Phytochemical and Anatomical Profiling of *Terminalia catappa* Linn., Fruit Rinds: A Multi-Targeted Approach to Resistant Human Pathogens

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Abstract

Terminalia catappa Linn. is a pharmaceutically recognized avenue tree belonging to the family Combretaceae. Various parts of the plant are recognized for their anti-diabetic, antioxidant and antibacterial potential. This study aimed to comprehensively analyse the anatomy, histochemical compounds, phytochemical compounds, secondary metabolites through the Gas Chromatography Mass Spectrometry (GC-MS) characterization and antibacterial properties. Different solvents, ranging from low-polarity to high-polarity solvents, were employed to extract various phytocompounds. Qualitative and quantitative phytochemical screening confirmed the presence of alkaloids, flavonoids, terpenoids, tannins and other secondary metabolites, with the aqueous extract showing the highest concentrations of phenols, flavonoids and tannins.

The antibacterial screening demonstrated efficient activity of ethanolic and aqueous extracts against a wide spectrum of Gram-positive and Gram-negative bacteria by following the agar-well method, with the ethanolic extract showing the highest zones of inhibition. The GC–MS analysis identified significant secondary metabolites, having potent antibacterial properties. This research underscores the pharmaceutical applications of T catappa fruit rinds (TCFR) and contributes to the developing interest in organic research by providing insights into the bioactive compounds of TCFR and the development of plant-based multi-drug-resistant (MDR) antibacterial therapeutics.

Keywords: *Terminalia catappa* Linn. (fruit rinds), Micro-morphology, Phytochemical mapping, Phytochemical evaluation, Antimicrobial, GC-MS.

Introduction

The MDR bacteria have become a threat to the public by diminishing the efficacy of conventional antibiotics, posing serious health issues[4]. Therefore, dependence on plant-based natural products has gained attention as a promising tool to counteract the proliferating MDR strains[1]. Such therapeutic plants are enriched with secondary metabolites including alkaloids, phenolics, flavonoids, tannins and glycosides, which exhibit potent antimicrobial activities[15,19,20]. India's rich biodiversity offers a therapeutic contribution to society. India's biodiversity profile reports that nearly 6,500 native plants are utilized by indigenous communities[8]. The climatic and ecological conditions affect the concentration of bioactive compounds in specific tissues of the medicinal plants. This necessitates the investigations for the various phytochemical components in plants to establish their roles in pharmacological functions[7].

The genus Terminalia belonging to the family Combretaceae), encompasses nearly 250 species of medium to large trees, geographically distributed across southern Asia, the Himalayan region, Madagascar, Australia and tropical to subtropical areas of Africa. Several species are prevalently used in the ethnomedical systems, including Ayurveda, traditional Chinese medicine and Tibetan medicine. Phytochemical investigations have been implemented on approximately 39 species so far, resulting in the isolation and identification of 368 chemical constituents, specifically terpenoids, tannins, flavonoids, phenylpropanoids and simple phenolics[40].

Different parts of *T. catappa* Linn. have been documented for their potential antimicrobial, antidiabetic, anti-inflammatory, anti-oxidant, liver-supportive, mitigation of neuronal dysfunction, cardioprotective lipid indices properties[2,3,16,21]. The medicinal properties of TCFR were documented for their antimicrobial, antiatherosclerosis properties[28,36]. Studies have shown that the leaves and bark of the plant demonstrate antioxidant, hepatoprotective, anti-inflammatory, antidepressant, antifungal and chemopreventive activities.

As a component in topical applications for wound healing, it has been reported that *T. catappa* Linn. extracts resulted in a 97% reduction in wound healing properties in rats compared to betadine ointment. The ethanol extract of *T. catappa* Linn. leaves exhibits anti-inflammatory activity in animal models[21]. Its fruits are utilized for potential medicinal properties[17]. Santos and his colleagues[30] have demonstrated the physico-chemical nature of pulp and nut oils. The findings highlighted that the pulp oils were enriched with unsaturated fatty acids, particularly omega-3 and omega-6 fatty acids, relative to the nut oils[31]. The fruit pulp was examined and confirmed for its potential in preventing diabetes[38]. The chemical profiling of the fruit and the peels has reported the presence of carbohydrates, alkaloids, glycosides, phenols and flavonoids[18].

The phytocomponents are enriched with antimicrobial potential. The pyrolyzed aqueous fractions of the fruits demonstrated the inhibition of bovine mastitis-causing bacteria efficiently[39]. In the present investigation, a detailed analysis of the micromorphology, anatomy and histochemistry of *T. catappa*, phytochemical profiling of the crude extracts was conducted using GC–MS as part of this study. The antimicrobial properties of TCFR extracts were further evaluated. Hitherto, no microscopy or anatomical investigations on the fruit rinds of *T. catappa* have been reported.

Material and Methods

Botanical material: Partially ripened TCFR were collected in Chennai, Tamil Nadu, India (coordinates 13° 03' 43.20" N and 80° 14' 2.40" E), in February 2024. A voucher specimen (T19022401C) was deposited in the Herbarium of Siddha Central Research Institute, Chennai. The ripened fruits without external defects were collected and analysed in this State to perform this procedure.

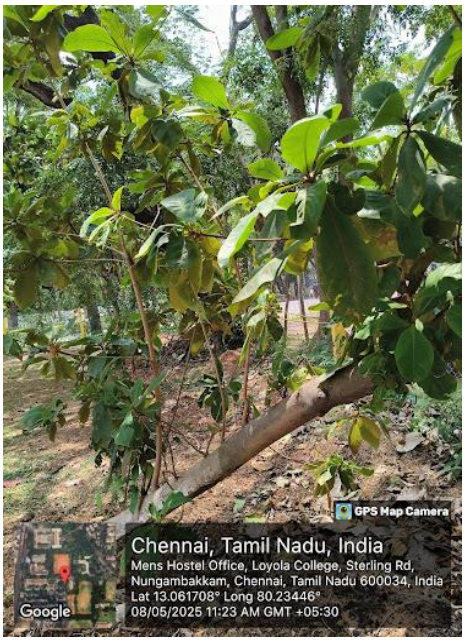


Fig. 1: *Terminalia catappa* Linn.

Morphological measurement: The external feature of TCFR was documented using a Nikon D-5600 Digital camera. Vegetative features e.g. colour, shape and the nature of the pericarp were examined and documented.

Microscopy procedure: The freshly collected TCFR samples were used to study the anatomy of the pericarp. The sample was preserved in fixative formalin-acetic acid-alcohol (FAA) for over 48 h. The preserved specimens were sliced into thin transverse sections (TS) using a sharp blade and the sections were stained with 0.8% safranin and 0.5% astra blue[23]. The sections were mounted on a glass slide, covered with a drop of 50% glycerin and a cover slip and photographed using Axiolab5 trinocular microscope attached with a Zeiss Axiocam208 color digital camera under bright field light. Magnifications were indicated by a scale bar.

Histochemical tests: The cross-sectioned pericarp was treated to examine the presence of crystals, oils, resins, starch, phenolic compounds, mucilage, lignified cell walls, suberized cell walls and alkaloids.

Maceration Techniques

Freshly harvested fruits were thoroughly washed with water to clear the dust and other debris. The rinds were separated from the hard endocarp, shade dried for 5 – 6 days and the dried material was blended in an electric blender to obtain a powder form. The fruit rind powder was stored at 4°C in an airtight container and utilized during further research studies. A 50 g portion of the powdered sample was sequentially extracted using solvents of increasing polarity: hexane, ethyl acetate, ethanol and water, at a ratio of 1:4 (w/v)[26]. The condensed extracts were used for the following assays.

Screening of Phytochemicals: The extracts were screened for the intended phytochemicals by following standard protocols[34].

Quantitative estimation of phytochemicals: The quantification of total phenols, flavonoids and tannins was evaluated by following the protocol established by Prasathkumar et al[25].

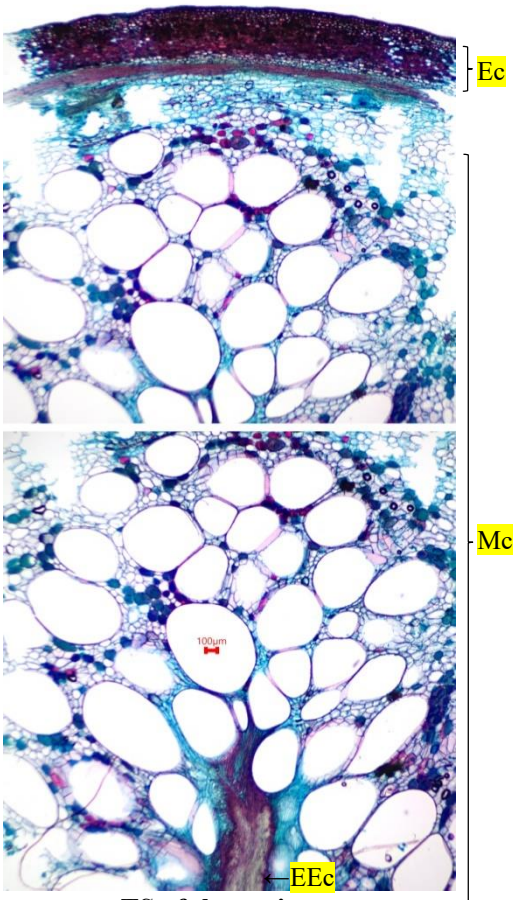
***In vitro* antibacterial activity:** Based on the pilot screening of phytochemical evaluation, major phytochemicals were found in the ethyl acetate, ethanol and aqueous extracts. Hence, these extracts of TCFR were utilized for antibacterial assessments. Anti-bacterial activity was investigated against *Clostridium sordellii* (ATCC 9714), *Enterococcus faecalis* (MTCC 439), *Escherichia coli* (MTCC 443), *Klebsiella pneumonia* (MTCC 109), *Micrococcus luteus* (MTCC 106), *Proteus mirabilis* (MTCC 1429), *Pseudomonas aeruginosa* (MTCC 424), *Salmonella typhi* (MTCC 98), Methicillin-resistant *Staphylococcus aureus* (MRSA) (MTCC 840) and *Staphylococcus aureus* (MTCC 96) using agar well diffusion method[22].

About 25 mL of molten Mueller-Hinton agar was transferred into the sterilized Petri plates and set aside to cool (Himedia, Mumbai, India). The culture plates were allowed to solidify and were seeded with 100 µl of the selected and subcultured bacterial strains. The plates were then punched with a sterile cork-borer and the open wells were inoculated with varying concentrations (50 µg/mL and 200 µg/mL) of the selected crude extract. Standard ciprofloxacin drug was used as a positive control. The prepared plates were incubated at 37°C for 24 h.

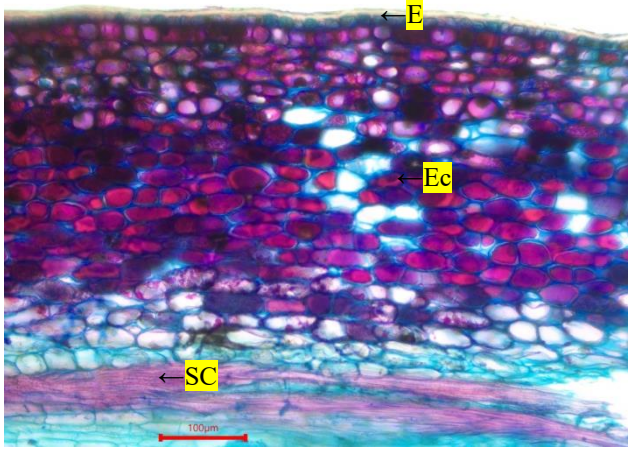
GC–MS analysis: GC-MS was used to identify the compounds present in the extract. It was performed on an Agilent GC 7890A carrying a triple quadrupole mass

spectrophotometer with a fused silica capillary column Agilent DB5MS (5%Diphenyl95% Dimethyl poly siloxane), 30m x 0.25 mm ID x 0.25 micron df. The column oven temperature program was as follows: 60°C hold for 1 min, up to 300°C at the rate of 15°C/min, injector temperature 280°C. Total GC running time was 23 min. The inlet temperature was set at 250°C and the ion source temperature was fixed at 230°C. Electron ionization was performed at 70 eV. For single scan analysis, the mass scan range was set from m/z 50 to 500. A solvent delay of 4 minutes was applied and the injection volume was 1 µL. The GC-MS/MS was performed at TÜV SÜD, Thirupur. Here, GC-MS/MS analysis was performed to identify the phytoconstituents of the extract at trace levels.

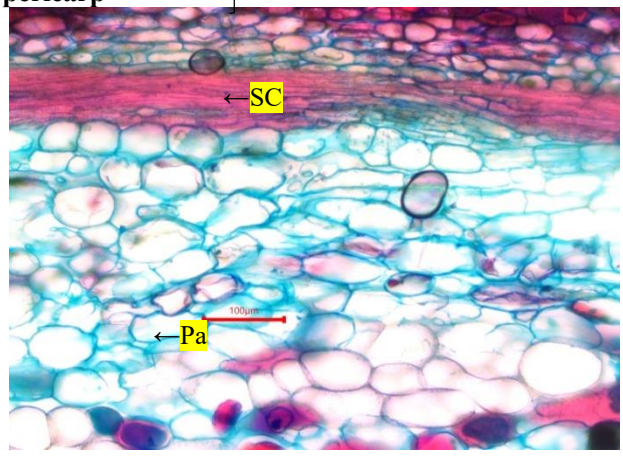
Figure 2: Macroscopy of *Terminalia catappa* fruit



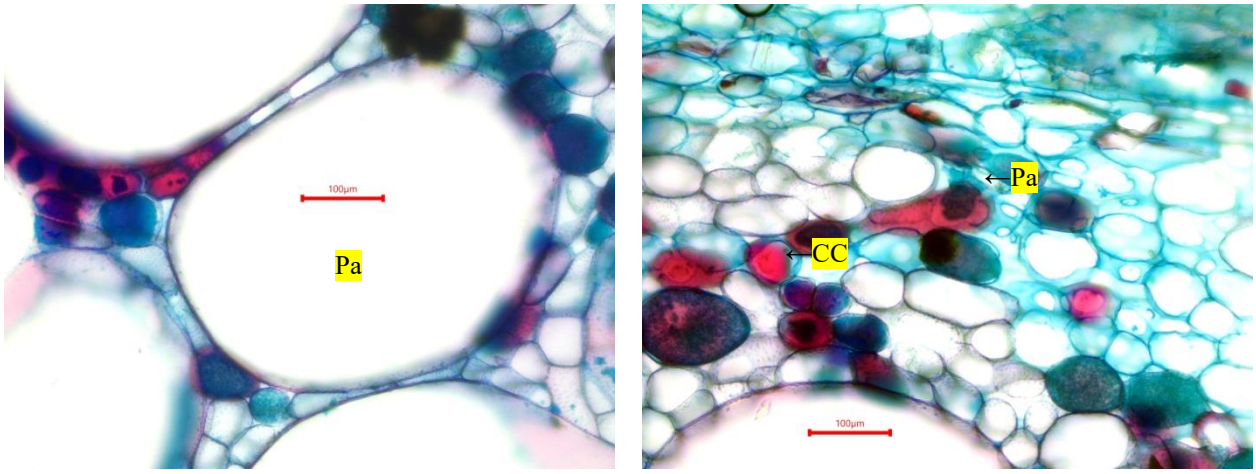
TS of the pericarp



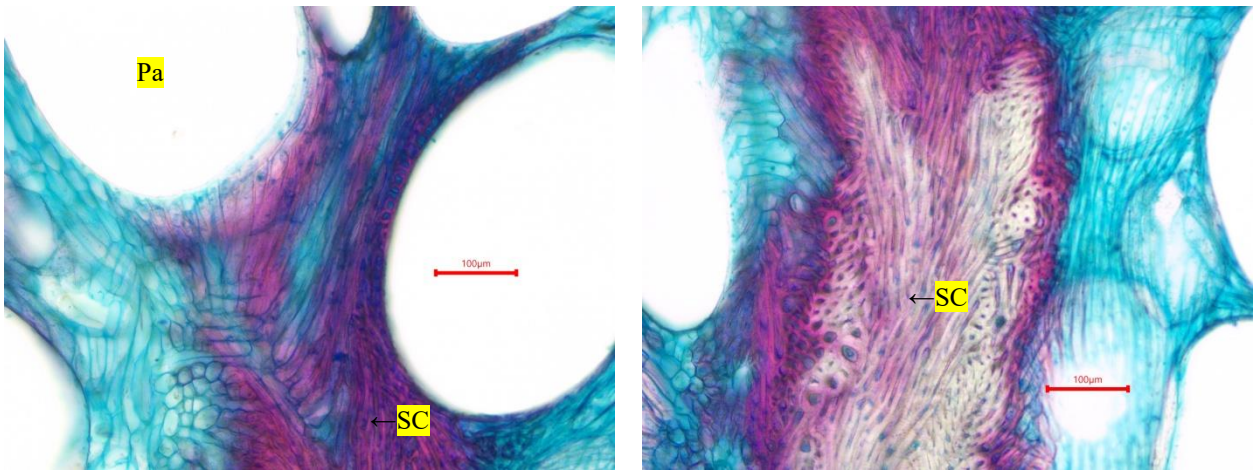
Enlarged view of epicarp



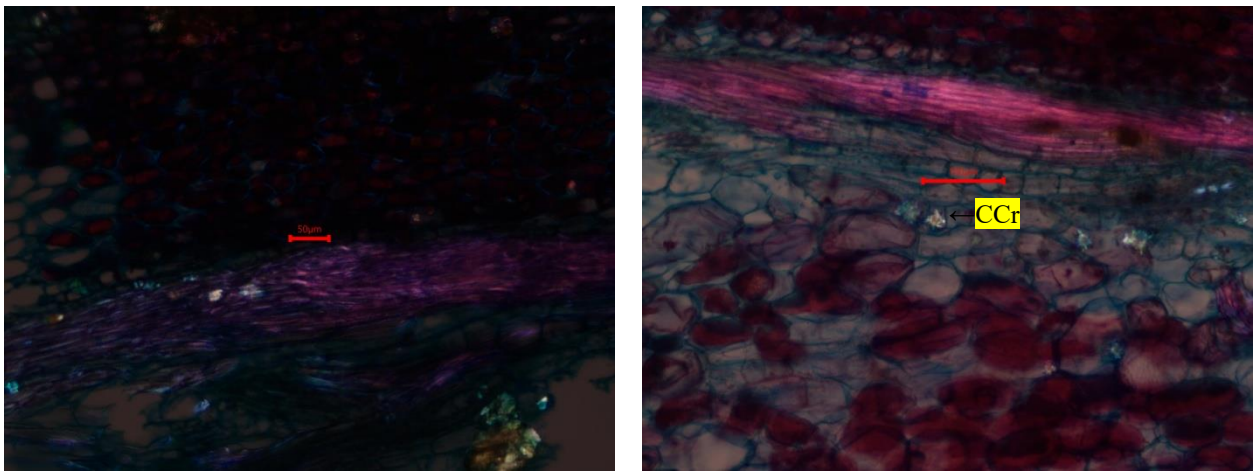
Enlarged view of mesocarp



Mesocarp parenchyma



Finger-like projections of the endocarp into the mesocarp



Mesocarp portion under polarizer field

Figure 3: TS of *Terminalia catappa* pericarp CC - cell content; CCr - cluster crystal; E - epidermis; Ec - epicarp; EEc - extension of endocarp; Mc - mesocarp; Pa - parenchyma; SC - stone cell

Table 1
Characteristic features

S.N.	Parameter	Observation
1	Colour	Green to reddish green coloured
2	Shape	Round to oval and flattened with two ridges
3	Taste	Astringent

For the compound separation, helium was used as a transport gas at a constant flow of 1 mL/min. During the chromatographic run, the injector temperature was set at 260°C and 1 mL of the extract was injected into the instrument.

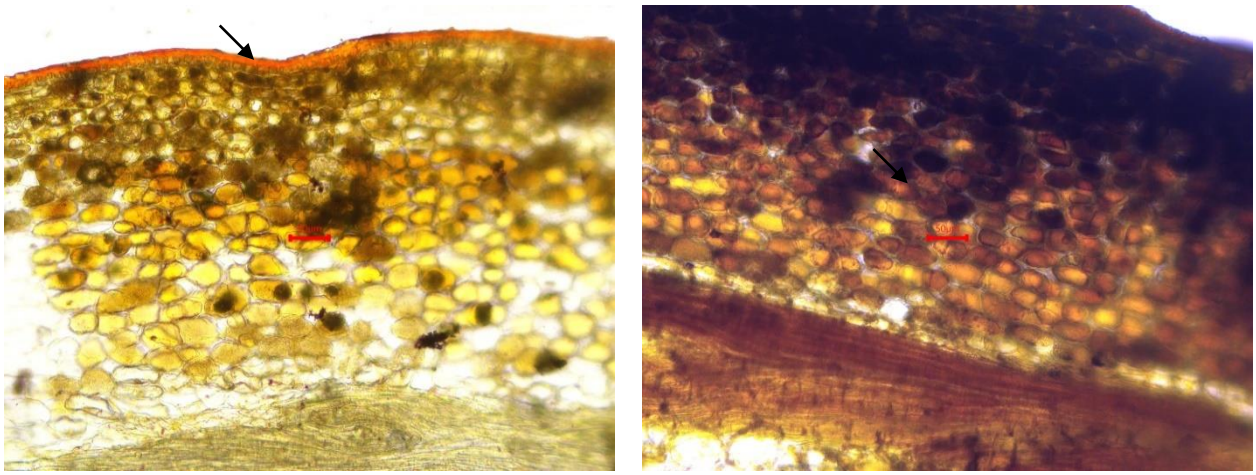
Results and Discussion

Morpho-macroscopic observation: The pharmacogenetic traits of TCFR were thoroughly evaluated using a range of analytical parameters. The organoleptic study of the fruit revealed characteristic colors, odors and tastes. The fruits were slightly ripened drupe, green to reddish green coloured, rounded to oval shaped and flattened with two ridges, 4 to 7 cm long and 2 to 5 cm wide, pericarp fleshy, husk is fibrous and tough, enclosing a single seed and odour characteristic with astringent taste (Table 1).

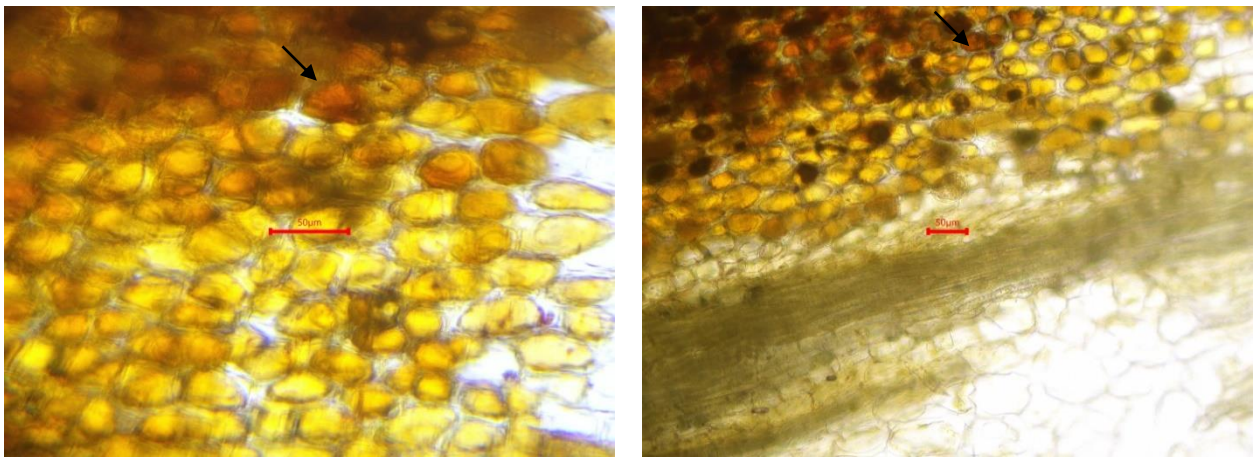
TS of pericarp shows outer epicarp, middle broad mesocarp and inner endocarp region; epicarp is formed of single layered epidermal cells followed by 14 to 16 layers of compactly arranged parenchyma cells filled with pigment contents; mesocarp region is broad and consists of 4 to 5 layers of thick stone cells which separate the epicarp region from mesocarp, inner to stony layer 6 to 7 layers of small parenchyma cells followed by broad inner mesocarp region of thick-walled parenchyma cells with wide lumen intermingled with small parenchyma cells. Some finger like projections of endocarp region can be seen penetrating to the mesocarp parenchyma cells. Several cluster crystals and some cell contents are scattered in mesocarp cells (Fig. 3).

Histochemical observation

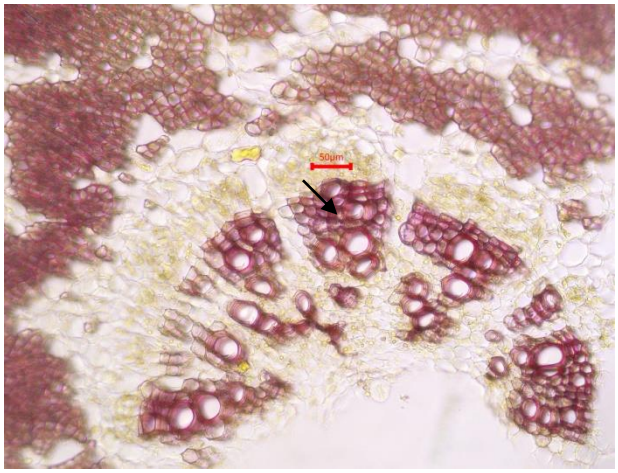
Pericarp: The treatment of the TS of the pericarp with Wagner's reagent transformed the yellow to reddish colour to brown color, confirming the presence of alkaloids. Alkaloids were detected in the epicarp and phenolic compounds were observed in the epicarp and mesocarp cells. The phenolic compounds in fruits are dietary components that play a crucial role in the human diet. The antibrowning effect of this compound makes it a good antioxidant[11]. Mucilage was detected in the mesocarp region and lignin was detected in the xylem and stone cells. Resins and starch grains are absent in the pericarp of *T. catappa.* Starch grains are well-packed storehouses of glucose sugar units. Mucilage is commonly found in fruits. Mucilage possesses the water retention ability, making it a potential gelling agent and it is widely utilized as a polymer in the cosmetic industry and food industry[13,37].



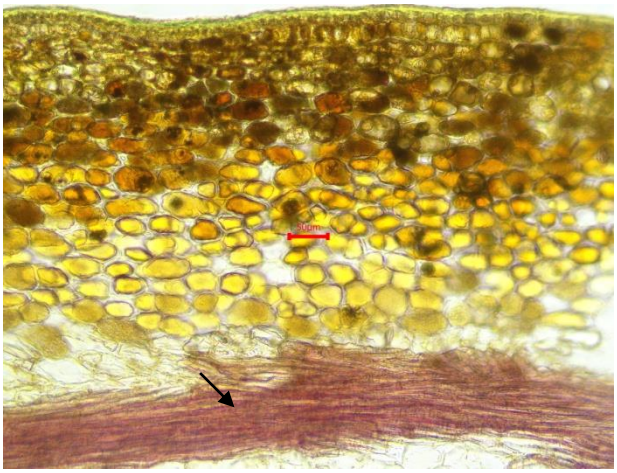
Alkaloid in epicarp



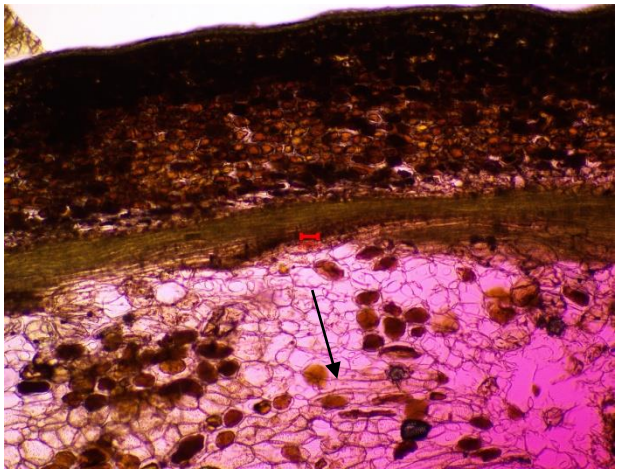
Alkaloid in mesocarp



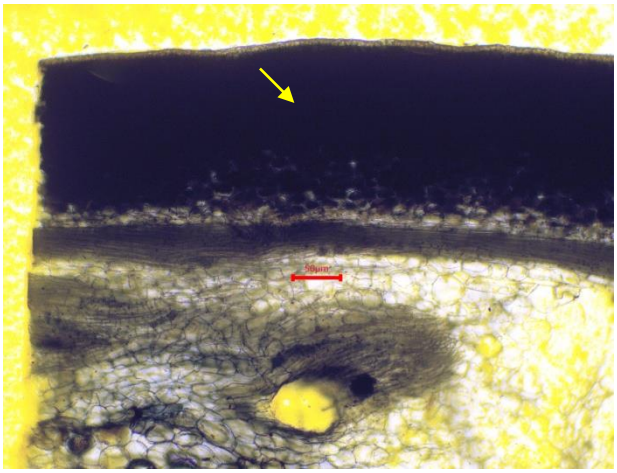
Lignin in vessels



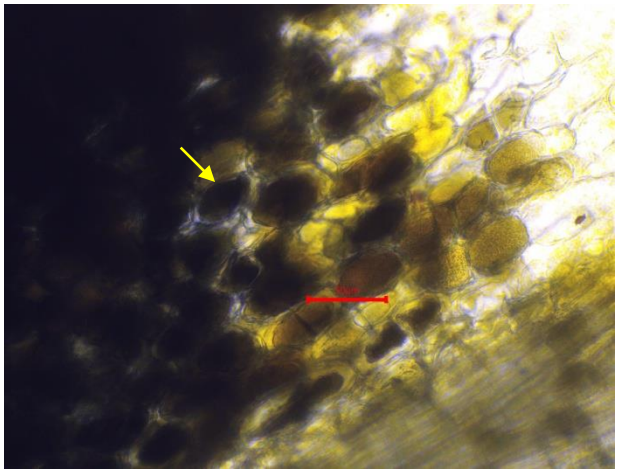
Lignin in stone cells



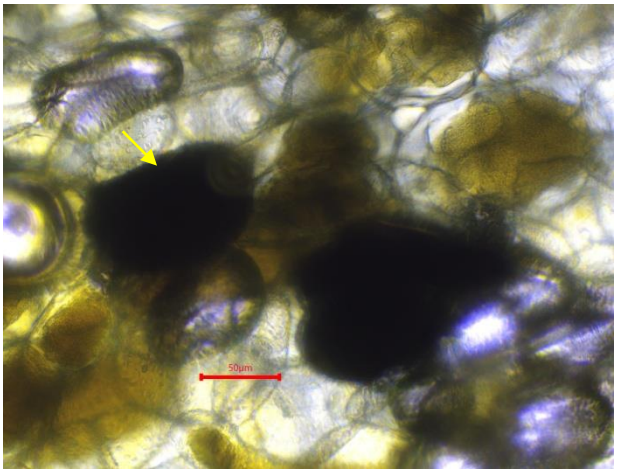
Mucilage in mesocarp cells



Phenolic compound in epicarp



Phenolic compound in the epicarp



Phenolic compounds in mesocarp

Figure 4: Histochemistry of *Terminalia catappa* fruit

Lignin is a polyphenolic polymer commonly confined to the parenchyma and the dermal regions of stems. The chromophores of lignin are secondary metabolites such as coumarins and stilbenes[24]. The presence of lignin indicates the localization of these secondary metabolites in the cells of TCFR.

Extraction: The crude was extracted from TCFR powder with solvents such as hexane, ethyl acetate, ethanol and aqueous solutions, following the maceration technique. The aqueous extract showed maximum extraction yield (17%) followed by ethanol (14%), ethyl acetate (11.3%) and hexane (2.74%) (Fig. 5).

Qualitative and quantitative screening of phytochemicals: The utilization of botanical products for treating various illnesses is widely documented. Each qualitative phytochemical test detects the medicinally

significant phytocompounds present in the plant sample[12]. Therefore, the screening of phytochemicals is necessary in the current research. The positive results confirmed the presence of various bioactive compounds including alkaloids, terpenoids, phenols, carbohydrates, saponins, glycosides, quinones, proteins, flavonoids and tannins across different solvent extracts (Table 1). However, the aqueous extract demonstrated the highest presence of phytoconstituents. Ethanol and ethyl acetate extractions identified eight major types of phytocompounds, while the hexane extract revealed relatively fewer phytochemicals compared to other solvents. Similarly, quantitative screening revealed that among the various solvent extracts, the aqueous extract contained a higher concentration of phytochemicals. In the aqueous extracts, total phenol, flavonoid and tannin content were estimated at 273.553 ± 0.33 mg GAE/g, 6.174 ± 0.008 mg GAE/g and 335.8 ± 0.09 mg GAE/g, respectively (Table 2). The presence of secondary metabolites is valued for the plant's medicinal value. Both the qualitative and the quantitative estimation imply that the fruit rinds are rich in polar compounds.

Table 2
Quantitative phytochemical analysis of TCFR extracts

Tests	Extracts			
	Hexane	Ethyl Acetate	Aqueous	Ethanol
TPC (mg GAE/g)	68.339 ± 0.35^{c}	273.24 ± 0.12^{b}	273.553 ± 0.33^{b}	323.973 ± 0.14^{a}
TFC (mg QE/g)	2.388 ± 0.41^{d}	36.443 ± 0.30^{a}	6.174 ± 0.008^{c}	21.683 ± 0.08^{b}
TTC (mg GAE/g)	82.537 ± 0.02^{d}	324.09 ± 0.20^{c}	335.8 ± 0.09^{b}	472.806 ± 0.08^{a}
Note. ^{abcd}Different letters in each row indicate that values are significantly different ($P<0.05$). Each value was expressed as mean±SD; n=3 independent experiments.				

Table 3
Qualitative phytochemical analysis of TCFR extracts

Tests	Solvent extracts			
	Hexane	Ethyl acetate	Aqueous	Ethanol
Alkaloids	+	+	-	+
Terpenoids	+	+	+	+
Phenols	+	+	+	+
Carbohydrates	+	+	+	+
Saponins	-	-	+	-
Glycosides	+	+	+	+
Quinone	-	+	+	+
Protein	+	-	+	-
Flavonoids	-	+	+	+
Tannins	+	+	+	+
– = Absent; + = Present				

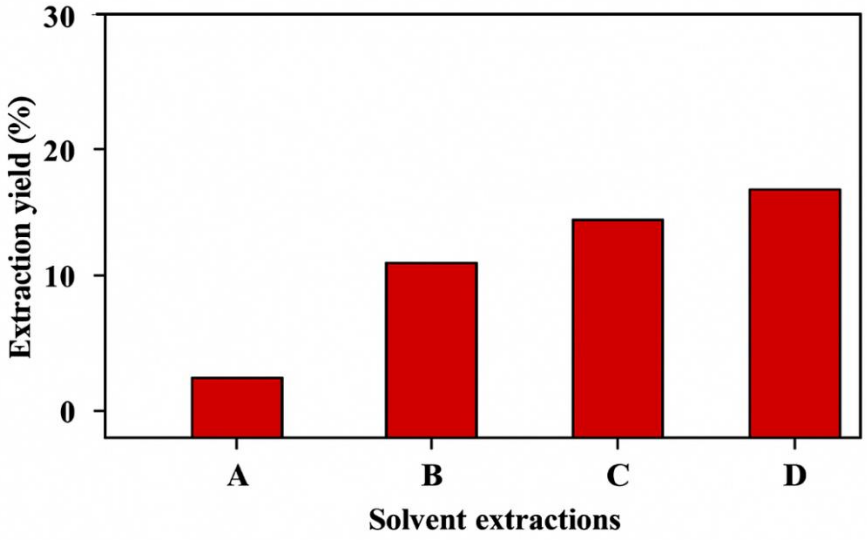


Fig. 5: Total yield (%) of TCFR extracts using different solvents. A) Hexane, B) Ethyl acetate, C) Ethanol, D) and E) Aqueous. The aqueous extract showed the highest yield compared to the other solvents.

***In vitro* antibacterial activity:** In the present study, crude ethyl acetate, ethanolic and aqueous extracts of TCFR were examined for their potential antibacterial efficiency against all the tested bacterial strains screened. The antimicrobial action of the extracts was observed against both Gram-positive and Gram-negative bacteria including *E. coli*, *Streptococcus mutans*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Clostridium sordelli* and *Enterobacter faecalis,* using the well diffusion method. The ethanolic extract demonstrated the maximum activity with a larger zone of inhibition against *E. faecalis* (25.16 mm), *Escherichia coli* (24.06 mm), MRSA (24.03 mm) and *K. pneumoniae* (23.2 mm) at the maximum drug concentration (200 µg/ml).

The ethanolic extract showed considerable activity. The rank of its activity against each strain is: *E. faecalis*> *Escherichia coli* > =MRSA>*K. pneumoniae* > *P. mirabilis* > *S.aureus* > *S. typhi* > *M. luteus* > *P. aeruginosa* > *C. sorelli* (Fig. 6). The aqueous extract also showed moderate activity against all the bacterial strains but its activity was higher compared to the ethyl acetate extract. Notably, the extract demonstrated its strongest antibacterial effect against *E. coli*, producing a substantial inhibition zone of 22.9 ± 0.05 mm at a concentration of 200 mg/ml (Fig. 7).

The crude ethyl acetate extract showed lesser effectiveness compared with the aqueous and ethanolic extracts (Fig. 8). These findings highlight ethanol as the most effective solvent for extracting antibacterial agents, outperforming both aqueous and ethyl acetate extracts. Although the ethyl acetate crude extract demonstrated significant antibacterial activity, its efficacy was comparatively lower than that of the ethanolic and aqueous extracts. Rank of solvents based on antibacterial activity of TCFR is: ethanol > aqueous > ethyl acetate. Álvarez-Martínez and his co-workers[5] have found that various plant secondary metabolites have been studied for their antibacterial potential against the common MDR pathogens. A cohort of studies has demonstrated that different parts and extracts of *T. catappa* were examined and documented for their antibacterial efficiency against various human pathogens, commonly *B. subtilis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa, Escherichia coli, Salmonella species,* Methicillin-Resistant *Staphylococcus aureus and S. aureus*, *E. coli* and *Pseudomonas aeruginosa*[21,27,34]. The current findings suggest that TCFR contains potent antibacterial phytoconstituents to combat common human pathogens. *T. catappa* is a versatile and nutritious plant with significant medicinal properties. The problem of microbial resistance is on the rise and the ultimate goal is to provide appropriate and effective antibiotics to the victims[29].

Before the introduction of allopathic medicines, disease treatment relied entirely on herbal remedies. In developing countries, the use of plant products to treat various infections is an age-old practice and there is a dependence on traditional medicines for different common diseases[6,10]. In the present investigation, the antibacterial potential, anatomy, histochemistry and phytochemistry of the fruit rinds of semi-ripened to ripened *T. catappa* were examined.

GC–MS analysis: Given the antibacterial activity, the ethanolic and aqueous extracts of TCFR showed better results compared to the ethyl acetate extracts. Hence, GC–MS analysis was conducted on these extracts, revealing the presence of distinct compounds in both the ethanolic and aqueous extracts. The chromatogram of both the extracts is presented in fig. 9 and fig. 10. The presence of the bioactive phytoconstituents, peak area (%), retention time, molecular formula and molecular weight are shown in tables 4 and 5. Several phytochemicals contained in TCFR have been reported to be potent antibacterial phytoconstituents. The GC-MS screening of both ethanolic and aqueous extracts reported a total of 37 and 35 secondary metabolites respectively.

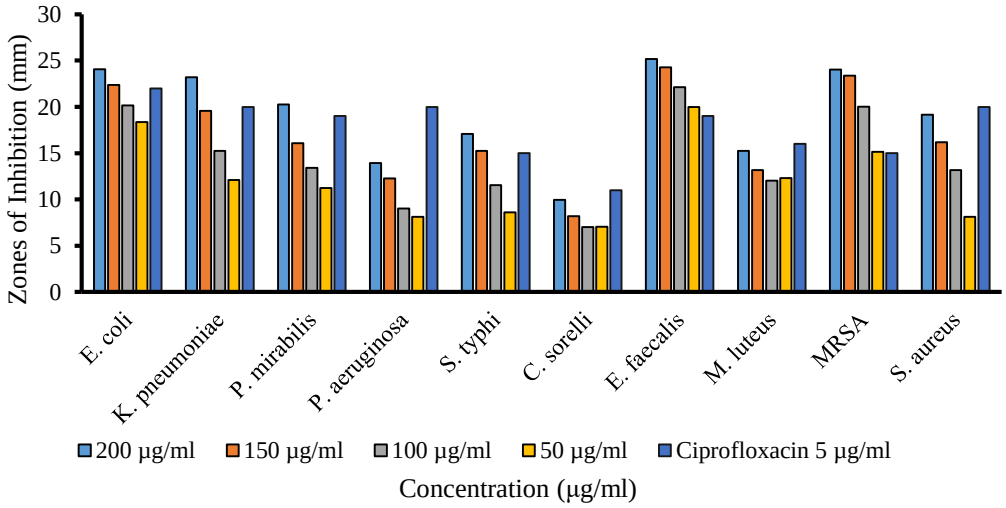


Fig. 6: Zones of inhibition (mm) showing antimicrobial activity of ethanolic extract

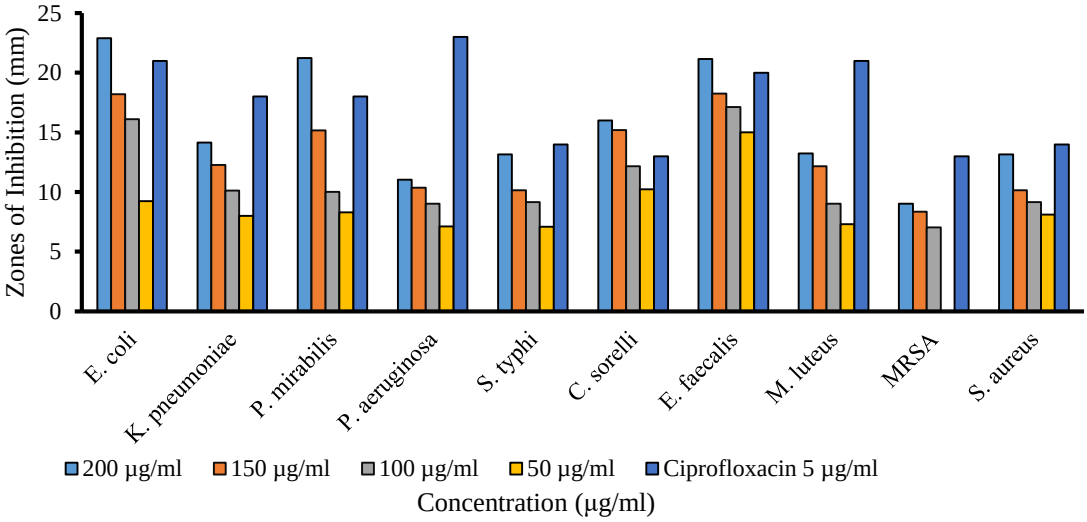


Fig. 7: Zones of inhibition (mm) showing antimicrobial activity of aqueous extract

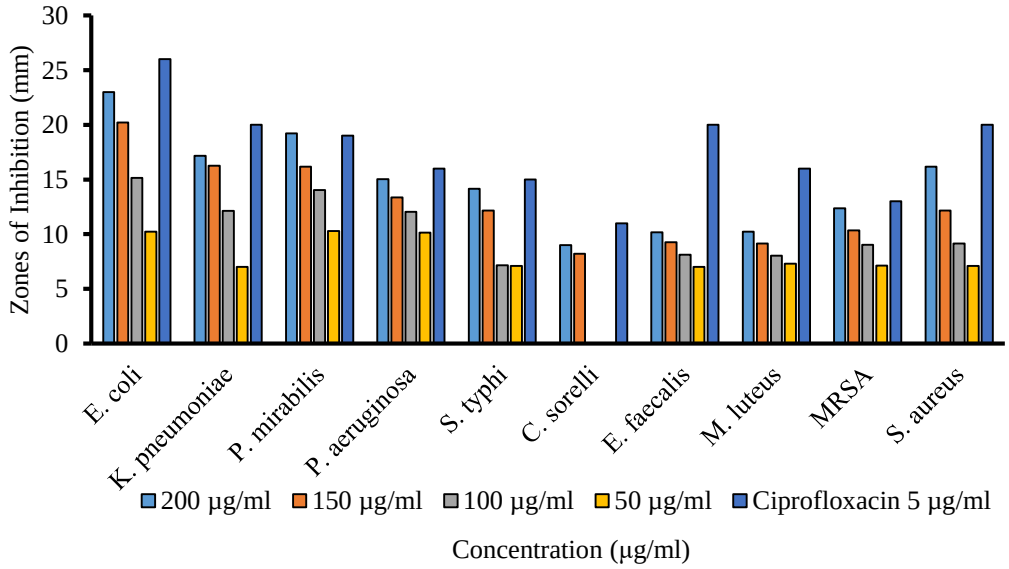


Fig. 8: Zones of inhibition (mm) showing antibacterial activity of ethyl acetate extract

Among the phytocompounds identified via GC–MS, octadecanoic acid, n-hexadecanoic acid, lupeol and vitamin E are noteworthy due to their documented occurrence in medicinal plants such as *Echinops echinatus* and *Tridax procumbens*, both of which have a long-standing ethnopharmacological reputation for managing infectious conditions[32]. Other species of Terminalia possess similar phytoconstituents. Literature has reported identical secondary metabolites present in different parts of *T. catappa*. However, some of the secondary metabolites such as Octadecanoic acid, 1H-Imidazole and Amyrin, are found to be confined to the fruit rinds. Among the array of detected secondary metabolites, 1H-pyrazole and 5-Amino-2-methyl-3H-imidazole-4-carboxylic acid ethyl ester were documented to have high potency as antibacterial agents[9,14,28,31]. Therefore, the study demonstrates that the ethanolic and aqueous extracts of the plant sample exhibit significant antibacterial properties.

Conclusion

The histochemical and anatomical studies of the TCFR demonstrated the presence of secondary metabolites localized within the cells. The phytochemical studies and GC-MS characterization have confirmed the rich source of antibacterial inhibiting secondary metabolites present in both ethanolic and aqueous extracts against some MDR human pathogens.

Table 4
GC-MS detection of different bioactive compounds in the ethanolic extract

S.N.	Compounds	Molecular formula	Molecular weight (mass) g/mol	Rt (min.)	Area%
1.	2,5-Furandione, dihydro-3-methylene-	$C_5H_4O_3$	112.0835	4.320 5.231	11.81 1.20
2.	1H-Imidazole	$C_3H_4N_2$	68.0773	4.320	11.81
3.	2-Furancarboxaldehyde, 5-methyl-	$C_6H_6O_2$	110.1106	4.498	1.22
4.	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	$C_6H_8O_4$	144.1253	4.698	2.77
5.	Thymine	$C_5H_6N_2O_2$	126.1133	5.687	0.83
6.	2H-Pyran-2,6(3H)-dione	$C_5H_4O_3$	112.0835	4.820	0.41
7.	2,5-Furandicarboxaldehyde	$C_6H_4O_3$	124.0942	5.720	0.30
8.	3,4-Difluoroanisole	$C_7H_6F_2O$	144.12	4.698	2.77
9.	2(1H)-Pyridinone, 6-hydroxy-	$C_5H_5NO_2$	111.0987	5.231	1.20
10.	2-Cyclohexen-1-one, 3-hydroxy-	$C_6H_8O_2$	112.13	4.820	0.41
11.	1,2-Benzenediol	$C_6H_6O_2$	110.1106	6.864	1.09
12.	1,2,3-Benzenetriol	$C_6H_6O_3$	126.1100	8.686	2.14
13.	Phenol, 4-methoxy-2-nitro-	$C_7H_7NO_4$	169.1348	11.397	10.76
14.	3-Furancarboxylic acid, methyl ester	$C_6H_6O_3$	126.1100	5.787	0.87
15.	Furyl hydroxymethyl ketone	$C_6H_6O_3$	126.1100	5.787	0.87
16.	3-Furancarboxylic acid, methyl ester	$C_6H_6O_3$	126.11	5.787	0.87
17.	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144.1253	6.464	3.26
18.	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4241	13.097	5.12
19.	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.4455	14.197	3.87
20.	6-Octadecenoic acid	$C_{18}H_{34}O_2$	282.4614	14.241	3.32
21.	Octadecanoic acid	$C_{18}H_{36}O_2$	284.4772	14.363	0.55
22.	2-Furancarboxaldehyde, 5-(hydroxymethyl)-				
23.	Benzoic acid, 3-hydroxy- (m-Hydroxybenzoic acid)	$C_7H_6O_3$	138.1207	9.608	4.31
24.	Benzoic acid, 4-hydroxy- (p-Hydroxybenzoic acid)	$C_7H_6O_3$	138.1207	9.608	4.31
25.	Propylparaben	$C_{10}H_{12}O_3$	180.2005	9.931	2.56
26.	Benzoic acid, 4-hydroxy-3-methoxy-, ethyl ester (Ethyl vanillate)	$C_{10}H_{12}O_4$	196.1999	10.208	0.44
27.	beta.-Amyrin	$C_{30}H_{50}O$	426.7174	15.608	1.75
28.	alpha.-Amyrin	$C_{30}H_{50}O$	426.7174	15.608	1.75
29.	dl-.alpha.-Tocopherol	$C_{29}H_{50}O_2$	430.7	20.396	0.36
30.	Stigmasterol	$C_{29}H_{48}O$	412.6908	21.885	0.64
31.	4-Dehydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene)tyramine	$C_{16}H_{14}N_2O_4$	298.29	21.885	0.64
32.	β-Sitosterol	$C_{29}H_{50}O$	414.7067	22.662	4.27
33.	γ-Sitosterol	$C_{29}H_{50}O$	414.7067	22.662	4.27
34.	Cholest-5-en-3-ol, 24-propylidene-, (3beta)-	$C_{30}H_{50}O$	426.72	22.862	1.80
35.	2-(3,4-Dimethoxyphenyl)-3,7-dihydroxy-4H-chromen-4-one	$C_{17}H_{14}O_6$	314.29	22.862	1.80
36.	Vitamin E	$C_{29}H_{50}O_2$	430.7061	20.396	
37.	Stigmasta-5,24(28)-dien-3-ol, (3β,24Z)-	$C_{29}H_{48}O$	412.6908	22.862	1.80

Table 5
GC-MS detection of different bioactive compounds in the aqueous extract

S.N.	Compound Name	Molecular Formula	Molecular Weight (g/mol)	Rt (min.)
1.	2,5-Furandione, dihydro-3-methylene-	$C_5H_4O_3$	112.08	4.265
2.	2-Furancarboxaldehyde, 5-methyl-	$C_6H_6O_2$	110.11	4.498
3.	4-Hydroxy-3-[[1,3-dihydroxy-2-propyl]oxy]benzoic acid	$C_{10}H_{12}O_3$	230.22	4.609
4.	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	$C_6H_8O_4$	144.13	4.687
5.	Maltol	$C_6H_6O_3$	126.11	5.653
6.	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	C_6H8O_4	144.13	6.431
7.	1,2-Benzenediol	$C_6H_6O_2$	110.11	6.864
8.	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	$C_6H_6O_3$	126.11	7.198
9.	Thymol	$C_{10}H_{14}O$	150.22	7.92
10.	Phenol, 2-methyl-5-(1-methylethyl)-	$C_{10}H_{14}O$	150.22	7.92
11.	Eugenol	$C_{10}H_{12}O_2$	164.2	8.453
12.	Phenol, 2-methoxy-3-(2-propenyl)-	$C_{10}H_{12}O_2$	164.2	8.453
13.	1,2,3-Benzenetriol	$C_6H_6O_3$	126.11	8.575
14.	2(3H)-Benzothiazolone	C_7H_5NOS	151.19	9.52
15.	Benzoic acid, 3-hydroxy	$C_7H_6O_3$	138.12	9.697
16.	Benzoic acid, 4-hydroxy-	$C_7H_6O_3$	138.12	9.753
17.	Phenol, 2-methoxy-4-(2-propenyl)	$C_{12}H_{14}O_3$	206.24	9.831
18.	Ethylparaben	$C_9H_{10}O_3$	166.17	9.931
19.	Tetradecanoic acid	$C_{14}H_{28}O_2$	228.37	11.664
20.	Pentadecanoic acid	$C_{15}H_{30}O_2$	242.4	11.664
21.	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	13.075
22.	Urs-12-en-24-oic acid, 3-oxo-	$C_{31}H_{48}O_3$	468.7	13.519
23.	β-Amyrin	$C_{30}H_{50}O$	426.72	13.519
24.	α-Amyrin	$C_{30}H_{50}O$	426.72	13.719
25.	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.45	14.186
26.	trans-13-Octadecenoic acid	$C_{18}H_{34}O_2$	282.46	14.219
27.	cis-13-Octadecenoic acid	$C_{18}H_{34}O_2$	282.46	14.219
28.	6-Octadecenoic acid	$C_{18}H_{34}O_2$	282.46	14.219
29.	Lupeol	$C_{30}H_{50}O$	426.72	15.608
30.	Benzo[b]naphtho[2,3-d]furan	$C_{16}H_{10}O$	218.25	15.608
31.	1H-Indole, 5-Methyl-2-Phenyl-	$C_{15}H_{13}N$	207.27	16.463
32.	1-Methyl-2-phenylindole	$C_{15}H_{13}N$	207.28	17.441
33.	N-Methyl-1-Adamantaneacetamide	$C_{13}H_{21}NO$	207.31	18.018
34.	Vitamin E	$C_{29}H_{50}O_2$	430.7061	20.396
35.	β-/γ-Sitosterol	$C_{29}H_{50}O$	414.71	22.662

Table 6
Common compounds present in both ethanolic and aqueous extracts

S.N.	Compound Name	Molecular Formula	Molecular Weight (g/mol)
1	1H-Pyrazole	$C_3H_4N_2$	68.08
2	2,5-Furandione, dihydro-3-methylene-	$C_5H_4O_3$	112.0835
3	2-Furancarboxaldehyde, 5-methyl-	$C_6H_6O_2$	110.1106
4	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	$C_6H_8O_4$	144.1253
5	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144.1253
6	1,2-Benzenediol	$C_6H_6O_2$	110.1106
7	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	$C_{18}H_{36}O_2$	284.4772
8	1,2,3-Benzenetriol	$C_6H_6O_3$	126.1100
9	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4241
10	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.4455
11	6-Octadecenoic acid	$C_{18}H_{34}O_2$	282.4614

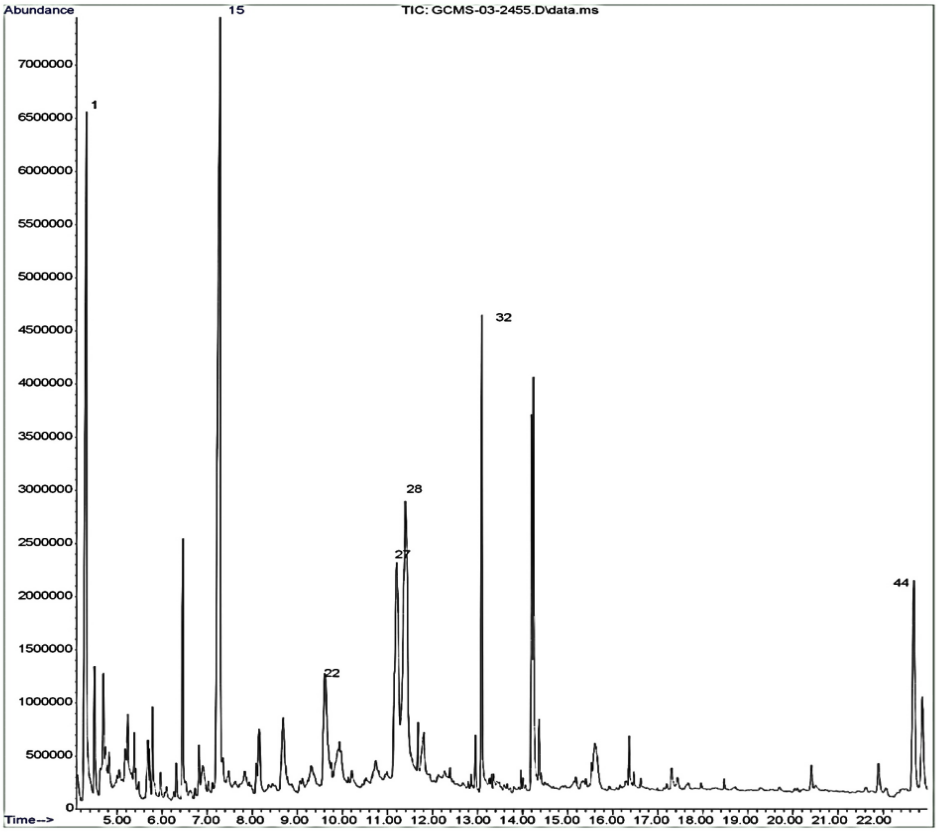


Fig. 9: GC–MS chromatogram of ethanolic extract. Each peak shows the relative abundance and area of a specific compound in the ethanolic extract.

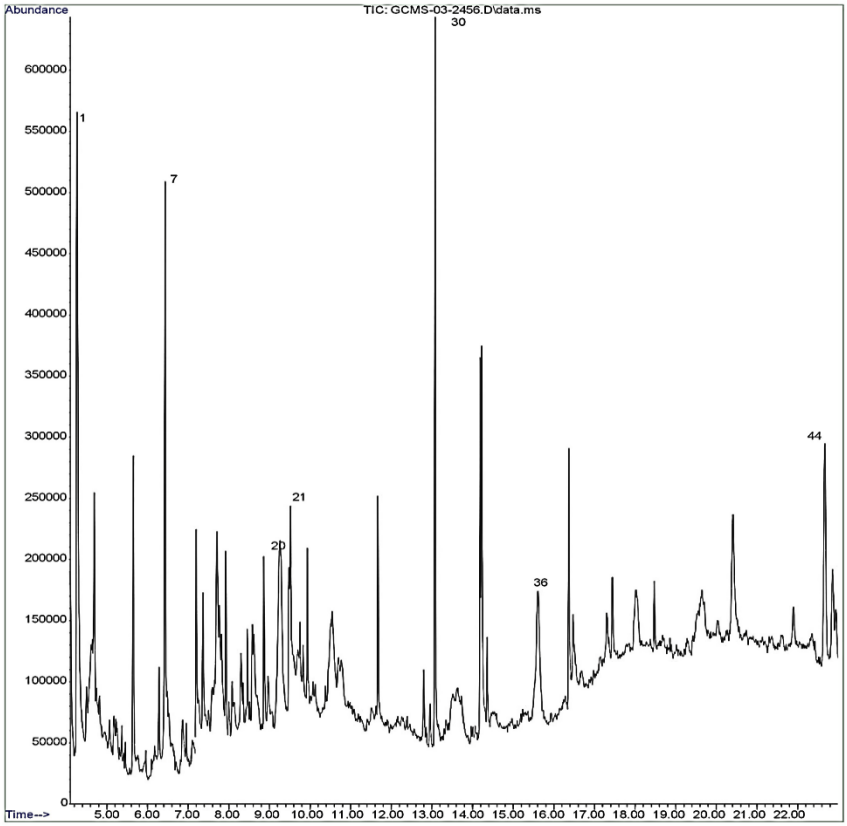


Fig. 10: GC–MS chromatogram of aqueous extract. Each peak shows the relative abundance and area of a specific compound in the ethanolic extract.

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