International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 25-01-2022, Accepted: 09-02-2022, Published: 25-02-2022
Volume 7, Issue 2, 2022, Page No. 569-573

Assessment of phytochemical constituents, trace metals, and antimicrobial efficacy of *Flacourtia indica*, Southern India

Marimuthu Koperuncholan*, Subramaniyan Kulandaivel
Department of Botany, Srimad Andavan Arts and Science College, (Affiliated to Bharathidasan University), Tiruchirappalli, Tamil Nadu, India

Abstract
Flacourtia indica leaves were studied for phytochemical components, trace metal concentrations, and antibacterial activity using three different extracts (petroleum ether, chloroform, and ethanol). Steroids, triterpenes, alkaloids, phenols, flavonoids, saponins, and tannins were found in the crude extract after the phytochemical screening. The trace metals in the leaves powder were analyzed using a 797 VA Computrace voltammetry, Metrohm. Cd, Cr, Cu, Fe, Ni, Pb, and Zn concentrations were BDL, 0.03, 0.33, 0.87, BDL, BDL, and 0.55 mg kg-1, respectively.The zone of inhibition of three distinct solvent extracts of F. indica was compared to positive controls such as Methicillin – 10 mcg and Itraconazole – 10 mcg for in-vitro antibacterial activity against various infections. *Enterococcus fecalis* and *Trichophyton rubrum* were the most sensitive (19 mm), while *Micrococcus luteus* and *Cryptococcus sp*. showed the least inhibition (7 mm).

Keywords: *Flacourtia indica*, antimicrobial activity, phytochemistry, trace metals

Introduction
Flacourtia indica is a tree or shrub that grows to be between 3 and 10 meters tall. The bark is normally pale, grey, powdery, and flaking, revealing pale orange areas [1]. It can turn brown to dark grey and flake. The appearance of the vegetative parts ranges from glabrous to densely pubescent. When young, the leaves are red or pink, varying in size from oval to round, up to 12 cm in diameter, edge serrated, and leathery; 4-7 pairs of veins clear on both surfaces; stalk to 2 cm. Flowers can be unisexual or bisexual (1 or several branches of a female specimen with perfect flowers, which, however, bear fewer stamens than the males). Male flowers in axillary racemes 0.5-2 cm long, with thin, pubescent pedicels up to 1 cm long and minute, caducous basal bracts. Sepals (min. 4) 5-6 (max. 7) mm long and broad, roughly oval, apex sharp to rounded, hairy on both sides [2]. Anthers are 0.5 mm long and filaments are 2-2.5 mm long. The disc is lobulate. Female flowers are solitary or in small racemes, with pedicles up to 5 mm long. Styles 4–8, central, connate at the base, spreading, up to 1.5 mm long; stigmas truncate; disc lobulate, clasping the base of the ovoid ovary; styles 4–8, central, connate at the base, spreading, up to 1.5 mm long When ripe, the fruit is spherical, reddish to reddish-black or purple in colour, juicy, and up to 2.5 cm wide, with persisting styles and up to ten seeds. Seeds are 5-8, 8-10 x 4-7 mm in size, with a rugose, pale brown testa.
In South Africa, the botanical name has historical and geographical significance. 'Flacourtia' celebrates E. de Flacourt (1607-60), a governor of Madagascar who visited the Cape before van Riebeeck, and indica suggests that this small Transvaal bushveld tree can also be found in the east. Before blossoming, the tree is normally leafless [3].
The blooms bloom in India from December to April, along with new leaves that are a lovely fresh green colour. From March until July, the fruits ripen. Birds consume them, thus the seeds are widely spread, explaining the species' vast distribution [4].

Materials and Methods
Plant Material
Between January and February 2022, fresh leaves of *F. indica* were harvested in the Tiruchirappalli district of Tamil Nadu. In the Soxhlet apparatus, the shade-dried plant powder (100 g) was loaded into the thimble. It was equipped with a condenser and a suitable round bottom flask with 250 mL petroleum ether, chloroform, and ethanol [5].
The Mantox heater has given constant heat for solvent recycling. The extract was placed into clean and pre-weighed universal tubes after complete extraction in a round bottom flask. The % yield was estimated after weighing and recording the weight of universal tubes containing extracts. The percentage yield was computed by dividing the beginning weight of raw material by the final weight of extract, and it was utilized as an antibacterial activity test sample.

Phytochemical screening

Qualitative analysis

To determine the nature of phytochemical ingredients present in the sample, the solvent extracts were submitted to routine qualitative chemical analysis [6]. Steroids: 3 ml of the test solution plus a little amount of chloroform, 3-4 drops of acetic anhydride, and one drop of concentrated H2SO4 was added. As a result, the purple colour changes to blue or green, indicating the presence of steroids. A piece of tin and two drops of thionyl chloride were introduced to a 3 ml test solution containing triterpenoids. The presence of triterpenoids is indicated by the production of a violet or purple colour. Reducing Sugars: 3 mL test solution, 2 mL Fehling's reagent and 2 mL water were added to a 3 mL test solution. The presence of reducing sugar is indicated by the production of a reddish-orange hue. Sugars: A 3 mL sample of the test solution was heated with a little amount of anthrone reagent and a few drops of concentrated H_2SO_4.The presence of sugars is indicated by the production of a green or purple tint. Alkaloids: A 3 ml test solution with 2N HCl was ingested. The created aqueous layer was decanted, and one or a few drops of Mayer's reagent were added. The presence of alkaloids is indicated by the production of white precipitate or turbidity. Phenols: To a 3 mL test solution in alcohol, one drop of neutral ferric chloride (5%) solution was added. The presence of phenols is indicated by the production of strong blue colour. Flavonoids: A 3 mL test solution in alcohol has been heated, along with a pinch of magnesium and one (or) two drops of strong HCl. The presence of flavonoids is indicated by the production of a red or orange tint. Saponins: Three milliliters of test solution were mixed with three milliliters of water and shacked. Saponins can be detected by the production of foamy lather. Tannins: A 3 mL test solution containing water and lead acetate was added. The presence of tannins is shown by the production of a white precipitate. Anthraquinones: Magnesium acetate was added to a 3 mL test solution. The presence of anthraquinones is indicated by the production of pink colour. Amino Acids: A 3 mL test solution containing 1% ninhydrin in alcohol was added. The presence of amino acids is indicated by the production of a blue or violet tint. Catechins: Ehrlich reagent and a few drops of strong HCl were added to a 3 mL test solution in alcohol. The presence of catechins is indicated by the production of a pink tint.

Quantitative analysis of phytoconstituents

The chlorophyll pigments in the leaves were calculated using Arnon's method [6]. The material was homogenized and extracted three times in cooled 80 percent acetone (v/v) after pre-cleaning weighted fresh leaves. The acetone extract was diluted to a predetermined volume, and the optical density was measured using a spectrophotometer at wavelengths of 645nm and 663nm. The chlorophyll pigment content was determined and represented in mg/g fresh weight. The ninhydrin technique was used to calculate amino acids, which was calorimetrically measured at 570nm [7]. The Bradford method was used to quantify protein concentrations, and the absorbance was measured at 595nm against a blank/sample [8]. The anthrone technique, which can be tested colorimetrically at 620nm (or) by employing a red filter [9], was used to determine carbohydrate content. All of the experiments were repeated three times, and the average results were provided.

Trace metal analysis

F. indica (Cg1) plant leaves were carefully plucked and cleaned in sterile distilled water. The cleansed leaves were smiled with an agate mortar and pestle after drying in the shade. The samples of powered plants were kept in a sterile plastic container. In a Teflon bomb, 1 g of powdered plant sample was treated with aqua-regia mixture and incubated for 2-3 days at 140 °C. The reaction mixture was filtered with Whatman No. 1 filter paper after incubation. The extraction was then analyzed by the 797 VA Computrace voltammetry, Metrohm, for trace metals (Fe, Cu, Zn, Pd, Cd, Cr, and Ni). Before the examination, the devices were washed in acidified water (10 percent HNO3) and weighed to dissolve metals to avoid contamination. All of the equipment and containers were also soaked in 10% HNO3 for 24 hours before being cleaned thoroughly in de-ionized water. Also, determine the instruments' below detectable limit.

Testing of antimicrobial activity

The organisms tested were Aeromonas liquefaciens MTCC 2645 (B1), Enterococcus faecalis MTCC 439 (B2), Klebsiella pneumonia NCIM 2883 (B3), Micrococcus luteus NCIM 2871 (B4), Salmonella typhimurium NCIM 2501 (B5), Vibrio cholerae MTCC 3906 (B6), Candida albicans MTCC 16 (F4). The cultures came from the MTCC in Chandigarh and the NCIM in Pune, India. The antimicrobial sensitivity of microbial strains was determined using the well diffusion method [10]. On Muller Hinton agar (MHA) and potato dextrose agar (PDA), the antibacterial and antifungal properties of test samples were assessed against a variety of pathogens. In concentrated DMSO, the solvent extracted samples were dissolved. The bacterial suspension was inoculated on the surface of the agar plate with a sterile cotton swab. Separately, the three different concentrations of the sample (2.5, 5, and 10 mg/ml) were put into wells (1 cm in diameter and 4 mm in depth) of the agar plates. The DMSO was employed as a negative control in the investigation. For bacteria, the plates were incubated at 37°C for 24–48 hours and 25°C for 48–72 hours (for fungi). The zone of inhibition was measured with a ruler after incubation. The assays were done in triplicate, and the average results are shown. Positive controls included 10mcg of methicillin (for bacteria) and 10mcg of itraconazole (for fungus). Hi-Media supplied all of the media, regular discs, and sterile discs.

Results and discussion

Phytochemical constituents of Secondary metabolites

Bioactive substances were discovered in medicinal plants such as *F. indica* in this investigation. Tables 1 and 2 show the results of screening phytochemical constituents using qualitative and quantitative approaches. In steroid study, the purple colour that has resulted turns blue or green, indicating the presence of steroids. Similarly, positive and negative outcomes are shown by the presence or absence of colour change. Positive results were achieved in these screens from three distinct extracts: petroleum ether, chloroform, and ethanol. Steroids, alkaloids, phenols, flavonoids, saponins, and tannins were found in the low polar solvent petroleum ether employed to screen the triterpenoids from *F. indica*, as well as medium and high polar solvents chloroform and ethanol extracts [11]. The cyclization of the triterpene squalene yields both lanosterol and cycloartenol [12]. Cell membranes are made up of steroid hormones and phospholipids. Cholesterol and other steroid hormones reduce membrane fluidity [13]. In warmer weather, trees emit more terpenes, which operate as a natural kind of cloud seeding. The forest can adjust its temperature by reflecting sunlight through the clouds [14]. Antibacterial berberine, anticancer compound vincristine, antihypertensionagen treserpine, cholinomimeric galantamine, spasmolytic agent atropine, vasodilator vincamine, anti-arrhythmia compound quinidine, anti-asthma medicinal ephedrine, and antimalarial medication quinine are all alkaloids. Even though alkaloids affect a wide range of metabolic processes in humans and other animals, they all have a bitter taste [15]. Flavonoids, commonly known as Vitamin P or citrin [16], are a type of secondary plant metabolite. These metabolites are mostly employed in plants to make yellow and other pigments that are important in plant coloration [17-18].

Trace metals analysis

Some trace elements are required for plant growth, whereas others have an impact on plant physiology [19]. The function of trace metal pollution in producing plant harm, either directly or through altering the host physiology, makes it more susceptible to infection [20]. As a result, the photosynthetic process, growth, and efficiency are all affected [21-22]. Metal concentrations in plant samples were BDL, 0.03, 0.33, 0.87, BDL, BDL, and 0.55 mg kg-1 for Cd, Cr, Cu, Fe, Ni, Pb, and Zn, respectively (Table 3).

Antibacterial and Antifungal screening

The antibacterial activity of *F. indica* was tested using the well diffusion test on a variety of microorganisms. Table 4 summarises the results of the antibacterial activity. On MHA and PDA plates, the three tested doses of 2.5, 5, and 10 mg/ml produce a zone of inhibition for bacteria and fungi, respectively. In most of the microorganisms studied, a higher (10 mg/ml) concentration of sample had greater sensitivity than a lower (2.5 & 5 mg/ml) concentration. The petroleum ether and chloroform extract samples were more efficient against *Enterococcus fecalis* (B2) bacteria, while *Micrococcus luteus* had a smaller effect (B4). In fungi, the test sample was efficient against *Trichophyton rubrum* (F4) in all extracts, although *Cryptococcus Sp*. had a smaller effect (F2). Against *Aeromonas liquefaciens*, the ethanol plant extracts were the most effective (B1). Except for B1, B3, and B4, all microbial strains show higher sensitivity to the higher dose (10 mg/ml) for the test sample when compared to the positive control. A solution devoid of sample employed as vehicle control (concentrated DMSO) has no antibacterial action, indicating that antimicrobial activity was directly tied to the sample.

Table 1: Qualitative phytochemical constituent of *F. indica*

Phytochemical Constituents	Petroleum ether	Chloroform	Ethanol
Steroids	-	-	+
Triterpenes	+	-	-
Reducing sugars	-	-	-
Sugars	-	-	-
Alkaloids	-	+	-
Phenolics	-	+	-
Catechins	-	-	-
Flavonoids	-	+	-
Saponins	-	-	+
Tannins	-	-	+
Anthraquinones	-	-	-
Amino acids	-	-	-

+ = Present; - =Absent

Table 2: Quantitative phytochemical constituent of *F. indica*

Biochemical constituents	*F. indica* (mg/g)
Chlorophyll A	0.119
Chlorophyll B	0.952
Total Chlorophyll	1.071
Amino acid	160.0

Protein	2.110
Carbohydrate	1.009
Phenol	0.026

Table 3: Trace metal concentrations in *F. indica*

Sampling Site Name	Sample Name/ Family	Sample No.	S. Code	**Cd**	**Cr**	**Cu**	**Fe**	**Ni**	**Pb**	**Zn**
Tiruchirappalli, Tamil Nadu	*Flacourtia indica*	P1	Cg1	BDL	0.03	0.33	0.87	BDL	BDL	0.55

BDL – Below detectable limit

Table 4: Antimicrobial activity of *F. indica* leaf extracts in various solvents

S. No	**Test Microorganisms**	**Zone of inhibition in the well diffusion method (mm) Sample (2.5, 5 & 10 mg/ml)**										**Diseases**	**Route of Transmission**
		Petroleum ether extract			Chloroform extract			Ethanol extract			PC		
	Bacteria	2.5	5.0	10.0	2.5	5.0	10.0	2.5	5.0	10.0	10 mcg		
1.	*Aeromonas liquefaciens* B1	8	11	12	10	12	12	7	10	11	14	Wound Infections / Gastroenteritis	Water / Food
2.	*Enterococcus fecalis* B2	12	15	19	13	15	18	11	14	15	8	Endocarditis / Epididymal Infections	Water / Food
3.	*Klebsiella pneumoniae* B3	7	10	13	8	12	13	10	12	13	28	Acute diarrhoea / Dysentery	Water / Food
4.	*Micrococcus luteus* B4	8	9	12	7	10	12	8	13	17	38	Skin & Pulmonary infections	Soil / Water / Air / Food
5.	*Salmonella typhimurium*B5	11	12	13	7	11	13	10	14	16	0	Typhoid	Water / Food
6.	*Vibrio cholarae* B6	11	12	14	10	13	15	10	13	17	16	Cholera	Water / Food
	Fungi												
7.	*Candida albicans* F1	12	13	16	11	14	16	10	13	16	10	Skin infection / Gastrointestinal tract Infection	Air / Wound / Soil / Water
8.	*Cryptococcus* sp. F2	7	9	13	7	10	11	12	13	15	9	Bronchiectasis / Endophthalmitis.	Air / Wound / Soil / Water
9.	*Microsporum canis* F3	12	16	17	13	15	17	13	14	16	9	Tinea capitis /Ringworm	Air / Wound / Soil / Water
10.	*Trichophyton rubrum* F4	9	15	19	13	14	19	13	16	19	7	Tinea corporis / Tinea pedis	Air / Wound / Soil / Water

PC – Positive Control (Antibiotic disc; Bacteria – Methicillin (10mcg/disc); Fungi – Itraconazole (10mcg/disc)
Samples – 2.5, 5, 10 mg/ml (well)

Conclusion

Except for B1, B3, and B4, this study found that the presence of steroids, triterpenes, alkaloids, phenols, flavonoids, saponins, and tannins aid antibacterial activity and are more efficient than the positive control. Heavy metals demonstrated that the plant was resistant to the trace metal, and their secondary metabolites were unaffected by these metals, suggesting that they may penetrate the human food chain. Traditional herbal treatments must, by necessity, be given the benefits of current science and technology to meet future world demands. Soon, *F. indica* could be used as an alternate antibiotic.

Acknowledgement

The authors gratefully acknowledge the Department of Botany, Srimad Andavan Arts and Science College [Autonomous], Tiruchirappalli – 620 005, Tamil Nadu, India, for Docking Studies.

Reference

1. Nelson EK, Wheeler DH. Some Constituents of the Cannonball Fruit (*Couroupita guianensis*, Aubl.). Journal of the American Chemical Society,1937:59(12):2499-2500.
2. Jones RL, Jiménez-Saa H, Risk AC. A survey of the woody and climbing plants of the Refugio de Aves Dr. Alexander Skutch," Los Cusingos," Pérez Zeledón Canton, *Costa Rica*. Journal of the Botanical Research Institute of Texas, 2015, 149-165.
3. Kumar CS, Naresh G, Sudheer V, Veldi N. A short review on therapeutic uses of *Couroupita guianensis* Aubl. International Research Journal of Pharmaceutical and Applied Sciences,2011:1(1):105-108.
4. Anjaneyulu ASR, Rao SS. A new ketosteroid from the bark of *F. indica* Aubl. Indian journal of chemistry section b-organic chemistry,1998:37:382-386.
5. Arnon DI. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. Plant physiology,1949:24(1):1.
6. Hwang MN, Ederer GM. Rapid hippurate hydrolysis method for presumptive identification of group B streptococci. Journal of Clinical Microbiology,1975:1(1):114-115.
7. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical biochemistry,1976:72(1-2):248-254.
8. Koperuncholan M, Ahmed John S. Biosynthesis of Silver and Gold Nanoparticles and Antimicrobial Studies of Some Ethno medicinal Plants in South-Eastern Slope of Western Ghats. IJPI'S Journal of Pharmacognosy and Herbal Formulations,2011:1(5):10-15.
9. Koperuncholan M, John SA. Antimicrobial and Phytochemical Screening in *Myristica dactyloides* Gaertn. Journal of Pharmacy Research,2011:4(2):398-400.
10. Fazal Mohamed MI, Arunadevi S, Koperuncholan M, Mubarak MS. Synthesis and antimicrobial activity of some naphthyl ether derivatives. De r Chemica Sinica,2011:2:52-57.
11. Koperuncholan M. Bioreduction of chloroauric acid (HAuCl4) for the synthesis of gold nanoparticles (GNPs): A special empathies of pharmacological activity. Int. J. Phytopharm,2015:5(4):72-80.
12. Ahmed John S, Koperuncholan M. Direct root regeneration and indirect organogenesis in *Silybum marianum* and preliminary phytochemical, antibacterial studies of its callus. The International Journal of Pharmaceutics,2012:2:52-57.
13. Koperuncholan M, Manogaran M. Edible plant mediated biosynthesis of silver and gold nanoflakes against human pathogens. World Journal of Pharmaceutical Research,2015:4(1):1757-1775.
14. Sinthiya A, Koperuncholan M. In-silico characterization for Multiple sclerosis: A special emphasis on Tetrakis (4-aminopyridine-kN1) dichloridocopper (II) monohydrate with sphingosine 1phosphate lyase. Crystal Research,2015:89:36824-36826.
15. Baskaran T, Kandavel D, Koperuncholan M. Investigation of trace metals and secondary metabolites from *Pavetta indica* and study their antimicrobial efficacy. Research Directions,2018:6(6):273-282.
16. Sinthiya A, Koperuncholan M. Synthesis and characterization of l-amino acid doped 2-aminopyridine co-crystals for anti-cancer activity. Life Science Informatics Publications-Research Journal of Life Sciences, Bioinformatics, Pharmaceutical, and Chemical Sciences, 2019:5(2):754-762.
17. John SA, Koperuncholan M. Antibacterial Activities of various solvent extracts from *Impatiens balsamina*. International Journal of pharma and bio sciences,2012:3(2):401-406.
18. Koperuncholan M, Sathish Kumar P, Sathiyanarayanan G, Vivek G. Phytochemical Screening and Antimicrobial Studies of Some Ethno medicinal Plants in South-Eastern Slope of Western Ghats. Int. J. Med. Res,2010:1:48-58.
19. Harinee S, Muthukumar K, Dahms HU, Koperuncholan M, Vignesh S, Banu RJ, James RA. Biocompatible nanoparticles with enhanced photocatalytic and anti-microfouling potential. International Biodeterioration & Biodegradation,2019:145:104790.
20. Santhakumar M, Koperuncholan M. Gold nano-drug design for antimicrobial activity. Life Science Informatics Publications-Research Journal of Life Sciences, Bioinformatics, Pharmaceutical, and Chemical Sciences,2019:5(2):720-731.
21. Ramesh T, Koperuncholan M, Praveena R, Ganeshkumari K, Vanithamani J, Muruganantham P, Renganathan P. Medicinal properties of some *Dendrobium* orchids–A review. Journal of Applied and Advanced Research,2019:4(4):119-128.
22. Ramesh V, Ahmed John S, Koperuncholan M. Impact of cement industries dust on selective green plants: a case study in Ariyalur industrial zone. International Journal of Pharmaceutical, Chemical & Biological Sciences,2014:4(1):152-158.