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Exploration of the Biomolecules in Roots of *Flacourtia indica* (*Burm. F*) Merr. Methanol Extract by Chromatography Approach

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Abstract: For thousands of years, medicinal plants have been zed in traditional remedies to treat various ailments. The biological substances in the plant parts used in medication preparation determine a plant's medicinal qualities. *Flacourtia* (Burm.f). Merr. is an indigenous medicinal plant commonly used ethnomedicine to treat various ailments. This work aimed to use preparative thin-layer chromatography and Gas Chromatography-Mass Spectrum (GC-MS) analysis to identify bioactive components in crude and purified fractions of methanol root extracts of *Flacourtia indica*. To isolate and purify the phytoconstituents in methanol root extract of *F. indica*, preparative thin layer chromatography was done using TLC plates coated with silica gel-G (TLC Silica gel 60, Merck) and GC-MS analysis. A VF-5ms fused silica capillary column and a gas chromatograph interfaced with a mass spectrometer were utilized in the GC-MS (GC-MS). From both crude and TLC purified fractions, GC-MS analysis indicated the presence of 41 distinct secondary metabolites, including Heneicosane (RT-25.945), Squalene (RT-20.51), Cholesterol (RT-33.525), Cycloheptasiloxane, tetradecamethyl-(RTP: 14.864), 2, 4-Di-tert-butylphenol-(RT: 16.032), Cycloheptasiloxane hexadecamethyl (RT: 16.848), Cyclononasiloxane octadecamethyl (RT: 20.733), and n-Hexadecanoic acid (RT: 22.092). All the chemicals found have antibacterial, antifungal, antioxidant, anti-cancer, and anti-proliferative properties. *Flacourtia indica* MeOH root extract has shown bioactive components can operate as a possible source of diverse pharmacological activities, according to the findings.

Keywords: *Flacourtia indica;* phytoconstituents; chromatography; ethnomedicine; biological; traditional.

1. Introduction

Medicinal plants have been used for thousands of years to cure various human diseases as the plants contain many constituents which have high therapeutic values [1,2]. In the developing world, almost 3.4 billion people rely on herbal medicine [3]. The World Health Organization (WHO) supports traditional medicine, provided it is proven to be productive and safe [4]. Most medicinal plants are distinctive in their ability to treat and cure various human ailments owing to the contribution of various valuable phytoconstituents in different plant parts [5,6]. In the pharmaceutical industry, phytochemicals are the key components for developing and preparing new drugs and therapeutic agents. The first step in preparing a new drug is identifying the active principles in natural sources. [7]. The initial screening of medicinal plants

by spectrometric and chromatographic methods provides basic information on chemical and pharmacological activities, which helps to select the biologically active plants [5]. Gas chromatography-mass spectroscopy (GCMS) is a combined analytical technique used to determine and identify compounds present in a plant sample. GCMS plays an essential role in the phytochemical analysis and chemotaxonomic studies of medicinal plants containing biologically active components [6]. GCMS is one of the fast, most accurate, and best techniques to identify the various bioactive constituents of long, long-chain hydrocarbons, alcohols, acids, esters, alkaloids, steroids, amino and nitro acids compounds, etc. Hence, Gas chromatography and Mass spectroscopy (GCMS) associated with particular detection techniques have become sophisticated means for analyzing various compounds [3,5]. Therefore, the present study uses GCMS analysis to identify bioactive compounds from methanol (MeOH) root extracts from Flacourtia indica roots. The present study reported the various bioactive principles of F. indica root extract for the first time.

Flacourtia indica (Burm F.) Merr is a small spinous tree or shrub belonging to the family Flacourtiaceae, an indigenous medicinal plant, generally known as 'Tuturi', or 'Baichi' or 'Katai'. It is native to South Africa and Asia and distributed throughout the Himalayas, and northern districts of Uttar Pradesh, Assam, Bengal, and Orissa [8]. The plant has been discovered to be useful in ethnomedicine for various ailments. The plant has a long folkloric history and is used as indigenous medicine for a variety of ailments: the fruits are used to treat jaundice and enlarged spleens, the seeds are combined with turmeric to prevent rheumatic pain, and the bark is applied to the body during intermittent fevers, and the root is used to treat nephritic colic [9]. Plant phytochemicals possess various biological properties, such as antibacterial, antifungal, antioxidant, and anticancerous activities. It is, therefore, important to identify the type of bioactive principles [3]. For analyzing such compounds, GCMS is found to be a sophisticated technique [10]. Hence, *Flacourtia indica* roots were selected, and the MeOH extract was subjected to analysis based on the previous results of their antifungal activity and phytochemical investigation obtained from this study. A detailed literature review on this plant under investigation has shown that, so far, there are no scientific reports related to the possible phytoconstituents. For the first time, the present study reports on bioactive volatile constituents of *F. indica* roots.

2. Materials and Methods

The roots of *Flacourtia indica* were collected from natural habitats in and around Hawajae, Udupi District, and Karnataka, India- latitude 13º.33N. The plant was identified taxonomically based on its typical characteristics by referring to standard flora [11] and authenticated by Dr. Shiddamallayya Mathapathi, Research Officer (Botany), at Regional Ayurveda Research Institute, Central Council for Research in Ayurvedic Sciences, Ministry of AYUSH and Govt. of India.

2.1. Preparation of root extract.

Freshly collected roots of *F. indica* were washed with running tap water several times to remove extraneous matter, shade dried for several weeks, and pulverized to powder using a mechanical grinder. The coarsely powdered materials were kept in air-tight containers and stored at room temperature until use. The required quantity of root powder of *F. indica* was weighed, transferred to a soxhlet extractor using 100 ml methanol, and extraction was carried

out for 72 hrs. The extract was filtered through Whatman no.41 filter paper separately, and the extracts were concentrated in a vacuum at 60ºC using a rotary evaporator to evaporate the methanol from it. After evaporation of methanol, dark brown color residues were obtained. The residues were kept separately in air-tight containers and stored at 4ºC for further use.

2.2. Preliminary phytochemical analysis.

Phytochemical profiling of crude root methanol root extract of *F. indica* was carried out and published [12] by adopting the procedures with slight modifications [12-15].

2.3. Isolation and identification of phytoconstituents from F. indica root extract by preparative thin layer chromatography (PTLC).

Following a preliminary investigation of phytochemicals in MeOH root extract of *F.indica,* preparative thin layer chromatography has been used further to isolate and identifies active principles using silica gel- G (TLC Silica gel 60, Merck) coated plates (20 cm x 20 cm) with a thickness of 2 mm. The different solvent systems of different polarities were prepared, and TLC studies were carried out. After loading with plant root extract, TLC plates were developed in two different solvent systems: n-butanol: acetic acid: water (80:20:20) and ethyl acetate: methanol: water (200:27:20), chosen as a mobile phase for the study. Two PTLC plates were run simultaneously in developing solvent systems, one plate was meant for chemical identification, and the other plate was meant for the isolation of phytoconstituents [16]. In the chemical identification method, the number of chemical reagents has been used to spry over one of the chromatograms for the detection of pharmacologically active principles, and from other chromatograms, phytoconstituents were isolated after calculating their Rf values. Elution can be done by scraping off the phytoconstituents and silica gel from a chromatogram, collected in air-tight containers and labeled as B1-B9 (Band 1-9) based on the separation of compounds in the form of bands on TLC plates. Eluted compounds were then dissolved in a suitable solvent, centrifuged, and the supernatant was decanted in separate containers. This process was repeated several times, and finally, all supernatants were pooled together and subjected to evaporation at room temperature. These PTLC fractions were then subjected to GCMS studies to identify phytoconstituents responsible for pharmacological properties [17].

2.2.1. Gas chromatography-mass spectroscopy (GCMS) analysis.

The GCMS (QP 2010, Indian Institute of Science, Bangalore, India) is equipped with a VF-5mscapillary column made of fused silica with a 30m length, 0.25mm diameter, and 0.25m film thickness. An electron ionization system with an ionization energy of 70eV was used for GCMS detection. Helium was used as a carrier gas at a constant flow rate of 1.51ml/min, with the injector and mass transfer line temperatures set to 200 °C and 240 °C, respectively. The oven was set to a temperature between 70 °C to 220 °C at 10 °C/min. And held isothermal for 1 min and raised to 300°C at 10°C/min. 2µl of the respective diluted sample was manually injected in the splitless mode, with a split ratio of 1:40 and a mass scan of 50-600 amu. The total running time of GCMS was 35 min. The relative percentage of the root extract constituents was expressed as a percentage with peak area normalization.

2.2.2. Identification of compounds.

The components were identified based on their retention indices, and the mass spectrum was interpreted using the National Institute of Standards and Technology's database (NSIT). The resulting spectra of the *Flacourtia indica* fraction's unidentified components were compared to the reference mass spectra of those components kept in the NIST library.

2.2.3. Calculations of retardation factor (Rf) value.

Rf (retention factor) value of all separated bands from both solvent systems as calculated using the following formula and the results were tabulated in Table 1.

$$\text{Retention Factor}(Rf) = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by the solvent}}$$

3. Results and Discussion

Phytochemical screening of the methanol fraction of *Flacourtia indica* root revealed the presence of alkaloids, phenols, terpenoids, steroids, tannins, saponins, pholabatannins, coumarins, proteins & amino acids, reducing sugars, anthraquinones, cardiac glycosides and the results of qualitative phytochemicals are presented in the (Table 1).

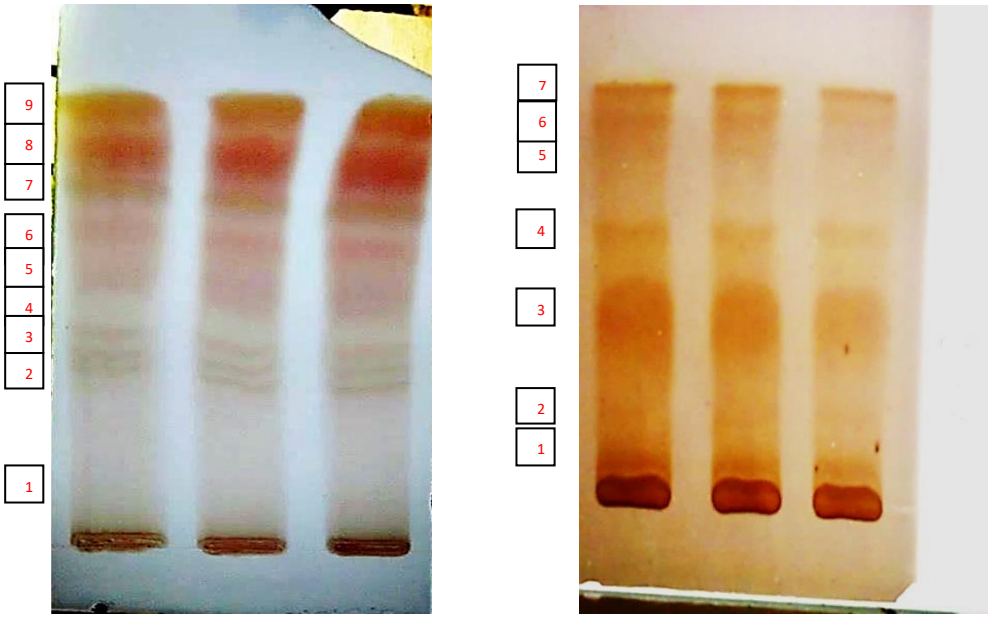
Table 1. Phytoconstituents of methanol fraction of *Flacourtia indica* root

Sl. No	Phytoconstituents	Result
1	Alkaloids	+
2	Phenols	+
3	Terpenoids	+
4	Steroids	+
5	Tannins	+
6	Saponins	+
7	Pholabatannins	+
8	Coumarins	+
9	Proteins & amino acids	+
10	Reducing sugars	+
11	Anthroqinones	+
12	Cardiac glycosides	+

Values are present as mean ± SD. "+" indicates present

3.1. Isolation and identification of phytoconstituents from F. indica root extract by preparative thin layer chromatography (PTLC).

As per the results of qualitative analysis, thin-layer chromatography was performed to identify phytochemicals in the MeOH root extract of *F. indica*. Following their identification, isolation has been done. For preparative TLC (PTLC), the solvent systems were standardized in different proportions. The two solvent systems, i.e., Ethyl acetate: Methanol: Water (A) and n-Butanol: Acetic acid: Water (B), have given good separation results and better resolution, as shown in (Figure 1).



A: Ethyl acetate : Methanol: Water B: n-Butanol : Acetic acid: Water

Figure 1. TLC Chromatogram of the crude MeOH root extract of *F. indica*, from solvent systems A & B, observed under visible light.

In the present investigation, TLC profiling of root extracts in the different solvent systems indicated the presence of different groups of phytochemicals. Among six solvent system screened for the screening, the solvent systems, ethyl acetate: methanol: water (200:27:20) and n-butanol: acetic acid: water (80:20:20), showed good separation and better resolution with retention factor values ranging between 0.15 to 0.92 cm as shown in (Table 2 and 3).

Table 2. Solute distance and solvent front

Ethyl acetate: Methanol: Water			**n -Butanol: Acetic acid: Water**		
Solvent Front	**Solute Distance**		**Solvent Front**	**Solute Distance**	
Length (cms)	**No. of Bands**	**Length (cms)**	**Length (cms)**	**No. of Bands**	**Length (cms)**
13	1	2.3	11.5	1	1.8
	2	3.5		2	4
	3	4.5		3	6.7
	4	7		4	7.2
	5	7.6		5	8.5
	6	8.5		6	8.9
	7	9.2		7	9.5
	8	10.7			
	9	12		----------	

Table 3. Rf values of separated bands.

Solvent System 1		**Solvent System 2**	
Ethyl acetate: Methanol: Water		**n-Butanol: Acetic acid: Water**	
No. of Bands	*** Rf values**	**No. of Bands**	*** Rf values**
1	0.17	1	0.15
2	0.26	2	0.34
3	0.34	3	0.58
4	0.53	4	0.62
5	0.58	5	0.73
6	0.65	6	0.77
7	0.7	7	0.82
8	0.82	-	-
9	0.92	-	-

* *Average* of 3 *Rf value (Rf =Retention factor)*

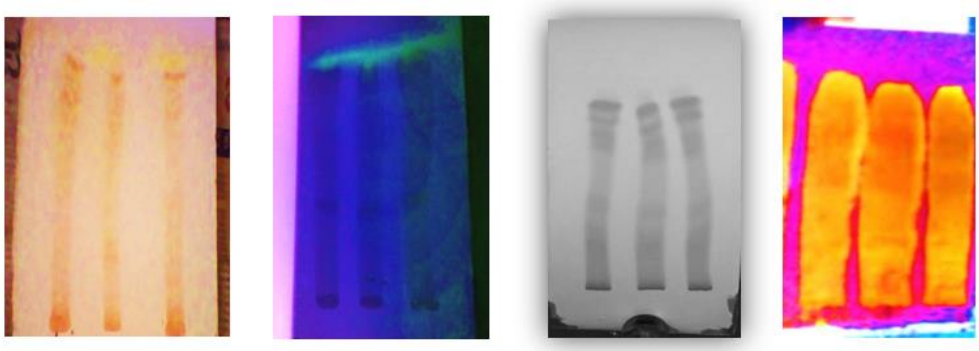
There are 9 bands separated on the PTLC plates in solvent system A (ethyl acetate: methanol: water), and 7 bands appeared on PTLC plates in the other solvent system B (n-butanol: acetic acid: Water), as shown in (Figure S1). In solvent system A, the first band is light in color, the second, third, and fourth bands are ash-colored, the sixth band is light pink, and the seventh, eighth, and ninth bands are intense pink. In solvent system B, bands 1 and 2 appear light in color, band 3 is thick and dark, while bands 4, 5, and 6 are light, and band 7^{th} is dark and distinct. The distance traveled by the solute from the origin was measured in centimeters from both the solvent systems and recorded as shown in Table 2 and 3).

Various phytochemicals have different Rf values in different solvent systems. Different Rf values of the compound also reflect their polarity. Compounds showing a high Rf value in the solvent system have low polarity, and low Rf value has high polarity. A mixture of solvents with variable polarity in different ratios can be used to separate the pure compound from plant extract [17]. This information will help select an appropriate solvent system for further separating and characterizing compounds from this plant.

3.2. Identification of phytoconstituents by chemical methods.

3.2.1. Identification of alkaloids.

Ethyl acetate: Methanol: Water (80:10:10) solvent system is used to develop TLC plates. The developed TLC plate was sprayed with Dragendroff's reagent and then heated for 5-6 minutes at 100°C. 2 orange colored bands were detected, indicating the presence of alkaloids, and the Rf value was found to be 0.82 cm and 0.85 cm, respectively (Figure 2).



a) Dragendorff test b) Borntrager test (UV-365) c) FC reagent d) VS- Reagent

Figure 2. Several chemical methods were used to localize separated root constituents on TLC plates. From left to right, spraying with Dragendorff reagent (alkaloids), Borntrager reagent (flavonoids), FC-reagent (phenols), and VS-reagents (glycosides, coumarins).

3.2.2. Identification of flavonoids.

Ethyl acetate: Formic acid: Glacial acetic acid: Water (100:11:11:26) solvent system is used to develop TLC plates loaded with sample and sprayed with, Borntrager reagent when plates observed under UV-365, intense green fluorescent colored zones observed which indicated the presence of flavonoids in the extract with Rf value of 0.96cm (Figure 2).

3.2.3. Identification of phenols.

Acetic acid: Chloroform (10:90) solvent system is used to develop TLC plate. When the developed TLC plate was sprayed with dilute 1:1 FC-reagent followed by 20%Na_2CO_3, blue-colored spots appeared at room temperature, indicating the presence of phenols with an Rf value of 0.98cm (Figure S2).

3.2.4. Identification of Steroids/Triterpenoids/Glycosides/Coumarins.

n-Butanol: Acetic acid**:** Water (80:20:20) solvent system is used for the detection of steroids, triterpenoids, and their glycosides, by spraying the developed TLC plates with Vanillin–Sulphuric acid (VS) reagent, resulting in the development of 4 pink colored bands with Rf value of band 1-4 ranging from 0.18cm, 0.57cm, 0.79cm, and 0.77cm respectively (Figure 2).

3.3. GCMS profiling of MeOH root extract of Flacourtia indica.

The GCMS chromatogram of the crude MeOH root extract shows 17 peaks indicating the presence of 17 metabolites exhibiting various biological activities. The compounds identified are represented in (Table 3) and (Figure 3). GCMS chromatogram reveals that**,** cycloheptasiloxane, tetradecamethyl-(RTP:14.864), 2,4-di-tert-butylphenol-(RT:16.032), cycloheptasiloxane hexadecamethyl (RT:16.848), and cyclononasiloxane octadecamethyl (RT: 20.733), n-hexadecanoic acid (RT: 22.092), Bis(2-ethylhexyl) phthalate (RT: 26.258), Squalene (RT-28.52), phenol, 2,4-bis (1,1-dimethylethyl)-, phosphite (RT-29.787), etc.

3.3.1. GCMS profiling of PTLC fractions of *Flacourtia indica* root extract.

Purified TLC fractions from two different solvent systems (n-Butanol; Acetic acid: Water and Ethyl acetate: Methanol: Water) yield 41 metabolites. From TLC B4, 15 different metabolites (Figure 3, Table 4), from TLC B6, five different metabolites (Figure 4, Table 4) from TLC B7, and seven different metabolites (Figure 5, Table 4) and from TLC B9, 18 different metabolites were identified (Figure 6, Table 4). The compounds were identified and authenticated using their Mass-Spectrum data by comparison with those of the National Institute Standard and Technology (NIST) of Mass Spectral Library. The absorption spectra of the unknown components and the known components stored in the NIST library and the retention times were compared. The test material's components were ascertained, including their nomenclature, molecular weights, and chemical structure. The retention time and percentage peak of various bioactive compounds are presented in (Figures 3-5). Tables 5-8, reveal the major bioactive principles present in crude and TLC-purified fractions in MeOH root extract.

The compounds identified from all TLC fractions B4, B5, B6, B7, B9) include diethyl phthalate (RT: 16.737), dibutyl phthalate (RT: 21.707), n-hexadecanoic acid (RT-22.096), octadecanoic acid (RT-24.032), heneicosane (RT-25.945), bis (2-ethylhexyl) phthalate (RT-26.258) cycloheptasiloxane, tetradecamethyl-(RT-14.864), 2,4-di-tert-butylphenol-(RT-16.032), squalene (RT-20.51), 2,4-di-tert-butylphenol(RT: 16.03) ethyl oleate (RT-23.8), cholesterol (RT-33.525), glycidyl palmitate (RT-26.022), 13-docosenamide, (Z)-(RT-28.417), DL-arabinitol (RT-5.5), trans-2,4-decadienol (RT-6.81), methyl nicotinate (RT-7.47), 4-nonylphenol (RT-14.2), 2,4-Di-tert-butylphenol (RT-16.03), and few others were also detected (Figure 7 and Table 5 to 8).

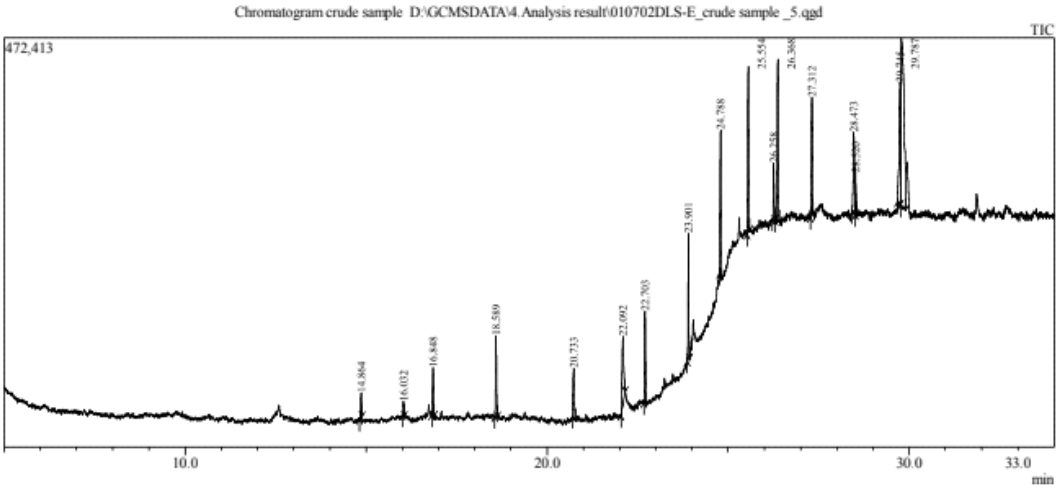


Figure 3. GCMS studies of crude MeOH root extract of *F. indica*.

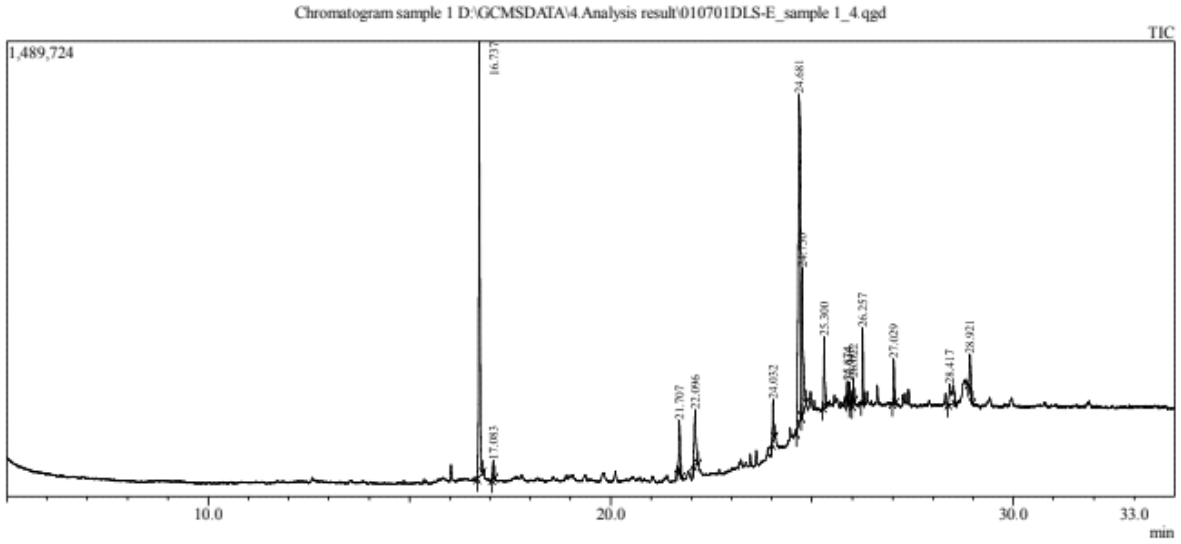


Figure 4. TLC Fraction B4 (Ethyl acetate: Methanol: Water).

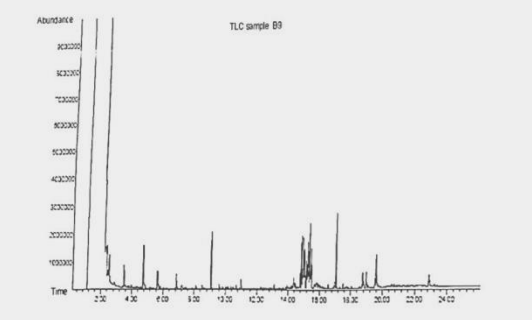


Figure 5. TLC Fraction B6 (n-Butanol: Acetic acid: water).

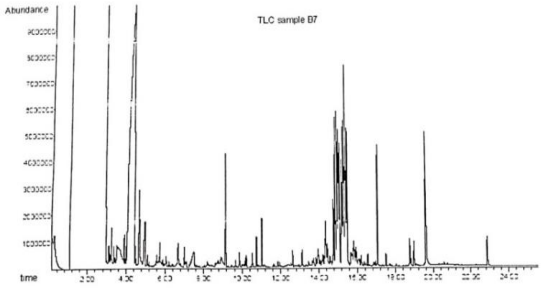


Figure 6. TLC Fraction B7 (n-Butanol: Acetic acid: water).

Table 4. Bioactive principles found in a crude methanol root extract of *F. indica*.

Sl. No.	RT (min)	Compound Name	MF	MW	Chemical Nature
1	14.864	Cycloheptasiloxane, tetradecamethyl-	$C_{14}H_{42}O_7Si_7$	518	Essential oil
2	16.032	2,4-Di-tert-butylphenol-	$C_{14}H_{22}O$	206	Alkylated phenol
3	16.848	Cyclooctasiloxane, hexadecamethyl-	$C_{16}H_{48}O_8Si_8$	592	Essential oil
4	16.848	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	666	Essential oil
5	20.733	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	666	Essential oil
6	22.092	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	Fatty acid Ester
7	22.703	Cyclooctasiloxane, hexadecamethyl-	$C_{16}H_{48}O_8Si_8$	592	Essential oil
8	23.901	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	666	Essential oil
9	24.788	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	666	Essential oil
10	25.554	Cyclononasiloxane, octadecamethyl	$C_{18}H_{54}O_9Si_9$	666	Essential oil

Sl. No.	RT (min)	Compound Name	MF	MW	Chemical Nature
11	26.258	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390	Phthalates
12	26.368	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	666	Essential oil
13	27.312	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	666	Essential oil
14	28.473	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	666	Essential oil
15	28.52	Squalene	$C_{30}H_{50}$	410	Triterpene
16	29.745	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite	$C_{42}H_{63}O_3P$	646	Alkylbenzene
17	29.787	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite	$C_{42}H_{63}O_3P$	646	Alkylbenzene

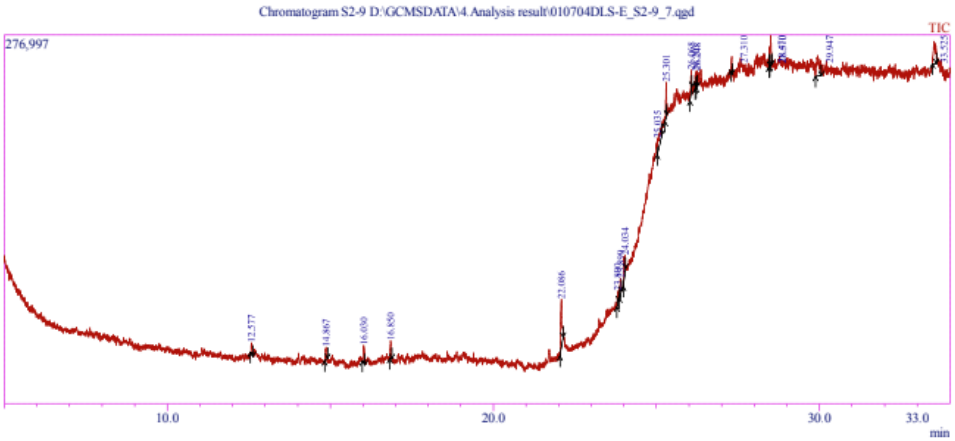


Figure 7. TLC fraction B9 (Ethyl acetate: Methanol: Water).

Table 5. Bioactive principles found in the purified TLC fraction B4 of MeOH root extract of *F. indica*.

Sl. No.	RT (min)	Compound Name	MF	MW	Nature of the compound
1	16.737	Diethyl Phthalate	$C_{12}H_{14}O_4$	222	Diethyl ester
2	17.083	3,4-Xylyl 3,5-di-tert-butylbenzoate	$C_{23}H_{30}O_2$	338	
3	21.707	Dibutyl phthalate	$C_{16}H_{22}O_4$	278	Phthalate ester
4	22.096	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	Fatty acid Ester
5	24.032	Octadecanoic acid	$C_{18}H_{36}O_2$	284	Saturated fatty acids
6	24.681	Phenol, 4,4'-(1-methylethylidene)bis-	$C_{15}H_{16}O_2$	228	Bisphenol
7	24.75	Glycidyl palmitate	$C_{19}H_{36}O_3$	312	Ester
8	25.3	Hexanedioic acid, bis(2-ethylhexyl) ester	$C_{22}H_{42}O_4$	370	Ester
9	25.874	9-Octadecenoic acid (Z)-, oxiranylmethyl ester	$C_{21}H_{38}O_3$	338	Fatty acid ester
10	25.945	Heneicosane	$C_{21}H_{44}$	296	Monoterpene
11	26.022	Glycidyl palmitate	$C_{19}H_{36}O_3$	312	Saturated fatty acid
12	26.257	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390	Aromatic Fatty ester
13	27.029	N,N'-Bis-(2,2,6,6-tetramethylpiperid-4-yl)pht	$C_{26}H_{42}N_4O_2$	442	
14	28.417	13-Docosenamide, (Z)-	$C_{22}H_{43}NO$	337	Docosenoic acid
15	28.921	Adiptic acid bis-(2,2,6,6-tetramethyl,-4-piperidyl	$C_{24}H_{46}N_4O_2$	422	

Table 6. Bioactive principles found in the Purified TLC fraction B6 (n-Butanol: Acetic acid: Water) of MeOH root extract of *F. indica.*

Sl. No.	RT(min)	Compound Name	MF	MW	Nature of the compound
1	7.47	Methyl Nicotinate	C_7H_7NO	137.14	Niacin
2	8.64	Methyl Nonanoate	$C_{11}H_{22}O_2$	186.29	Fatty acid methyl ester
3	10.92	Hexyl neopentanoate	$C_{11}H_{22}O_2$	186.29	Fatty acid methyl ester
4	14.2	4-Nonylphenol	$C_{15}H_{24}O$	220.35	Alkyl phenol
5	18.53	1-Docosanol	$C_{22}H_{46}O$	326.61	Fatty alcohol

Table 7. Bioactive principles found in the purified TLC fraction B7 (n-Butanol: Acetic acid: water) of MeOH root extract of *F. indica.*

Peak	RT	Compound Name	MF	MW	Nature of the molecule
1	4.47	2-Methly-3-haxanol	$C_7H_{16}O$	116.2	Aliphatic 2°Alcohol
2	5.5	DL-Arabinitol	$C_5H_{12}O_5$	152.15	Sugar alcohol
3	5.95	Methyl heptanoate	$C_{10}H_{16}O$	144.21	Fatty aldehyde
4	6.81	trans-2,4-Decadienol	$C_{10}H_{16}O$	152.23	Fatty aldehyde
5	10.51	Fumaric acid, diallyi ester	$C_4H_4O_4$	196.2	Fumaric acid
6	12.38	Hept-2-ulopyranose	$C_7H_{14}O_7$	210.18	Fumaric acid
7	14.9	4-Nonylphenol	$C_{15}H_{24}O$	220.35	Alkyl phenol

Table 8. Bioactive principles found in the purified TLC fraction B9 of MeOH root extract of *F. indica*.

Peak	RT (Min)	Compound Name	MF	MW	Nature of Compound
1	12.577	Benzaldehyde, 4-propyl-	$C_{10}H_{12}O$	148	
2	14.867	Cycloheptasiloxane, tetradecamethyl-	$C_{14}H_{42}O_7Si_7$	518	Essential oil
3	16.03	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	206	Alkylated phenol
4	16.85	Cyclooctasiloxane, hexadecamethyl-	$C_{16}H_{48}O_8Si_8$	592	Essential oil
5	22.086	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	Fatty acid Ester
6	23.8	Ethyl Oleate	$C_{20}H_{38}O_2$	310	Fatty acid Ester
7	23.899	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	666	Essential oil
8	24.034	Octadecanoic acid	$C_{18}H_{36}O_2$	284	Saturated fatty acids
9	25.035	2-Chlorothiophene-3,4-diamine	$C_4H_5ClN_2S$	148	Heterocyclic compound
10	25.301	Diisooctyl adipate	$C_{22}H_{42}O_4$	370	Diester
11	26.068	3,3,5-Trimethylcyclohexyl ethyl ether	$C_{11}H_{22}O$	170	
12	26.201	Cyclopentanecarboxylic acid, 1-methyl-3-(1-methylethyl	$C_{10}H_{18}O_2$	170	
13	26.248	2-Methoxyethyl-, pinacolyl-, methylphosphonate	$C_{10}H_{23}O_4P$	238	
14	27.31	(Trimethylsilyl)methyl stearate	$C_{22}H_{46}O_2Si$	370	Methyl Ester
15	28.47	9-Oxa-bicyclo[3.3.1]nonane-2,7-diol	$C_8H_{14}O_3$	158	Phenolic compound
16	28.51	Squalene	$C_{30}H_50$	410	Triterpene
17	29.947	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	666	Essential oil
18	33.525	Cholesterol	$C_{27}H_{46}O$	386	Steroid

Most of these compounds have not been reported from this plant so far. However, there may be variations in the chemical composition based on topography. Most of the identified compounds possessed many biological properties. n-hexadecanoic acid and 9-octadecenoic acid (Z)-methyl esters were reported to have hypocholesterolemic, antifungal, antioxidant, anti-carcinogenic, potent antimicrobial, nematicidal, pesticidal, anti-androgenic, hemolytic, 5-alpha reductase inhibitory activities, potent mosquito larvicidal [17-21]. The hexadeconoic acid ethyl ester was also reported by Perera *et al.* [22] I, in the ethanol bark. Other identified metabolites include the extract of *F. indica*. Bis (2-ethylhexyl) phthalate, diethyl phthalate, and dimethyl phthalate are the derivatives of phthalic acid reported having antimicrobial, anti-inflammatory, anti-tumor, and other pharmacological activities. These compounds have been used to treat chronic cardiovascular and cerebrovascular diseases. Docosanol (n-docosanol or behenyl alcohol) is saturated aliphatic alcohol. Its interference with viral fusion to host cell membranes early in replication and at millimolar concentrations inhibits lipid-enveloped viruses, especially HSV-1 and HSV-2. It also exhibits anti-inflammatory effects; a non-antiviral action has been described. Historically used in cosmetics as an emollient, emulsifier, and thickener. Herpes labialis can be treated using a lotion containing 10% docosanol [23]. Docosanol has just received FDA approval as a pharmaceutical antiviral treatment for shortening the duration of cold sores brought on by the herpes simplex virus [24]. Alkyl-phenols, a class of closely related chemical compounds, include nonylphenols (NPs). They are employed in the production of emulsifiers, solubilizers, lubricating oil additives, laundry and dish detergents, and antioxidants [24]. A fatty acid ester called ethyl oleate is utilized as a solvent in pharmaceutical medicinal formulations that contain lipophilic compounds like steroids. According to the Food and Drug Administration's list of "Food Additives Permitted for Direct Addition to Food for Human Consumption" [25] it is also used as a plasticizer and lubricant. It has been found to be a honeybee-priming pheromone. The major phytoconstituents in the MeOH crude extract of *F. indica* roots are cycloheptasiloxane, tetradecamethyl, cyclooctasiloxane, hexadecamethyl, and

cyclononasiloxane octadecamethyl. These essential oils have antioxidant and antimicrobial activities [26-31]. 2, 4-Di-tert-butylphenol or 2, 4-bis (1, 1-dimethylethyl)-phenol (2, 4-DTBP) showed remarkable cytotoxicity against HeLa cells. The antioxidant and anti-inflammatory activities of 2, 4-DTBP have been emphasized in many publications [32].

4. Conclusions

Medicinal plants have been used to treat various human illnesses, and the healing power of medicinal plants depends on their phytochemical constituents. *Flacourtia indica* is rich in secondary metabolites and may have valuable pharmacological properties. Our investigation reveals the presence of 62 bioactive constituents in crude methanol root extract and TLC-purified fractions. The identified major compounds possess some important biological potential for future drug development. The relationship between phytochemical substances and their biological actions is becoming more widely recognized.

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Conflicts of Interest

The authors declare no conflict of interest.

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