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Research Article

PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF ETHANOLIC EXTRACT OF TERMINALIA CATAPPAFLOWERS

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ABSTRACT

*TerminaliaCatappa*is a well known medicinal plant belonging to the family of *Combretaceae*. Due to its effective biological activities, it is used as a folk medicine in Southeast Asia. It is well known for Indian system of medicines. *TerminaliaCatappa*is one of the plants having a rich Phytoconstituents like *Flavonoids, Sterols, Taninns and Saponins.*

The aim of the present study is toPrepare the ethanolic extract of the flowers of *TerminaliaCatappa,* to Identify the compounds present in the extract and to Evaluate the antibacterial activity of ethanolic extract.GCMS analysis helps to identify the compounds present in the extract. The antibacterial activity was done by well diffusion method. It shows good activity against *Staphylococcus aureus, Escherichia coli* and *Pseudomonas putida*.

Keywords: TerminaliaCatappa, Ethanolic extract, GCMS, Antibacterial activity.

INTRODUCTION

*TerminaliaCatappa*is a well known traditional medicinal plant from the family of *Combretaceae*. It is one of the plants which are widely used in Ayurvedic medicinal systems. Various biological applications like anticancer, antioxidant, anti HIV, reverse transcriptase, antidiabetic, anti-inflammatory and hepatoprotective activity have been reported for various parts of this plant[1]. The methanolic extract fromthe leaves of this plant shows very good antitumor activity against Ehrlich Ascetic Lymphoma [EAL] cell lines[2].The presence of various bioactive constituents like alkaloids, flavonoids, cardiac glycosides, tannins, saponins and cyanogenic glycosides in the decoction of *Terminaliacatappa* in place of water shows very good biochemical results which proves the efficacy of the plant in medicinal systems[3]. Antimicrobial activity of the aqueous extract of leaves of *TerminaliaCatappa* was evaluated against*Klebsiellapneumoniae*,*Staphylococcus aureus*,*Escherichia coli* and *Candida albicans*. It shows good activity against *Klebsiellapneumoniae* and further it has more microbes potency than standard antibiotics like pencillin and ampicillin also reported[4]. Five carotenoids *viz.*,violaxanthin, lutein epoxide, lutein, two lutein isomers andbeta cryptoxanthin were isolated and characterized from the leaves of this plant[5].The water extract of this plant leaves has better *in vitro* bacterialactivity against the bacteria isolated from the aquatic animals like *Pasteurellapneumotropica*(0.8 mg/ml), *Photobacteriumdamselae*and*Enterococcus faecal*[6].Two new flavones glycosides

*apigenin6-C-(2''-O-galloyl)-beta-D-glucopyranoside*and*apigenin 8-C-(2''-O-galloyl)-beta -D-glucopyranoside* together with four known flavone glycosides called isovitexin, vitexin, isoorientin, and rutinwere also isolated from the dried fallen leaves of *Terminaliacatappa*[7].Antioxidant and anthelmintic potential was evaluated fromthe leaves of *Terminaliacatappa*[8]andthen the leaves extracts also inhibit MMP-9 expression and HCC cell metastasis[9].Alcoholic and aqueous extract from the fruits of this plant shows very good antihyperglycemic activity in alloxaninduced diabetic in rats[10.] From the seeds of this plant, essential oil was extracted and physicochemical characterization of this oil was evaluated[11].To be the part of research works on *Terminaliacatappa*, this study shows the antibacterial activity and screening of compounds in the ethanolic extract of flowers of *Terminaliacatappa* using well diffusion method and GC-MS.

MATERIALS AND METHODS

Collection and preparation of flower extract

Fresh flowers of *Terminaliacatappa* were collected fromVirudhunagar, Tamil Nadu, India during August 2015. About 100 ml of ethanol was added to about 50 grams of coardly powdered flowers and the extraction carried out for 5 hours at 50^0C in Soxhletapparatus. The crude extract was pooled to the volume of 200 mland then filtered using Whatman filter paper. Then the extract was concentrated in a rotator evaporator and kept in air tight amber color bottle. The non-soluble portion of the extracted solid remains in the thimble, and is discarded[12].

Phytochemical screening

The following preliminary phytochemical tests were carried out using the above mentioned ethanolic extract to detect the presence of different phytoconstituents.The phytochemical screenings were performed using standard procedures.

a) **Test for terpenoids [Salkowski test]:** Tosmall amount of the extract solution, 1 ml of chloroform and 5ml concentrated H_2SO_4 was carefully added to form a layer. A reddish brown colouration of the interface indicates the presence of terpenoids[13].
b) **Test for flavonoids:** About1mlconc.HCl andpiece of magnesium ribbon were added to 2 ml of the ethanolic extract. The development of pink-tomato red colour indicated the presence of flavanoids[13].
c) **Test for saponins:** To 1 ml of the extract, 5 ml of distilled water was added and kept for few minutes.Frothing persistence indicated the presence of saponins[13].
d) **Test for alkaloids:** To about 3 ml of extract, a few drops of Mayer's reagent [prepared by dissolving 1.36g ofmercuric chloride and 5g of potassium iodide in 100ml of water] were added along the sides of the test tube. Development of cream coloured precipitate inferred the presence of alkaloids[14].
e) **Test for tannins:** About200 mg of flower material was extracted with distilled water and then filtered. To the filtrate, drops 0.1 % of $FeCl_3$ was added. A blue-black colouration, indicatethe presence of tannins[14].
f) **Test for quinones:** To 1 ml of the ethanolic extract, 1 ml of concentrated sulphuric acid was added. Presence of quinines was indicateby development of red colour[15].
g) **Test for coumarins:** Above 1 ml of extract was mixed with a few drops of 10% sodium hydroxide. Development of yellow colour exhibited the presence of coumarins[15].

GC-MS analysis

The GC-MS study was carried out using Clarus500 GC Perkin Elmer instrument in Food Testing Laboratory at Indian Institute of Crop Processing Technology, Thanjavur. The analysis was carried out for 72 minutes with Turbo mass detector[14].

Antibacterial activity

Antibacterial activity of the ethanolic extract was tested against *Staphylococcus aureus, Escherichia coli, and Pseudomonas putida*by well diffusion method. The extract was injected into the well in different volume of 100µL, 200µL, 300µL and 400µL using sterile syringe. The plates were incubated at 37^oC for 24 hours for bacterial growth. The plates were then observed for the zone of clearance around the well and measured in mm. The diameter of the inhibition zone was taken in four different fixed directions[15, 16].

RESULTS AND DISCUSSION

In the recent years, research on medicinal plants has attracted a lot of attention globally. Large body of evidence has accumulated to demonstrate the promising potential of medicinal plants used in various traditional, complementary and alternate systems of

treatment of human diseases. Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids and flavonoids etc, which have been found in vitroto have antibacterialand antifungal properties.[17, 18]

Preliminary phytochemical screening of ethanolic extract

The phytoconstituents present in the ethanolic extract of *Terminaliacatappa*flowers were identified by preliminary phytochemical screening and GC-MS analysis. The screening of ethanolic extract was carried out for different class of compounds and the results indicate the presence of *terpenoids*, *flavonoids* and *alkaloids*in theextract. The extraction and analysis of chemical constituents is an eco-friendly approach[19, 20].

Compounds identified from GCMS analysis

The ethanolicextract was subjected to GCMS analysis for the identification of compounds present in it. GCMS analysis shows that n-Hexadecanoic acid (33.67%), Tetradecanoic acid (16.37%) and 9, 12 – Octadecadienoic acid (14.5%) are present in higher level while the other volatile compounds like 2,6,6-Trimethyl-2,4-cycloheptadien-1-one , 4,6,6-Trimethylbicyclo(3.1.1)hept-3-en-2-one, 2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropane-1-carboxylic acid, squalene and cedrane, 8-propoxy- are present in very low level .

The GCMS spectrum of ethanolic extract of *TerminalicaCatappa*indicating the presence of chemical constituents [Table 1] is given in Figure 1.

Antibacterial activity of ethanolic extract of *Terminalicacatappa* flowers

The antibacterial activity of ethanolic extract was tested in different concentrations against *Staphylococcus aureus, Escherichia coli, and Pseudomonas putida.* The zone of inhibition was measured after 24 hours and the results presented in Table 2.

Significant antibacterial activity was observed for 300 µL and 400 µL of ethanol extract against all the pathogens tested. Maximum inhibition activity was found for 400 µL with the inhibition zone of 8 mm against *Escherichia coli*followed by 7 mm against *Streptococcus aureus*[Figure 2]. The inhibition activity for 100 µl was minimum for the all the organism.

The phytochemical analysis reveals that the bioactive compounds n- hexadecanoic acid, tetradecanoicacid and 9, 12 – octadecadienoicacidin the extract are also responsible for antibacterial activity.

Table 1: Compounds identified from *GCMS* analysis

S.No	Compound	Molecular Formula	Molecular Weight in g/mol	Retention Time	Peak Area %
1.	2,6,6-Trimethyl-2,4-cycloheptadien-1-one	$C_{10}H_{14}O$	150.21	6.74	1.35
2.	4,6,6-Trimethylbicyclo[3.1.1]hept-3-en-2-one	$C_{10}H_{14}O$	150.22	6.92	1.13
3.	5-Hydroxymethyl-2-furaldehyde	$C_6H_6O_3$	126.11	7.77	1.15
4.	2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropane-1-carboxylic acid	$C_{10}H_{16}O_2$	168.232	10.05	6.56
5.	Dodecanoic acid [Lauric acid]	$C_{12}H_{24}O_2$	200.32	11.00	2.38
6.	Tetradecanoic acid [Myristic acid]	$C_{14}H_{28}O_2$	228.37	13.42	16.34
7.	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.43	16.23	33.67
8.	Ethyl hexadecanoate [Ethyl palmitate]	$C_{18}H_{36}O_2$	284.48	16.46	4.04
9.	Tetradecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethylester [Synonyms: Myristic acid β-monoglyceride]	$C_{17}H_{34}O_4$	302.45	17.22	0.46
10.	9,12-Octadecadienoic acid(Z,Z)-	$C_{18}H_{32}O_2$	280.44	18.72	14.54
11.	Octadecanoic acid [Stearic acid]	$C_{18}H_{36}O_2$	284.48	19.17	7.80
12.	1,2- Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390.55	24.66	1.68
13.	3,5,5-trimethyl-4-(3-oxobutyl)cyclohex-2-en-1-one	$C_{13}H_{20}O_2$	208.29	27.64	3.35
14.	Squalene	$C_{30}H_{50}$	410.73	28.83	3.79
15.	8-propoxycedrane,	$C_{18}H_{32}O$	264.44	31.35	1.73

Table 2: Antibacterial activity of ethanolic extract of *Terminaliacatappa*flowers

Volume of ethanolic extract per well (µl)	Diameter of Inhibition zone (mm)		
	Staphyloccusaureus	*Escherichia coli*	*Pseudomonas putida*
100	2	3	4
200	4	5	5
300	5	6	5
400	7	8	5

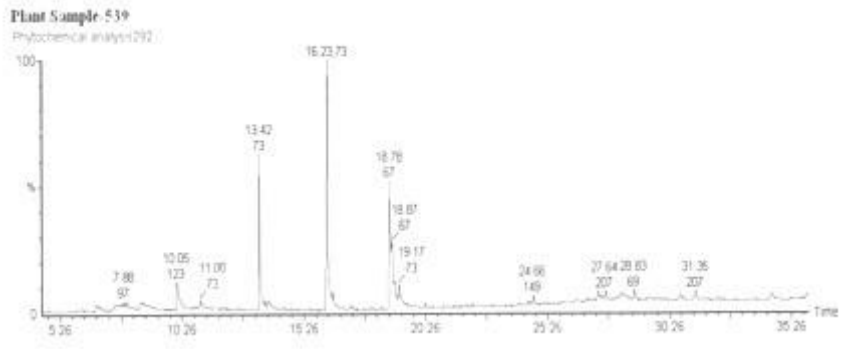

Fig. 1: GCMS spectrum of the ethanolic extract of the *Terminaliacatappa* flower

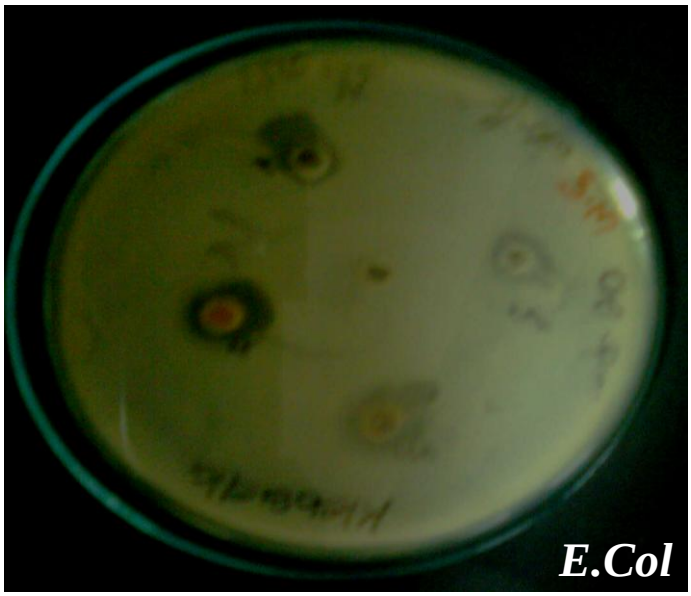

Fig. 2: Zone of Inhibition for ethanolic extract against *Escherichia coli*

CONCLUSION

Terminaliacatappa is an important tropical plant grown in many parts of Tamil Nadu, India. The Terminaliacatappa possess good nutritional, biological and medicinal value.

In the present study the ethanolic extract of*Terminaliacatappa*flowers was prepared and it was found to

1. Contain many organic compounds which cannot be easily synthesized.
2. Have good antibacterial activity. The phytochemicals present in the *Terminaliacatappa*flower contribute for its antibacterial properties.

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