

Research Article



Phytochemical Profiles, *In Vitro* Antioxidant, Anti Inflammatory and Antibacterial Activities of *Terminalia catappa*

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ABSTRACT

The aim of the study was to analyze the antibacterial, antifungal, antioxidant and anti inflammatory potential of three different extracts (acetone, chloroform and petroleum ether) of *Terminalia catappa* leaves. Dried leaves powder of *T. catappa* was used to perform the phytochemical analysis and three different solvent extracts were subjected for gas chromatography-mass spectrometry (GC-MS) analysis to identify its bioactive components. Antibacterial and antifungal potential was analyzed using agar well diffusion method against *Staphylococcus aureus* (MTCC: 3160) and *Proteus mirabilis* (MTCC: 3310). Acetone, chloroform and pet ether extracts derived from the leaves of *T. catappa* was assessed for its antioxidant and anti-inflammatory activity by *in vitro* method. Antioxidant capability was studied using 2,2-diphenyl-1-picrylhydrazyl scavenging assay and *in vitro* anti-inflammatory activity was evaluated using albumin denaturation assay. Phytochemical screening of leaves powder revealed the presence of bioactive compounds like, phenols, alkaloids, tannins, anthraquinone glycosides and flavonoids. All the three extract demonstrated a significant antibacterial activity in the well diffusion assay against *S. aureus* and *P. mirabilis*, acetone extract showed highest inhibition zone of about 31mm against both the pathogens at 10mg concentration. Antifungal activity against *Aspergillus niger* and *Aspergillus flavus* was not observed for all the three extracts. The pet ether extract demonstrated 73% of free radical scavenging activity followed by acetone 64% and chloroform 63% at the concentration of 150µg/ml.

Keywords: *Terminalia catappa*, Ornamental plant, Indian almond, Antibacterial activity, *In vitro* anti-inflammatory activity.

INTRODUCTION

Terminalia catappa is a shade and salt tolerant street tree, which was primarily used as an ornamental plant¹. They are most commonly found on tropical and subtropical beaches. In India and Philippines, the leaves of this plant were used as folk medicine in dermatitis and hepatitis treatment². In India, it is known to be as Malabar almond, Indian almond and tropical almond³. *T. catappa* is a species belonging to combertaceae family and it is widely known in Brazil as "cuca", "guarda-sol", "castanheirada Índia", "castanhola", "amendoeira", "amendoeira-da-Praia", "amendoeira-da-Índia"⁴. They are widely seen throughout the warmer parts of India and also in other countries like Australia, Srilanka, Malaysia, Pakistan and many other south Asian countries³. The plant grows mostly in well aerated, freely drained, sandy soils and they can withstand strong winds, the salt sprays and also moderately to high salinity in the root zones⁵. By providing a wide range of nonwood products and services, it plays a vital role in coastal communities⁵. The bark, leaves and fruit of the *T. catappa* were used in different countries like India, Malaysia and Philippines to cure dermatitis and also for hemostatic and antipyretic purposes⁶. In hepatoma and hepatitis treatment, the leaves of *T. catappa* have been widely used in Tiawan by shredding and drying⁶.

Taxonomic Classification of *Terminalia catappa*

Kingdom :	Plantae
Division :	Angiospermae
Class :	Dicotyledones
Order :	Myrtales
Family :	Combretaceae
Genus :	Terminalia
Species :	catappa

Antioxidant, anti-inflammatory, hepatoprotective activity were reported in this plant and antidiabetic property usually seen in fruit^{2,7}. Aphrodisiac, antioxidant, anti-HIV reverse transcriptase and anticancer were also reported³. India is the native for *T. catappa* tree and for the reforestation and ornamental purpose, it was introduced in south america⁷. *T. catappa* contains two third seed and one-third pulp. In developed countries, the human intake the seeds of *T. catappa* which are rich in fat (51.8%) composed mostly of oleic (up to 31.5%) and linoleic (up to 29%) acids and Mg (8%), carbohydrates (16%), crude protein (23.8%), K (9.3%) and Ca (8.3%)⁷. *T. catappa* L. commonly used as a traditional medicine in Taiwan and it has been believed to have a beneficial effect on liver-related diseases. Naturally, the leaves of *T. catappa* which were fallen from the tree were boiled in water and used for drinking purposes. Many hydrolyzable tannins except caffeine were found in leaves of *T. catappa* and



some of them are granatin B, certain, chebulagicacid, corilagin, punicalagin, punicalin and geraniin⁸.



Figure 1: The leaves of *Terminalia catappa*

The leaves were used for treating several diseases like eye problems, leprosy and different types of cancer as they exhibit anti-clastogenic, antioxidant and anti-cancer properties⁹. Travel nausea can be lowered with these leaves and it also used to get rid of intestinal parasites, for stopping bleeding during teeth extraction⁹. Consumption of almond will help in adduction of risk related to the heart disease in addition to its LDL and cholesterol-lowering effects. Thereby consumption of almond has beneficial health function which attributes to the antioxidant activity of vitamin E and mono unsaturated fats and also the presence of phenolic compound such as 2-prenyl-4-O-β-D-glucopyranosyl-oxy-4-hydroxybenzoic acid, 2-prenylated benzoic acid, catechin, procatechuic¹⁰. In infected H9 lymphocytes, this punicalin and punicalagin exhibited inhibition of HIV replications with little cytotoxicity⁸. Hepatitis, pyresis, diarrhea and dermatitis are treated using this leaves in Asian counties⁴. Some previous study has been reported that bleomycin-induced genotoxicity of Chinese hamster ovary cells and C14 induced hepatotoxicity can be suppressed by *T.catappa* water extracts¹¹.

In Caribbean region, this plant is also listed in pharmacopeia vegetables, in which the leaves were used in a distillation for gastric and urinary infection⁴. Acid and prenylated benzoic acid are found in the stem, root and leaf part of the almond plant¹⁰. The antiasthmatic compound, cyanidin 3-glucoside, corilagin, ellagic acid. (anti-HIV), gallic acid, pentosans and xanthine oxidase inhibitor are present in the fruit part of *T.catappa* and the gum has been reported to contain xylose (1%), mannose (1%), uronic acids (19%), D-galactose (20%) and L-arabinose (59%)³. As the plant leaves do not carry any Agro-economical and food value, they are used for synthesizing the AgNPs as an alternative bioresource and also used for channeling the bioactive components that are present in the biomass waste. Gold nanoparticles were also synthesized using this leaf extract¹². Several research has been reported that the leaves serve as an ideal source for synthesizing the AgNPs. Antioxidant, flavonoid like Kaemferol, phenols, polyphenols and tannins are the some of the bioactive components are

known to be present in these leaves, that remain unharnessed but they can bring about the reduction of Ag^+ ions to Ag^0 , which results in the formation of AgNPs¹²

MATERIALS AND METHODS

Preparation of leaf extracts

The fresh leaves of *Terminalia catappa* were collected from Vellore Institute of Technology, Vellore and rinsed with tap water then the leaves were dried under shade at room temperature for 2 weeks. The dried leaves were powdered using an electric grinder. 20 grams of leaves powder was dissolved in 200ml of different solvents acetone, chloroform and pet ether and kept in the shaker for overnight. Using Whatman filter paper, the content was filtered twice and the filtrate was evaporated to obtain the leaf crude extract.

Qualitative analysis of phytochemicals constituents

The presences of phytochemicals were qualitatively analysed using following protocol. Three different extracts were analyzed to determine the presence of tannins, phenol, saponin, flavonoid, alkaloid and anthraquinone Glycoside¹³.

Water Extract

2grams of leaf powder was introduced to 10ml of distilled water. Using mantle the content was boiled, then filtered using Whatman filter paper to collect the water extract.

Tannin Test

Add 2ml of filtrate along with few drops of 1 M $FeCl_3$ in the test tube. The presence of condensed tannin was confirmed by the appearance of green color and blue color indicates the presence of hydrolyzable tannin.

Phenol Test

Add 5% of $FeCl_3$ solution to 1ml of water extract in a test tube. The presence of phenolic compounds can be confirmed by the appearance of dark green color.

Saponin test

In a test tube, take 2 ml of aqueous filtrate and shake the content vigorously. The presence of saponins was confirmed by the formation of froth.

Acid Extract

A volume of 6ml concentrated HCl was mixed with 1g of leaves powder in a glass beaker and kept undisturbed for 20min. Using Whatman filter paper, the acid extract was filtered.

Flavonoids

Take 2ml of acid extract in two separate test tube. Add 2ml of NaOH in one tube and 2ml of distilled water in another tube. The presence of flavonoids was confirmed by the appearance of yellow color.

Alcohol Extract

To the 8ml of methanol, 2grams of leaf powder was added and kept undisturbed for 30mins. Then the content was filtered using Whatman filter paper after 30mins. Using mantle the filtrate was evaporated and resuspended by adding 3ml of chloroform

Alkaloid

Dragendorff's reagent was sprayed over the filter paper containing few drops of alcohol extract. The presence of alkaloids was confirmed by the appearance of reddish brown color.

Anthraquinone Glycoside

1ml of ammonia and 2 ml of alcohol extract was taken in a test tube and shaken vigorously. The presence of anthraquinone was confirmed by the appearance of green color in the bottom and reddish color in aqueous layer.

Determination of antioxidant capacity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) reduction assay

DPPH radical scavenging assay is a standard method used to determine the antioxidant activity of three different extracts (acetone, chloroform and pet ether) of *T. catappa*. The crude extracts were dissolved in methanol to prepare 1mg /ml concentration extracts. The DPPH (4mg) was dissolved in 100ml of methanol. 2ml of methanolic DPPH was added to the test tubes containing 1ml of different concentrations (50, 100 and 150 µg/ml) of crude extracts. The reaction mixtures were shaken well and incubated for 30mins in dark region. For each test samples, triplicates were done. After the incubation period, the absorbance was measured at 570nm and gallic acid was used as the reference sample.¹⁴

The percentage inhibition was calculated using the following equations:

Percentage of inhibition = $\frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$

Abs control – Absorbance of the sample at 570nm

Abs sample – Absorbance of the control at 570nm

Determination of antibacterial activity using agar well diffusion method

Antibacterial activity was performed on the three different leaves extracts using agar well diffusion method. Bacterial pathogens such as *Staphylococcus aureus* (MTCC: 3160) and *Proteus mirabilis* (MTCC: 3310) were cultured in nutrient broth (Hi-media). Using sterile cotton swab, the bacterial lawn culture was made on Muller Hinton Agar (Hi-media) culture plate. The appropriate wells were made using Sterile cork borer. To the wells, different concentrations (2.5, 5, 10mg/ml) of leaf extracts were added respectively. Then the plates were incubated overnight at 37°C. The zone of inhibition around the well

containing leaf extract was observed and measured. Streptomycin disc was used as positive control.¹⁵

Determination of antifungal activity using agar well diffusion method

In vitro antifungal activity of acetone, chloroform and pet ether extract of *T. catappa* leaves were examined by agar well diffusion method. *Aspergillus niger* and *Aspergillus flavus* were inoculated on sterile SDA plates and spread over the media using sterile swabs. Wells were made on the SDA plates using sterile cork borer. In order to test the antifungal activity of leaf extract, 1mg of each extract were dissolved in 1ml of distilled water. 100 µl volumes of leaf extracts were loaded into the well and kept undisturbed for few minutes for extract diffusion. Then the plates were incubated at room temperature for 3-7 days. After the incubation period, the plates were observed for zone of inhibition around the well. Fluconazole disc was used as the positive control for antifungal activity test.¹⁶

Determination of anti inflammatory activity using *in vitro* albumin denaturation method

Inhibition of albumin denaturation by *T. catappa* was studied using a standard protocol. The reaction mixture contains various concentrations of the three different test extracts (acetone, chloroform, pet ether) and 1% of bovine albumin fraction (aqueous solution). The pH of the resulting mixture was adjusted to 6.8 using 1N HCl. Then the contents were incubated respectively at 37°C for 20mins followed by heating at 57°C for 20mins. After heating the samples were cooled and the turbidity was measured at 660nm using spectrophotometer. Aspirin was used as the standard.¹⁷

The experiment was carried in triplicates for each extract and the protein denaturation inhibition percentage was calculated using the formula:

Percentage of inhibition = $\frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$

Abs control – Absorbance of the sample at 570nm

Abs sample – Absorbance of the control at 570nm

Thin layer chromatography

Samples were prepared by dissolving crude extracts in acetone. Different extracts were spotted on the silica gel TLC plate using capillary tube and it is allowed to dry for few seconds. Pet ether and acetone was used as the solvent system in the ratio of 9:1(v/v). TLC plates were placed in TLC glass chamber after spotted with crude extract and then mobile phase was allowed to move through adsorbent phase up of the plate. Once the mobile phase reached 3/4th of the plate, the plates were removed from solvent system and allowed to dry. Then the plates were visualized under UV fluorescence lamp at 254nm to identify various compounds.¹⁸



Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

Acetone, chloroform and pet ether extracts obtained from *T. catappa* containing different compounds were subjected to analysis of GC-MS. Each extract were analyzed in Perkin Elmer Clarus 680 equipped with mass spectrometer Clarus 600 (electron ionization) which was fitted with Elite-5MS Capillary column (30m, 0.25mmID, 250µmID). The GC oven was maintained at an initial temperature of 60°C for 2 mins ramp 10°C/min-300°C, for about 6mins. The temperature was maintained at 300°C. Carrier gas used here was helium with constant flow rate 1mL/min, mass transfer line and source temperature was set at 240°C. For spectral analysis, turbo mass version 5.4.2 software was used. By comparing the components spectrum with the database of the spectrum of known components stored in the NIST-2008 library, the structures were determined^{13,19}.

RESULTS

Phytochemical Analysis

The leaves of *T. catappa* were subjected to the phytochemical analysis to identify the presences and absences of phenols, alkaloids, tannins, anthraquinone glycosides, saponins, and flavonoids. The phytochemical analysis results in Table 1 revealed the presences of phenols, alkaloid, tannins, anthraquinone glycosides and flavonoids in the *T. catappa* leaves. The preliminary qualitative screening showed phytochemical constituents which are recognized to exhibit medicinal and physiological activities.

Table 1: Qualitative phytochemical composition of *Terminalia catappa* leaves

Phytochemicals	Inference
Phenols	+
Alkaloids	+
Tannins	+
Anthraquinone glycosides	+
Saponins	-
Flavonoids	+

'+' = Shows the presence : '-' = Shows the absence of phytochemical

In vitro antioxidant activity

For evaluating the antioxidant activity of test samples, DPPH radical is a most commonly used substrate for its simplicity and rapid process. The pet ether leaves extract had the strongest DPPH scavenging ability followed by acetone and chloroform. The pet ether leaf extract showed the strongest 73% of the DPPH free radical at 150µg/ml followed by acetone 64% and chloroform 63%. The human diseases associated with free radical mechanism can be treated by developing the drug using

the compounds which have the strong antioxidant ability. Figure 2 showed the graphical representation of the DPPH assay results.

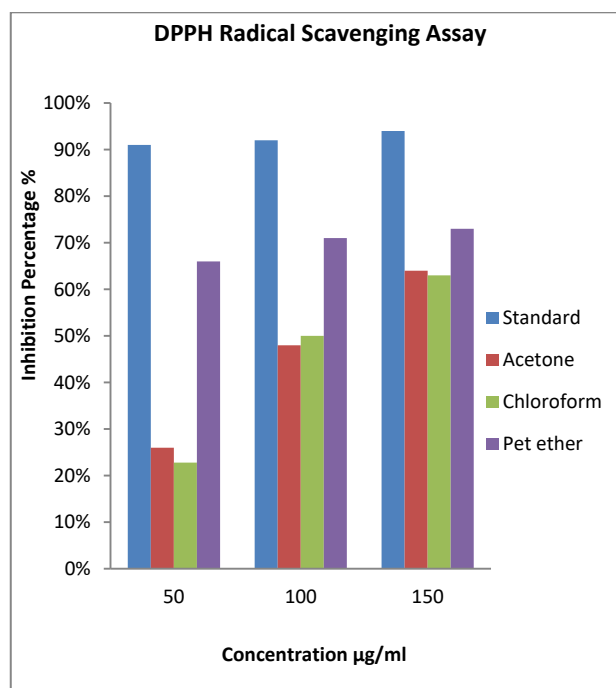


Figure 2: The results for DPPH assay of different extracts of *T. catappa*

In vitro antibacterial activity

Antibacterial activity was performed for the three different extracts (acetone, chloroform and pet ether). All the three extracts showed the strong activity against test pathogens *P. mirabilis* and *S. aureus*. Compared to chloroform and pet ether extracts, acetone extract showed maximum inhibition zone of about 31mm against both the pathogens at 100mg/ml concentration. As the concentration of the extract increases, the extracts also showed a linear increase in their antibacterial activity. Similar result was also shown by Sumitra Chanda *et al.*, 2011. Methanol, acetone and N, N-dimethylformamide extracts of *T. catappa* are active against *S. aureus*, *B. subtilis* and *B. cereus*. The experiments were done in triplicates and the mean values of antibacterial activity of extracts and standard drugs were shown in the given Table 2.

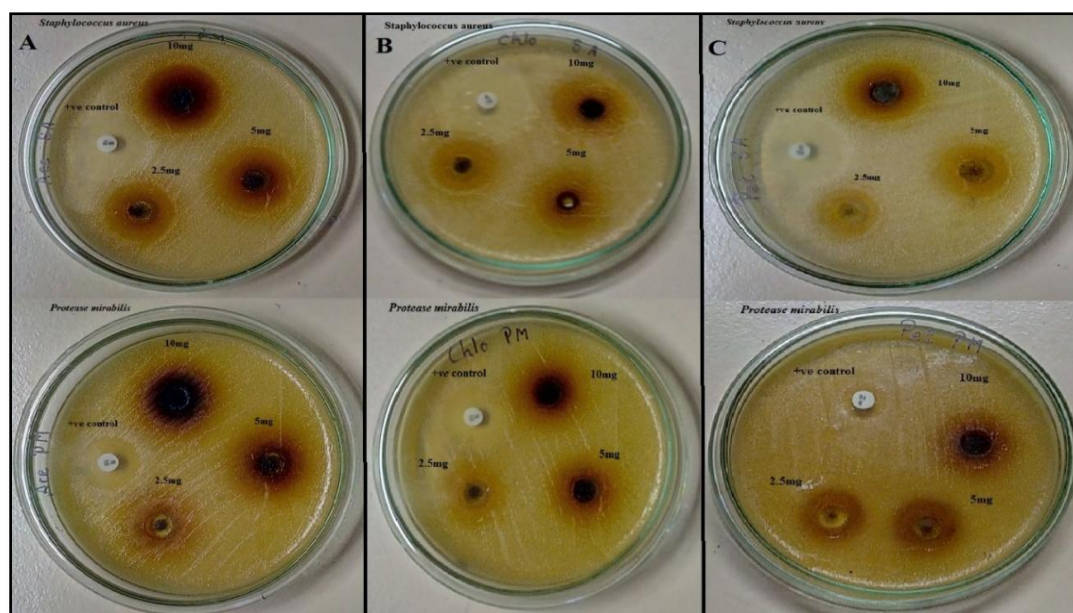
Antifungal activity

Antifungal activities of three different extracts against two fungi *Aspergillus niger* and *Aspergillus flavus* were investigated by agar well diffusion method. All the three Acetone, Chloroform and Pet ether extracts of *T. catappa* leaves does not showed antifungal activity against both *Aspergillus niger* and *Aspergillus flavus*. Sumitra Chanda *et al* 2011 also reported that the N, N-dimethylformamide (DMF) extract, Acetone extract and Methanol extract of *T. catappa* has no activity against *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus candidus*.



Table 2: Antibacterial activity of different extracts of *T. catappa*

Plants extracts	Concentration (mg)	Inhibition zone in mm	
		Gram positive bacteria	Gram negative bacteria
		<i>Staphylococcus aureus</i>	<i>Protease mirabilis</i>
Acetone	10	31	31
	5	26	27
	2.5	24	26
Choloform	10	26	25
	5	25	23
	2.5	24	22
Petroleum ether	10	29	23
	5	20	22
	2.5	19	21
Streptomycin	10	28	23

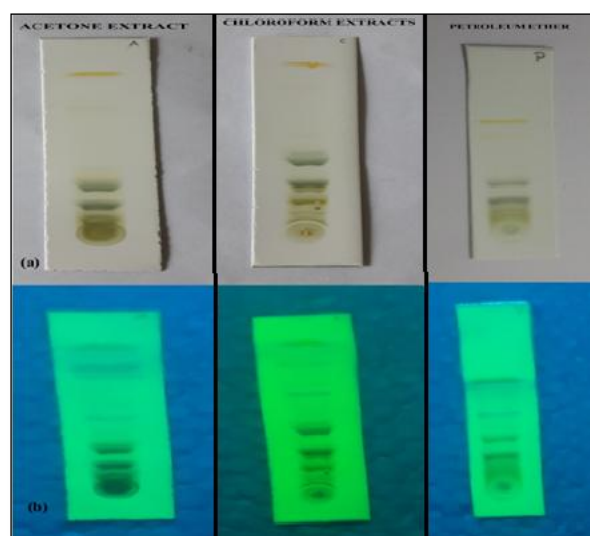
**Figure 3:** Zone of inhibition was observed for all the three extracts of *T. catappa*

Anti inflammatory activity using *in vitro* albumin denaturation method

Inflammation is mainly caused by protein denaturation. *In vitro* anti-inflammatory activity was performed for three different solvent extracts of *T. catappa* to determine its ability to inhibit protein denaturation. Due to the high colour intensity, anti-inflammatory activity of leaves extracts could not be identified.

Thin layer chromatography

The TLC studies of the acetone, chloroform and petroleum ether extracts of *T. catappa* were done in the solvent system of petroleum and acetone 9:1 (v/v). The result of thin layer chromatography of different extracts of *T. catappa* leaf was shown in below Figure 4.

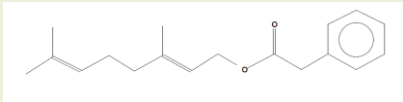
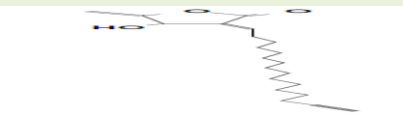


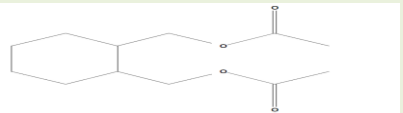
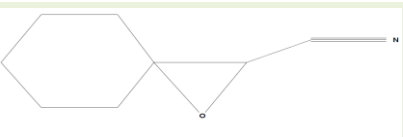
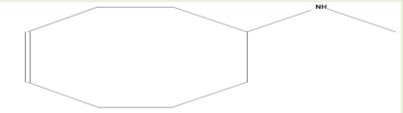


**(a)** TLC sheets under normal visible light **(b)** TLC sheets under UV 254nm**Figure 4:** TLC analysis of acetone, chloroform and petroleum ether extracts of *Terminalia catappa*

GC-MS analysis


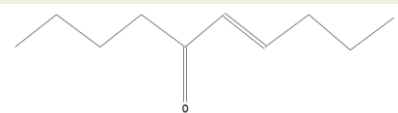
Acetone, chloroform and pet ether extracts were subjected to GC-MS analysis and chromatogram with percentage peak area of acetone, chloroform and pet ether were shown in Figure 5A, Figure 5B and Figure 5C respectively. The result of GC-MS illustrated in Table 3 represents the retention time, chemical structure and physiological activities of the plant *T. catappa*. In the acetone extract of the plant, (3R,2E)-2-(HEXADEC-15-YNYLIDENE)-3-HYDROXY-4-METHYLENEBUTANOLIDE is

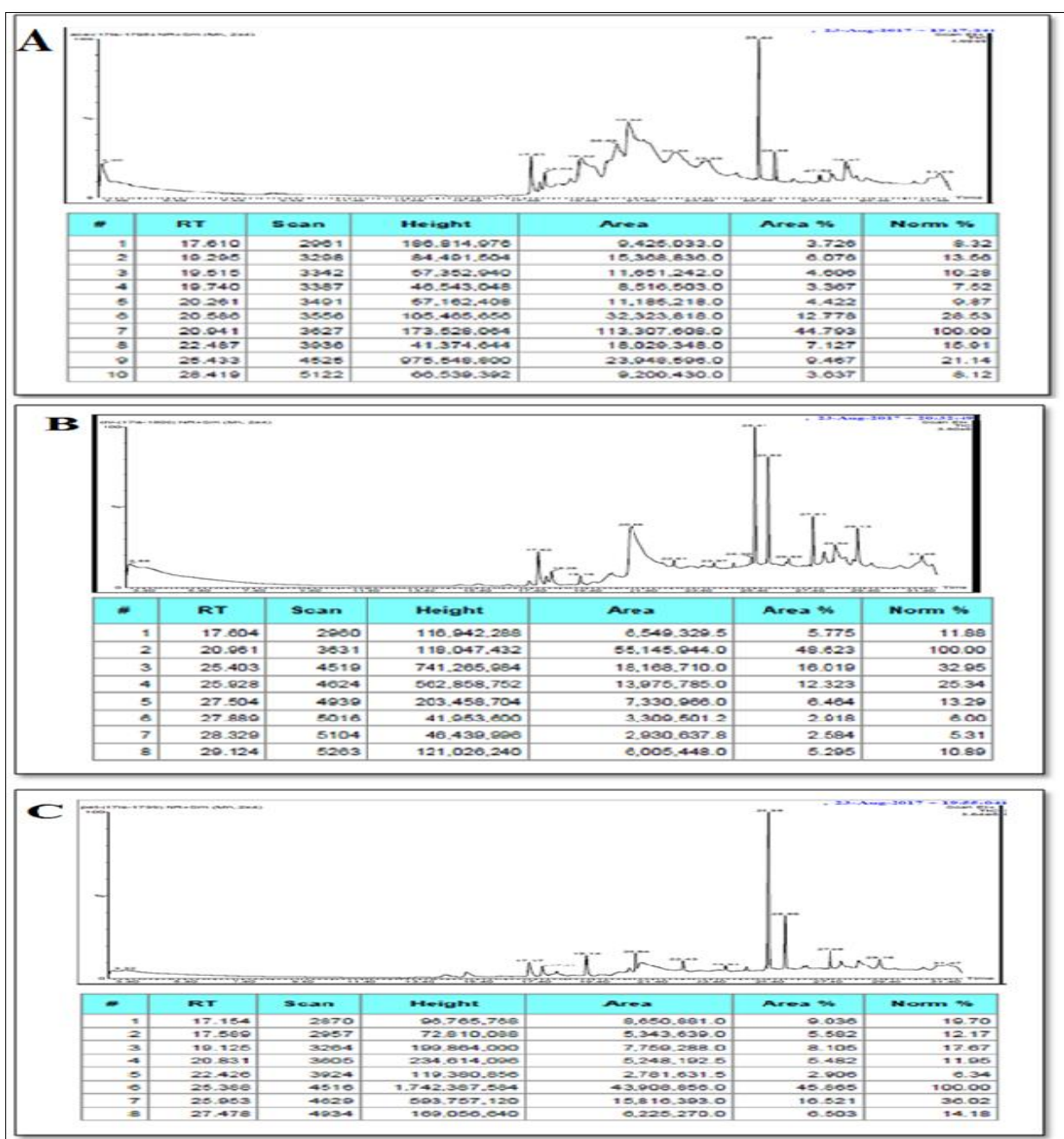
the major compound present in the Rt 20.94 min with the area percentage of 44.7% and it could be the possible active ingredient for the plants antibacterial activity. The unidentified compound with percentage peak area of 48.6% is a major compound in chloroform extract in Rt 20.96 min and its an potential novel compound unexplored from this plant. Other novel compound in Rt 25.38 with 45.8% is a major compound present in the petroleum ether extracts of *T. catappa*, which could be the active moiety for the observed activity and further studies on novel compounds are needed.

Table 3: List of NIST library matches for compounds present in crude extracts

R.t	Reverse	Forward	Compound name	Structure
Acetone extracts				
19.29	–	–	–	Unknown
19.515	–	–	–	Unknown
19.740	–	–	–	Unknown
20.261				Unknown
20.536	615	377	GERANYL PHENYLACETATE	
20.946	784	462	(3R,2E)-2-(HEXADEC-15-YNYLIDENE)-3-HYDROXY-4-METHYLENEBUTANOLIDE	
25.438	963	936	SQUALENE	
22.487	–	–	–	Unknown
25.983	920	893	HEPTACOSANE	
27.919	621	330	CYCLOHEXANE-1,2-DIMETHANOL, DIACETATE	
28.444	718	530	1-OXASPIRO[2.5]OCTANE-2-CARBONITRILE	
Chloroform extract				
17.619	549	428	BICYCLO[2.2.1]HEPTAN-2-OL, 6-TERT-BUTYL-	
18.075	727	673	1,2-DIHEPTYLCYCLOPROPENE	
19.170	647	451	2-PROPENOIC ACID, ETHYL ESTER	

20.961	–	–	–	Unknown
25.413	551	318	1,5-CYCLOOCTADIEN-4-ONE	
25.928	–	–	–	Unknown
27.509	917	873	HEPTACOSANE	
27.889	–	–	–	Unknown
28.329	649	346	3-METHYL-2-(2-OXOPROPYL)FURAN	
29.129	708	381	3,4-UNDECADIENE-2,10-DIONE, 6,6-DIMETHYL-	
31.455	569	322	CIS-1-METHYL-3-N-NONYLCYCLOHEXANE	
Pet ether extract				
14.998	689	623	CYCLOPENTANE, BUTYL-	
17.164	546	370	1H-ISOINDOLE-1,3(2H)-DIONE, HEXAHYDRO-	
17.594	845	791	CIS-9,10-EPOXYOCTADECAN-1-OL	
19.145	543	406	CYCLOPROPANECARBOXYLIC ACID, UNDEC-2-ENYL ESTER	
20.831	969	952	CYCLOTETRACOSANE	
22.426	961	948	3-EICOSENE, (E)-	
25.388	–	–	–	Unknown
25.953	–	–	–	Unknown
27.478	–	–	–	Unknown
28.444	602	348	PSI., PSI.-CAROTENE, 7,7',8,8',11,11',12,12',15,15'-DECAHYDRO-	

29.149	641	550	E-11(13-METHYL)TETRADECEN-1-OL ACETATE	
31.500	571	347	6-DECEN-5-ONE	



(A) acetone extract (B) chloroform extract (C) pet ether extract

Figure 5: Chromatogram for plant extracts with percentage peak area

DISCUSSION

A study by N'GUESSAN Koffi et al 2011, demonstrated that administration of aqueous decoction from leaves of *Terminalia catappa* significantly reduces hyperglycaemia to normal glycaemia value in hyperglycaemic rabbits at 40

mg/ml. Laís Pinheiro Silva et al 2014, demonstrated that oral treatment of aqueous fraction from *T. catappa* leaves (25mg/kg) showed good gastroprotective effect by effectively reducing the ulcerative lesions induced by ethanol and reperfusion injury. The aqueous and

methanol extracts of *Terminalia catappa* were given daily to alloxan induced hyperglycemic rats for three weeks, they showed good anti-hyperglycemic activities by reducing the blood sugar levels of 25–62% (A.N. Nagappa et al 2003). A study by Shu-Chen Chu et al 2006 revealed the significant inhibitory effect of ethanolic extracts of *T. catappa* leaves on the invasion and motility of highly metastatic A549 and Lewis lung carcinoma (LLC) cells.

A previous study by Sumitra Chanda et al 2011, evaluated that 63% of Gram negative and 70% of the total Gram positive bacteria studied were inhibited by the methanol, acetone and N, N-dimethylformamide extracts of *T. catappa*. Our results have shown the best antibacterial effect of the acetone, chloroform and pet ether extracts obtained from the leaves of *Terminalia catappa*.

CONCLUSION

The Present study highlights that the extracts (acetone, chloroform and pet ether) from *T.catappa* serves as a potential antibacterial agent against *Protease mirabilis* and *Staphylococcus aureus* under *in vitro* condition. Among all the extracts, acetone extract showed the significant antibacterial activity. Thereby the study concluded that antibacterial activity of acetone extract of *T.catappa* and its active constituents may be helpful in interacting with various kinds of plant disease and human allergies. Further research could elucidate the possibilities of *T.catappa* in commercial use for the benefits of patients suffering from bacterial infections.

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