin standard stock solution was prepared by dissolving 1 mg of each marker in 1 ml HPTLC grade methanol in an Eppendrof tube and vortexing for 2-3 minutes. Gallic acid and quercetin stock solution was further diluted with methanol to yield a standard gallic acid and quercetin solution (200 µg/ml).

2.6.2 Instrumentation and chromatographic conditions

It consisted of a Linomat 5 automatic applicator with a 100µl Hamilton syringe, twin trough plate development chamber, a Camag TLC scanner 3, and win CATS software. The system was manufactured

by CAMAG (Muttenz, Switzerland).

2.6.3 Development of HPTLC fingerprinting of gallic acid and quercetin

The extracts were dissolved with 0.5 ml of corresponding solvent. The solutions as obtained were used in HPTLC system, comprising of a Camag TLC scanner, along with linomat 5 automatic applicator. Approximately 2µl sample solutions and 2µl standard were taken as bands of 5 mm band width. Precoated silica gel 60 F_{254} plates (10 x 4 cm, 250µm thick; E. Merck, Mumbai, India) with margins of 8 mm from the bottom and 15 mm from the side borders were used for the spotting. The plate was kept in vertical position in a twin trough glass chamber (Camag, Switzerland) for the compounds' isolation, which was saturated with the appropriate mobile phase (ethylaceate and benzene, 1:5) for phenolics and flavonoids, respectively. At room temperature, the optimal chamber saturation time for the mobile phase was 20 minutes. The chromatographic preparation had a run length of 90 mm from the starting point.

2.6.4 Derivatization and scanning

The plate was developed by immersing sample HPTLC plate in a glass chamber (20 cm × 10 cm) containing the solvent system ethyl acetate: benzene 1:5 (v/v). After complete development, the plate was oven dried at 60ºC for 5 minutes for complete evaporation of the mobile phase. Scanning was carried out in densitometer by linear scanning at 254nm and 366nm. The scanned photographs of the HPTLC plate were taken in photo-documentation chamber (CAMAG REPROSTAR 3) under visible light, 550 nm. Peaks of the densitogram were identified along with their height and area.

2.7 FTIR (Fourier Transform Infra-Red Spectrometer) analysis.

Fourier analysis of the sample extract of *F. indica* was done within a range starting from 400 to 4000 cm^{1} and resolution of 4 cm^{1} in FTIR spectroscope (Shimadzu, IR affinity 1 Japan) to analyse the presence of functional groups in the extracts based on peak values in the range of 400-4000 nm.

Statistical Analysis

For every independent experiment, three replicates were obtained, and the values are shown as the mean±standard error. The data were subjected to analysis of variance. Duncan's multiple range test at $P \leq 0.05$ was used to confirm the differences with the least degree of significance.

3. Results

3.1 Phytochemical composition of extracts

The outcome of phytochemical screening of different solvent extracts of *F. indica* revealing the presence and absence of phytochemicals is shown in Table 1.

Table 1: Qualitative phytochemical composition of different extracts.

Phytochemicals	Solvents				
	N-Hexane	Benzene	Chloroform	Ethanol	methanol
Phenols	---	---	+++	+++	+++
Flavonoids	---	---	+++	+++	+++
Tannins	---	±	++	++	++
Alkaloids	---	++	----	----	---
Coumarin	---	---		++	++
Terpenoids	---	++		++	+++

Steroids	---	++	-----	----	+++

3.2 Quantitative phytochemical composition of different extracts.

Quantitative analysis for secondary metabolites in the plant extract of *F. indica* in the solvents like ethanol, methanol, distilled water, chloroform, benzene and n- hexane are shown in the Table 2.

Table 2: Quantitative phytochemical composition of different extracts.

Solvents	Phenolics	Flavonoids	Tannins
Ethanol	16.10 ± 0.33^{ab}	8.36 ± 0.21^{de}	17.2 ± 0.42^{a}
Methanol	15.23 ± 0.32^{ab}	7.45 ± 0.18^{e}	16.32 ± 0.35^{ab}
DistilledWater	14.03 ± 0.30^{bc}	6.81 ± 0.16^{ef}	16.02 ± 0.33^{ab}
Chloroform	9.17 ± 0.24^{d}	5.28 ± 0.16^{ef}	14.42 ± 0.31^{b}
Benzene	8.12 ± 0.21^{de}	2.81 ± 0.13^{ef}	12.26 ± 0.28^{c}
N-Hexane	7.43 ± 0.18^{de}	2.54 ± 0.12^{f}	9.06 ± 0.24^{cd}

The data represent mean±SE of replicates (n=3). Values in the table carrying different letters are significantly different at $P\leq0.05$ by Duncan's multiple range test.

3.3 Antibacterial activity by agar cup method (ACPM)

The antibacterial susceptability of *F. indica* plant extracts in polar solvents like methanol, ethanol and distilled water, as well as non-polar solvents like benzene, n-hexane, and chloroform with standard antibiotic ciprofloxacin in contrary to the test pathogenic bacteria was carried out by agar cup method (Fig 1.a-f). Out of the six test bacteria, *Streptococcus pneumoniae* (Fig1-b), *Vibrio cholerae* and *Escherichia coli* (Fig1-d, a) were found to be of greater sensitivity to chloroform plant extract producing wide zone of inhibition of 26±0.2, 23±0.3 and 23±0.1mm respectively. *Corynebacterium kroppenstedtii* (fig1-f) showed 17±0.1mm in comparision to ciprofloxacin with a zone of 32±0.1. Further, the ethanolic extract also showed greater sensitivity producing wide zone of inhibition of 24±0.2, 20 ±0.3 and 22 ±0.1mm respectively for the test bacteria *Streptococcus pneumoniae*, *Vibrio cholerae* and *Escherichia coli*. All tested bacteria were moderately sensitive to methanolic extract whereas N-hexane extract had no antibacterial susceptibility for the tested bacteria. The tested bacteria were also found to be resistant for aqueous and benzene extract with a zone of inhibition within a range of 7 to 8 ±0.1mm only. However, extract prepared in chloroform and ethanol steadily showed better antibacterial activity when compared to methanol, aqueous, benzene and n-hexane extract.

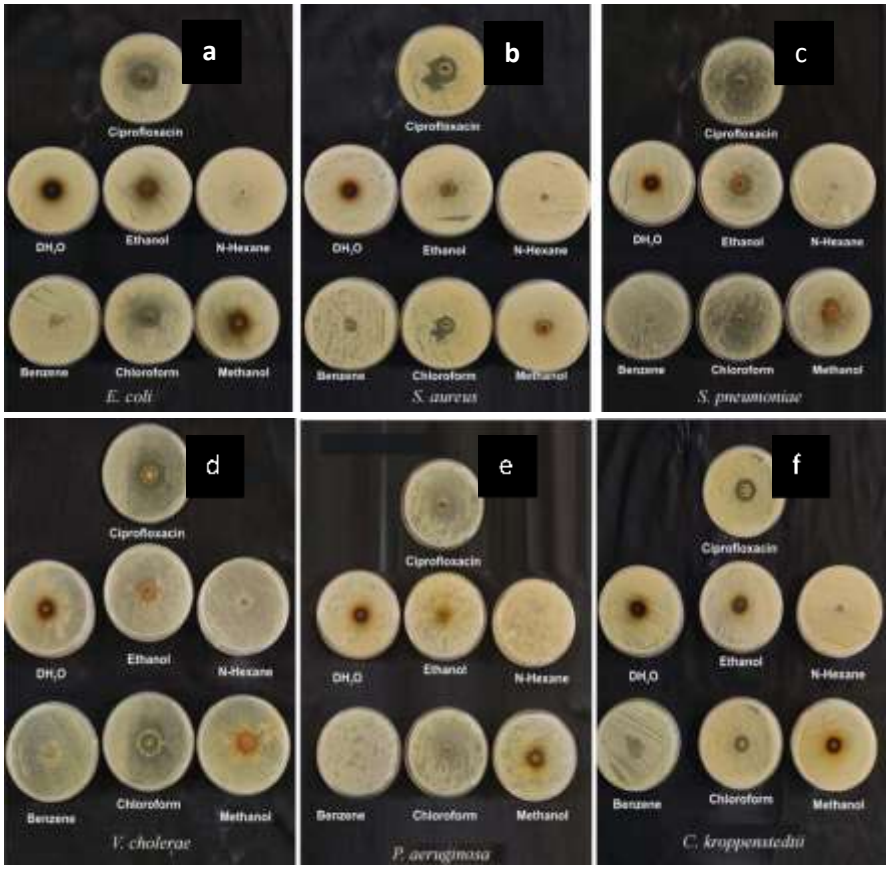
Table 3: Inhibitory effect of different plant extracts studied through ACPM

Zone of Inhibition (in mm)						
Extracts	***E. coli***	***S. aureus***	***S. pneumoniae***	***V. cholerae***	***P. aeruginosa***	***C. kroppenstedtii***
Methanol	20 ± 1.2^{c}	18 ± 1.2^{cd}	16 ± 1.2^{cd}	18 ± 1.2^{cd}	16 ± 1.2^{cd}	16 ± 1.2^{cd}
Chloroform	23 ± 1.3^{bc}	21 ± 1.2^{bc}	26 ± 1.3^{b}	23 ± 1.3^{bc}	19 ± 1.2^{cd}	17 ± 1.2^{cd}

Ethanol	22±1.3^{bc}	16±1.2^{cd}	24±1.3^{bc}	20±1.3^{c}	17±1.2^{cd}	19±1.2^{cd}
N hexane	-----	-----	-----	-----	-----	-----
Benzene	7±1.0^{e}	8±1.1^{de}	8±1.1^{de}	7±1.0^{e}	12±1.2^{d}	8±1.1^{de}
Distilled Water	7±1.0^{e}	7±1.0^{e}	8±1.1^{de}	7±1.0^{e}	8±1.1d^{e}	7±1.0^{e}
Ciprofloxacin (Standard)	32±1.4^{ab}	31±1.3^{ab}	33±1.4^{a}	32±1.4^{ab}	30±1.3^{ab}	26±1.3^{b}

The data presented displays the mean±SE of three replicates. According to Duncan's multiple range test, values in the table with different letters are substantially different at P≤0.05.

Fig 1: Inhibitory effect of plant extract on(a) *Escherichia coli,*(b) *Staphylococcus aureus* ,(c) *Streptococcus pneumoniae,* (d) *Vibrio cholerae*,(e) *Pseudomonas aeruginosa*,(f) *Corynebacterium kroppenstedtii* showing Zone of Inhibition.



3.4 MIC and MBC of the plant extract: Various concentrations of solvent extracts (chloroform and ethanol ranging from 0-400 g/ml) has been utilized to estimate the MIC values against the pathogens. From the result of MIC of the extracts against different organisms it was observed that the effective concentration ranged between 25-200 µg/ml (Table 4). Chloroform extract exhibited significant antibacterial activity against different human pathogens during screening through ACPM. Amongst all the extracts, the values of MIC for chloroform extract were found to be more effective with 25±1.0 µg/ml, 50±1.4 µg/ml, 100±1.6 µg/ml against *S. pneumoniae, V. cholerae* and *E. coli* respectively. The ethanolic

extract was also observed to be effective, with MIC of 50±1.1µg/ml, 25±1.5 µg/ml, 200±1.3 µg/ml against *S. pneumoniae, V. cholerae* and *E. coli* respectively which were less than the control ciprofloxacin (200±1.5µg/ml). MBC value was calculated to be 50 µg/ml for both chloroform extract and ethanolic extract against Streptococcus *pneumoniae*. From the above results it was evidenced that MIC is more effective in chloroform extract (50 ±1.0 µg/ml) followed by ethanolic extract (100.±1.0 µg/ml) but was found to be less effective as compared to the synthetic antibiotic Ciprofloxacin **(**200±1.5µg/ml**).**

Table 4: Minimum inhibitory concentration by two fold tube dilution method

(MIC) Minimum Inhibitory Concentration (µg/ml) of the extract against the microorganisms		
	Chloroform extract	**Ethanol extract**
Control (Ciprofloxacin)	200±1.3^{a}	200±1.2^{a}
Streptococcus pneumoniae	25±1.0^{c}	50±1.1^{b}
Vibrio cholerae	50±1.1^{b}	25±1.0^{c}
Escherichia coli	100±1.2^{ab}	200±1.3^{a}

The data presented displays the mean±SE of three replicates. According to Duncan's multiple range test, values in the table with different letters are substantially different at P≤0.05.

Table 5: Minimum bactericidal concentration by two fold tube Dilution Method

(MBC) Minimum Bactericidal Concentration(µg/ml) of the extract against the microorganisms		
	Chloroform extract	**Ethanol extract**
Control (Ciprofloxacin)	200±1.4^{a}	200±1.4^{a}
Streptococcus pneumoniae	50±1.0^{b}	50±1.0^{b}
Vibrio cholerae	50±1.0^{b}	100±1.3^{ab}
Escherichia coli	100±1.2a^{b}	200±1.4^{a}

The data presented displays the mean±SE of three replicates. According to Duncan's multiple range test, values in the table with different letters are substantially different at P≤0.05.

Fig 2: Microdilution method using Resazurin to determine the MIC of the plant extract against the test bacteria.

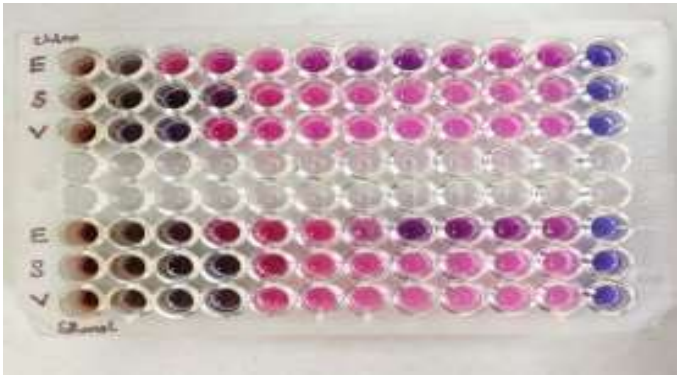


Table 6: Determination of MIC and MBC by microdilution method.

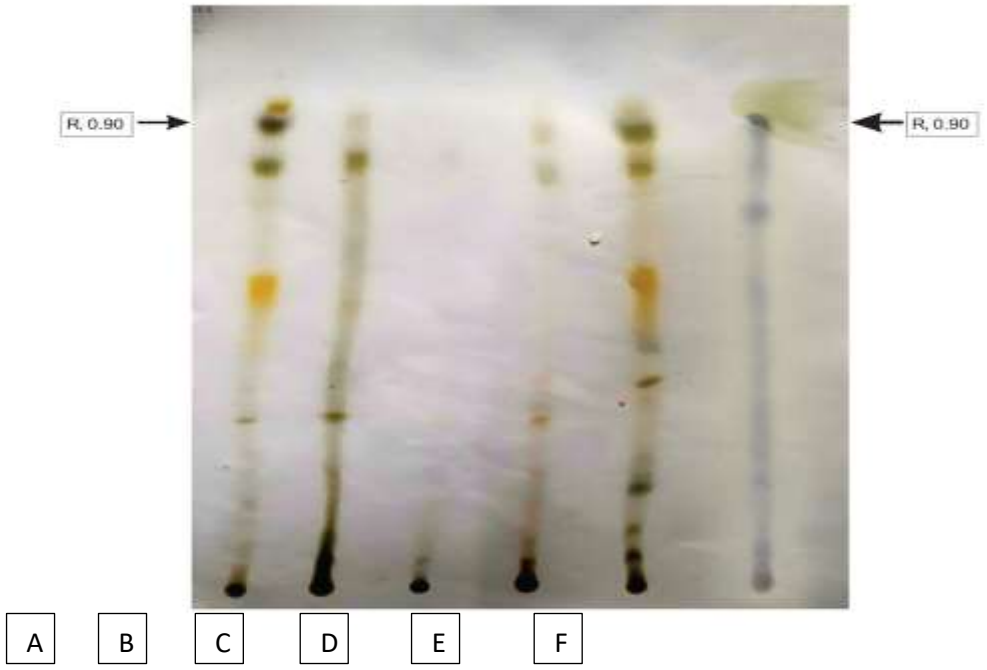
Extract	Chloroform extract		Ethanolic extract	
	MIC	MBC	MIC	MBC
Control (Ciprofloxacin)	200±1.0^{ab}	200±1.2^{a}	200±1.0^{a}	200±1.3^{a}
Streptococcus pneumoniae	25±1.1^{c}	50±1.3^{bc}	50±1.0^{bc}	50±1.2^{bc}
Vibrio cholerae	50±1.1^{bc}	50±1.1^{bc}	25±1.2^{c}	100±1.4^{b}
Escherichia coli	100±1.3^{b}	100±1.5^{b}	200±1.4^{a}	200±1.6^{a}

The data presented displays the mean±SE of three replicates. According to Duncan's multiple range test, values in the table with different letters are substantially different at P≤0.05.

3.5 TLC Profile

In the TLC profiling of the plant extract of chloroform, five bands were detected in the chromatogram depicting R_f values of 0.29, 0.54, 0.77, 0.90, and 0.92 with the solvent system ethyl acetate: benzene (1:5). Along with the plant extract gallic acid was taken as standard compound for identification of phenol. To locate the spot FCR was sprayed over the plate after removing from solvent system. A spot with R_f value 0.90 was observed which coincides with the spot developed by standard gallic acid having same R_f 0.90 and marked as phenol (Fig 3).

Fig 3: TLC of *Flacourtia indica* of different extracts; A: Chloroform, B: Ethanolic, C: Methanol, D: Benzene, E: n-Hexane, F: Gallic acid

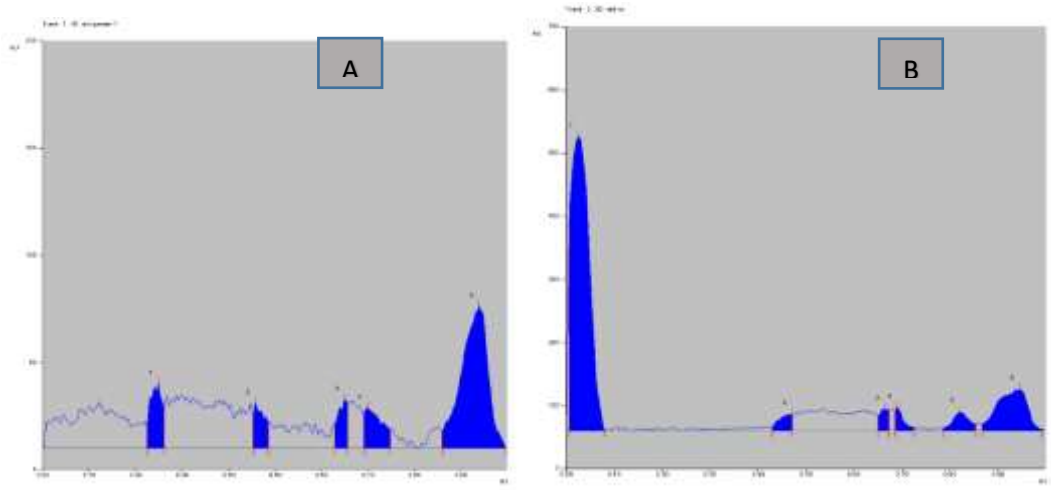


3.6 HPTLC profile:

Remarkable bands were observed in chloroform extracts of *F. indica* as compared to standard gallic acid and quercetin. HPTLC profile of soxhlated chloroform extract of *F. indica* under 254 nm scanning

exhibited ten polyvalent phyto-constituents (Fig-5) with R_f values from 0.02 to 0.99 with optimum phyto-constituents of 49.43% whereas the soxhlated ethanolic extract of *F. indica* under 254 nm scanning exhibited five polyvalent phyto-constituents (Fig-7) with a range of R_f values from 0.02 to 0.94 with optimum phyto-constituents of 71.48%. Similarly, soxhlated chloroform extract of *F. indica* under 366 nm exhibited five polyvalent phyto-constituents (Fig-5) with R_f values from 0.02 to 0.91 with optimum phyto-constituents of 70.07%. However, the ethanolic extract exhibited only two polyvalent phyto-constituents (Fig-7) with R_f values from 0.02 to 0.92 with optimum phyto-constituents of 94.35%. Both the extracts of *F. indica* were studied and compared with the standard gallic acid and quercetin. Plant extracts showed remarkable peak at the UV range similar to the standard gallic acid and quercetin. Under 254nm, the chloroform extract of *F. indica* showed the presence of both Gallic acid and Quercetin with R_f values 0.94 and 0.91 respectively (Table-7) whereas the ethanolic extract showed the presence of Gallic acid with R_f value 0.94 (Table -7). Similarly under 366nm, the chloroform extract of *F. indica* evidenced the presence of Gallic acid with R_f value 0.24 (Table-8). From HPTLC chromatogram it was evidenced that gallic acid in phenolic form and quercetin in flavonoid form are the constituents in various solvent extracts of *F. indica* extract.

Fig 4: HPTLC Densitogram for different solvent extracts of *F. indica* at 254nm for detection of Phenolics and Flavonoids. Phenol/Gallic acid (A), flavonoids/Rutin (B), Flavonoids/Quercetin (C), chloroform extract (D), ethanolic extract (E) .



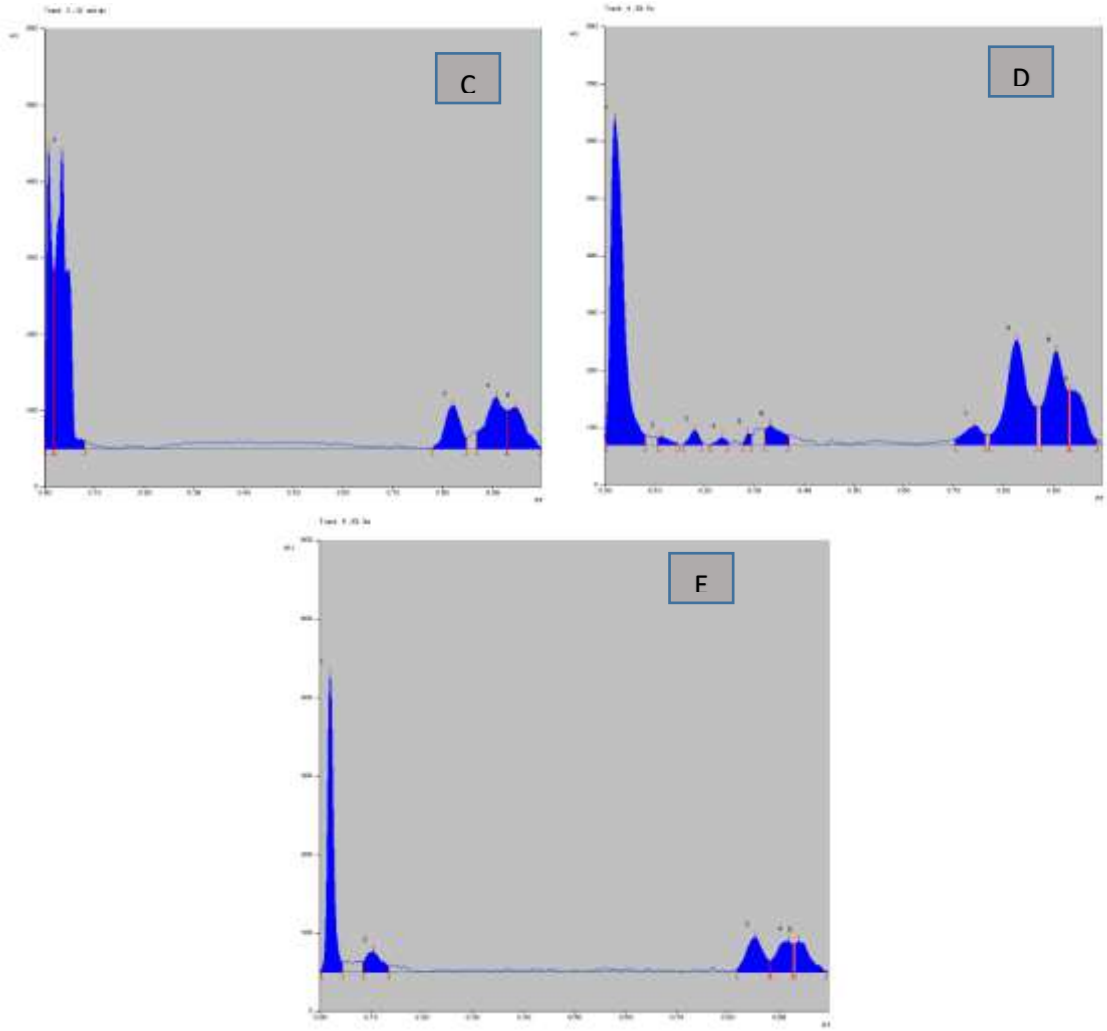


Fig 5: 3D HPTLC densitogram for different solvent extracts of *F. indica* at 254nm

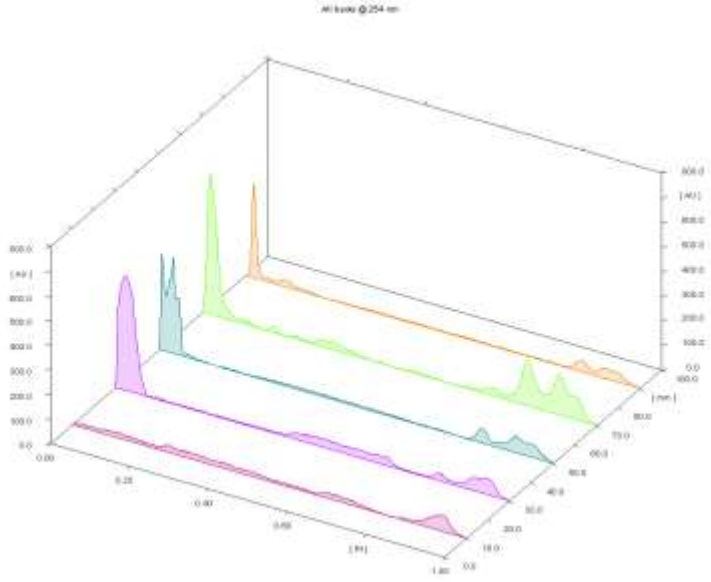
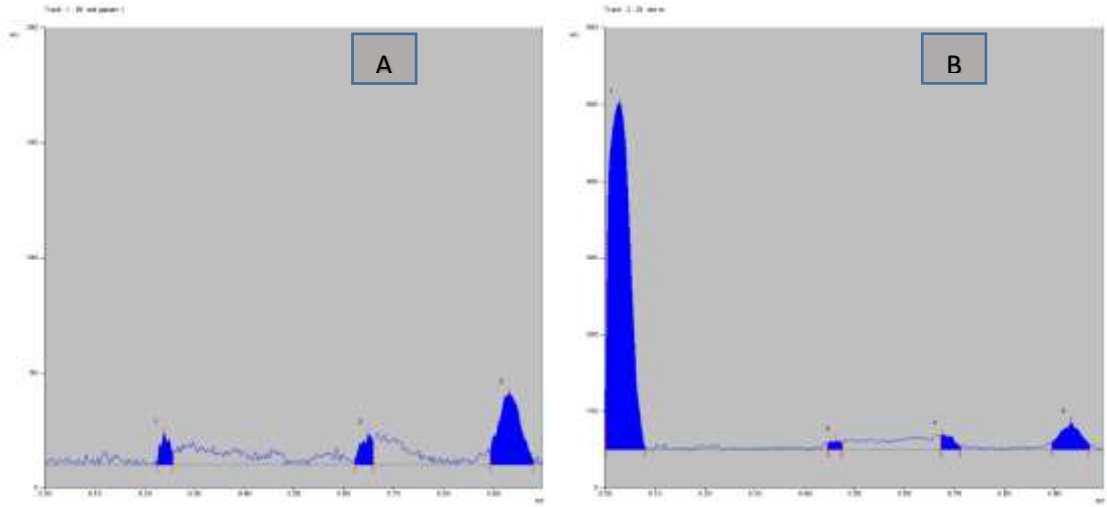


Table 7: HPTLC profiling of *F. indica* extract (Under 254nm)

	A		B		C		D		E	
Peak	**R_f Value**	**Area**	**R_f Value**	**Area**	**R_f Value**	**Area**	**R_f Value**	**Area**	**R_f Value**	**Area**
1	0.25	699.8	0.01	16739.3	0.01	3660.2	0.02	13584.6	0.02	4005.1
2	0.46	457.5	0.47	688.6	0.04	8464.1	0.11	299.5	0.11	760.2
3	0.65	435.4	0.67	617.2	0.82	1619.0	0.18	433.5	0.85	1390.1
4	0.70	663.3	0.69	582.4	0.91	2316.4	0.23	219.4	0.92	1149.9
5	0.94	3483.2	0.82	953.6	0.95	794.6	0.29	221.9	0.94	1026.4
6	------	------	0.95	3820.9	------	------	0.33	1016.6	------	------
7	------	------	------	------	------	------	0.74	1157.5	------	------
8	------	------	------	------	------	------	0.83	7198.4	------	------
9	------	------	------	------	------	------	0.91	5457.2	------	------
10	------	------	------	------	------	------	0.94	2607.8	------	------

HPTLC profiling of *F.indica* ethanolic extract with R^f values, area of Phenol/Gallic acid (A), flavonoids/Rutin (B), Flavonoids/Quercetin (C), chloroform extract (D), ethanolic extract (E) of *F. indica*.

Fig 6: HPTLC Densitogram for different solvent extracts of *F. indica* at 366nm for detection of Phenolics and Flavonoids. Phenol/Gallic acid (A), flavonoids/Rutin (B), Flavonoids/Quercetin (C), chloroform extract (D), ethanolic extract (E)



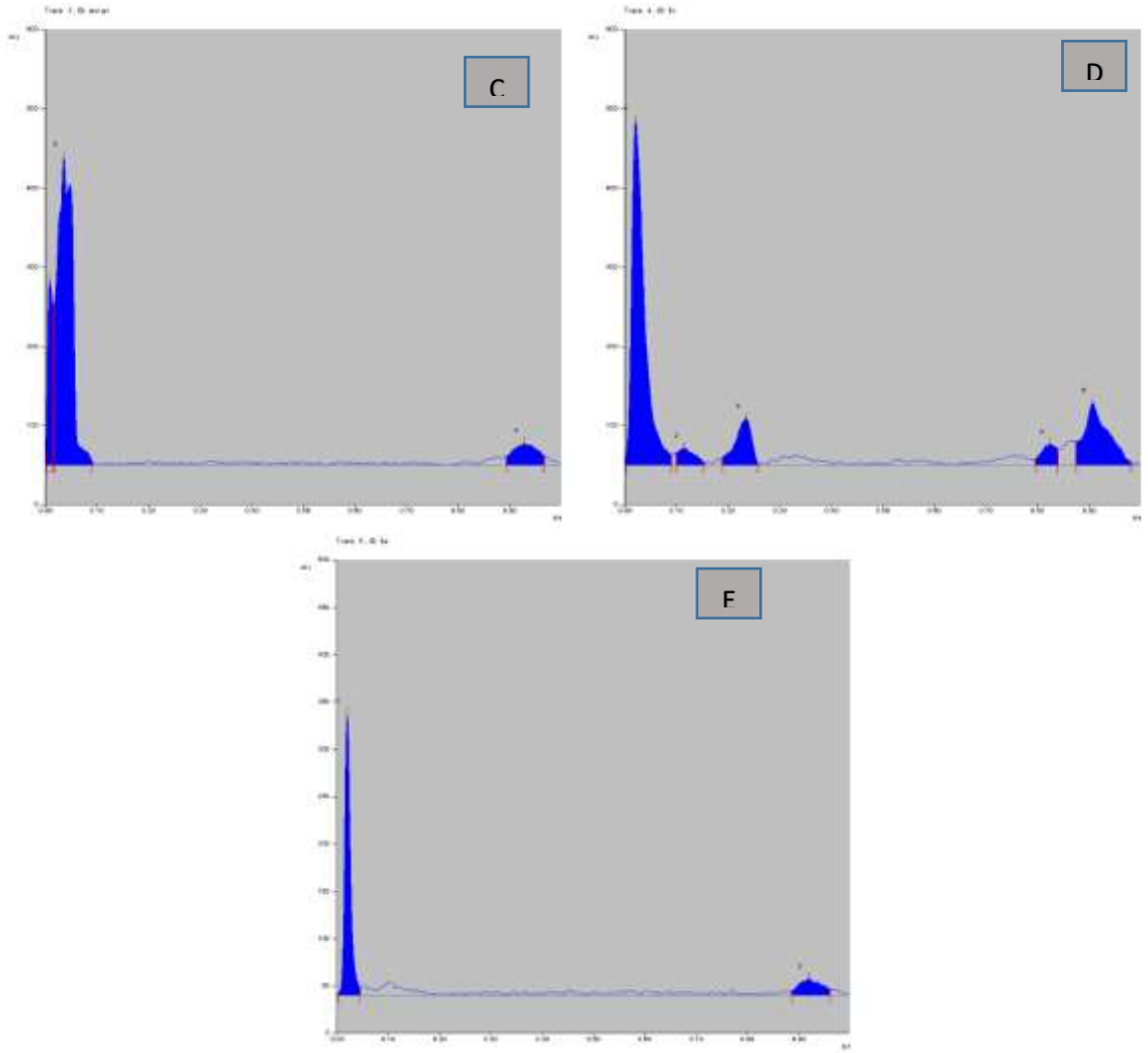
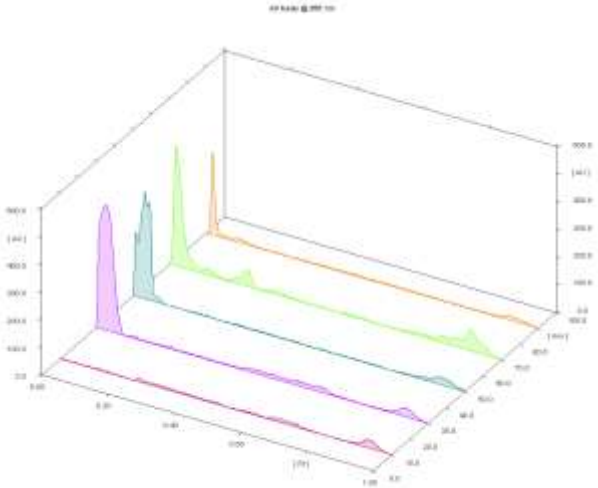


Fig 7: 3D HPTLC densitogram for different solvent extracts of *F. indica* at 366nm



Peak	A		B		C		D		E	
	R_f Value	Area	R_f Value	Area	R_f Value	Area	R_f Value	Area	R_f Value	Area
1	0.24	227.4	0.03	17411.2	0.01	2103.7	0.02	10072.3	0.02	3075.6
2	0.65	285.7	0.46	247.7	0.04	10907.3	0.12	642.2	0.92	710.7
3	0.93	1269.5	0.68	439.1	0.82	1220.5	0.24	1728.9	------	------
4	------	------	0.94	1301.8	0.93	-----	0.82	709.9	------	------
5	------	------	------	------	------	-----	0.91	3404.9	------	------

Table 8: HPTLC profiling of *F. indica* extract (under 366nm)

HPTLC with R_f values, area of Phenol/Gallic acid (A), Flavonoids/Rutin (B), Flavonoids/Quercetin, (C), chloroform extracts (D), ethanolic extract (E) of *F. indica*.

3.7 FTIR analysis:

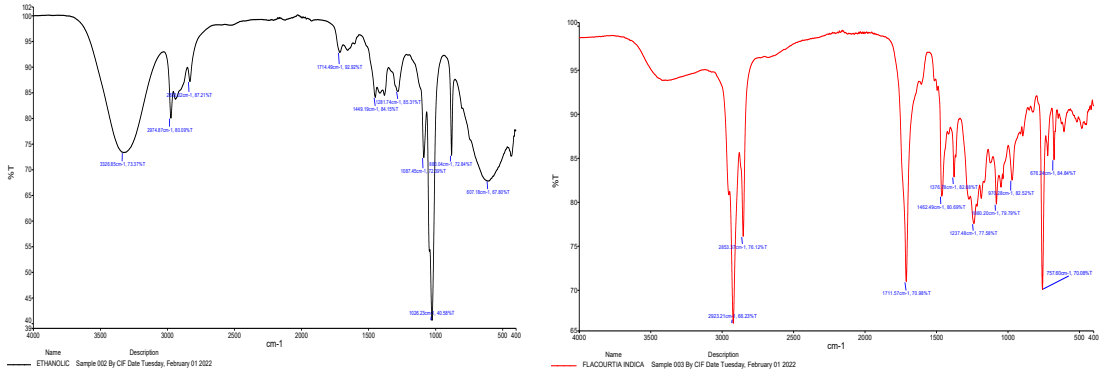
The results of the FTIR analysis of *F. indica* extracts were presented in the table 9. Through FTIR analysis the presence of Alcohol phenols, Alkenes, Aromatics, Alcohol, Aliphatic amines, Carboxylic acid, Esters, Ethers, Nitro compounds, Primary, Secondary amines and Alkyl halides were confirmed.

Sl No.	Frequency Range	Observed frequency in chloroform extract of *F. indica*	Observed frequency in ethanolic extract of *F. indica*	Functional groups	Bond
1	3500-3200	3326.85	----------	Alcohol phenols	O-H
2	3000-2850	2833.02	2923.21	Alkenes	C-H
3	3300-2500	2974.87	2853.37	Carboxylic acid	C-H
4	2260-2100	----------	----------	Alkynes	C-C
5	1750-1700	1714.49	1711.57	C-O stretching consolidant	C-O
6	1680-1640	----------	----------	Alkenes	C-H
7	1550-1475	----------	----------	Nitro compounds	N-O
8	1470-1450	----------	----------	Alkenes	C-H
9	1500-1400	1449.19	1462.49	Aromatics	C-C
10	1400-1290	----------	1376.78	Nitro compounds	N-O
11	1320-1000	1281.74	1237.48	Alcohol, Carboxylicacid, Esters, Ethers	C-O
12	1300-1150	----------	----------	Alkyl halides	C-H
13	1250-1020	1087.45, 1026.23	1080.2, 970.28	Aliphatic amines	C-N
14	910-665	880.04	757.6	Primary,Secondary amines	N-H

15	700-610	----------	----------	Alkynes	C (triple bond) C-H:C-H
16	690-515	607.18	676.24	Alkyl halides	C-Br

Table 9: FTIR analysis of the extracts.

Figure 8: FT-IR spectral analysis of chloroform extract and ethanolic extract of *F. indica*.



4. Discussion

The present investigation aimed to authenticate some traditional uses of the plant *F. indica* for further exploration. The exploration was carried out for phytochemical analyses and antimicrobial activities of *F. indica*.

The research work involves collection of leaves of *F. indica*, preparation of leaf extracts, screening and quantification of phytochemicals, to evaluate the antibacterial activity and the structural analysis through HPTLC, FTIR. Eramma *et al*., (2013) revealed the existence of flavonoids, saponins, alkaloids, tannins, terpenoids, glycosides and phenolic compounds in the root extract of *F. indica* as observed in the current investigation during phytochemical screening. Ndhlala *et al*., (2007) also reported the presence of similar phytochemicals like phenol, flavonoids and tannin in the fruit extract of *F. indica*. The methanolic extract of stem bark also showed the presence of Phenolic Glucosides (Madan *et al.,* 2009) which may be responsible for the antibacterial and antifungal properties shown by the methanolic root extract of *F. indica.* Presence of tannin and flavonoids in the aqueous extract of *F. indica* as reported by Chingwaru *et al*., (2020) contribute towards subsequent development of the present study.

Ample investigations have been carried on the leaves of *Flacourtia indica* [Saxena *et al.,* 2010., Islam *et al*.,2022, Eramma *et al*.,2023, Bashera *et al.,* 2024], however, not many studies are available on the antimicrobial and pharmacological potency of *Flacourtia indica* aerial parts. Aqueous extracts of bark of *Flacourtia indica, Swartzia madagascariensis* and *Ximenia caffra* have showed antibacterial/ antidiarrhoeal activitis against *Shigella* spp., *Salmonella typhi* and *Escherichia coli* O157 when compared to ethanolic extract (Chingwaru *et al.,* 2020).The present investigations revealed that chloroform extract (nonpolar solvent) of *F. indica* exhibited highest zones of inhibition against all tested microorganisms followed by ethanolic extract (polar solvent). Significant antimicrobial activity of chloroform extract was observed against *Streptococcus pneumoniae* (with 26mm ZOI), *Vibrio cholerae* with (23mm ZOI) and *E. coli* with (23mm). Methanolic root extract of *F. indica* revealed for poor antibacterial activity against

Vibrio cholerae with (8mm ZOI) and *E. coli* with (6mm) by Eramma *et al*. in 2013. Moderate ZOI was observed in Benzene and aqueous extract for the six tested bacteria like *Streptococcus pneumoniae, Staphylococcus aureus, Corynaebacterium kropenstedtii, Pseudomonas aeruginosa, Vibrio cholerae and Escherichia coli* while N- hexane extract showed no profound result. Moreover, such effectiveness was also confirmed by Eramma *et al*., (2013) in the methanolic root extract against the test bacteria like *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Proteus sp., Klebsiella pneumoniae, Shigella dysenteriae* and *V. cholerae sp.* The results inferred that the antimicrobial compound present in the extracts of *F.indica* were effective against both Gram positive (*Streptococcus pneumoniae*) and Gram negative bacteria (*E.coli, Vibrio cholerae*). High range of MIC values were evidenced possibly due to the nature and level of antimicrobial compounds present in the plant extract.

Besides phytochemical screening, Chromatographic techniques and the HPTLC analysis confirm the presence of the phytochemicals like phenol and flavonoids in chloroform and ethanolic extract of the plant whereas no significant result was obtained in the aqueous, methanolic, benzene and N-hexane extract.

To reveal secondary metabolites in the plant extract of *F. indica*, an effort was also made by TLC in the present study, The TLC profiling in ethyl acetate: benzene gave remarkable result indicating the presence of number of phytoconstituents. Better separation of chloroform extract with different R_f value with gallic acid as standard demonstrated the highest relative antibacterial activity; advocating that, chloroform solvent might have had higher solubility for antimicrobial phytoconstituents.

For the simultaneous quantitative determination and validation of scopoletin and gallic acid in the methanolic fraction of *Jatropha glandulifera* L., a High-performance thin-layer chromatography (HPTLC) method was carried out by Dwivedi *et al.,* (2020). Similarly Padhiari *et al., (*2020) also went for rapid and simultaneous determination of vasicine and vasicinone from the plant parts of *Justicia adhatoda* L. The simultaneous analysis of quercetin and gallic acid in *Eclipta alba* and *Guiera senegalensis* was done by Khalid *et al*. in 2021. Gajjar *et al.* (2022) conducted a fast and simple quantitative investigation of *C. mukul* in both unpurified and purified guggul using high-performance thin-layer chromatography (HPTLC).

HPTLC profile of ethanol extract of stem bark and small branches of *F. indica* was also carried out by Srivastava *et al*., (2015) which showed five bands at R_f 0.44, 0.62, 0.71, 0.74, 0.77, specifying the existence of at least four similar compounds in the plant extract. HPTLC profile of soxhlated chloroform extract of *F. indica* under 254 nm scanning exhibited ten polyvalent (10 peaks) phyto-constituents with R_f values from 0.02 to 0.99, whereas the soxhlated ethanolic extract of *F. indica* under 254 nm scanning exhibited five polyvalent phyto-constituents with R_f values ranging from 0.02 to 0.94. Similarly, soxhlated chloroform extract of *F. indica* under 366 nm exhibited five polyvalent phyto-constituents with R_f values from 0.02 to 0.91 whereas the ethanolic extract exhibited only two polyvalent phyto-constituents with R_f values from 0.02 to 0.92. From the chromatogram it was evident that gallic acid in phenolic form and quercetin in flavonoid form are the constituents in chloroform and ethanol solvent extracts of *F. indica.*

Through FTIR analysis in the range of 400 to 4000 cm^{-1}, the presence of phenols, alkenes, carboxylic acid, aromatics, nitro compounds, Alcohol, esters, ethers, aliphatic amines, primary and Secondary amines, alkyl halides were confirmed with peaks at 3326.85 cm^{-1} , 2974.87 cm^{-1}, 2853.37 cm^{-1}, 1462.49 cm^{-1} . Earlier it was confirmed by Ahmad *et al*., (2020), who obtained the peaks for phenolic hydroxyl groups (3503 $cm^{-1,)}$, 2969 cm^{-1} (C–H bonds), flavonoids and aromatic groups (2353 cm^{-1} and 663 cm^{-1}). Moreover thirteen phenolic, four flavonoids together with fourteen various known compounds, were isolated from the methanolic extract of leaves of *Flacourtia indica,* Nguyen *et al*., (2021). Our findings

suggested that the chloroform and ethanolic extract of *Flacourtia indica* stem/leaf extract possessed antibacterial activity due to the presence of various phyto-constituents which may be contributing to the antimicrobial activity. Hence, additional study must be conducted to evaluate the individual phyto-constituents as well as the pharmacological profile of the plant.

5. Conclusion:

The outcomes of this study on the extracts of *Flacourtia indica* in ethanol and chloroform proved their broad spectrum antibacterial properties. The existence of some biologically active phytochemical elements has proved their effectiveness in the plant through antibacterial screening, MIC determination and MBC determination utilising the two fold dilution method and resazurin dye. Following phytochemical screening, TLC, HPTLC fingerprinting, and FTIR profiles of this plant, the principal compounds like phenols and flavonoids, with a specific solvent system may be responsible for the antibacterial potency of the chloroform extract and ethanolic extract of *Flacourtia indica*. Therefore, the plant's extract may be helpful in the management of numerous bacterial illnesses, and a step towards for newer drug development. Though it is a preliminary study, studies such as this is a prerequisite to apply the antimicrobial potential of medicinal and aromatic plants.

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