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Antimicrobial activities of different solvent extracts of *Tithonia diversifolia* (Hemsely) A. Gray

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ABSTRACT

In vitro efficacy of three organic solvent (petroleum ether, chloroform and methanol) extracts of Tithonia diversifolia leaves were tested against nine plant pathogenic fungal species (Alternaria alternata, Alternaria solani, Aspergillus flavus, Aspergillus niger, Cuvularia lunata, Drechslera oryzae, Fusarium oxysporum, Penicillium expansum and Penicillium italicum), one antagonist fungus(Trichoderma viride) and four human pathogenic bacteria (Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus). Among the extracts, petroleum extract showed the highest antifungal activity followed by methanol and chloroform extracts. All the three extracts showed inhibitory effect against all the tested bacteria.

Key words: *Tithonia diversifolia*, antifungal, antibacterial, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC)

INTRODUCTION

Tithonia diversifolia (Hemsley) A. Gray commonly known as Mexican sunflower or Mexican arnica is a member of the family Asteraceae. It is an annual weed growing aggressively in abandoned lands, road-sides, river banks and cultivated farmlands. The plant is adaptable to most soils [1]. Though a native of Central America, it has become naturalized in many tropical countries including the North-East regions of India. In Manipur the plant is widely grown in wild, especially at the foothills and roadsides. There had been reports of *Tithonia* being used for a wide variety of purposes [2] including medicinal and ethnobotanical values [3]. The local healers used the flower heads for treatment of wounds and bruises [4]. Looking into the abundance and varied uses of this plant, the present investigation was made to evaluate the antifungal and antibacterial activities of *T. diversifolia* against 9 plant pathogenic and 1 antagonistic fungal species and 4 human pathogenic bacteria.

MATERIALS AND METHODS

Fungal species

The fungal species used for the study, the sources of their isolation and the respective plant diseases they caused are listed in Table 1.

Table 1. Test fungal species

Fungal species	Source of isolation	Type of infection
Alternaria alternata (Fr.) Keissler ITCC Id no. 8246.11	French bean leaf	Leaf spot disease
Alternaria solani Sorauer	Potato leave	Early blight disease
Aspergillus flavus Link NFCCI 2791	Groundnut seed	Aflatoxin contamination
Aspergillus niger Van Tieghem ITCC Id no. 8241.11	Groundnut seed	Black mold disease
Curvularia lunata (Wakker) Boedijn	Rice grain	Grain discoloration
Drechslera oryzae Breda de Haan ITCC Id no. 8240.11	Rice grain	Brown spot disease
Fusarium oxysporum (Schl.) emend. Snyder & Hansen NFCCI 2790	Tomato root	Wilt disease
Penicillium expansum Link ex. Fries	Apple fruit	Soft rot
Penicillium italicum Wehmer	Lemon fruit	Blue mould rot
Trichoderma viride Pers. ITCC Id no. 8229.11	Field soil	Biocontrol agent

The fungal species were isolated using standard methods and the pure cultures were maintained by sub culturing periodically in Czapeck Dox Agar (CDA) medium and preserved at 4ºC prior to use. The identities of some of the fungal isolates were reaffirmed from ITCC, IARI, New Delhi and NFCCI, Pune.

Bacterial species

The bacterial species used for testing antibacterial activities include two gram positive bacteria *Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 29212 and two gram negative bacteria *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. The bacterial cultures were obtained from the Microbiology Department, Regional Institute of Medical Sciences, Imphal, Manipur, India. The cultures were maintained by sub culturing periodically using nutrient agar medium and were preserved at 4ºC prior to use.

Preparation of plant extracts

Healthy and matured leaves of *T. diversifolia* were collected, washed, cut into small pieces, dried in shade and ground into powder using a mortar and pestle. The dried plant powder was extracted successively with petroleum ether (PE), chloroform (CH) and methanol (ME) in a Soxhlet extractor (60-80ºC). The extracts were concentrated under vacuum at 40-50ºC using a rotary flash evaporator. Extracts were stored at -20ºC and were freshly dissolved in suitable solvents prior to screening for antimicrobial activity.

Antifungal activity

The antifungal activities of the plant extracts were evaluated by employing the poisoned food technique [5]. The plant extracts were reconstituted with minimum amount of dimethyl sulphoxide (DMSO) and mixed with sterile CDA medium to get a concentration of 0.5mg/ml at a temperature of 40º-45ºC. Plant extract impregnated media were poured into sterile Petri dishes (90mm dia). Plates without any treatment served control. After cooling, the Petri dishes were inoculated with 5mm diameter mycelial discs removed from the margins of actively growing colonies of the test fungal species and incubated at 25±1ºC. Radial mycelial growth (mm) was recorded after 72 hr incubation period. Each experiment was carried out in triplicate and the percent inhibition of radial growth (PIRG), if any was determined by the following formula [6]:

$$\text{PIRG} = \frac{(dc - dt)}{dc} \text{x } 100$$

where dc = average increase in mycelial growth (mm) in control plates, dt = average increase in mycelial growth (mm) in treated plates. The average increase in mycelial growth was obtained by subtracting the inoculum diameter (5mm) from the colony diameter.

Antibacterial activity

Antibacterial activity of each plant extract was determined using the Kirby-Bauer disc diffusion method [7] with slight modification [8]. Bacterial cell suspensions were prepared in fresh normal saline.The turbidity of the resulting suspensions was adjusted to 0.5 McFarland turbidity standard. Sterile Mueller Hinton Agar (MHA) plates were swabbed with the respective culture of the organisms using sterile cotton swabs and kept for 15 min. in laminar chamber for absorption to take place. The sterile discs (6 mm dia) of Whatman's No. 1 filter paper were impregnated with 20 µl of different extract solutions to achieve desired concentrations of 0.5 and 1.0mg/disc and placed separately in the inoculated agar plates. Streptomycin (10µg/disc) was used as positive control and disc

impregnated with DMSO was used as negative control. Each experiment was carried out in 5 replicates. The antibacterial assay plates were incubated at 37°C for 24hr and mean diameters of the inhibition zones were recorded.

Determination of MIC and MBC

Based on the preliminary screening the extracts showing potent antifungal activities were further subjected to minimum inhibitory concentration (MIC) assay. Various concentrations ranging from 1 to 10 mg/ml of the extracts were prepared by adding appropriate quantities of each extract to pre sterilized molten CDA medium and thoroughly mixing with the medium. Twenty ml of treated medium having a particular extract concentration was poured in a 90 mm sterilized Petri plate. Plates were separately inoculated with the fungal species, incubated for 72 hr and observed for growth inhibition as described earlier. Each treatment was replicated thrice. MIC was recorded as the concentration at which no visible mycelial growth was observed after the incubation period.

The extracts which showed antibacterial activity in the disc diffusion method were subjected to MIC assay. Determination of MIC was carried out using the broth dilution method [9]. One ml of reconstituted extract solution (conc. 100 mg/ml) was added in a test tube containing 1 ml of sterile broth so as to obtain a concentration of 50 mg/ml. One ml of this dilution was transferred to another test tube in the same manner and the dilution series was continued till the 7th test tube was reached. The 8th test tube did not contain any extract, but a solution of pure solvent and served as negative control. Then 1 ml of an 18 hr old broth culture of each of the bacterial species (earlier adjusted at 10^8cfu/ml) was put into each tube containing broth with plant extract and thoroughly mixed on a vortex mixer. The tubes were incubated at 37°C for 24 hr and observed for growth in form of turbidity. The test tube with the lowest dilution with no detectable growth by visual inspection was considered as the MIC. The minimum bactericidal concentration (MBC) values were determined by removing 0.1 ml of bacterial suspension from the MIC tubes that did not show any growth and sub-cultured in MHA plates and incubated at 37ºC for 24 hr. The concentration without any resultant bacterial growth was recorded as MBC.

RESULTS AND DISCUSSION

The percent yields of *T. diversifolia* leaf extraction with PE, CH and ME were 3.16, 2.71 and 7.32%, respectively. The effect of the three extracts on colony growth of the ten test fungi is shown in Table 2. The results revealed that each fungal species may show differential growth responses to different solvent extracts of the same plant species. The PE extract could inhibit the growth of nine fungal species while the CH and ME extracts inhibited the growth of 3 and 5 fungal species, respectively. As seen from the table, colony growth of *A. alternata* could not be inhibited by any one of the extracts. CH and ME extracts were ineffective against *A. solani, A. flavus, A. niger, P. expansum* and *P. italicum*. The highest inhibitory effects were observed with PE extract against *D. oryzae* (55.1%), *A. solani* (51.6%) and *A. flavus* (50.7%). Both CH and ME extracts showed highest growth inhibition against *C. lunata* i.e. 33.6% and 40.0%, respectively. While determining the MIC values of the three extracts against those fungi which have shown more than 25% growth inhibition, it was found that the MIC of PE extract against *A. solani, A. flavus, C. lunata and D. oryzae* was 8mg/ml, and that of CH extract against *C. lunata* and ME extract against *C. lunata* and *D. oryzae* was 16mg/ml (Table 3).All the three extracts of *P. diversifolia* showed inhibitory effects against the four test bacteria representing both Gram-positive and Gram-negative species (Table 4). However, the highest inhibitory effect was induced by PE and CH extracts against *S. aureus*, the inhibition zones being 11.6 and 11.4mm, respectively. The lowest value of MIC was recorded as 0.39mg/ml of CH extract against *S. aureus*.

A large number of references on antimicrobial activities of several plant species are available. Most workers have reported narrow- or broad-spectrum and less or more effective antimicrobial activities of plants especially those species which have medicinal values. The plants are rich sources of antimicrobial compounds. It is estimated that the total number of plant chemicals may exceed 4,00,000 of which 10,000 plant chemicals have defensive functions [10]. Many previous studies [11-18] have shown that a plant species may not be equally effective against all the test fungal or bacterial species. The basic principle of target specificity of a metabolite or the susceptibility of a microbial species or its related group to a specific compound is of practical importance in designing an antibiotic or a pesticide for control of specific pathogens or groups of pathogens. Extract-organism specific antimicrobial activities of different solvent extracts of leaf, bark and branch wood extracts and latex of *Himatanthus articulates* had been observed [19]. Some of the extracts were very effective against some organisms while others were totally inactive. The methanolic and ethanolic extracts of *Sesame radiatum* showed broad spectrum antibacterial activity while the aqueous extract was ineffective [20]. It was also found that methanolic extract of *Abrus pulchellus* was more active against Gram-positive bacteria as compared to Gram-negative bacteria. There was further observation

that among different solvent extracts of *Solanum seaforthianum* stem, the methanolic extract exhibited high degree of antibacterial activity [22]. These observations in corroboration with the present finding show that different solvent extracts of a plant species may have different spectra of antimicrobial activity that can be explained by the solubility or insolubility of the active compound(s) in the solvent used for extraction.

The present observation that *T. diversifolia* has antifungal and antibacterial properties is supported by the work of Ogunfolakan [23] who concluded that the leaf extract had promising broad spectrum antimicrobial activity. Kareru [24] also reported that soap made from leaf extract of *T. diversifolia* was effective against *E. coli.*

Table 2. Effect of different solvent extracts of *Tithonia diversifolia* (conc. 0.5mg/ml) on radial growth of different fungal species

Fungal species	Colony diameter (mm)			
	PE	CH	ME	Control
Alternaria alternata	29.6(-)	37.3(-)	32.3(-)	27.6
Alternaria solani	14.6(51.6)	27.0(-)	23.0(-)	25.0
Aspergillus flavus	15.3(50.7)	31.0(-)	32.0(-)	26.0
Aspergillus niger	23.6(8.2)	27.3(-)	43.0(-)	25.3
Curvularia lunata	26.0(42.7)	29.3(33.6)	27.0(40.0)	41.6
Fusarium oxysporum	31.3(8.1)	33.0(2.3)	32.0(5.8)	33.6
Drechslera oryzae	18.0(55.1)	27.6(21.8)	26.0(27.5)	34.0
Penicillium expansum	18.0(7.1)	20.6(-)	22.3(-)	19.6
Penicillium italicum	11.3(24.0)	13.6(-)	21.0(-)	13.3
Trichoderma viride	80.0(11.7)	83.0(8.24)	74.0(18.82)	90.0

	Fungal species	Extracts	Interaction
S.Ed.(±)	0.02	0.02	0.05
CD (0.05)	0.03	0.05	0.10

PE=Petroleum ether extract; CH=Chloroform extract; ME=Methanol extract
Values in parenthesis indicate per cent growth inhibition.
(-) indicates no growth inhibition

Table 3. MIC of different solvent extracts of *Tithonia diversifolia* against susceptible fungal species

Fungal species	Solvent	MIC (mg/ml)
A. solani	PE	8
A. flavus	PE	8
C. lunata	PE	8
C. lunata	CH	16
C. lunata	ME	16
D. oryzae	PE	8
D. oryzae	ME	16

Table 4. Antibacterial activity of *Tithonia diversifolia* leaf extracts (conc. 1mg/disc)

Bacterial species	Inhibition zone(mm)			
	PE	CH	ME	ST
E. coli	7.2	7.0	6.8	14.8
E. faecalis	7.4	6.6	7.0	12.2
P. aeruginosa	7.0	6.8	6.8	10.2
S. aureus	11.6	11.4	6.8	16.4

S.Ed.(±)	Bacterial species	Extracts	Interaction
	0.29	0.29	0.59
CD (0.05)	0.59	0.59	1.17

ST = Streptomycin (10µg/disc)

Table 5. MIC(mg/ml) and MBC(mg/ml) of different solvent extracts of *Tithonia diversifolia* against susceptible bacterial species

Bacterial species	PE		CH		ME	
	MIC	MBC	MIC	MBC	MIC	MBC
E. coli	1.56	1.56	3.13	3.13	6.25	6.25
E. faecalis	0.78	1.56	0.78	3.13	6.25	6.25
P. aeruginosa	3.13	6.25	6.25	6.25	6.25	6.25
S. aureus	0.78	1.56	0.39	0.78	6.25	6.25

CONCLUSION

Leaf extracts of *Tithonia diversifolia* showed broad spectrum antifungal and antibacterial activities, although different solvent extracts showed differential effectiveness against the tested microbial species. Further studies can be made to identify the bioactive principles and also to exploit the products of this widely distributed weed species.

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