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Antimicrobial Activity of *Terminalia catappa* L. Leaf Extracts against Some Clinically Important Pathogenic Microbial Strains

Sumitra Chanda*, Kalpna Rakholiya, Rathish Nair
[1]*Phytochemical, Pharmacological and Microbiological Laboratory, Department of Biosciences, Saurashtra University, Rajkot, India*
E-mail: *svchanda@gmail.com
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Abstract

Objective: The present study was undertaken to evaluate *in-vitro* antimicrobial activity of methanol, acetone and N, N-dimethylformamide extracts from leaf of *Terminalia catappa* L. (Combretaceae). **Methods:** *In vitro* antimicrobial activity of all the extracts was done by agar disc diffusion assay. 91 clinically important strains were used for the study, which were both clinical isolates as well as identified strains. Piperacillin and gentamicin were used as standards for antibacterial assay, while nystatin and flucanazole were used as standards for antifungal assay. Antimicrobial activity was determined by measurement of inhibition zone around each paper disc. For each extract three replicate trials were conducted against each organism. **Results:** The antibacterial activity was more pronounced against bacteria than fungal strains. The Gram positive bacteria were more susceptible than Gram negative bacteria. The methanol extract showed best antibacterial activity. *T. catappa* leaf extracts showed better antibacterial activity than commercially used antibiotics. **Conclusion:** Demonstration of antimicrobial activity of *T. catappa* provides the scientific basis for the use of this plant in the traditional treatment of diseases and may help to discover new chemical classes of antibiotic substances that could serve as selective agents for infectious disease chemotherapy and control. This investigation has opened up the possibility of the use of this plant in drug development for human consumption possibly for the treatment of various infections caused by microbes.

Keywords: *Terminalia catappa*, Antibacterial Activity, Antifungal Activity, Clinical Strains, Organic Solvents

1. Introduction

Traditional medicine has been practiced for many centuries in many parts of the world, including India especially in rural areas due to availability and low cost. Nature has provided a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine [1]. There has been an increasing incidence of multiple resistances in human pathogenic microorganisms, largely due to the indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases [2]. The development of bacterial resistance to presently available antibiotics has necessitated the search for new antibacterial agents. Numerous studies have been conducted with the extracts of various plants, screening antimicrobial activity as well as for the discovery of new antimicrobial compounds [3-6]. The efforts of scientists in establishing plants with promising antimicrobial property is yielding fruitful results as a number of plants with high antimicrobial property have been elucidated [7-13].

Terminalia catappa L. belongs to the family Combretaceae. *T. catappa* is used primarily as an ornamental, shade, and salt-tolerant street tree, but the leaves provide food for the Tasar silkworm, and the seeds are edible like almonds with similar oils. On the Malay peninsular and through the Canary islands this tree is known as the tropical almond. *T. catappa* has been claimed to have therapeutic effects for liver related diseases [14]. In Java, it is attributed with cholagogue action. In India, it is used as cardiac stimulant. Its leaves are widely used as a folk

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medicine in Southeast Asia for the treatment of dermatosis and hepatitis [15]. More and more pharmacological studies have reported that the extract of *T. catappa* leaves and fruits have anticancer, antioxidant, anti-HIV reverse transcriptase, anti-inflammatory, antidiabetic effects and hepatoprotective activities [16-19] but the effective components and related mechanisms remain unknown.

In the present work, antimicrobial activity of *T. catappa* leaf extracts were investigated against an array of clinically isolated as well as standard microbial cultures.

2. Material and Methods

2.1. Plant Material

The leaves of *T. catappa* were collected in February, 2005 from Rajkot in the State of Gujarat Western India and identified by comparison with specimens PSN 291 available at the Herbarium of the Department of Biosciences, Saurashtra University, Rajkot, Gujarat, India.

2.2. Extraction

The leaves of *T. catappa* were air dried and then powdered in a homogenizer and 10 g was used for different solvent extraction N, N-dimethylformamide (DMF), acetone and methanol, the sample was extracted in solvent kept on a rotary shaker overnight, and then the filtrate was collected and centrifuged at 5000 rpm. The solvent was then evaporated to dryness under reduced pressure and the extracted compound left was used for the antimicrobial assay. The percentage yield of N, N-dimethylformamide (DMF), acetone and methanol extracts were 20.92, 4.96 and 14.48 respectively.

2.3. Microorganisms Studied

91 clinically important microbial strains which included 20 Gram positive, 55 Gram negative and 16 fungal strains were studied for the antimicrobial activity. These strains included both clinical isolates as well as identified strains. The identified strains were obtained from National Chemical Laboratory (NCL), Pune, India and clinical isolates were obtained from Spandan Diagnostic and Microcare Diagnostic Laboratory, Rajkot, Gujarat, India (**Tables 1-5**). The bacteria were grown in the nutrient broth and maintained on nutrient agar slants at 4˚C while fungal strains were grown in Sabouraud dextrose broth and maintained on MGYP slants for yeast and potato dextrose agar slants for mould at 4˚C.

Table 1. Antibacterial activity of *Terminalia catappa* leaf extracts against some Gram positive bacteria.

Sr. No.	Strain (Location of collection)	Zone of inhibition (mm)[a]				
		TME	TAE	TDE	G	Pc
1	Staph-1 (Sputum)	14.67 ± 0.33	9.66 ± 0.33	10 ± 0.58	-	-
2	*S. aureus* (Pus)	14 ± 0	11± 0.58	9 ± 1.15	18.67 ± 0.33	17.33 ± 0.33
3	*S. aureus* (Urine)	13 ± 0.58	9 ± 0.58	8 ± 0.58	-	-
4	*S. aureus* (Pus)	16 ± 0.58	8 ± 0.58	14 ± 0.58	-	-
5	Staph-2 (Pus)	-	-	-	-	-
6	*S. aureus* (Sputum)	-	-	-	-	-
7	*S. aureus* (Tracheal)	15 ± 0.58	10 ± 0.58	9.67 ± 0.33	-	-
8	*S. aureus* (Tracheal)	15 ± 0.58	12 ± 0.59	13 ± 0.58	-	-
9	Staph-3 (Sputum)	14.33 ± 0.66	12.33 ± 0.88	10 ± 1.73	14.67 ± 0.33	-
10	*S. aureus* (Ear swab)	16.67 ± 1.53	14 ± 2.89	10 ± 1.73	-	-
11	*S. aureus* (Sputum)	18.67 ± 0.33	14 ± 0.58	13 ± 0.58	20.67 ± 0.33	-
12	*S. aureus* (Pus)	-	-	-	-	-
13	*S. aureus* (Pus)	-	-	-	10.33 ± 0.33	-
14	*S. aureus* (ATCC25923)	14.5 ± 0.28	8.5 ± 0.86	10 ± 1.73	-	-
15	*S. epidemidies* (ATCC12228)	11 ± 0.58	-	-	-	-
16	*S. subflava* (NCIM2178)	19 ± 0.58	13.5 ± 1.44	11.5 ± 0.28	-	20.17 ± 0.44
17	*B. cereus* (ATCC11778)	11.5 ± 0.28	9.5 ± 0.28	11 ± 0.58	20.17 ± 0.16	18.83 ± 0.16
18	*B. subtilis* (ATCC6633)	9 ± 1.15	8.5 ± 0.86	-	18.33 ± 0.33	17.83 ± 0.93
19	*B. mega* (ATCC9885)	-	-	-	-	-
20	*M. flavus* (ATCC10240)	14 ± 0.58	8.5 ± 0.86	15 ± 1.15	27.67 ± 0.33	12.67 ± 0.33

[a]Values are Mean ± SEM, n = 3, zone includes disc diameter 7 mm; G—Gentamicin (10 µg/disc); Pc—Piperacillin (100 µg/disc); TME—Methanol extract; TAE—Acetone extract; TDE—N, N-dimethylformamide (DMF) extract; "-" means no activity; Staph—*Staphylococcus* species.

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Table 2. Antibacterial activity of *Terminalia catappa* leaf extracts against some *Pseudomonas* species.

Sr. No.	Strain (Location of collection)	Zone of inhibition (mm)[a]				
		TME	TAE	TDE	G	Pc
1	*Ps. aeruginosa* (ATCC27853)	-	-	-	17 ± 1.15	12.33 ± 0.66
2	*Ps. aeruginosa* (Sputum)	-	-	-	16.67 ± 0.67	-
3	*Ps. aeruginosa* (Pus)	-	-	-	19.67± 0.33	-
4	*Ps. fluorescence* (Tracheal)	8.67 ± 0.33	-	12.67± 1.44	-	-
5	*Ps. fluorescence* (Pus)	13.67 ± 3.18	8 ± 0.58	-	-	-
6	*Ps. fluorescence* (Urine)	-	-	-	-	-
7	*Ps. testosterone* (NCIM5098)	-	-	-	22.33 ± 0.66	-
8	*Ps. pseudoalcaligenes* (ATCC17440)	15.5 ± 0.28	12.5 ± 0.86	14.5 ± 028	19.33 ± 0.6	-
9	Pseudo-1 (Sputum)	11 ± 2.31	13 ± 0.58	11.67 ± 0.33	14 ± 0.58	-
10	Pseudo-2 (Pus)	13.67 ± 3.18	8 ± 0.58	-	-	-
11	Pseudo-3 (Urine)	14.67 ± 1.45	16 ± 0.58	14.67 ± 0.33	-	-
12	Pseudo-4 (Pus)	14 ± 0.58	10.6 ± 2.34	9.33 ± 1.23	-	-
13	Pseudo-5 (Tracheal)	-	-	-	-	-
14	Pseudo-6 (Wound swab)	-	-	-	-	-
15	Pseudo-7 (Pus)	16 ± 0.56	10 ± 0.58	12 ± 1.15	-	-
16	Pseudo-8 (Tracheal secretion)	14 ± 1.15	9 ± 1.15	9 ± 1.15	-	-
17	Pseudo-9 (Pus)	11.67 ± 0.88	9.33 ± 1.20	-	-	-
18	Pseudo-10 (Sputum)	17 ± 0.58	12 ± 0.33	13.67 ± 0.88	-	-
19	Pseudo-11 (Sputum)	18.33 ± 0.33	16.33 ± 1.45	13 ± 0.58	20 ± 0.58	-

[a]Values are Mean ± SEM, n = 3, zone includes disc diameter 7 mm; G—Gentamicin (10 µg/disc); Pc—Piperacillin (100 µg/disc); TME—Methanol extract; TAE—Acetone extract; TDE—N, N-dimethylformamide (DMF) extract; "-" means no activity; Pseudo—*Pseudomonas* species.

Table 3. Antibacterial activity of *Terminalia catappa* leaf extracts against some *E. coli* isolates.

Sr. No.	Strain (Location of collection)	Zone of inhibition (mm)[a]				
		TME	TAE	TDE	G	Pc
1	*E. coli* (Pus)	10 ± 1.53	8.66 ± 0.88	7.66 ± 0.33	-	-
2	*E. coli* (Urine)	12.33 ± 2.73	9.66 ± 1.45	-	-	-
3	*E. coli* (Urine)	16 ± 0.58	12.33 ± 0.88	11.67 ± 0.33	-	-
4	*E. coli* (Urine)	15 ± 0.88	10 ± 0.33	13 ± 0.58	-	-
5	*E. coli* (Urine)	15 ± 0.88	11 ± 0.58	14 ± 0.33	-	-
6	*E. coli* (Pus)	10 ± 0.58	14 ± 0.88	13 ± 1.15	-	-
7	*E. coli* (Urine)	14.33 ± 1.20	12 ± 0.58	14 ± 1.15	-	-
8	*E. coli* (Stool)	15.67 ± 0.33	10.67 ± 0.33	13 ± 0.58	21 ± 0.58	-
9	*E. coli* (Pus)	12 ± 0.58	11.33 ± 0.88	14.67 ± 0.33	-	-
10	*E. coli* (Urine)	14.33 ± 0.33	10.67 ± 0.33	14 ± 0.58	18.67 ± 0.33	-
11	*E. coli* (Pus)	12.67 ± 0.66	11.67 ± 0.33	11.33 ± 0.66	-	-
12	*E. coli* (Urine)	15.33 ± 0.88	12.67 ± 0.33	14 ± 0.58	20.33 ± 0.33	-
13	*E. coli* (Vaginal swab)	13.5 ± 0.28	-	12.67 ± 0.33	-	-
14	*E. coli* (Urine)	-	-	-	-	-
15	*E. coli* (Blood)	14.5 ± 0.28	-	-	-	-
16	*E. coli* (ATCC25922)	14 ± 0.58	10 ± 1.73	-	17.83 ± 0.16	14.5 ± 0.50

[a]Values are Mean ± SEM, n = 3, zone includes disc diameter 7 mm; G—Gentamicin (10 µg/disc); Pc—Piperacillin (100 µg/disc); TME—Methanol extract; TAE—Acetone extract; TDE—N, N-dimethylformamide (DMF) extract; "-" means no activity.

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Table 4. Antibacterial activity of *Terminalia catappa* leaf extracts against some Gram negative bacteria.

Sr. No.	Strain (Location of collection)	Zone of inhibition (mm)[a]				
		TME	TAE	TDE	G	Pc
1	Ent-1 (Tracheal)	8.33 ± 0.88	-	-	-	-
2	Ent-2 (Tracheal)	11 ± 1.15	-	8 ± 0.58	19.67 ± 0.88	-
3	*E. aerogenes* (ATCC 13048)	-	-	-	-	-
4	Kleb-1 (Urine)	13.67 ± 0.88	11 ± 0.58	11 ± 0.58	22 ± 0.58	-
5	Kleb-1 (Sputum)	14 ± 0.58	10.33 ± 0.33	10 ± 0.58	-	-
6	*K. aerogenes* (Pus)	8 ± 0.58	-	8.67 ± 0.88	-	-
7	Kleb-2 (Urine)	14 ± 0.58	12.33 ± 0.33	14.67 ± 0.33	-	-
8	*K. aerogenes* (Urine)	13.67 ± 0.33	10.67 ± 0.33	13.33 ± 0.33	-	-
9	*K. pneumoniae* (NCIM2719)	-	-	-	-	24.67 ± 0.33
10	*P. mirabilis* (Wound swab)	18 ± 1.20	10.33 ± 0.33	12.67 ± 0.33	-	14 ± 0.58
11	Prot-1 (Pus)	14.67 ± 0.33	10 ± 0.58	13.33 ± 0.33	-	-
12	*P. mirabilis* (NCIM2241)	-	-	-	18.67 ± 0.33	-
13	*P. vulgaris* (NCTC8313)	14.5 ± 0.28	-	-	18 ± 1.00	-
14	*P. morganii* (NCIM2040)	-	-	-	-	-
15	*P. rettgeri* (Pus)	16.33 ± 0.88	10.67± 0.33	11.67 ± 0.33	-	-
16	Citro-1 (Pus)	12 ± 0.58	9 ± 0.58	10 ± 1.16	-	-
17	*C. freundii* (Pus)	-	-	-	12.33 ± 0.33	-
18	*C. freundii* (ATCC10787)	-	-	-	-	-
19	*A. fecalis* (ATCC8750)	-	-	-	18.33 ± 0.66	-
20	*S. typhimurium* (ATCC23564)	12 ± 0.58	8.5 ± 0.86	10.5 ± 0.86	18.5 ± 0.28	-

[a]Values are Mean ± SEM, n = 3, zone includes disc diameter 7 mm; G—Gentamicin (10 µg/disc); Pc—Piperacillin (100 µg/disc); TME—Methanol extract; TAE—Acetone extract; TDE—N, N-dimethylformamide (DMF) extract; "-" means no activity; Ent—*Enterobacter* species; Kleb—*Klebsiella* species; Citro—*Citrobacter* species; Prot—*Proteus* species.

Table 5. Antifungal activity of *Terminalia catappa* leaf extracts.

Sr. No.	Fungus (Location of collection)	Zone of inhibition (mm)[a]				
		TME	TAE	TDE	Fu	Ns
1	*Candida* spp. (Sputum)	-	-	-	-	14 ± 0.58
2	*C. albicans* (Urine)	-	7.5 ± 0.29	10 ± 1.73	-	11.33 ± 0.33
3	*C. albicans* (Sputum)	-	-	-	-	18 ± 0.58
4	*Candida* spp. (Sputum)	-	-	-	-	14 ± 0.58
5	*Candida* spp. (Urine)	-	-	-	-	10 ± 0.58
6	*C. albicans* (ATCC2091)	8.5 ± 0.87	8.5 ± 0.87	-	17.67 ± 0.33	13 ± 0.58
7	*C. albicans* (ATCC18804)	-	-	-	-	14.33 ± 0.33
8	*C. glabrata* (NCIM3448)	-	-	-	39.67 ± 0.88	22 ± 0.58
9	*C. tropicalis* (ATCC4563)	-	-	-	-	8.33 ± 0.33
10	*C. apicola* (NCIM3367)	19.33 ± 0.33	13 ± 1.15	14.33 ± 0.33	-	21.33 ± 0.88
11	*C. neoformans* (ATCC34664)	-	-	-	21.33 ± 0.33	17 ± 0.58
12	*C. luteolus* (ATCC32044)	17.5 ± 2.60	8.5 ± 0.86	-	23.66 ± 0.88	17.66 ± 0.88
13	*T. beigelii* (NCIM3404)	12 ± 0.58	12 ± 0.58	7.5 ± 0.29	-	-
14	*A. flavus* (NCIM538)	-	-	-	-	-
15	*A. candidus* (NCIM883)	-	-	-	-	-
16	*A. niger* (ATCC6275)	-	-	-	-	-

[a]Values are Mean ± SEM, n = 3, zone includes disc diameter 7 mm; Ns—Nystatin (100 units/disc); Fu—Fluconazole (10 µg/disc); TME—Methanol extract; TAE—Acetone extract; TDE—N, N-dimethylformamide (DMF) extract; "-" means no activity; Fu—Fluconazole; Ns—Nystatin.

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2.4. Antimicrobial Assay

The N, N-Dimethyl formamide extract (TDE), acetone extract (TAE) and methanol extract (TME) were dissolved in DMSO. The antimicrobial activity was evaluated at a concentration of 250 µg/disc. Antimicrobial activity was performed by agar disc diffusion method [20,21]. The bacterial strains were grown in nutrient broth while fungal strains were grown in MGYP (Malt glucose yeast peptone) broth. Mueller Hinton agar No. 2 was the media used to study the antibacterial susceptibility while Sabouraud dextrose agar was used to study the antifungal susceptibility test. The cultures were grown for 24 h, and the turbidity of the culture was maintained according to the 0.5 MacFarland standards. The inoculum's size was 1×10^8 cells/ml. The media Mueller Hinton Agar No. 2 and MRS media and the test bacterial cultures were poured into Petri dishes Hi-Media. The test strain 200 µl was inoculated into the media inoculums size 10^8 cells/ml when the temperature reached 40˚C - 42˚C. The test compound 20 µl was impregnated in to sterile discs 7 mm Hi-Media and was then allowed to dry. The disc was then introduced into medium with the bacteria. For each microbial strain negative controls were maintained where pure solvent DMSO was used instead of the extract since it does not possess any antimicrobial effect [22] and for positive control the standard antimicrobics Gentamicin 10 µg/disc and piperacillin 100 µg/disc for bacteria, nystatin 100 units/disc and flucanazole 10 µg/disc Himedia Labs for fungus were used for comparative studies. The plates were incubated overnight at 37˚C for bacterial strains and 42˚C for fungal strains. The experiment was performed under strict aseptic conditions. Microbial growth was determined by measuring the diameter of the zone of inhibition. The experiment was performed in triplicates and the mean values of the result are shown in **Tables 1-5**.

3. Results and Discussion

Herbal medicine in developing countries is commonly used for the traditional treatment of health problems [23]. In recent years multiple drug resistance in human pathogenic microorganisms have developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases [24]. In addition to this problem, antibiotics are sometimes associated with adverse effects on host including hypersensitivity, immunosuppression and allergic reactions [25]. Therefore there is a need to develop alternative antimicrobial drugs for the treatment of infections obtained from various sources such as medicinal plants [26, 27].

In the present study *T. catappa* leaf extracts extracted in DMF (TDE), acetone (TAE) and methanol (TME) were investigated for their antimicrobial potentiality against 91 clinically important microbial strains. Drug resistance is a new problem, but it is not a new phenomenon. Soon after the introduction of penicillin, *Staphylococci* were found to be very resistant to many of the antibiotics. Although recognized earlier that antibiotics resistance was only in the hospitals, now resistance in the community is also seen. Bacteria such as *Staphylococcus* have emerged with resistance to six and more different antibiotics [28].

All the three extracts of *T. catappa* TDE, TAE and TME were active against 70% of the total Gram positive bacteria studied while only 63% of Gram negative bacteria were inhibited Tables 1-4, on the other hand, the three extracts of *T. catappa* were active against only 25% of fungal strains Table 5. The best antibacterial activity was shown by the methanol extract. Similar results were also shown by Babayi *et al.* [29] and Kaneria *et al.* [30]. The Gram positive bacteria were more susceptible than Gram negative bacteria. This is in agreement with previous reports that plant extracts are more active against Gram positive bacteria than Gram negative bacteria [31-33]. These differences may be attributed to the fact that the cell wall in Gram positive bacteria is of a single layer, whereas the Gram negative cell wall is multilayered structure [34].

The most striking feature of the present findings is that many of the clinical isolates were resistant to the standard antimicrobics used while the plant extracts showed moderate to good antibacterial activity. The need of the hour is to find new antimicrobics because the microorganisms are getting resistant to the existing antibiotics [35,36]. The persistent increase in multi drug resistant strains compels the search for more potent new antibiotics. Thus there is a need for a continuous search for new effective and affordable antimicrobial drugs. The results of present study signify the potentiality of *T. catappa* leaf as a source of therapeutic agents which may provide leads in the ongoing search for antimicrobial botanicals.

4. Conclusions

Present study showed that the *T. catappa* leaf extracts possessed significant *in vitro* antimicrobial property against 91 clinical isolate as well as identified strains. The methanol extract exhibited strongest inhibitory effect on bacteria as compared to standard antibiotics against the tested microorganisms. It is necessary to carry out a bioassay guided fractionation of the extract in a bid to isolate and identify the compounds responsible for the antimicrobial activity. An elucidation of the mechanisms

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of action of these extract must be followed by toxicity and *in vivo* tests to determine the therapeutic applicability of such compounds in combination therapy. These are subjects of on-going investigation in our research group.

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