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Antibacterial Activity of Leaf Extracts of *Euphorbia heterophylla* L. and *Tamilnadia uliginosa* (Retz.) Tirveng. & Sastre against *Xanthomonas campestris* pv. *citri*

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Abstract

Acetone, ethanol, methanol and petroleum ether leaf extracts of the plants, *Euphorbia heterophylla* and *Tamilnadia uliginosa* were tested for their antibacterial activity against *Xanthomonas campestris* pv. *citri* using disc diffusion technique. The leaf extract concentrations tested were 0 (control), 125, 250, 500 and 1000 ppm for each plant. The control (0 ppm) received no extracts and chloramphenicol (30 µg) was used as standard antibiotic. The antibacterial activity of the extracts used in the present study was found to be concentration dependent. The highest zone of inhibition, 2.8 cm was recorded in 1000ppm ethanolic leaf extract of *Tamilnadia uliginosa* with an activity index of 0.90 against *Xanthomonas campestris* pv. *citri*. At 125 ppm concentration, ethanolic leaf extracts of *Euphorbia heterophylla* showed zone of inhibition (0.8 cm) and the other solvent extracts showed no activity. However, at 125 ppm concentration, acetone, ethanol and methanol leaf extracts of *Tamilnadia uliginosa* showed least inhibition zones, 1.2 and 0.8 cm respectively.

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Introduction

Xanthomonas, a Gram negative bacterium, with its different species and varieties serve as causative agent of diseases in many plants. Among which the species, *Xanthomonas campestris* pv. *citri* causes citrus canker on most *Citrus* spp. (Graham, 2001; Gottwald et al., 2002; Das, 2003). Controlling of this pathogen is done by chemical methods worldwide as this disease in *Citrus* spp. causes considerable loss in plant growth and fruit yield (Graham et al., 2004).

As an alternate control measure, researchers also tried to control this disease using plant derived products and extracts. Leksomboon et al. (2001) tried aqueous extracts of five plant extracts of *Hibiscus subdariffa Psidium guajava*, *Punica granatum*, *Spondias pinnata* and *Tamarindus indica* for controlling citrus canker disease of *Citrus aurantifolia* (lime) caused by *Xanthomonas axonopodis* pv. *citri* under greenhouse condition and reported that a significant reduction in disease incidence. More than 200 plant diffusates have been tested against *Xanthomonas campestris* pv. *citri* by Akhtar et al. (1997) and reported that the diffusates from higher plants are possessing potential antimicrobial agents to be used against citrus canker. Similarly, the essential oils of 24 higher plants tested against *Xanthomonas arboricola* pv. *juglandis* causing walnut bacterial blight clearly showed that antibacterial activity against the bacterium (Soltani and Aliabdi, 2013). These studies clearly indicate that the plant derived products or extracts show promising results in controlling plant pathogenic

bacteria, in particular *Xanthomonas* spp. In the present study, four different solvent extracts of leaves of *Euphorbia heterophylla* and *Tamilnadia uliginosa* against *Xanthomonas campestris* pv. *citri* using disc diffusion assay.

Materials and methods

The leaves of the medicinal plants selected for the present study were collected from Sirumalai hills (Eastern Ghats), Dindigul, Tamil Nadu and the identification was confirmed using standard local floras (Gamble and Fischer, 1957; Matthews, 1983). The names of the plants identified were *Euphorbia heterophylla* L. (Euphorbiaceae) and *Tamilnadia uliginosa* (Retz.) Tirveng. & Sastre (Family: Rubiaceae) (Fig. 1). The leaves collected were transported to the laboratory for further processing. The cold extraction procedure was used for extracting leaves with solvents as per the procedure given below (Prakash and Karmegam, 2012; Vigneshwari et al., 2014). The leaves of the plants collected were individually washed with tap water, blotted with filter paper and spread over news paper for air drying under shade. After complete dryness, the leaves of individual plants were powdered using a mixer grinder. A known quantity of leaf powder (100 g) of each plant was taken in a 250 ml conical flask and added with 100-200 ml of acetone, ethanol, methanol and petroleum ether individually. The solvent-leaf powder mixtures were kept at room temperature for 48 hrs and rapidly stirred using glass rod every 8 hrs. After 48 hrs, the extract of each plant was filtered through Whatmann No.1 filter paper to exclude the leaf powder. Then each filtrate was kept in beaker on a water bath at 45ºC until the solvent gets evaporated. A greasy final material (crude extract) obtained for each plant was transferred to screw cap tubes and stored under refrigerated condition till use.

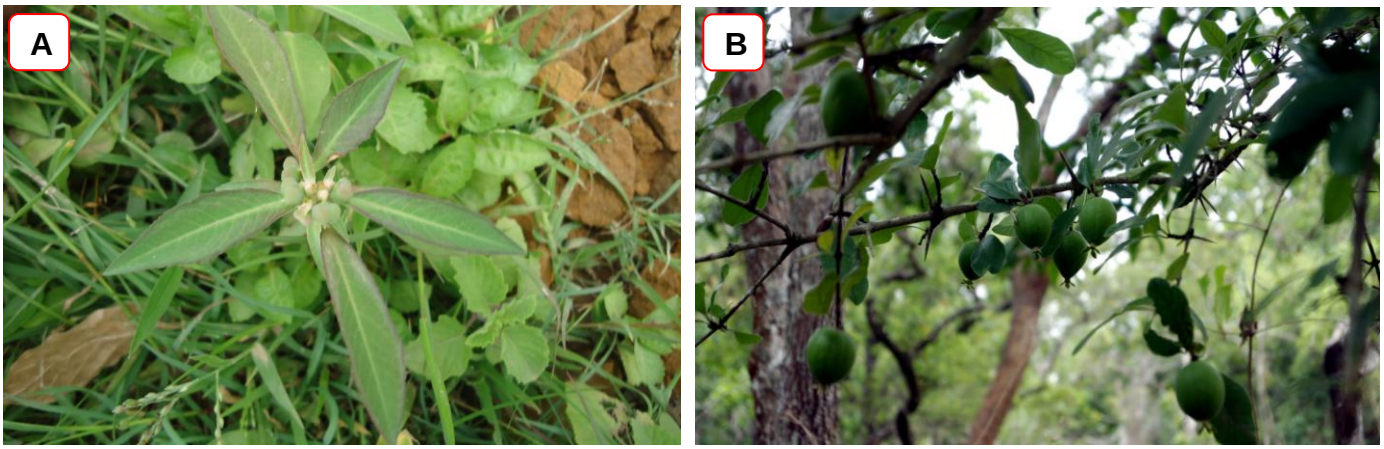


Fig. 1: The plants selected for the present study: (A) *Euphorbia heterophylla*; (B) *Tamilnadia uliginosa*.

By using digital electronic balance, 200 mg of each crude extract was carefully taken in a standard measuring flask and 5 ml of ethanol was added to dissolve the extract and 1-2 drops of emulsifier (Triton-X100) was added to completely dissolve the extract. Then it was made up to 200 ml by adding distilled water. This forms the stock solution of 1000 ppm (i.e., 1mg/ml), from which different concentrations of test solutions, 125, 250, 500 and 1000 ppm were prepared and used for antibacterial assay. Disc diffusion method of antibacterial assay was used to test the sensitivity of selected test organisms to the ethanolic extracts individually and in combination as described above (Bauer et al., 1966).

The test bacteria, *Xanthomonas campestris* pv. *citri* maintained in the Laboratory of Department of Microbiology, Kanchi Shri Krishna College of Arts and Science, Kilambi, Kanchipuram which was originally obtained from the Microbial Type Culture Collection (MTTC) of Institute of Microbial Technology (IMTECH), Chandigarh were used for the present study. The Petri plates of 100mm diameter with Trypticase Soy Agar (TSA) were swabbed with broth culture of the test bacteria in separate plates by using sterile swab. Over this, prepared antimicrobial discs were placed under aseptic conditions. Three discs of each extract were placed in triangle. Chloramphenicol (30 µg) was used as standard antibiotic. Also the discs without plant extract (discs prepared using 200 ml distilled water + 5 ml appropriate + one or two drops of emulsifier) were also maintained as control. The plates were then incubated at 37ºC for 24 hrs and the zone of inhibition (ZI) was measured in diameter (cm) around the discs and recorded. The assays were performed with three

replicates. From the results activity index was calculated by comparing the ZI of leaf extracts with standard antibiotic as follows:

$$\text{Activity Index} = \frac{\text{Inhibition area of test sample}}{\text{Inhibition area of standard antibiotic}}$$

Results and discussion

Antibacterial activity of leaf extracts of *Euphorbia heterophylla* and *Tamilnadia uliginosa* against *Xanthomonas campestris* pv. *citri* is shown in Table 1. The standard antibiotic, chloramphenicol (30 µg) showed a zone of inhibition (ZI) of 3.1 cm against *Xanthomonas campestris* pv. *citri*. The four different solvent extracts of leaves of *Euphorbia heterophylla* and *Tamilnadia uliginosa* showed various degrees of antibacterial activity, no activity, ZI around the disc and ZI from 0.7-2.8 cm.

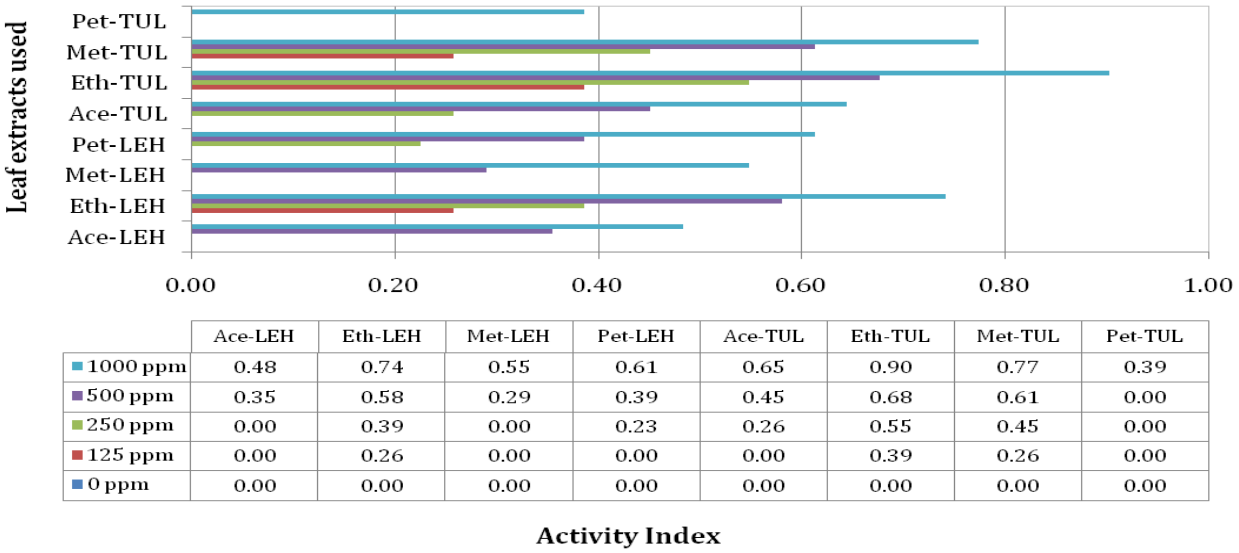
Among the four different solvent extracts of *Euphorbia heterophylla* leaf extracts, ethanol extract showed higher activity than acetone, methanol and petroleum ether extracts. The 1000 ppm ethanolic leaf extracts of *Euphorbia heterophylla* recorded 2.3 cm ZI, followed by the same concentration of petroleum ether-leaf extract (ZI = 1.9 cm), methanol-leaf extract (ZI = 1.7 cm) and acetone-leaf extract (ZI = 1.5 cm) (Table 1).

The highest ZI of 2.8 cm was observed in 1000 ppm ethanolic leaf extract of *Tamilnadia uliginosa* against *Xanthomonas campestris* pv. *citri*. The ethanolic leaf extract of *Tamilnadia uliginosa* showed antibacterial activity in all the extract concentrations, i.e., 125, 250, 500 and 1000 ppm followed by methanolic leaf extract. Comparatively, *Tamilnadia uliginosa* leaf extracts showed higher activity than *Euphorbia heterophylla* leaf extracts (Table 1).

Table 1. Antibacterial activity of leaf extracts of *Euphorbia heterophylla* and *Tamilnadia uliginosa* against *Xanthomonas campestris* pv. *citri*.

Plant leaves used	Solvent	Zone of inhibition (cm)[#]					
		Std.*	0 ppm[$]	125 ppm	250 ppm	500 ppm	1000 ppm
Euphorbia heterophylla L.	Acetone	3.1	-	-	AD	1.1	1.5
(Family: Euphorbiaceae)	Ethanol	3.1	-	0.8	1.2	1.8	2.3
	Methanol	3.1	-	-	-	0.9	1.7
	Petroleum ether	3.1	-	-	0.7	1.2	1.9
Tamilnadia uliginosa	Acetone	3.1	-	AD	0.8	1.4	2.0
(Retz.) Tirveng. &	Ethanol	3.1	-	1.2	1.7	2.1	2.8
Sastre	Methanol	3.1	-	0.8	1.4	1.9	2.4
(Family: Rubiaceae)	Petroleum ether	3.1	-	-	-	AD	1.2

[#] - Values are mean of three replicates; [$] - Control (without extract); *Std. - Standard antibiotic, Chloramphenicol (30 µg); AD – Around the disc.



	Ace-LEH	Eth-LEH	Met-LEH	Pet-LEH	Ace-TUL	Eth-TUL	Met-TUL	Pet-TUL
1000 ppm	0.48	0.74	0.55	0.61	0.65	0.90	0.77	0.39
500 ppm	0.35	0.58	0.29	0.39	0.45	0.68	0.61	0.00
250 ppm	0.00	0.39	0.00	0.23	0.26	0.55	0.45	0.00
125 ppm	0.00	0.26	0.00	0.00	0.00	0.39	0.26	0.00
0 ppm	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Fig. 2: Activity index of leaf extracts of *Euphorbia heterophylla* and *Tamilnadia uliginosa* against *Xanthomonas campestris* pv. *citri* (Standard antibiotic used-Chloramphenicol 30 µg; Ace-Acetone; Eth-Ehanol; Met-Methanol; Pet-Petroleum ether; LEH-Leaf extract of *Euphorbia heterophylla*; TUL-Leaf extract of *Tamilnadia uliginosa*).

The ZI has been expressed in terms of activity index with reference to the standard antibiotic, chloramphenicol at 30 µg (Fig. 2). A maximum activity index of 0.90 was found in 1000 ppm ethanolic leaf extract of *Tamilnadia uliginosa* followed by the same concentration of methanolic extract of *Tamilnadia uliginosa* leaves>ethanolic extract of *Euphorbia heterophylla* leaves>acetone extract of *Tamilnadia uliginosa* leaves.

Studies conducted world-wide on the antibacterial activity of plant derivatives support the favourable and safety control of *Xanthomonas* spp. which fall in line with the present study findings. The citrus canker disease incidence in lime has reduced from 18-52% while applying aqueous extracts of *Hibiscus subdariffa, Punica granatum, Spondias pinnata* and *Tamarindus indica* (Leksomboon et al., 2001). The diffusates obtained from various parts of *Phyllanthus emblica*, *Acacia nilotica*, *Sapindus mukorossi* and *Terminalia chebula* exhibited the inhibition zone measuring 4.83-6.00 mm at 50 g/l concentration has been reported by Akhtar et al. (1997) as most effective against *Xanthomonas campestris* pv. *citri*. Similarly, another species of *Xanthomonas*, *Xanthomonas arboricola* pv. *juglandis* was found to be effectively controlled by essential oils of *Ziziphora persicae*, *Mentha piperita*, *Mentha spicata* and *Achillea vermiculatus* in *in vitro* studies (Soltani and Aliabaadi, 2013). The present study findings indicate that there is a possibility of utilizing ethanolic extract of *Euphorbia heterophylla* and *Tamilnadia uliginosa* leaves for the control of *Xanthomonas campestris* pv. *citri* infection in citrus plants with proper field efficacy trials.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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