

Original Research Article

<http://dx.doi.org/10.20546/ijcmas.2016.512.053>

Antimicrobial Activity of Aqueous Extracts of Different Parts of *Terminalia catappa L.*

P. Venkatalakshmi^{1*} and P. Brindha²

¹Department of Biochemistry, S.T.E.T. Women's College, Sundarakkottai, Mannargudi, India

²Centre for Advanced Research in Indian System of Medicine, SASTRA University, Thirumalaisamudram, Thanjavur, India

*Corresponding author

ABSTRACT

Keywords

Anti microbial activity, Disc diffusion method, *Terminalia catappa L.*

Article Info

Accepted:
18 November 2016
Available Online:
10 December 2016

Infectious diseases have become a major cause of morbidity and mortality worldwide. Antimicrobial resistance is a serious threat to global public health. Plant chemicals are useful for infection control and, until the advent of antibiotics, were the only remedies available. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found *in vitro* to have antimicrobial properties. Tropical almond, botanically equated as *Terminalia catappa L.* is a large spreading tree. In the present study, attempts were made to evaluate the antimicrobial activity of aqueous extract of different parts of this plant against bacterial and fungal species using disc diffusion method. Anti bacterial activity of bark was more against gram negative bacteria than gram positive. Aqueous extract of fruit revealed more efficacy towards gram positive organisms where as wood extract was equipotent against gram positive as well as gram negative organisms. Antifungal activity was more for fruits than bark and wood extracts. Activity of the extracts was compared with standard antibiotics.

Introduction

Infectious diseases have become a major cause of morbidity and mortality worldwide. Antimicrobial resistance is a serious threat to global public health. Plant chemicals are useful for infection control and, until the advent of antibiotics, were the only remedies available. The use of and search for drugs and dietary supplements derived from plants have accelerated in recent years. Ethnopharmacologists, botanists, microbiologists, and natural-products chemists are combing the Earth for phytochemicals and “leads” which could be

developed for treatment of infectious diseases (Cowan, 1999). In India, medicinal plants are widely used by all sections of people both directly as folk medicines in different indigenous systems of medicine like Siddha, Ayurveda and Unani and indirectly in the pharmaceutical preparations (Srinivasan *et al.*, 2001). India has about 4.5 million plant species and among them, several thousands have been claimed to possess medicinal properties against human diseases.

Terminalia catappa L., a large spreading tree belongs to the family Combretaceae, is distributed throughout the tropics in coastal environments. The plant has been reported to have many pharmacological activities (Venkatalakshmi *et al.*, 2016). The dried leaves are used as an alternative to antibiotics to control fish pathogens (Chitmanat *et al.*, 2005). Various extracts of different parts of *T. catappa* have been reported to exhibit antimicrobial (Neelavathi *et al.*, 2013; Sangavi *et al.*, 2015; Mathiyarai *et al.*, 2015; Parimalagandhi *et al.*, 2015), anti-inflammatory (Lin *et al.*, 1992; Chen *et al.*, 2000; Sivaranjani *et al.*, 2014; Venkatalakshmi *et al.*, 2015), antioxidant and anti-tumor (Venkatalakshmi *et al.*, 2014; Venkatalakshmi *et al.*, 2016), anti-HIV (Tan *et al.*, 1991) and hepato-protective (Chen *et al.*, 2000) and anti-diabetic properties (Nagappa *et al.*, 2003) besides being aphrodisiac (Ratnasooriya and Darmasiri, 2000). The moderate consumption of the seed kernel is useful in treating sexual dysfunction among men, primarily for premature ejaculation (Ratnasooriya and Darmasiri, 2000). In the present study, attempts were made to evaluate the antimicrobial activity of the aqueous extract of bark, fruits and wood of this plant against bacterial and fungal species using disc diffusion method.

Materials and Methods

Collection and authentication of plant materials

Selected plant parts such as bark, fruits and wood of *Terminalia catappa* Linn. for the proposed study were collected from in and around Mannargudi, Tamilnadu, India. The identity of the plant specimens were confirmed using Flora of Presidency of Madras (Gamble, 1997). The botanical identity was authenticated by comparing

with the herbarium specimen deposited at RAPINAT Herbarium, St. Joseph's College, Tiruchirappalli, Tamilnadu, India (Voucher specimen number P.N.001/2012). Medicinally useful parts of the plants were studied in both fresh and dried conditions as per the standard textual methods (Trease and Evans, 1983).

Preparation of the extract

Powdered samples of bark, wood and fruit of *T. catappa* L. were used for the preparation of aqueous extracts. Plant powder (50 g) was taken with 250 ml water and incubated for 36 h. Then, it was filtered and the filtrate was allowed to evaporate at 56°C until semi solid consistency is obtained. Then the aqueous extract was re-dissolved in water at 1 mg/ml ratio and used for the assessment of antimicrobial activity.

Antimicrobial Assays

Disc preparation

6mm (diameter) discs were prepared from Whatmann No. 1 filter paper. The discs were sterilized by autoclaving at 121°C. After sterilization the moistened discs were dried in a hot air oven at 50°C and impregnated with different concentrations of the test substances.

Microorganisms

Three Gram-positive bacteria *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Enterococcus faecalis*; three Gram negative bacteria *Salmonella typhi*, *Vibrio cholerae*, *Escherichia coli* and four fungi *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus terreus* and *Candida albicans* were selected for antimicrobial efficacy studies. The bacterial isolates were first sub cultured in a nutrient broth and incubated at

37⁰C for 18h while the fungal isolates were sub cultured in Potato dextrose agar for 72h at 25⁰C.

Antibacterial activity

Antibacterial activity was carried out following the modified method originally described by Bauer *et al.*, (1966). Muller Hinton agar was prepared and autoclaved at 15 lbs pressure for 20 minutes and cooled to 45⁰C. The cooled media was poured on to sterile petriplates and allowed for solidification. The plates with media were seeded with the respective bacterial suspensions using sterile swab. The test drug coated discs were placed suitably on petriplates along with control and standard (Gentamicin (10 µg) for Bacteria) discs. The plates were incubated at 37⁰C for 24 hrs. After incubation period, the diameter of the zone formed around the paper disc were measured and expressed in mm.

Antifungal activity

Antifungal activity test was carried out following the modified method originally described by Bauer *et al.*, (1966). Potato Dextrose Agar (PDA) was prepared and autoclaved at 15 lbs pressure for 20 minutes and cooled to 45⁰C. The cooled media was added with 10ml/L tartaric acid (10%) which act as an antibacterial agent and poured on to sterile petriplates and allowed for solidification. The plates with media were seeded with the respective fungal suspensions using sterile swab. The test drug coated discs were placed suitably on petriplates along with control and standard (Amphotericin B (20 µg)) discs. The plates were incubated at 28⁰C for 72 hrs. After incubation period, the diameter of the zone formed around the discs were measured and expressed in mm.

Results and Discussion

Protection against infectious diseases is considered as the primary function of the immune system. A well functioning immune system resists harmful invasions of pathogens through its innate and adaptive arms of immune responses. In immunocompromised conditions, herbal supplements can be used as adjuvants to enhance the function of the immune system. In this way, herbal drugs which show promising antimicrobial activity can be considered as immune enhancers. Hence in the present study, antimicrobial activity of the selected plant drugs was evaluated against some bacterial and fungal pathogens.

Antibacterial activity

In the present study antibacterial activity of aqueous extracts of bark, fruits and wood of *T. catappa* was evaluated against 3 gram positive and 3 gram negative organisms such as *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Enterococcus faecalis*, *Salmonella typhi*, *Vibrio cholerae* and *Escherichia coli*. Four different concentrations (50 µg, 100 µg, 150 µg, 200 µg) of the test drugs were chosen.

Aqueous extract of bark produced maximum inhibition zone of 22 mm for *Salmonella typhi* among the tested organisms. It was identical to the value obtained for standard antibiotic gentamicin. It produced inhibition zone of 18mm for *Vibrio cholerae* and *E. coli*. The aqueous extract of bark was found to be more potent in controlling the growth of gram negative bacteria. Aqueous extract of fruits produced maximum inhibition zone against *Staphylococcus aureus* (17 mm) and *E. coli* (17 mm). When compared to bark, fruit extract was found to be effective against gram positive organisms.

Table.1 Antibacterial activity of aqueous extracts of different parts of *T.catappa* L.

Bacteria	Zone of inhibition (mm in diameter)													
	Control	Gentamicin (10µg)	<i>T. catappa</i> L.											
			Bark				Fruit				Wood			
			50µg	100µg	150µg	200µg	50µg	100µg	150µg	200µg	50µg	100µg	150µg	200µg
<i>Enterococcus faecalis</i>	-	22	10	12	12	14	12	13	15	16	13	16	17	21
<i>Staphylococcus aureus</i>	-	26	12	15	16	14	10	14	16	17	14	15	17	17
<i>Streptococcus pneumoniae</i>	-	21	16	14	12	16	07	09	11	15	10	12	13	13
<i>Escherichia coli</i>	-	21	15	17	18	18	10	11	13	17	12	16	18	20
<i>Salmonella typhi</i>	-	22	18	18	21	22	07	08	10	10	14	15	20	22
<i>Vibrio cholerae</i>	-	26	17	18	20	18	08	08	10	11	13	15	19	22

Table.2 Antifungal activity of aqueous extracts of different parts of *T.catappa* L.

Fungi	Zone of inhibition (diameter in mm)													
	Control	Amphotericin (20µg)	<i>T. catappa</i> L.											
			Bark				Fruit				Wood			
			50µg	100µg	150µg	200µg	50µg	100µg	150µg	200µg	50µg	100µg	150µg	200µg
<i>Aspergillus niger</i>	-	16	-	-	-	15	13	14	14	29	-	10	12	18
<i>Aspergillus flavus</i>	-	18	-	-	12	18	13	13	14	22	-	-	13	15
<i>Aspergillus terreus</i>	-	13	-	14	14	20	-	14	14	15	-	08	09	18
<i>Candida albicans</i>	-	14	-	-	08	18	-	12	08	16	-	12	08	19

In the case of wood extract maximum zone of inhibition was obtained for *Salmonella typhi* (22 mm) and *Vibrio cholerae* (22 mm). Like bark extract wood extract was also efficacious in controlling the growth of gram negative organisms.

From the data of the results obtained, it was evident that the aqueous extracts of test drugs produced dose dependent bacteriostatic activity against the bacteria under study. Maximum zone of inhibition was obtained at a concentration of 200 µg. Among the test drugs, bark and wood extracts were effective against gram negative organisms, where as fruit extract was found to be effective against gram positive organisms.

Antifungal activity

Antifungal activity of bark, fruits and wood of *T.catappa* was evaluated against four fungal species namely *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus terreus* and *Candida albicans*. The activity of the extracts was compared with Amphotericin B. Four different concentrations (50µg, 100µg, 150µg, 200µg) of the extracts were selected. Aqueous extract of bark at a concentration of 50 µg, did not produce inhibition zone against all the tested organisms. At a concentration of 100 µg, it inhibited the growth of *A.terreus* with an inhibition zone of 14 mm. No activity was found against *A.niger* even at a concentration of 150 µg. The aqueous extract of bark was found to be effective in inhibiting all the four organisms at a concentration of 200 µg. Maximum activity was shown against *A.terreus* (20mm).

Aqueous extract of fruit showed better antifungal activity than bark. At 50 µg, it was ineffective against *A.terreus* and *C.albicans*. All the other concentrations

found to be effective in controlling fungal growth. Maximum activity of the extract was found at 200 µg. In the case of wood extract, 50 µg was found ineffective, since it did not produce inhibition zone. The extract was effective in controlling the growth of selected organisms at a concentration of 150 µg and 200 µg. Among the three extracts, aqueous extract of fruit was found to have more antifungal activity followed by wood and bark.

In conclusion, from the data of the results obtained in the present study, it can be concluded that, among the three parts selected, bark and wood extracts produced inhibition zones against the selected bacterial strains in a much better way than fruit extract. Fruit extract is found to be effective towards gram positive organisms than gram negative. Antifungal activity was more for fruits than bark and wood extracts.

Acknowledgement

Authors record their sincere gratitude to the managements of S.T.E.T. Women's college, Mannargudi and SASTRA University, Thanjavur.

References

- Bauer, A.W., Kirby, M.M., Sherris, J.C., and Turck, M. 1966. Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Pathol.*, 45: 493-496.
- Chen, P.S., Li, J.H., Liu, T.Y., and Lin, T.C., 2000. Folk medicine Terminalia catappa and its major tannin component, punicalagin, are effective against bleomycin-induced genotoxicity in Chinese hamster ovary cells. *Cancer Letters*, 152: 115-122.
- Chitmanat, C., Tongdonmuan, K., Khanom, P., Pachontis, P., Nunsong, W., 2005. ISHS Acta Horticulturae 678: WOCMAP Congress on medicinal and aromatic plants. Vol4. Targeted screening of Medicinal and

- Aromatic Plants Economics and Law.
- Cowan, M.M. 1999. Plant Products as Antimicrobial Agents. *Clinical Microbiol. Rev.*, 2(4): 564-582.
- Gamble, J.S. 1997. *Flora of Presidency of Madras*. India: Botanical Survey of India, 2: 665-667.
- Lin, C.C., Chen, Y.L., Lin, J.M., and Ujiie, T. 1992. Evaluation of the antioxidant and hepatoprotective activity of *Terminalia catappa*. *Am. J. Chin. Med.*, 25(2): 153-161.
- Mathiyarasi, V., Venkatalakshmi, P., and Brindha, P., 2015. Efficacy of *Terminalia catappa* L. wood against some bacterial pathogens. *World J. Pharm. Res.*, 4(12): 1377-1383.
- Nagappa, A.N., Thakurdesai, P.A., Venkat Rao, N., and Singh, J., 2003. Antidiabetic activity of *Terminalia catappa* Linn fruits. *J. Ethnopharmacol.*, 88: 45-50.
- Neelavathi, P., Venkatalakshmi, P., and Brindha, P., 2013. Antibacterial activities of aqueous and ethanolic extracts of *Terminalia catappa* leaves and bark against some pathogenic bacteria. *Int. J. Pharmacy and Pharmaceutical Sci.*, 5(1): 114-120.
- Parimala Gandhi, P., Venkatalakshmi, P., and Brindha, P., 2015. Efficacy of *Terminalia catappa* L. wood and bark against some fungal species. *Int. J. Cur. Microbiol. App. Sci.*, 4(9): 74-80.
- Ratnasooriya, W.D., and Dharmasiri, M.G., 2000. Effects of *Terminalia catappa* seeds on sexual behaviour and fertility of male rats. *Asian J. Androl.*, 2(3): 213-220.
- Sangavi, R., Venkatalakshmi, P., and Brindha, P., 2015. Anti-bacterial activity of *Terminalia catappa* L. bark against some bacterial pathogens. *World J. Pharm. Pharm. Sci.*, 4(9): 987-992.
- Sivaranjani, C., Venkatalakshmi, P., and Brindha, P., 2015. *In vitro* anti-inflammatory and antioxidant activities on fruits of *Terminalia catappa* L. *Res. J. Pharm. Tech.*, 8(10): 1409-1411.
- Srinivasan, D., Nathan, S., Suresh, T., Perumalsamy, P.L. 2001. Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. *J. Ethnopharmacol.*, 74: 217-220.
- Tan, G.T., Pezzuto, J.M., Kinghorn, A.D. and Hughes, S.H. 1991. Evaluation of natural products as inhibitors of human immunodeficiency virus type 1 (HIV-1) reverse transcriptase. *J. Natural Products*, 54: 143-154.
- Trease, G.E., and Evans, W.C., 1983. *Textbook of Pharmacognosy*. 12th ed. London: Balliere, Tindall, pp.57-59.
- Venkatalakshmi, P., Brindha, P., and Induja, K., 2014. *In vitro* antioxidant and anti-tumour activities of *Terminalia catappa* bark. *Int. J. Pharm. Pharm. Sci.*, 6(1): 1-3.
- Venkatalakshmi, P., Brindha, P., and Vadivel, V. 2015. *In vitro* antioxidant and anti-inflammatory studies on bark, wood and fruits of *Terminalia catappa* L. *Int. J. Phytomed.*, 7(3): 246-253.
- Venkatalakshmi, P., Vadivel, V., and Brindha, P., 2016. Identification of Flavonoids in different parts of *Terminalia catappa* L. Using LC-ESI-MS/MS and investigation of their anticancer effect in EAC Cell Line Model. *J. Pharm. Sci. & Res.*, 8(4): 176-183.
- Venkatalakshmi, P., Vadivel, V., and Brindha, P. 2016. Phytopharmacological significance of *Terminalia catappa* L.: An updated review. *Int. J. Res. Ayurveda Pharm.*, 7(2): 130-137.

How to cite this article:

Venkatalakshmi, P., and Brindha, P. 2016. Antimicrobial Activity of Aqueous Extracts of Different Parts of *Terminalia catappa* L. *Int.J.Curr.Microbiol.App.Sci*. 5(12): 493-498.
doi: <http://dx.doi.org/10.20546/ijcmas.2016.512.053>