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Original Article

FOOD VALUE ANALYSIS, PHYTOCHEMICAL PROFILING, LC/MS ANALYSIS, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF METHANOLIC EXTRACT OF *FLACOURTIA INDICA* (BURM.F.) MERR. FRUITS OF LATERITIC WEST BENGAL

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ABSTRACT

This study aims to evaluate the phytochemical profile, nutritional value and biological activities of methanolic extracts of *Flacourtia indica* fruits. The extract was analyzed for total phenolic content (34.72 ± 2.03 µg/mg), total flavonoid content (9.56 ± 1.21 µg/mg), ß-carotene and lycopene content at 0.0615 ± 0.01 (µg/mg) and 0.0235 ± 0.005 (µg/mg) respectively. Nutritional analysis of the fruit revealed total moisture content (41.56 ± 1.62 g/100 g), protein content (1.46 ± 0.17 g/100g), carbohydrate content (21.78 ± 0.37 g/100 g), fibre content (2.61 ± 0.13 g/100 g), and lipid content (1.89 ± 0.04 g/100 g). Antioxidant activity showed as IC_{50} values was 124.65 ± 1.38 µg/ml in case of ABTS assay and 189.14 ± 1.08 µg/ml in case of DPPH assay. Antibacterial assays showed strong activity against Gram positive bacteria *Staphylococcus aureus* (MTCC 96) and Gram negative bacteria *Pseudomonas aeruginosa* (MTCC 542), while antifungal activity against *Candida albicans* (MTCC 317) is minimal. MIC values of *Staphylococcus aureus* and *Pseudomonas aeruginosa* are 0.35 mg/ml and 0.32 mg/ml respectively, and 23 mg/ml in case of *Candida albicans*. LC/MS analysis identified nine compounds, with sparteine (anticonvulsant drug) being predominant. The results highlight the potential of *Flacourtia indica* as a rich source of natural antioxidants, high nutritional value and effective antimicrobials, offering potential applications in mitigating oxidative stress.

KEYWORDS: Antimicrobial activity, Antioxidant property, *Flacourtia indica,* Food value, LC MS, Phytochemicals.

1. Introduction

Flacourtia indica (Burm.f.) Merr., commonly referred to as 'Tuturi,' ' Boichi,' or 'Katai,' is a valuable medicinal plant belonging to the Flacourtiaceae family, noted for its effectiveness in addressing a range of health conditions. The fruits of *F. indica*, which represent the mature ovaries or seeds of the plant, are safe for consumption and are rich in natural antioxidants [1]. Recent literature emphasizes that a consistent intake of fruits is linked to a significant reduction in the risk of chronic diseases, including cancer [2], cardiovascular diseases, diabetes, and neurodegenerative conditions [3]. In traditional medicine, the edible parts of *F. indica* are utilized for managing various health conditions, such as roundworm infections, digestive disorders, jaundice, intermittent fever, age related disorders and immune system deficiencies [4], nephritic colic, cholera, and wounds, showcasing the plant's broad therapeutic potential.

The health benefits attributed to these fruits are primarily due to their rich antioxidant content, which protects biomolecules from oxidative damage induced by free radicals and reactive oxygen species [5]. Phenolic compounds, a type of phytochemical found in *F. indica*, play a crucial role in these protective effects [6], as studies have established a direct correlation between total phenolic content and antioxidant activity [7]. Polyphenolic antioxidants are known to prevent skin damage [8], slow aging [9], and protect cellular components such as DNA, protein and lipids from oxidative stress [10]. Antioxidants are compounds that can inhibit, delay or prevent the oxidation of oxidizable components [11] by scavenging and neutralizing free radicals. Additionally, flavonoids present in the fruits exhibit various beneficial properties, including anti-

inflammatory, antiviral, cytokine regulating [12], antimutagenic [13] and anti-carcinogenic effects [14].

Despite the extensive use of medicinal plants in traditional medicine, there is still a need for scientific validation of their therapeutic claims, particularly in tropical developing countries like India, where these plants serve as a vital resource for indigenous medicine. Previous phytochemical investigations of *F. indica* have identified a range of bioactive compounds, including tannins, saponins, alkaloids, flavonoids, and terpenoids [15], which contribute to their antioxidant and anti-inflammatory properties [16].

This study aimed to analyze the food value of *F. indica* fruits by assessing their total carbohydrate, protein, lipid, and fibre content, alongside phytochemical profiling, including total phenolic and flavonoid content, as well as ß-carotene and lycopene levels. Furthermore, the research employed LC-MS analysis to identify bioactive compounds and evaluated the antimicrobial (antibacterial and antifungal) and antioxidant (DPPH and ABTS assays) activities of the fruits. The aims of the paper have been achieved. The findings are intended to contribute to understanding nutritional and therapeutic potential of *F. indica*, providing a foundation for further research and possible applications in health-promoting formulations.

2. Materials and methods

The medicinal plant utilized in the present study is *Flacourtia indica* (Burm.f.) Merr. belonging to the Flacourtiaceae family, which was authenticated by the Botanical Survey of India (BSI), Howrah with voucher specimen number VU/ BM-01.The samples were collected from their natural habitat at Godapiasal Khirkul jungle in Paschim Medinipur district, Rospal jungle in Bankura district and Kankrajhore jungle in Jhargram district. Fruits were cleaned with tap water and shade-dried for 15-20 days. Dried fruits were crushed into powder form by using an electrical blender. Sample powder was stored in a zipper bag and kept at 4ºC for future use. Powered plant materials were taken in conical flasks and macerated in methanol (1:10 w/v) and stirred continuously for 72 hours on a shaker. The samples were filtered by using filter paper. Then the extracts wereevaporated utilizing a rotary evaporator under reduced pressure and kept at 4°C in airtight containers for future applications.

2.1. Phytochemical analysis

2.1.1. Estimation of Total Phenolic Content

The total phenolic content was estimated using Phuyal et al. [17] method. Gallic acid was utilized as standard. Optical density (OD) was determined at 760 nm spectrophotometrically. The total phenolic content was calculated as µg in gallic acid equivalents (GAE) per mg of sample.

2.1.2. Estimation of Total Flavonoid Content

The total flavonoid content was estimated using Phuyal et al. [17] method. Quercetinwas utilized as standard. Optical density (OD) was determined at 510 nm spectrophotometrically. The total flavonoid content was given as µg in quercetin equivalents (QE) per mg of sample.

2.1.3. Estimation of ß-carotene and lycopene content

ß-carotene and lycopene contents were evaluated using Nagata and Yamashita methods with slight modification [18]. 0.1 mL of the extract was subjected to strong agitation with 10 mL of hexane: acetone mixture (6:4) for 2 mins and immediate absorbance was measured at 663 nm, 453 nm, and 505 nm wavelengths.

ß-carotene and lycopene content were calculated utilizing the following formula:

ß-carotene (µg/mg) = 0.216×A663 - 0.304×A505 + 0.452×A453.

Lycopene (µg/mg) = 0.0458×A663 + 0.307×A505 - 0.452×A453.

2.2. Analysis by LC/MS

The isolation of phytochemicals was performed using solvent extraction, and the compounds were subsequently identified and profiled using a LC-MS system (Shimadzu, Japan LC-MS 2020), which included an integrated degasser (DGU-20A3R), a dual binary pump (LC-20ADXR), an autosampler (SIL-20AXR), a column heater (CTO-20AC), and a diode array detector (SPD-M20A), constructed with an electrospray ionization (ESI). The registration of chromatogram was implemented using an AQUASIL C18 analytical column (150 mm × 3 mm × 3 mm), maintained at 40º C temperature. The mobile phase comprised of 0.1% formic acid in methanol (solvent B) and water (solvent A) at a fluid velocity of 0.4 mL/min. The elution commences with 70% A/10% B from 0-30 min, 90% B from 30-45 min, from 45-55 min 100% B, from 55-60 min 90% A/10% B. Chromatograms were acquired using a photodiode array detector that was set to 350 nm. 10 µL volume was deposited and peaks were detected at 350 nm. By comparing the Rt (retention time) and the UV spectra, peak identification was achieved of fraction phenolic chromatogram. Mass spectrometric analysis was conducted using a Shimadzu massspectrometer. Mass spectral data were obtained in ionization mode within a mass range of m/z 50–1500. The instrument conditions were as follows: nebulizing gas pressure at 40 psi, drying gas flow at 12 L/min, drying gas temperature set to 400 °C, and nebulizing gas flow at 1.5 L/min. Negative ionization mode (m/z [M-H]−) was employed for accurate identification and quantification of compounds. The negative ionization mode was selected because phenolic compounds, such as flavonoids and phenolic acids, typically exhibit higher ionization efficiency in this mode. This ensures optimal sensitivity for detecting and quantifying these compounds, which were the main emphasis of the study. Negative ionization (m/z $[M-H]^-$) was commonly used for analyzing phenolic compounds because of their potential to lose a proton, which leads to more stable and consistent ionization compared to positive modes [19].

2.3. Proximate composition

50 grams of fresh fruit were placed into a moisture analysis container and left to air-dry in the shade for 10 to 15 days. The moisture content was then measured using the Thiex [20] method. Total carbohydrate content was estimated using the method of Miller [21] with slight modification. The amount of protein content was determined applying Lowry et al. [22] method. Total lipid content was estimated using the method of Itoh and

Kaneko [23]. Crude fibre was estimated using the method of Thiex [20].

2.4. Antioxidant activity

2.4.1. DPPH assay

The antioxidant activity of the sample was evaluated through the discoloration of DPPH. The sample was prepared at different concentrations. Ascorbic acid was utilized as standard. After incubation, OD value was assessed at 517 nm spectrophotometrically against the control. The IC50 value was determined to reflect the extract concentration required to scavenge 50% of the DPPH radicals [24].

DPPH radical scavenging (%) = ⌊(Abscontrol-Abssample) /Abscontrol⌋× 100

Abscontrol= Absorbance of the control (without sample)

Abssample = Absorption of the extract with the reagent

2.4.2. ABTS assay

ABTS assay was performed using Leong and Shui [25] method. As a standard Trolox was utilized. From the calibration curve, the IC50 value was calculated.

ABTS radical scavenging (%) = ⌊(Abscontrol-Abssample) /Abscontrol⌋× 100

Abscontrol= Absorbance of the control (without sample)

Abscontrol= Absorption of the extract with the reagent

2.5. In vitro antimicrobial activity

2.5.1. Antibacterial activity

The antibacterial activity was determined by agar well diffusion method. Approximately 25 ml of nutrient agar was deposited onto Petri plates and left to solidify. To create five wells in the Petri plates, a sterilized cork borer was utilized. Standardized inoculums of test organisms (*Pseudomonas aeruginosa* MTCC 542 and *Staphylococcus aureus* MTCC 96) were spread over nutrient agar using an L-shaped sterile spreader. Different concentrations of fruit extract were utilized to evaluate the antibacterial effect. Amoxicillin was utilized as a positive control and as a negative control DMSO (20%) was utilized. In an incubator, the Petri plates were placed for 24 hours at 37°C for optimal growth conditions. After incubation, the diameter of zone of inhibition was measured in millimetres. Minimum inhibitory concentration (MIC) was determined using the broth dilution method [26]. Total Bacterial Count (TBC) was calculated against *Pseudomonas aeruginosa* using the formula:

$$\text{Bacterial Count } \left(\frac{\text{CFU}}{\text{ml}}\right) = \frac{\text{Number of colonies counted}}{(\text{Dilution factor} \times \text{Volume of culture plated})}$$

2.5.2. Antifungal activity

Candida albicans (MTCC 317) was utilized for determination of the antifungal activity by disc diffusion assay. Approximately 25 ml of nutrient agar was poured onto Petri plates and allowed to solidify. Fungus was inoculated into Sabouraud Dextrose Agar (SDA) medium and incubated for seven days to obtain an active culture for the antifungal assay. Inoculated fungus (10^5 CFU/ml) was swabbed using a sterile swab. Clotrimazole (30 µg/ml) was utilized as antifungal agent. In an incubator, the Petri plates were incubated for 48 to 72 hours. After incubation, the diameter of zone of inhibition was measured in millimetres. Minimum inhibitory concentration (MIC) was measured utilizing the broth dilution method [27]. Total Fungal Count (TFC) was calculated against *Candida albicans* by applying the formula:

$$\text{Number of fungus } \left(\frac{\text{CFU}}{\text{ml}}\right) = \frac{\text{Number of colonies counted}}{(\text{Dilution factor} \times \text{Volume of culture plated})}$$

2.6. Statistical analysis

All experimental results were demonstrated as mean ± standard deviation (SD) and statistical calculation was executed using Microsoft® Office Excel (Microsoft®, USA). Variances among samples were compared using ANOVA, with p-values of <0.05 and <0.001 considered statistically significant.

3. Results

3.1. Phytochemical analysis

The methanolic extract showed high phenolic (34.72±2.03 µg/mg) and flavonoid (9.56±1.21 µg/mg) content. However, the quantity of ß-carotene (0.0615±0.01µg/mg) and lycopene (0.0235±0.005 µg/mg) are very low. The results are shown in Table 1.

Table 1. Total phenol, total flavonoid, ß-carotene and lycopene content of methanolic extract obtained from *F. indica* fruits. Results are presented as mean ± SD (n=3).

Total Phenol (µg/mg)	Total Flavonoid (µg/mg)	ß-carotene (µg/mg)	Lycopene (µg/mg)
s 34.72±2.03	9.56±1.21	0.0615±0.01	0.0235±0.005
e			

3.2. Liquid chromatography and mass spectrometry (LC/MS)

The LC/MS analysis exhibited nine phenolic compounds such as citronellol (retention time 1.176 min and area 8.10%), sparteine (retention time 1.434 min and area 13.88%), 8-prenylnaringenin (retention time 1.484 min and area 16.12%), capsidiol (retention time 1.658 min and area 7.44%), catechin-3-O-gallate (retention time 1.658 min and area 13.70%), harmine (retention time 2.136 min and area 17.60%), kaempferol-3-O-ß-d-glucuronide (retention time 3.658 min and area 32.64%), peonidin (retention time 4.015 min and area 20.35%) and delphinidin (retention time 4.113 min and area 5.59%). Citronellol is recognized for its antioxidant and antimicrobial activity. Sparteine is used as anticonvulsant drug. 8-Prenylnaringenin is noted for its potent phytoestrogenic activity. Capsidiol demonstrates antifungal properties, particularly against plant pathogens. Catechin-3-O-gallate exhibits strong antioxidant and antimicrobial properties, helping to mitigate oxidative stress. Harmine has shown promising antitumor and anti-inflammatory properties. Kaempferol-3-O-ß-d-glucuronide is noted for its antioxidant and anti-inflammatory activities. Peonidin is known for its anticancer activities. Delphinidin exhibits anticancer and cytoprotective activities. Presence of compounds and their pharmacological property with references are shown in Table 2. The graph is shown in Figure 1.

Table 2. Name of the pharmacologically active compounds found by LC/MS analysis along with their molecular formula, retention time, area and pharmacological property.

Peak	Name of the compound	Molecular formula	Retention time (min)	Area (%)	Pharmacological property	Reference
1	Citronellol	$C_{10}H_{20}O$	1.176	8.10	Antioxidant activity	[28], [29]
2	Sparteine	$C_{15}H_{26}N_2$	1.434	13.88	Anticonvulsant drug	[30]
3	8-Prenylnaringenin	$C_{20}H_{20}O_5$	1.484	16.12	Phytoestrogen activity	[31], [32]
4	Capsidiol	$C_{15}H_{24}O_2$	1.658	7.44	Antifungal activity	[33], [34]
5	Catechin-3-O-gallate	$C_{22}H_{18}O_{10}$	1.658	13.70	Antioxidant, antimicrobial activity	[35], [36]
6	Harmine	$C_{13}H_{12}N_2O$	2.136	17.60	Antitumor, anti-inflammatory activity	[37], [38]
7	Kaempferol -3-O-ß-d-glucuronide	$C_{21}H_{18}O_{12}$	3.658	32.66	Antioxidant, anti-inflammatory activity	[39]
8	Peonidin	$C_{16}H_{13}0_6$	4.015	20.35	Anti-cancer activity	[40]
9	Delphinidin	$C_{15}H_{11}O_7$	4.113	5.59	Anti-cancer, Cytoprotective activity	[41]

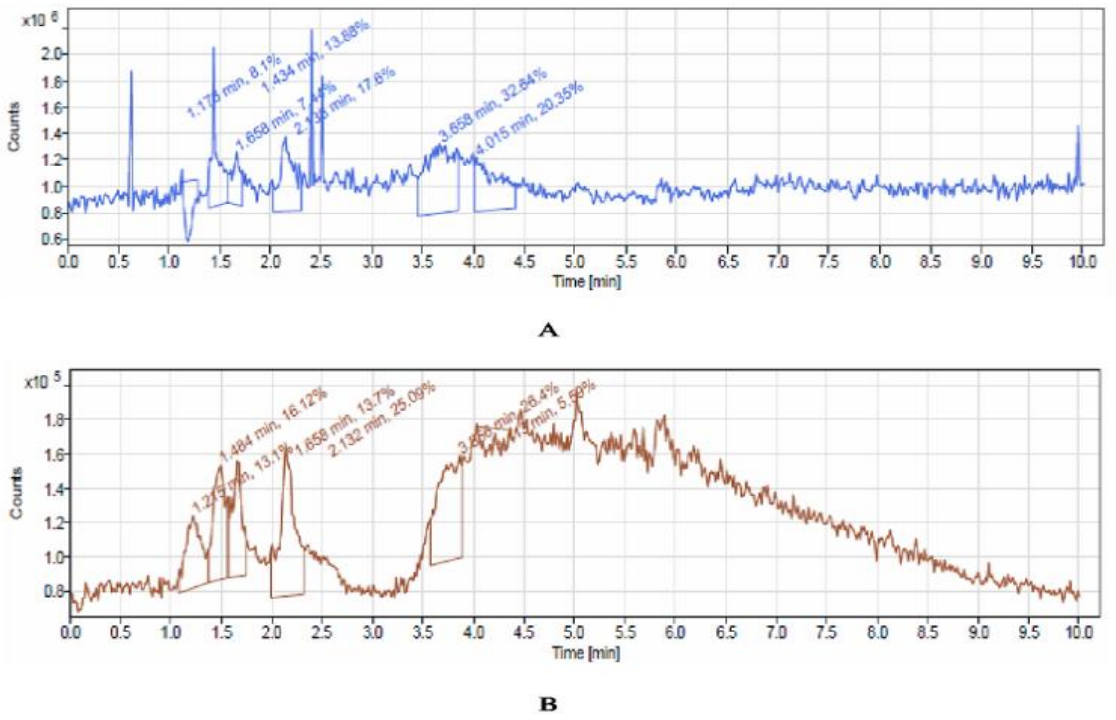


Fig. 1. LC/MS chromatogram of the methanolic extract of *Flacourtia indica* fruits identifying various bioactive compounds exhibiting retention times (RT), peak areas (%): (A) Signal description (MS1 +TIC SCAN ESI Frag=100V Gain=1.0) with Rt (retention time) and area % ; (B) Signal description (MS1 -TIC SCAN ESI Frag=100V Gain=1.0) with Rt (retention time) and area %

3.3. Proximate composition

High moisture content (41.56±1.62 g/100 g) is present. The amount of total carbohydrate content (21.78±0.37 g/100g), protein content (1.46±0.17 g/100 g), lipid content (1.89±0.04 g/100 g) and crude fibre content (2.61±0.13 g/100 g) are also satisfactory. Results are shown in Table 3.

Table 3. Moisture, carbohydrate, protein, lipid and crude fibre content obtained from *F. indica* fruits. Result are presented as mean ± SD (n=3).

Moisture content (g/100g)	Carbohydrate content (g/100g)	Protein content (g/100g)	Lipid content (g/100g)	Crude fibre content (g/100g)
41.56±1.62	21.78±0.37	1.46±0.17	1.89±0.04	2.61±0.13

3.4. Antioxidant activity

Methanolic extract showed high antioxidant activity. The IC_{50} value of DPPH is 189.14±1.08 µg/ml and ABTS is 124.65±1.38 µg/ml. Results are shown in Table 4 and the graphs are shown in Figure 2.

Table 4. Analysis of antioxidant activity of methanolic extract of *F. indica* fruits by estimation of IC_{50} value of DPPH and ABTS radical scavenging assay with respect to standard. Result are presented as mean ± SD (n=3).

Antioxidant activity	Fruits extract	Standard
DPPH radical scavenging assay (IC_{50}=µg extract/ml)	189.14±1.08[a]	11.54±0.02[a]
ABTS radical scavenging assay (IC_{50}=µg extract/ml)	124.65±1.38[b]	9.29±0.01[b]

Different letters in the column show statistically significant differences ($p<0.05$) according to ANOVA.

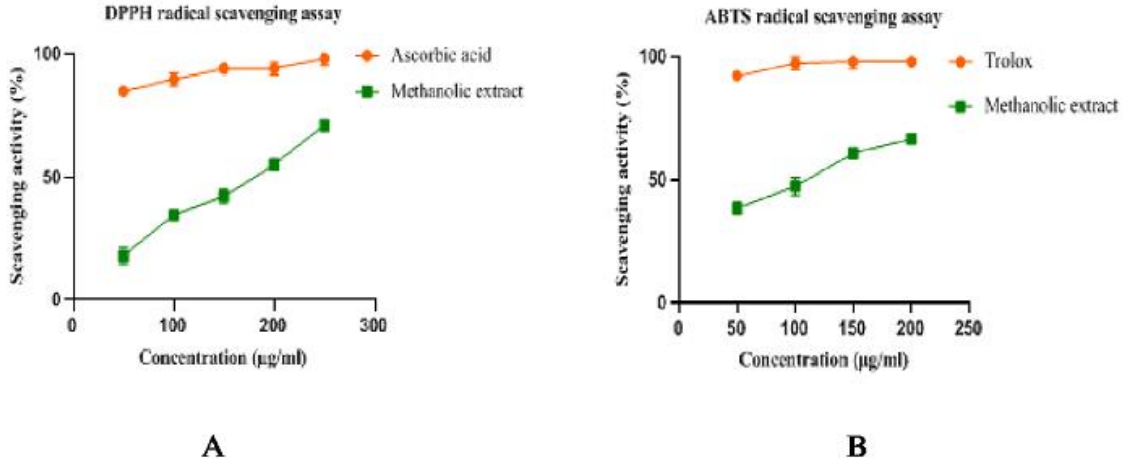


Fig. 2. Percentages of radical scavenging indicating antioxidant capacity compared to the standard (ascorbic acid and Trolox respectively) of methanolic extract obtained from *F. indica* fruits. (A) DPPH assay (B) ABTS assay. Values are presented as mean ± SD (n=3).

3.5. Antimicrobial activities

The methanolic extract obstructed the growth of Gram negative bacteria *Pseudomonas aeruginosa* (MTCC 542) at 0.32 mg/ml, Gram positive bacteria *Staphylococcus aureus* (MTCC 96) at 0.35 mg/ml and the fungal strain *Candida albicans* (MTCC 317) at 23 mg/ml. The zone of inhibition (ZOI) and minimum inhibitory concentration (MIC) are shown in Table 5 and Table 6, respectively. The inhibition zone is displayed in Figure 3. The result of Total Bacterial Count (TBC) and Total Fungal Count (TFC) are shown in Table 7 and Table 8, respectively.

Table 5. Antibacterial (*Staphylococcus aureus* and *Pseudomonas aeruginosa*) and antifungal (*Candida albicans*) activities of methanolic extract obtained from *F. indica* fruits

Test organism	Cone. (µg/ml)	Zone of Inhibition (ZOI) (mm)		
		Extract	Positive control	Negative control
Gram positive bacteria				
Staphylococcus aureus (MTCC 96)	50	17	40	No Zone
	100	22		
	150	32		
Gram negative bacteria				
Pseudomonas aeruginosa (MTCC 542)	50	16	40	No Zone
	100	21		
	150	33		
Fungal strain				
Candida albicans (MTCC 317)	50	No Zone	40	No Zone
	100	07		
	150	12		

Table 6. Determination of Minimum Inhibitory Concentration (MIC) values against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans* of methanolic extract obtained from *F. indica* fruits.

Test Organism	Minimum Inhibitory Concentration (MIC)		
	Extract (mg/ml)	Amoxicillin (mg/ml)	Clotrimazole (mg/ml)
Staphylococcus aureus (MTCC 96)	0.35	0.1	----
Pseudomonas aeruginosa (MTCC 542)	0.32	0.1	-----
Candida albicans (MTCC 317)	23	----	0.1

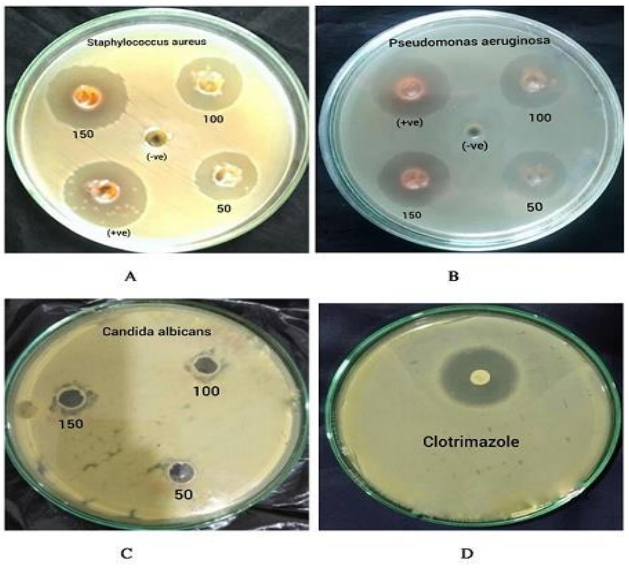


Fig 3. Antimicrobial activity of methanolic extracts obtained from *F. indica* fruits: (A) Antibacterial activity shown against the Gram positive bacteria *Staphylococcus aureus*; (B) Antibacterial activity shown against the Gram negative bacteria *Pseudomonas aeruginosa*. '-ve' denotes negative control (DMSO); '+ve' denotes positive control (Amoxicillin); (C) Antifungal activity shown by the sample in different concentration against the fungi *Candida albicans*; (D) Antifungal activity shown by the standard against the fungi *Candida albicans*.

Table 7. Determination of Total Bacterial Count (TBC) and Total Fungal Count (TFC) after 24 hrs of incubation of methanolic extract obtained from *F. indica* fruits

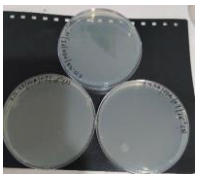
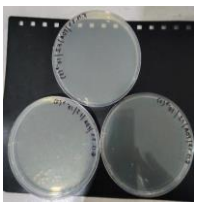
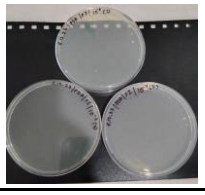
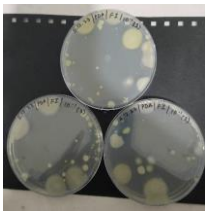
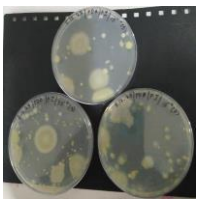
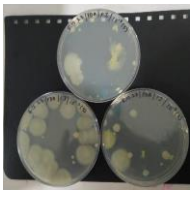
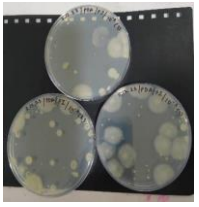
Media used	Dilution	Total Bacterial Count CFU/ml(Average of triplicates)	Total Fungal Count CFU/ml(Average of triplicates)	Photos
PDA	10^1	No colonies	No colonies	
PDA	10^2	No colonies	No colonies	
PDA	10^3	No colonies	No colonies	
PDA	10^4	No colonies	No colonies	

Table 8. Determination of Total Bacterial Count (TBC) and Total Fungal Count (TFC) after 72 hrs of incubation of methanolic extract obtained from *F. indica* fruits

Media used	Dilution	Total Bacterial Count CFU/ml (Average of triplicates)	Total Fungal Count CFU/ml (Average of triplicates)	Photos
PDA	10^1	1.96×10^1	1.06×10^1	
PDA	10^2	4.26×10^2	1.36×10^2	
PDA	10^3	0.96×10^3	0.96×10^3	
PDA	10^4	1.5×10^4	1.96×10^4	

The phytochemical analysis of methanolic extract of *Flacourtia indica* fruit revealed significant phenolic and flavonoid content. LC/MS analysis identified several phenolic compounds with notable antioxidant, antimicrobial, and anticancer properties. The extract exhibited strong antioxidant activity and effective antimicrobial properties, particularly against Gram-negative and Gram-positive bacteria. These findings support the potential of *Flacourtia indica* as a source of bioactive compounds with diverse pharmacological applications.

4. Discussion

The phytochemical analysis of the methanolic extract of *Flacourtia indica* fruits revealed a diverse range of bioactive compounds, with notably high levels of phenolic content (34.72±2.03 µg/mg) and flavonoid content (9.56±1.21 µg/mg). These compounds are known to play an essential role in the defense mechanisms of plants and have been associated with various health benefits because of their antioxidant properties. The low levels of ß-carotene (0.0615 ± 0.01 µg/mg) and lycopene (0.0235 ± 0.005 µg/mg), however, suggest that while the fruit may not be a rich source of these particular carotenoids, it still holds potential due to its phenolic richness. The LC/MS analysis revealed nine phenolic compounds with varied retention times and peak areas, each exhibiting distinct biological activities. Research has demonstrated that citronellol exhibits significant antibacterial activity against various bacterial strains, including *Escherichia coli* and *Staphylococcus aureus*. A study assessing the antimicrobial effects of essential oil components found that citronellol is the most effective molecule against both pathogens, causing changes in hydrophobicity, surface charge, and membrane integrity, leading to potassium leakage from the bacterial cells [28]. Additionally, citronellol has been shown to possess antioxidant properties. A study investigating its protective effects against doxorubicin-induced cardiotoxicity in rats found that treatment with citronellol improved redox status and prevented heart damage caused by reactive oxygen species [29]. Sparteine, a quinolizidine alkaloid, has demonstrated significant pharmacological properties, particularly in the modulation of ion channels and neurotransmission. Sparteine has been investigated for its possible anticonvulsant properties in the central nervous system. Research indicates that sparteine can reduce locomotor activity and has mild analgesic effects, suggesting its potential in managing neurological conditions such as epilepsy. Sparteine shows anticonvulsant effects by modulating ion channels and neurotransmission. Villalpando-Vargas and Medina-Ceja [30] evaluated sparteine's potential as an anticonvulsant drug, highlighting its effects on the central nervous system. 8-Prenylnaringenin (8-PN) is a potent phytoestrogen, primarily found in hops (*Humulus lupulus*), known for its significant estrogenic activity. Research indicates that 8-PN can bind to estrogen receptors, potentially influencing hormone-related health benefits [31]. Due to its estrogenic effects, 8-PN has been investigated for its potential in alleviating menopausal and post-menopausal symptoms, such as hot flashes and bone density loss. Studies suggest that 8-PN may help preserve bone density and reduce menopausal symptoms [32]. Capsidiol is a sesquiterpenoid phytoalexin produced by plants in response to pathogen attack, known for its antifungal properties [33]. In *Capsicum* species, capsidiol accumulates in infected ripe pepper fruits, contributing to the plant's defense mechanisms against fungal pathogens [34]. Catechin-3-O-gallate, a flavonoid derivative, exhibits potent antioxidant and antimicrobial activities. Its antioxidant activity is attributed to its ability to scavenge free radicals, thereby reducing oxidative stress and preventing cellular damage. This process resembles that of other catechins, such as epigallocatechin-3-gallate (EGCG), which possess antioxidant activities due to their phenolic groups sensitive to oxidation [35]. In terms of antimicrobial effects, catechins have been shown to possess moderate antibacterial activity, potentially aiding in the treatment of topical and oral infections. They may also influence the composition of gastrointestinal flora, contributing to overall well-being [36]. Harmine, a ß-carboline alkaloid, has undergone extensive investigation for its therapeutic potential, particularly its antitumor and anti-inflammatory effects. Research indicates that harmine exhibits strong cytotoxic and antiproliferative properties. Research suggests that it suppresses the proliferation of multiple cancer cell lines, including breast cancer, by inducing apoptosis and arresting the cell cycle. Yao et al. [37] reported the ability of harmine to suppress malignant phenotypes and promote apoptosis in breast cancer cells. Harmine also exhibits significant anti-inflammatory effects. It has been demonstrated to inhibit the NF-κB signalling pathway, leading to a reduction in the expression of pro-inflammatory cytokines. Liu et al. [38] reported that harmine exerts its anti-inflammatory effect by inhibiting the NF-κB signalling pathway. Kaempferol-3-O-ß-d-glucuronide, a metabolite of kaempferol, exhibits notable antioxidant and anti-inflammatory properties. As an antioxidant, it effectively scavenges free radicals, protecting cells from damage. Additionally, it exerts anti-inflammatory effects by suppressing the synthesis of pro-inflammatory cytokines and enzymes, such as cyclooxygenase (COX) and lipoxygenase (LOX), that are involved in inflammatory pathways. These actions contribute to its potential therapeutic benefits in conditions like arthritis and cardiovascular diseases. Lim et al. [39] demonstrated that kaempferol-3-O-ß-d-glucuronide exhibits anti-inflammatory effects in LPS-stimulated RAW264.7 cells and mice models. Peonidin, an anthocyanin pigment, is recognized for its potent antioxidant and anticancer properties. As an antioxidant, it effectively scavenges free radicals, and protects cells from damage. Additionally, peonidin has demonstrated the ability to suppress the proliferation of several cancer cell lines by inducing apoptosis and arresting the cell cycle at different phases. Moreover, it exhibits anti-inflammatory effects, which further enhance its anticancer activity by reducing inflammation-associated cancer promotion. Kowalczyk et al. [40] demonstrated that peonidin exhibits antioxidant properties, contributing to the neutralization of free radicals, and protects cells from damage. Delphinidin, an anthocyanidin, is recognized for its potent antioxidant and anticancer properties. As an antioxidant, it effectively scavenges free radicals, thus protecting cells from damage. Additionally, delphinidin has demonstrated the ability to suppress the proliferation of several cancer cell lines by inducing apoptosis and arresting the cell cycle at different phases.

Moreover, it exhibits anti-inflammatory effects, which further enhance its anticancer activity by reducing inflammation-associated cancer promotion. Hafeez et al. [41] demonstrated that delphinidin exhibits antioxidant properties, assisting in the neutralization of free radicals and protecting cells from damage. However, more investigation is required to confirm the concentration of these compounds in *Flacourtia indica* and their specific health effects.

The potent antioxidant activity observed in the DPPH and ABTS assays may be ascribed to compounds such as catechin-3-O-gallate and kaempferol-3-O-ß-d-glucuronide, which are well known for their ability to scavenge free radicals. Similarly, the ability of the fruit extracts to combat microorganisms is likely influenced by citronellol, sparteine, and catechin-3-O-gallate, known for their microbial inhibitory properties. The presence of harmine and peonidin, with their antitumor activities, suggests that *Flacourtia indica* fruits may have potential applications in cancer prevention and therapy.The identification of these bioactive compounds underscores the health-promoting potential of *Flacourtia indica* fruits. This study not only adds to the phytochemical understanding of the species but also paves the way for further research into its applications in nutraceuticals and pharmaceuticals. The diversity of biological activities associated with these compounds highlights the ability of the fruit as a functional food and a valuable resource for bioactive compounds.

Beside advantages, there are some limitations of the LC-MS method. The choice of negative ionization mode for analyzing phenolic compounds, while suitable for many phenolic classes, may not be optimal for all compounds present in the sample. Some phenolic compounds may have poor ionization efficiency in this mode, leading to suboptimal detection or even underrepresentation of certain compounds. As a result, the overall profiling of phenolic compounds might be incomplete, with lower sensitivity for compounds that do not ionize well in the negative mode. This could influence the quantification and subsequent comparison of phenolic content across different samples.The existence of additional compounds in the sample (such as sugars, proteins, or fatty acids) could interfere with the ionization of phenolic compounds. The matrix effects may result in ion suppression or enhancement, influencing the accuracy of the quantification. Since the method uses a gradient elution and PDA for initial detection, the possibility of matrix-related interference is higher during complex sample analysis. This could result in variability in peak areas or inaccurate detection and measurement of phenolic compounds, especially when comparing different extraction solvents. The AQUASIL C_{18} column used for chromatographic separation may not fully resolve all isomeric or closely related phenolic compounds. Overlapping peaks due to poor separation can complicate the detection and measurement of phenolic compounds, leading to the potential loss of detail in the phenolic profile. For example, structurally similar flavonoids or phenolic acids may co-elute, rendering it challenging to distinguish individual compounds based solely on retention times and UV spectra.The LC-MS system utilized in this study has a mass range of 50–1500 m/z, which is adequate for detecting a broad range of compounds. However, compounds with extremely low molecular weights (less than 50 m/z) or very high molecular weights (over 1500 m/z) may not be detected, limiting the scope of the analysis. The sensitivity of the apparatus, although high, may also be a limiting factor for detecting trace amounts of certain compounds, especially those with low ionization efficiencies. Over time, especially with repeated sample injections, retention times can drift due to slight changes in system conditions, such as column degradation or pump inconsistency. This could impact reproducibility, particularly when analyzing a substantial number of samples or when comparing results across different runs. Standardization and careful calibration of the system are necessary to mitigate this issue, but small shifts in retention times are sometimes unavoidable. In result, there are some effects of limitation, such asincompleteprofiling, the limited ionization efficiency of certain compounds, matrix effects, and poor chromatographic separation which could result in incomplete profiling of phenolic compounds, with some compounds potentially going undetected or misidentified. This could affect the interpretation of the biological activity of the fruits being studied, as the composition of phenolic compounds might not be fully captured. Reduced quantification accuracy, the matrix effects and overlapping peaks could influence the accuracy of quantification, potentially leading to either overestimation or underestimation of certain phenolic compounds. This would limit the reliability of the quantitative results, especially when comparing concentrations across different samples or experimental conditions. Reproducibility concerns, retention time drift and system variability could affect the reproducibility of results across different runs. This may make it harder to reach consistent conclusions regarding the profiles of phenolic compounds over time, especially when samples are processed on different days or using slightly different system settings.

There are several implications for clinical applications.The detection of phenolic compounds with strong antioxidant and antimicrobial activities, such as those found in *Flacourtia indica* fruits, has direct implications for clinical use. The antioxidant activity of phenolics could support their role in preventing oxidative stress-related diseases such as cardiovascular disorders, neurodegenerative diseases, and cancer. Clinical studies may explore these compounds as possible treatment options or as adjuncts for the prevention and management of chronic diseases associated with oxidative damage.As resistance to traditional antibiotics continues to rise, plant-derived phenolic compounds with demonstrated antimicrobial activity could serve as alternative or complementary therapeutic agents. The identification of specific phenolics with antimicrobial properties could lead to the development of novel, plant-based antimicrobial drugs or supplements. Clinical applications may involve topical or oral formulations in the management of infections caused by drug-resistant bacteria or fungi.The antioxidant and antimicrobial phenolic compounds from fruits such as *Flacourtia indica* could be utilized in the formulation of nutraceuticals, which are products derived from food sources that offer health benefits beyond basic nutrition. Clinical applications of such nutraceuticals might include the prevention of aging, improving immune function, and supporting skin health. Clinical trials could further

evaluate the safety, bioavailability, and efficacy of these substances in human health. If phenolic compounds are consistently linked to specific disease prevention (such as oxidative stress biomarkers in cardiovascular health), they could be developed into diagnostic or prognostic biomarkers. Clinical research may focus on establishing reliable biomarkers for monitoring the efficacy of treatments or preventing disease progression through dietary interventions.

In industrial applications there are some implications.The health-promoting potential of phenolic compounds position them as valuable ingredients in functional foods and beverages. The food industry could use extracts from fruits like *Flacourtia indica* as natural antioxidants to preserve the storage longevity of food products or as functional additives in health-oriented food products. This might extend to the formulation of fortified juices, teas, and snack foods that promote overall health, particularly by targeting oxidative stress. Owing to their antimicrobial and antioxidant characteristics, phenolic-rich plant extracts are valuable in the cosmetic industry. They can be used in anti-aging skincare products, lotions, serums, and sunscreens. These compounds may help guard the skin against oxidative damage induced by UV radiation and environmental pollutants, reducing the visible signs of aging and improving skin health. The cosmetic industry could incorporate these phenolic extracts in formulations aimed at promoting skin rejuvenation, wound healing, and antimicrobial protection. The isolation of bioactive phenolic compounds could pave the way for the development of pharmaceutical products, particularly those targeting oxidative stress, inflammation, or microbial infections. Industrial-scale extraction and purification of these compounds could be scaled up for use in the pharmaceutical industry, offering more sustainable, plant-based alternatives to synthetic drugs. Additionally, phenolic compounds could be utilized as excipients or active ingredients in drug formulations, enhancing their stability and bioavailability. Antimicrobial phenolic compounds could also have applications in agriculture. As natural alternatives to chemical pesticides, these compounds could be used to develop eco-friendly solutions for controlling plant diseases and pests. Moreover, the development of bio-pesticides derived from phenolic-rich plant extracts could offer a sustainable and environmentally friendly alternative to synthetic pesticides, benefiting organic farming practices. The extraction and utilization of phenolic compounds from fruits like *Flacourtia indica* align with the growing trend of green chemistry and sustainable practices in industry. By sourcing bioactive compounds from renewable plant sources, industries can reduce their environmental impact and dependence on synthetic chemicals. This can play a role in growing demand for sustainable, natural products in various sectors, from food to cosmetics to pharmaceuticals.

The proximate composition is also satisfactory. The moisture content (41.56 ± 1.62 g/100 g) is considerably higher than in the fruit of *Antidesma bunius* (4.53 ± 0.35 g/100 g) [42]. The quantity of total carbohydrate content (21.78 ± 0.37g/100 g) is very much higher than in the *Psidium guajava* (12.06 ± 2.04 g/100 g) fruits [43]. The amount of total protein content (1.46 ± 0.17 g/100 g), total lipid content (1.89 ± 0.04 g/100 g) and crude fibre (2.61 ± 0.13 g/100 g) are higher than in the *Borassus sp*. (0.24 ± 0.05 g/100 g), *Carissa* sp. (0.11 ± 0.03 g/100 g) and *Syzygium* sp. (0.86 ± 0.10 g/100 g) [43]. The proximate composition of the fruit supports its nutritional value. High moisture content, combined with significant levels of carbohydrates, proteins, lipids, and fibre, underlines the potential of this fruit as a nutritious food source. These findings suggest that *Flacourtia indica* can contribute meaningfully to dietary requirements, particularly in regions where it is commonly consumed.

The IC_{50}value of DPPH of the fruit extract (189.14 ± 1.08 µg/ml) is lower than *Antidesma bunius* fruit (395.002 ± 3.605 µg/ml) [42] and the IC_{50} value of ABTS (124.65 ± 1.38 µg/ml) is lower than *Psidium cattleyanum*fruits (242.30 ± 4.08 µg/ml) as reported by Pereira et al. [44]. The antioxidant activity of the fruit extract, as demonstrated by its strong DPPH and ABTS radical scavenging abilities, highlights its potential in neutralizing free radicals. When compared to other fruit species, *Flacourtia indica* exhibited superior antioxidant capacity, indicating its promise as a natural source of antioxidants that could be useful in mitigating oxidative stress.

The Minimum Inhibitory Concentration (MIC) values are crucial in determining the effectiveness of antimicrobial agents. They provide a quantitative measure of the lowest concentration of an antimicrobial compound required to inhibit the growth of a microorganism. From a practical perspective, MIC values guide clinicians in selecting appropriate concentrations for treatment, ensuring both efficacy and minimizing potential resistance development. Additionally, MIC values help in evaluating the potency of novel antimicrobial compounds, aiding in the development of more effective therapies. Understanding MIC values is also vital in respect to environmental safety, as they inform appropriate dosages and application strategies to minimize harmful effects on beneficial microorganisms.

The MIC value against the Gram-negative bacteria *Pseudomonas* sp. is 0.32 mg/ml and Gram-positive bacteria *Staphylococcus* sp. is 0.35 mg/ml, which is stronger than the fruit extract of *Adansonia digitata* (6.3 mg/ml) as reported by Tshikalange et al. [45]. The methanolic extracts of *Diospyros melanoxylon* Roxb. fruits did not show antifungal activity against *Candida albicans* as reported by Mal et al. [46]. However, this extract showed little amount of antifungal activity against *Candida albicans*. The antimicrobial activity of the methanolic extract further emphasizes the fruit's bioactive potential, with strong antibacterial effects against both Gram-negative and Gram-positive bacteria. This suggests potential applications in developing natural antibacterial agents, especially considering its efficacy compared to other plant-based extracts. Additionally, the observed antifungal activity, though limited, indicates that the fruit may possess compounds with potential antifungal properties, meriting further investigation.

Overall, the findings underscore the importance of *F. indica* fruits as not just valued for their nutritional content but also as a promising candidate for further research into their therapeutic potentials, particularly in combating microbial resistance and promoting health.

These findings create new opportunities for exploring its use in food and pharmaceutical applications. However, more investigation is needed in the quest for discovery of new bioactive components.

5. Conclusion

The present study showed that fruits of *Flacourtia* sp. have good quantity of nutritional (carbohydrate, protein and lipid) contents which could be beneficial as a dietary supplement other than conventional fruits available in the market. Methanolic extract exhibited high antibacterial activity and moderate antifungal activity. Antioxidant potential is also more satisfactory than other fruits. These outcomes support the utility of *F. indica* fruits in folk medicine, particularly as a potential aid in treating of skin damage, aging, snake bites, jaundice, scabies, arthritis and other disorders.

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