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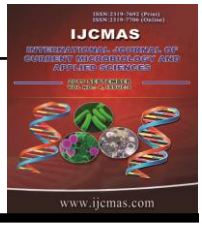

Original Research Article

Efficacy of *Terminalia catappa* L. Wood and Bark against Some Fungal Species

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K e y w o r d s

Antifungal activity, Infectious disease, *Candida albicans* and *Ganoderma* sp.

A B S T R A C T

The present study was carried out to evaluate the antifungal activity of the aqueous, ethyl acetate and hexane extracts of *Terminalia catappa* wood and bark against some fungal species. Antifungal activity was assessed by agar disc diffusion method. Among the three extracts, hexane extract exhibited potent antifungal activity against all the selected fungal species. The activity is compared with a standard antibiotic Clotrimazole. The extracts exhibited the growth inhibitory activity in a dose dependent manner.

Introduction

Infectious diseases have become the leading cause of morbidity and mortality worldwide. The harmful side effects of the synthetic drugs and their high cost produced a gradual revival of interest in the use of medicinal and aromatic plants as antimicrobial agents in developed as well as developing countries. Consequently plant research has been intensified now days to develop potential antimicrobial drugs of plant origin.

Medicinal plants are plants containing inherent active ingredients used to cure disease or relieve pain. The use of traditional medicinal plants in most developing countries as therapeutic agents for the maintenance of good health has been widely observed (UNESCO, 1996).

Modern pharmacopoeia still contains at least 25% drugs derived from plants and many others, which are synthetic analogues, built on prototype compounds isolated from plants.

Terminalia catappa, is an important medicinal plant with diverse pharmacological spectrum. There are a number of phytochemicals present in this plant such as gallic acid, ellagic acid, corilagin and unidentified tannins which are responsible for many of the pharmacological activities. Due to the presence of number of phytoconstituents, the different extracts have exhibited antimicrobial (Chanda *et al.*, 2013), antioxidant, antibacterial (Neelavathi *et al.*, 2013), antidiabetic (Ahmed and Beg,

2005; Nagappa *et al.,* 2003) antitumor (Venkatalakshmi *et al.,* 2014) activities. Punicalagin and punicalin, from the leaves are used to treat dermatitis and hepatitis as both have strong anti oxidative activity (Lin *et al.,* 1999). Hence in the present study, attempts were made to evaluate the antifungal activities of aqueous, ethyl acetate and hexane extracts of *T. catappa* wood and bark against selected fungal species.

Materials and Methods

Collection of plant material

Fresh wood and bark of *Terminalia catappa,* were collected from Mannargudi, Thiruvarur Dt, Tamilnadu, India, which were carefully identified and authenticated in the department of CARISM, SASTRA University, Thirumalaisamudhram. The collected plant materials were cut into pieces and washed thoroughly 2–3 times with running water and once with sterile distilled water, then the plant material was air-dried on sterile blotter under shade.

Microorganisms

Microorganisms such as *Aspergillus fumigatus, Candida albicans, Ganoderma sp., Microsporum gypseum, Mucor sp., Pencillium sp., Rhizopus* sp., *Scopulariops* sp., *Sporothrix scheckii* and *Trichoderma* sp. were obtained from the Department of Microbiology, S.T.E.T. Women's college, Mannargudi, were used as antifungal test organisms. Each fungal strain was suspended in Muller Hinton broth and incubated for 48 hrs at room temperature.

Extraction of plant material

The coarse powder of *Terminalia catappa* wood and bark was used for the extraction purposes. Extraction process was carried by soaking the coarse powder in Distilled water, Ethyl acetate and N - Hexane kept in shaker for 48 hrs, the extracts were filtered through Whattman filter paper and evaporated the extracts using water bath.

Determination of antimicrobial activity (Perez *et al.,* 1990)

Agar well diffusion method was followed to determine the antimicrobial activity. Muller Hinton agar (Hi media) medium plates were prepared by sterilizing the medium with the use of autoclave at 121^{o} C and 15 lbs pressure for 15 minutes; petriplates were also sterilized using autoclave. After sterilization, about 25 ml the cooled medium was poured into Petriplates and allowed to solidify. Muller Hinton agar medium plates were swabbed (sterile cotton swabs) with 48 hrs broth culture of fungi. Four wells (10mm diameter) were made in each of these plates using sterile cork borer. The test solution was prepared by dissolving 1mg of extract in 1 ml of DMF. About 50, 100, 150 µl from 1µg / l µl concentration of aqueous, ethyl acetate and N - Hexane extracts of *T. catappa* wood and bark, DMF (control) were added using micropipette into the wells and allowed to diffuse at room temperature for 2 hours. Clotrimazole was used as standard antibiotic. The plates were incubated at room temperature for 48 hours. Diameters of the inhibition zones were recorded in mm.

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 medicine. Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids and

flavonoids etc, which have been found *in vitro* to have antimicrobial properties. (Dahanuker *et al.,* 2000; Carneiro *et al.,* 1999).

Considering the vast potentiality *of T. catappa*, the current investigation has been undertaken to screen the antifungal activities of three different extracts of *T. catappa* wood and bark against *Aspergillus fumigatus, Candida albicans, Ganoderma sp., Microsporum gypseum, Mucor sp., Penicillium sp., Rhizopus* sp., *Scopulariops* sp., *Sporothrix scheckii* and *Trichoderma* sp.

Disc diffusion method is the most widely used procedure for testing antimicrobial susceptibility (Sambath Kumar *et al.,* 2006). Aqueous extract of *Terminalia catappa* wood at various concentrations (50, 100, 150µl) were found to be effective against *Aspergillus fumigatus, Microsporum gypseum, Mucor sp., Penicillium sp., Rhizopus* sp., *Scopulariops* sp. and *Sporothrix scheckii* (Table 1). The maximum inhibitory activity was observed for *A.fumigatus, Mucor* sp., (19mm) and then for *Penicillium* (17mm). The extract has no effect against *C. albicans* and *Ganodema* sp. at all the three concentrations. The activity of the herbal extract was compared with a standard antibiotic Clotrimazole (10µg). The antifungal activity of the aqueous extract was found to be equivalent to the antibiotic in inhibiting the growth of *Scopulariops* sp. (15mm for 150µl of plant extract; 14mm for standard antibiotic) and *Sporothrix scheckii* (14mm for 150µl of plant extract; 13mm for standard antibiotic). The extract showed maximum activity than the antibiotic to inhibit the growth of *Mucor* sp. (19mm for 150µl of plant extract; 15mm for standard antibiotics). The present findings prove the efficacy of the wood extract of *T. catappa* as an antifungal agent.

Maximum inhibitory activity of the ethyl acetate extract was against *A. fumigatus, Scopulariops* sp. and *M. gypseum* (28mm/150µg/ml). The extract has no effect against *S. schenckii* at all the three concentrations (Table 2). The inhibitory activity of the extract has been found to be high for all the tested organisms *A. fumigatus, Scopulariops* sp. and *M. gypseum* (28mm), *Mucor* sp.(19mm), *Rhizopus* sp. and *Ganoderma* sp. (16mm), *C. albicans* (20mm), *Trichoderma* sp. (25mm). The ethyl acetate extract proved its efficacy more than the standard antibiotic clotrimazole (10µg) against *Trichoderma* sp., for which the antibiotic produced an inhibitory zone of 17mm where as the extract produced 25mm.

In the case of hexane extract, there is no inhibitory activity against *S. schenckii*. Maximum activity of the hexane extract was revealed for *M. gypseum* (29mm), *A. fumigatus* (28mm), *Penicillium* sp. (28mm), *Rhizopus* sp. (24mm), *Scopulariops* sp. (22mm). The extract is found to be equipotent as the antibiotic in controlling the growth of *Ganoderma* (16mm). It is highly potent than the antibiotic in controlling the growth of *A.fumigatus* (Inhibitory zone produced by the extract - 28mm; Inhibitory zone produced by the antibiotic - 22mm) and *M. gypseum* (Inhibitory zone produced by the antibiotic - 19mm), *C. albicans* (Inhibitory zone produced by the extract – 23 mm; Inhibitory zone produced by the antibiotic - 17mm), *Mucor* (Inhibitory zone produced by the extract - 26mm; Inhibitory zone produced by the antibiotic - 15mm), *Penicillium* sp. (Inhibitory zone produced by the extract - 26mm; Inhibitory zone produced by the antibiotic - 21mm), *Rhizopus* sp. (Inhibitory zone produced by the extract - 24mm; Inhibitory zone produced by the antibiotic – 20mm) and *Scopulariops* sp. (Inhibitory zone produced by the extract - 22mm; Inhibitory zone produced by the antibiotic – 14mm) (Table 3).

Table.1 Antifungal activity of the aqueous extract of *T. catappa* wood

S. No.	Fungal Species	Zone of Inhibition (mm)				
		50µl	100µl	150µl	DMF (Control)	Clotrimazole (10µg/ml)
1	*Aspergillus fumigatus*	15	16	19	_	22
2	*Candida albicans*	_	_	_	_	17
3	*Ganoderma*	_	_	_	_	16
4	*Microsporum gypseum*	_	8	9	_	19
5	*Mucor*	13	15	19	_	15
6	*Pencillium*	19	15	17	_	21
7	*Rhizopus*	13	13	16	_	20
8	*Scopulariops*	12	15	15	_	14
9	*Sporothrix schenckii*	11	11	14	_	13
10	*Trichoderma*	_	11	8	_	17

Table.2 Antifungal activity of the ethyl acetate extract of *T. catappa* wood

S. No.	Fungal Species	Zone of Inhibition (mm)				
		50µl	100µl	150µl	DMF (Control)	Clotrimazole (10µg/ml)
1	*Aspergillus fumigatus*	24	26	28	_	22
2	*Candida albicans*	_	_	20	_	17
3	*Ganoderma*	10	12	16	_	16
4	*Microsporum gypseum*	25	26	28	_	19
5	*Mucor*	15	16	19	_	15
6	*Pencillium*	20	21	23	_	21
7	*Rhizopus*	11	16	16	_	20
8	*Scopulariops*	25	26	28	_	14
9	*Sporothrix schenckii*	_	_	_	_	13
10	*Trichoderma*	21	22	25	_	17

Table.3 Antifungal activity of the hexane extract of *T. catappa* wood

S. No.	Fungal Species	Zone of Inhibition (mm)				
		50µl	100µl	150µl	DMF (Control)	Clotrimazole (10µg/ml)
1	*Aspergillus fumigatus*	23	24	28	_	22
2	*Candida albicans*	13	18	23	_	17
3	*Ganoderma*	13	13	16	_	16
4	*Microsporum gypseum*	25	27	29	_	19
5	*Mucor*	17	23	26	_	15
6	*Pencillium*	22	24	26	_	21
7	*Rhizopus*	19	22	24	_	20
8	*Scopulariops*	12	19	22	_	14
9	*Sporothrix schenckii*	_	_	_	_	13
10	*Trichoderma*	17	23	26	_	17

Table.4 Antifungal activity of the aqueous extract of *T. catappa* bark

S. No.	Fungal species	Zone of Inhibition (mm)				
		50µl	100µl	150µl	DMF (Control)	Clotrimazole (10µg/ml)
1	*Aspergillus fumigatus*	_	8	_	_	22
2	*Candida albicans*	12	13	15	_	17
3	*Ganoderma*	13	13	15	_	16
4	*Microsporum gypseum*	_	13	15	_	19
5	*Mucor*	_	_	_	_	15
6	*Pencillium*	9	_	16	_	21
7	*Rhizopus*	_	_	_	_	14
8	*Scopulariops*	_	15	16	_	21
9	*Sporothrix schenckii*	11	14	17	_	20
10	*Trichoderma*	11	15	17	_	13

Table.5 Antifungal activity of the ethyl acetate extract of *T. catappa* bark

S. No.	Fungal Species	Zone of Inhibition (mm)				
		50µl	100µl	150µl	DMF (Control)	Clotrimazole (10µg/ml)
1	*Aspergillus fumigatus*	20	22	26	_	22
2	*Candida albicans*	15	18	19	_	17
3	*Ganoderma*	14	17	19	_	16
4	*Microsporum gypseum*	20	21	24	_	19
5	*Mucor*	12	14	16	_	15
6	*Pencillium*	13	15	18	_	21
7	*Rhizopus*	_	_	15	_	20
8	*Scopulariops*	18	18	21	_	14
9	*Sporothrix schenckii*	16	19	21	_	13
10	*Trichoderma*	12	14	19	_	17

Table.6 Antifungal activity of the hexane extract of *T. catappa* bark

S. No.	Fungal Species	Zone of Inhibition (mm)				
		50µl	100µl	150µl	DMF (Control)	Clotrimazole (10µg/ml)
1	*Aspergillus fumigatus*	23	24	26	_	22
2	*Candida albicans*	15	17	19	_	17
3	*Ganoderma*	14	15	17	_	16
4	*Microsporum gypseum*	24	27	29	_	19
5	*Mucor*	21	25	26	_	15
6	*Pencillium*	19	24	27	_	21
7	*Rhizopus*	20	21	24	_	20
8	*Scopulariops*	22	25	28	_	14
9	*Sporothrix schenckii*	_	_	_	_	13
10	*Trichoderma*	21	20	25	_	17

Aqueous extract of *Terminalia catappa* bark at various concentrations (50, 100, 150µl) were found to be effective against *Candida albicans, Ganoderma sp., Sporothrix scheckii* and *Trichoderma* species (Table 4). The maximum inhibitory activity was observed for *Sporothrix scheckii* and *Trichoderma* sp (17mm).The extract has no effect against *Rhizopus* and *mucor* sp. at all the three concentrations. The activity of the herbal extract was compared with a standard antibiotic clotrimazole (10µg). The antifungal activity of the aqueous extract was found to be equivalent to the antibiotic in inhibiting the growth of *Ganoderma* sp. (15mm for 150µl of plant extract; 16mm for standard antibiotic). The extract showed maximum activity than the antibiotic to inhibit the growth of *Trichoderma* sp. (17mm for 150µl of plant extract; 13mm for standard antibiotics). The present findings prove the efficacy of the bark extract of *T. catappa* as an antifungal agent.

Maximum inhibitory activity of the ethyl acetate extract of bark was against *A.fumigatus* (26mm/150µg/ml) and *M.gypseum* (24mm/150 µl). The inhibitory activity of the extract has been found to be high for all the tested organisms *A. fumigatus* (26mm). The extract proved its efficacy more than the standard antibiotic clotrimazole (10µg) against *A.* fumigatus, for which the antibiotic produced an inhibitory zone of 22mm where as the extract produced 26mm, *C. albicans* for which the antibiotic produced an inhibitory zone of 17mm where as the extract produced 19mm, *Ganoderma* sp. for which the antibiotic produced an inhibitory zone of 16mm where as the extract produced 19mm, *M. gypseum* for which the antibiotic produced an inhibitory zone of 19mm where as the extract produced 24mm, *Mucor* sp. for which the antibiotic produced an inhibitory zone of 15mm where as the extract produced 16mm, *Scopulariops* sp. for which the antibiotic produced an inhibitory zone of 14mm where as the extract produced 21mm, *Trichoderma* sp. for which the antibiotic produced an inhibitory zone of 17mm where as the extract produced 19mm, *S. schenckii* for which the antibiotic produced an inhibitory zone of 13mm where as the extract produced 21mm (Table 5).

In the case of hexane extract, there is no inhibitory activity against *S. schenckii*. Maximum activity of the hexane extract was revealed for *M. gypseum* (29mm). The extract is found to be highly potent than the antibiotic in controlling the growth of *A. fumigatus* (Inhibitory zone produced by the extract - 26mm; Inhibitory zone produced by the antibiotic - 22mm), *M. gypseum* (Inhibitory zone produced by the extract - 29mm; Inhibitory zone produced by the antibiotic - 19mm), *C.albicans* (Inhibitory zone produced by the extract - 19mm; Inhibitory zone produced by the antibiotic - 17mm), *Ganoderma* sp. (Inhibitory zone produced by the extract - 17mm; Inhibitory zone produced by the antibiotic - 16mm), *Mucor* sp. (Inhibitory zone produced by the extract – 26 mm; Inhibitory zone produced by the antibiotic – 15 mm), *Penicillium* (Inhibitory zone produced by the extract – 27 mm; Inhibitory zone produced by the antibiotic – 21 mm), *Rhizopus* sp. (Inhibitory zone produced by the extract - 24 mm; Inhibitory zone produced by the antibiotic – 20 mm), *Scopulariops* sp. (Inhibitory zone produced by the extract – 28 mm; Inhibitory zone produced by the antibiotic – 14 mm) (Table 6).

The results of the present study showed that hexane extracts of *T. catappa* wood and bark have more antifungal activity than ethyl acetate and aqueous extracts. This might have been due to the capacity of hexane to extract the antifungal principles present in *T. catappa* wood and bark.

The need of the hour is to find new antimicrobials (Bhattacharya *et al.,* 2005). The persistent increase in multi drug resistant strains compels the search for new effective and affordable antimicrobial drugs. The results of the present study signify the potentiality of *Terminalia catappa* wood and bark as a source of therapeutic agent which may provide leads in the ongoing search for antimicrobial botanicals.

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