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Research Article

IN VITRO ANTIMICROBIAL ACTIVITY OF LEAF, STEM AND ROOT EXTRACTS OF THE MEDICINAL PLANT SPECIES, *THALICTRUM JAVANICUM* BLUME AGAINST CERTAIN HUMAN PATHOGENS

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ABSTRACT

Antimicrobial activity of methanolic extracts of leaf, stem and root parts of the plant species, *Thalictrum javanicum was* evaluated against certain pathogenic species of bacteria (*Bacillus subtilis, B. thuringiensis, Enterococcus faecalis, Staphylococcus aureus, S. pyogenes, Streptococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus vulgaris, P. mirabilis, Serratia marcescens, , Salmonella paratyphi, S. paratyphi*-A and *S. paratyphi*-B.) and fungal species (A*spergillus fumigatus, A. niger*, *Azospirullum lipoforum*, *Candida albicans, Fusarium* sp.*, Mucor* sp., *Penicillum* sp., *Pacilomyces litacinus*, *Trichoderma viride* and *Verticillum lecanii*) by disc diffusion method. It was observed that the methanolic extracts had potent antimicrobial activity against both human pathogenic Gram$^+$ and Gram$^-$ bacterial species and fungal species. Among the three parts studied, methanolic stem extract showed higher activity against the bacterium, *Proteus mirabilis* (diameter of inhibition zone, 26 mm). The bacterium, *E. coli* has been controlled moderately by the extracts of all the studied parts of *T. javanicum* (for leaf diameter of inhibition zone, 12 mm, for stem it was about 15 mm, and for root it was 12 mm). Similarly for fungal species also the methanolic stem extracts were effective against *Mucor* sp., (diameter of inhibition zone, 38 mm). The minimum inhibitory concentration of methanolic leaf, stem and root extracts were determined to be ranging between 200 and 500 µg/mL for both bacteria and fungi studied. The results of this study support that the plant species, *Thalictrum javanicum* had potential antimicrobial activity and it may be used for the commercial production of drugs to treat dreadful diseases caused by various pathogens.

Keywords: *Thalictrum javanicum* , Ranunculaceae, Antimicrobial activity.

INTRODUCTION

In recent years, multiple drug resistances in human pathogenic microorganisms have developed due to the indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. The undesirable side effects of certain antibiotics and the emergence of previously uncommon infections are the disadvantages of commercial antimicrobial drugs [1, 2]. The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of new anti–infective agents [3].

The genus, *Thalictrum* (Ranunculaceae) distributed in certain temperate habitats of India is having many therapeutic uses like tonic aperient, diuretic, stomachic, antiseptic and for the treatment of snake bite, jaundice, rheumatism etc. [4]. To date investigators have identified 290 *Thalictrum* alkaloids in about 80 species of this genus [5]. *Thalictrum* plants are generally rich in benzylisoquinoline derived alkaloids, atleast 250 have been isolated from 60 species and most of them with strong biological activities [5]; alkaloid isomers from *Thalictrum* are known to exhibit various pharmacological activities including antitumour, antimicrobial, antidiamebic and HIV antiviral activities [6]. However, studies in the species, *T. javanicum* are limited and no studies were carried out for its antimicrobial properties. Hence, the present study was aimed at to know the antimicrobial properties of various parts of *T. javanicum* such as leaf, stem and root.

MATERIALS AND METHODS

Collection and processing of plant materials

The perennial herb, *T. javanicum* was collected from the forest margins at high hills of Nilgiris, the Western Ghats, Tamil Nadu, India. The plants were thoroughly washed in running tap water with sodium chloride and then in sterile water before being shade dried for 20 days. The dried leaf, stem and root were ground into fine texture using pulverizer, then stored in sealed and labeled sterilized glass container.

Preparation of plant extracts

About 50g of whole plant powdered plant materials (50 g /350 mL) was extracted in soxhlet apparatus for 8-10 hours, sequentially with the alcoholic solvents *viz.,* petroleum ether, chloroform, methanol and water. Then the extract was evaporated to dryness by using vaccum rotary evaporator and stored in vials kept in 4°C for further use.

Microbial strains

Gram positive bacterial strains *viz.*, *Bacillus subtilis, B. thuringiensis, Enterococcus faecalis, Streptococcus faecalis, Staphylococcus aureus* and *S. pyogenes* and gram negative bacterial strains such as *viz., Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus vulgaris, P. mirabilis, Serratia marcescens*, *Salmonella paratyphi, S. paratyphi*-A and *S. paratyphi*-B and fungal species *viz.,* A*spergillus fumigatus*, *A. niger*, *Azospirullum lipoforum*, *Candida albicans*, *Fusarium* sp., *Mucor* sp., *Penicillum* sp., *Pacilomyces litacinus*, *Trichoderma viride* and *Verticillum lecanii* were obtained from the Department of Microbiology, Hindustan College of Arts and Science, Coimbatore and they were maintained at 4˚C on the slants of nutrient agar and potato dextrose medium respectively for further use.

Antibacterial activity

In vitro antibacterial activity was analysed for the crude extracts of leaf, stem, and root parts of the study species, *Thalictrum javanicum* against the above mentioned bacterial species selected. For this, the bacterial strains were subcultured periodically 2-3 days interval [7]. An inoculum of each of the pathogenic bacterial strain was suspended in 5 mL nutrient broth and incubated at 37˚C for 18 hours. This inoculum was spread over nutrient agar medium with sterile glass spreader. The alcoholic extracts were tested for their effect against the growth of pathogenic bacteria by disc diffusion method [8]. Small circular paper discs (6 mm diameter) impregnated with known amount of each extract was placed upon the surface of the inoculated plates. Ampicillin is used as positive control. The plates were kept at room temperature for the absorption of extract in the medium and then incubated at 37˚C for 24 hours. The antibacterial activity was evaluated by measuring the diameter of inhibition zone.

Antifungal activity

To evaluate antifungal activity, the fungal species for experiments were prepared by seeding a loopful of the respective fungus into

potato dextrose broth and incubated without agitation for 48 hours at 25°C [9]. Antifungal activity of plant extracts against different species were checked by disc diffusion method (Bauer *et al.*, 1996). Then the PDA medium was poured into the Petri plates and after solidification the fungal species were streaked on the PDA medium separately. Circular paper discs (6 mm diameter) impregnated with known amount of each extract were placed upon the surface of inoculated plates. Tetracycline is used as positive control. The plates were kept at room temperature for 48 hours for absorption of plant extracts in the medium. Then the zone of inhibition was measured.

Minimum inhibitory concentration (MIC)

As the methanolic extract exhibited prominent control over the growth of the bacteria and fungi, MIC was determined for methanolic extract of leaf, stem and root parts of *Thalictrum javanicum* against the control of growth of bacteria and fungi studied. (MIC) was determined through the broth dilution method [10]. Bacteria were grown in nutrient broth for 6 hrs and then 200 µL of 10^6 cells/mL broth were inoculated in tubes with 1800 µL nutrient broth supplemented with eight different concentrations from 100 to 800 µg/mL of leaf, stem and root extracts separately. Ampicillin 100 µg/mL was used as positive control and the pure solvent, methanol, 100 µL was used as negative control. All the tubes were incubated at 37°C for 24hrs and they were examined for visible turbidity. The MIC values were identified as the lowest concentration that inhibited the visible growth of the tested bacteria [11,12].

For fungi also determaination of MIC was carried out by using methanolic leaf, stem and root extracts. Tetracycline at 100 µg/mL was used as positive control and DMSO at 100 µg/mL was used as negative control. All the tubes were incubated at 37°C for 72hrs and they were examined for visible turbidity.

Statistical analysis

The antimicrobial activity of leaf, stem and root extracts of *T. javanicum* were indicated by clear zones of growth inhibition. All experiments were performed in triplicates and results were presented as Mean ± SD (Standard deviation). The significance in the difference of mean was determined according to Duncan's Multiple range test [13].

RESULTS AND DISCUSSION

The results of the study showed that the aqueous and alcoholic solvent extracts (petroleum ether, chloroform and methanol) of leaf, stem and root parts of the study species, *T. javanicum* had prominent antimicrobial activity against the human pathogenic bacteria and fungi studied (Tables 1 and 2). The effect of various alcoholic solvent extracts of *T. javanicum* for the antibacterial activity was determined to be varied across the bacterial species tested. Among the four extracts studied, methanolic extract showed significantly higher activity in all the parts of *T. javanicum*. On the other hand, chloroform extract had moderate activity against the growth of bacteria and fungi. Certain early studies have supported that the methanolic extract of some Ranunculaceae members had potential antimicrobial activity like *Thalictrum minus* [14], *Aconitum heterophyllum* [15] and *Clematis brachiata* [7]. This may be due to high polarity of methanolic solvent which may resulted in drawing more variety of phytochemicals like alkaloids, flavonoids, glycosides, phytosterols, saponins, steroids, tannins, triterpenoids etc from plant source [16] and some of which might have been associated with antimicrobial activities and thus have curative properties against pathogens [17]. Higher zone of inhibition has been effected by the methanolic leaf extract of *T. javanicum* against the bacterium, *Proteus mirabilis* (20 mm diameter), by the methanolic stem extract against the two bacteria, *Proteus mirabilis* and *Bacillus subtillus* (26 mm and 23 mm diameter respectively), and by the methanolic root extract against the bacterium, *Streptococcus faecalis* (22 mm diameter). Similar trend of results were reported for some other species of *Thalictrum* elsewhere (*T. orientale, T. rhynchocarpum, T. longistylum T. revolutum, T. minus, T. decipiens, T. cultarum T. foliosum, T. delavayi* and *T. fortunei* and *T. rugosum*) [14, 18-26]. Among the three parts studied, the methanolic stem extract had potent activity against the bacterium, *Proteus mirabilis* (26 mm diameter) than that of the other parts. However, the colonial growth of the bacterium, *E. coli* has been controlled moderately by the methanolic extract of all the three parts (leaf, stem and root) of *T. javanicum* (Table 1). It may be due to the presence of certain specific secondary metabolites that can interfer the growth of disease causing pathogens. *T. javanicum* reported to have some specific protoberberine alkaloids such as magnoflorine, jatrorrhizine, demethyleneberberine, palmatine, columbamine, thalifendine, berberine, oxyberberine, thalrugosaminine, thalisopine and rugosinone [27, 28] which may play role for specific antibacterial activity. Especially protoberberine alkaloids display a great variety of biological and pharmacological activities which include the inhibition of DNA synthesis, protein synthesis, inhibition of membrane permeability and uncoupling of oxidative phosphorylation and these activities likely to contribute for allelochemical and toxic effects on the growth of bacteria, fungi, insects and invertebrates and other plants [29, 30].

For antifungal activity the highest zone of inhibition was noted against the fungi, *Verticillum lecanii* by methanolic leaf extract (22 mm diameter) and *Mucor* sp. by methanolic stem and root extracts (38 mm and 29 mm diameter respectively) (Table 2). Nishukera *et al.* (2012) reported that the species, *Thalictrum foliosum* had prominent antifungal activity against the fungi, *Aspergillus flavus* by stem extract and *Aspergillus niger* and *Microsporum gypsum* by root extract [31].

Methanolic extracts of leaf, stem and root parts of the study species, *Thalictrum javanicum* were studied to determine minimum inhibitory concentration (MIC) (Tables 3and 4). The results showed that the MIC value of leaf, stem and root were ranging between 200 and 500 µg/mL for both bacteria and fungi. It was noted that 200 µg/mL extract of all the three parts were most effective to control the growth of bacteria and fungi. Other species of the genus, *Thalictrum* such as *T. minus* and *T. orientale* have also been reported for their MIC value around 200 µg/mL [14, 32].

Similar to bacteria, for various fungal species also, the methanolic extracts of all the three studied parts of *Thalictrum javanicum* exhibited the MIC value, 200 µg/mL for the suppression of colonial growth. Other species of the studied family, Ranunculaceae such as *Thalictrum minus*, *Narvelia zeylanica* and *Hepatica nobilis* were also reported to have the MIC value, around 200 µg/mL against the growth of certain pathogenic fungi [33-35]. It was explained that the alkaloid, jatrorrhizine may serve as a leading compound for potent antifungal activity present in Ranunculaceae members in general and *Thalictrum* species in particular [27,36].

Table 1: Antibacterial activity of various alcoholic and aqueous extracts leaf, stem and root of *Thalictrum javanicum.*

Bacteria type	Diameter of the inhibition zone (mm)														
	Leaf					Stem					Root				
	C	P	CH	M	W	C	P	CH	M	W	C	P	CH	M	W
Gram-Positive															
BS	6±0.2^{d}	-	-	6±0.1^{d}	-	20±0.12^{b}	-	22±0.26^{a}	23±0.35^{a}	-	8±0.22^{e}	8±0.13^{c}	9±0.01^{d}	7±0.24^{d}	8±0.15^{a}
BT	6±10.23^{d}	-	-	13±0.08^{c}	-	19±0.09^{c}	-	23±0.02^{a}	20±0.32^{b}	7±0.25^{a}	6±0.09^{f}	-	-	6±0.03^{d}	-
EF	6.5±0.02^{d}	-	-	6±0.15^{d}	-	16±0.11^{c}	-	-	17±0.05^{c}	-	6±0.23^{f}	-	6±0.23^{e}	7±0.01^{d}	-
SA	-	-	-	-	-	-	-	-	11±0.13^{d}	-	15±0.11^{c}	-	11±0.19^{c}	12±0.08^{b}	8±0.23^{a}

SF	6±0.12[d]	-	-	-	-	-	-	-	-	-	16±0.11[ab]	13±0.23[a]	24±0.32[a]	25±0.16[a]	7±0.02[b]
SP	-	-	-	-	-	25±0.21[a]	-	12±0.25[d]	16±0.15[C]	6±0.16[b]	22±0.12[a]	10±0.22[b]	22±0.33[a]	10±0.17[b]	6±0.18[b]
Gram-Negative															
EC	11±0.32[c]	-	-	12±0.21[c]	-	13±0.26	-	15±0.32	15±0.35[C]	-	16±0.12[a]	-	19±0.01[b]	12±0.32[b]	-
KP	12±0.25[c]	-	-	12±0.35[c]	-	16±0.36[c]	10±0.06[b]	20±0.30[b]	20±0.20[b]	-	16±0.01[ab]	-	9±0.06[d]	8±0.32[cd]	-
PA	11±0.32[c]	-	6±0.32[b]	13±0.43[c]	-	14±0.32[d]	-	17±0.39[C]	12±0.43[cd]	-	6±0.42[f]	-	-	6±0.21[d]	-
PV	-	-	-	-	-	-	-	-	7±0.42[e]	-	11±0.24[d]	-	10±0.35[d]	10±0.43[b]	7±0.42[b]
SM	-	-	-	-		11±0.33[e]	-	-	13±0.06[d]	-	16±0.35[ab]	7±0.09[d]	9±0.02[d]	9±0.32[c]	7±0.24[b]
PM	35±0.25[a]	9±0.32[a]	11±0.35[a]	20±0.06[a]	8±0.04[a]	22±0.36[a]	-	11±0.09[d]	25±0.39[a]	-	16±0.26[ab]	-	10±0.39[d]	7±0.35[d]	-
SPA	-	-	-	-	-	26±0.32[a]	-	-	-	-	17±0.06[b]	8±0.35[c]	13±0.45[c]	11±0.35[b]	-
SPA1	21±0.25[b]	-	-	17±0.06[b]	-	23±0.03[ab]	11±0.20[a]	23±0.35[a]	7±0.38[e]	-	7±0.25[ef]	-	-	6±0.38[d]	-
S PA2	11±0.35[c]	-	-	11±0.06[c]	-	24±0.08[a]	11±0.38[a]	22±0.34[a]	21±0.49[b]	-	7±0.25[af]	-	-	6±0.03[d]	-

Control- Ampicillin,

P-Petroleum ether, CH-Chloroform, M-Methanol, W-Water.

BS- *Bacillus subtilis,* BT- *B. thuringiensis,*ET- *Enterococcus faecalis, SA-Staphylococcus aureus,* SP-*S. pyogenes,* SF-*Streptococcus faecalis* EC- *E. coli,* KP-*Klebsiella pneumoniae,*PA -*Pseudomonas aeruginosa,* PV- *Proteus vulgaris,* SM-*Serratia marcescens,* PM-*Proteus mirabilis,* SPA-*Salmonella partyphi,* SPA1 *S. paratyphi-A,* SPA2 *S. paratyphi-B.*

Table 2: Antifungal activity of various alcoholic and aqueous extracts of leaf, stem and root extracrs of *Thalictrum javnicum*..

Fungi	**Diameter of the inhibition zone (mm)**														
	Leaf					**Stem**					**Root**				
	C	**P**	**CH**	**M**	**W**	**C**	**P**	**CH**	**M**	**W**	**C**	**P**	**CH**	**M**	**W**
AF	50±0.2[a]	15±0.6[a]	16±0.05[a]	21±0.03[a]	-	45±0.02[a]	15±0.09[b]	16±0.06[b]	26±0.09[b]	7±0.3[d]	41±0.08[a]	16±0.09[ab]	18±0.04[a]	19±0.09[b]	6±0.01[b]
AN	20±0.06[c]	11±0.09[c]	8±0.06[b]	13±0.01[b]	8±0.0[1b]	20±0.05[d]	6±0.0[6d]	8±0.1[2d]	25±0.03[b]	8±0.0[8d]	20±0.02[d]	11±0.01[b]	8±0.0[5c]	15±0.08[c]	8±0.07[a]
AL	50±0.06[a]	13±0.01[b]	16±0.1[a]	22±0.08[a]	8±0.0[8b]	46±0.06[a]	16±0.03[b]	16±0.09[b]	22±0.09[c]	8±0.0[1d]	40±0.08[a]	21±0.06[a]	15±0.09[b]	20±0.08[b]	8±0.01[a]
CA	-	-	-	10±0.01[c]	-	-	-	-	16±0.06[e]	-	-	-	-	9±0.0[2d]	-
FS	49±0.05[a]			12±0.03[b]		35±0.03[b]	8±0.0[6c]	8±0.0[2d]	10±0.03[f]	-	8±0.0[5e]	7±0.1[2bc]	8±0.1[4c]	11±0.03[cd]	-
MS	-	-	-	-	-	35±0.01[b]	20±0.06[a]	25±0.03[a]	38±0.06[a]	25±0.01[a]	35±0.04[b]	10±0.01[b]	8±0.0[4c]	29±0.01[a]	6±0.01[b]
PS	40±0.12[ab]	-	-	12±0.06[b]	-	35±0.03[b]	8±0.0[4c]	8±0.0[6d]	22±0.02[c]	-	35±0.05[b]	12±0.04[b]	12±0.06[ab]	18±0.01[b]	6±0.01[b]
PL	20±0.01[c]	10±0.12[d]	8±0.2[1b]	12±0.03[b]	8±0.0[6b]	20±0.03[d]	6±0.0[4d]	7±0.0[4d]	23±0.02[c]	8±0.0[3d]	20±0.02[d]	10±0.03[b]	8±0.0[1c]	15±0.01[c]	8±0.01[a]
TV	30±0.01[b]	6±0.0[6e]	8±0.3[1c]	9±0.0[9d]	7±0.0[5c]	25±0.04[c]	6±0.0[6d]	8±0.0[3d]	9±0.1[g]	9±0.1[0c]	25±0.06[c]	8±0.0[3c]	9±0.0[3c]	14±0.21[c]	8±0.05[a]
VL	49±0.03[a]	11±0.05[c]	16±0.02[a]	22±0.01[a]	17±0.08[a]	46±0.04[a]	11±0.06[bc]	12±0.02[c]	19±0.09[d]	10±0.02[b]	40±0.1[a]	6±0.2[c]	8±0.0[4c]	19±0.02[b]	6±0.06[b]

C-Control Tetracycline,

P - Petroleum ether, CH-Chloroform, M-Methanol, W-Water.

AF-A*spergillus fumigatus,* AF- *Aspergillus niger*, AL- *Azospirullum lipoforum*, CA- *Candida albicans,* FS- *Fusarium sp*, MS-*Mucor sp*, PS-*Penicillum sp*, PL-*Pacilomyces litacinus*, TV-*Trichoderma viride* , VL-*Verticillum lecanii.*

Table 3: Minimum inhibitory concentration (MIC) of methanolic leaf, stem and root extracts of *Thalictrum javanicum* against certain pathogenic bacteria.

Plant parts	**Minimum inhibitory concentration (µg/mL)**														
	Gram-Positive bacteria						**Gram-Negative bacteria**								
	BS	**BT**	**EF**	**SA**	**SP**	**SF**	**EC**	**KP**	**PA**	**PV**	**SM**	**PM**	**SPA**	**SPA1**	**SPA2**
Leaf	300	200	400	300	300	300	300	200	300	300	300	3000	300	200	200
Stem	200	300	300	200	200	200	300	300	200	300	300	200	300	300	300
Root	300	300	300	300	200	300	300	300	300	300	300	300	300	500	300

Positive control - Ampicillin, Negative control - Methanol, P - Petroleum ether, CH - Chloroform, M - Methanol, W - Water. BS - *Bacillus subtilis,* BT - *B. thuringiensis,* ET - *Enterococcus faecalis,* SA - *Staphylococcus aureus,* SP - *Staphylococcus pyogenes,* SF - *Streptococcus faecalis* EC - *E. coli,* KP - *Klebsiella pneumoniae,* PA -*Pseudomonas aeruginosa,* PV - *Proteus vulgaris,* SM - *Serratia marcescens,* PM - *Proteus mirabilis,* SPA – *Salmonella paratyphi,* SPA1 - *S. paratyphi*-A, SPA2 - *S. paratyphi*-B.

Table 4: Minimum inhibitory concentration (MIC) of methanolic leaf, stem and root extracts of *Thalictrum javanicum* against human pathogenic fungi.

Plant parts	Minimum inhibitory concentration (µg/mL)									
	Fungus									
	AF	AN	AL	CA	FS	MS	PS	PL	TV	VL
Leaf	300	300	300	300	400	200	400	300	200	200
Stem	300	300	300	300	300	200	200	200	200	300
Root	300	400	300	500	300	300	300	500	200	200

Positive control - Tetracycline, Negative Control – DMSO. P - Petroleum ether, CH - Chloroform, M - Methanol, W - Water. AF- A*spergillus fumigatus,* AF - *Aspergillus niger*, AL - *Azospirullum lipoforum*, CA - *Candida albicans,* FS - *Fusarium* sp., MS - *Mucor* sp., PS - *Penicillum* sp., PL - *Pacilomyces litacinus*, TV - *Trichoderma viride* , VL - *Verticillum lecanii.*

CONCLUSION

As the extracts of all the major parts of *Thalictrum javanicum* effectively controlled the growth of many pathogenic bacteria and fungi, they can be used in the treatment of various infectious diseases. Bioactive compounds from *T. javanicum* can therefore be employed in the formulation of antimicrobial agents for the treatment of various bacterial and fungal infections including gonorrhea pneumonia, eye infections and mycotic infections.

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