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Screening for antipathogenicity of weeds

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ABSTRACT

A total of 11 weeds were collected and screened for their antimicrobial activity against plant and human pathogens. Antimicrobial compounds of weeds were extracted with three different solvent *viz*. methanol, chloroform and petroleum ether of varying polarity. The results revealed that the petroleum ether extract of *Lucas aspera* was most effective against the *Fusarium oxysporum, Colletotricum falcatum* and *C. capsici*. The methanol extract of *Phyllanthus niruri* was showing highest antimicrobial activity against the human pathogens such as *Entrococcus coli, Bacillus subtilis, Bacillus licheniformis, Bacillus amylovorus*. Similarly, chloroform extract of *L. aspera* recorded the highest inhibition zone against the *Vibrio cholerae* and *Salmonella typhi.* Among the 11 weeds, *L. aspera* and *P. niruri* were found having more antimicrobial activity against most of the selected plant and human pathogens.

Key words : Weed extract, Antimicrobial activity, Biocontrol.

The use of higher plants and their extracts to treat infections is an age-old practice. Herbal medicines are gaining greater interest because of their cost effectiveness and eco-friendly attributes (Dwivedi and Abu-Ghazaleh 1997). Development of resistance to the synthetically manufactured antibiotics is a well known fact (Geyid *et al*. 2005). However hardly any report is available to show this phenomenon against the herbal medicines. Hence, more studies pertaining to the use of plants as therapeutic agents should be emphasized. There are many plants growing as weed. If these plants are utilized for meaningful purpose it may provide an additional income to farmers.

The use of natural products to control fungal disease in plants is considered as an interesting alternative to synthetic fungicides due to their less negative impacts on the environment (Nwachukwe 2001). Free flavonoids and sterols extracted from *Tridex procumbens* and bound flavonoids of *Lantana camara* fully inhibited spore germination of the *Fusarium oxysporum* (Sharma and Kumar 2009). *P. aeruginosa*, *A. niger*, *F. solani* and *C. albicans were* found more sensitive against the essential oils of *Lantana camara* (Deena and Thoppil 2000). Similarly aqueous extract of *Datura stramonium* and *Sida acuta* have recorded significant antifungal activity against eight pathogenic *Aspergillus* species (Satish *et al*. 2007). *Physalis minima* leaf extracts showed maximum antimicrobial activity against both human and plant pathogenic bacteria including *Bacillus subtilis*, *E. coli*, *Pseudomonas solanacearum*, *Xanthomonas axonopodis* pv. *malvacearum, X. vesicatoria* and fungi like *Aspergillus niger*, *A. flavipes*, *Fusarium verticillioides* and *Penicillium* sp. (Shariff *et al*. 2006). The present study was undertaken to explore the hidden potential of commonly available weeds of Jabalpur region of Madhya Pradesh against some selected plant and human pathogens.

MATERIALS AND METHODS

Collection of available weeds

A detailed survey was conducted at various places of Jabalpur for collection of available medicinally important weedy plants from the crop fields. Leaves of 11 weedy plants *viz., Calotropis gigantea, Physalis minima, Emilia sonchifolia, Lucas aspera*, *Abelmoschus moschatus, Sida acuta***,** *Tridex procumbens, Datura stramonium*, *Lantana camara, Malvastrum coroman-delianum* and *Phyllanthus niruri* were collected based upon the anecdotal evidence and availability.

Microorganisms used

Plant pathogen cultures (*Aspergillus niger, Colleto-trichum falcatum, C.capsici, Fusarium oxysporum)* were obtained from Institute of Microbial Technology (IMTECH), Chandigarh and the human pathogens (*Pseudomonas aeruginosa, Vibrio cholerae, Escherichia coli, Streptococcus faecalis, Staphylococcus aureus, Salmonella typhi, Entrococcus* sp.*, Bacillus subtilis, B. licheniformis, B. amylovorus*) were collected from Kovai Medical College Hospital, Coimbatore (Tamilnadu).

Preparation of plant extracts

Leaves of the weed samples were shade dried at room temperature (28 ± 2^0C) for 3-4 days, ground and stored in airtight containers in the dark. Powdered leaf samples were extracted with 80 % methanol (polar solvent), chloroform (medium polar solvent) and petroleum ether (least polar solvent) at 1:5 sample: solvent ratio for overnight. The extracts were then filtered and

concentrated under vacuum in rotary evaporator to get gummy residue (Geyid *et al*. 2005). All the extracts were kept in tightly stoppered bottle in a refrigerator until used for the anti-microbial testing.

Preparation of test samples, test controls and culture media

Hundred mg of dried weed extract was dissolved in 1 ml of absolute ethanol and used for the antifungal and antibacterial assays against the plant and human pathogens. Absolute ethanol without the test compound was used as the negative control. Where as the antibacterial agent ampicillin and the antifungal agent ketaconazole at a concentration of 1 mg/ml were used as the positive controls. The bacteria and fungi were cultured and maintained on nutrient agar and potato dextrose agar medium respectively. For the bioassay, a loopful of the culture was inoculated into 100 ml of the respective broth and incubated at 37^{0}C for 24 hours for bacteria and 3-6 days for fungi.

Agar well diffusion assay

One per cent of culture broth was added into the sterilized medium and poured into the petriplates and allowed to solidify. Then each petriplate was divided into four equal quarters using a marker pen. Using a sterile cork borer, wells of 6 mm in diameter were made in each quadrat. For each organism, 20µl of the prepared plant sample was loaded in each well using sterilized dropping pipette. Two replications were maintained for each treatment. For each microorganism, the positive control and the negative control (two replications each) were also loaded in a separate well. The plates were incubated for 24-48 hrs for bacteria and 3-4 days for fungi and the observations were taken by measuring the inhibition zone (or halo like area), which indicates the absence of microbial growth around the well. The diameter of inhibition zone (DIZ) was measured and the mean D1Z was calculated (Iqbal *et al.* 1998).

RESULTS AND DISCUSSION

The selected weed plants exhibited varying degree of antimicrobial activity against the selected plant and human pathogens. The inhibition zones produced by the weed extract were compared with the inhibition zones of standard antibiotics. In the present study, the chloroform extract of *L. aspera* (1.8 cm) showing highest inhibitory activity against the plant pathogen *F. oxysporum*. This was followed by petroleum ether extract of *C. gigantea* (1.6 cm) (Table 1). Khan *et al*. (1996) observed that the leaf extracts from *Calotropis procera* reduced the number of seed mycoflora of *F. oxysporum* on chickpea and increased their germination percentage.

The same extract showed remarkable inhibition zone (1.5 cm) against the *C. capsici*. The extracts of *P. minima*, *E. sonchifolia* and *A. moschatus* have not shown any activity against the *C. capsici.* Similarly, *C. falcatum* was found to sensitive and were inhibited by petroleum ether extract of *L. aspera* (1.4 cm) (Table 1). Rahman *et al.* (2007) reported that the chloroform and ether extract of *L. aspera* posses the antifungal activity and he mentioned that, the free radical scavenging property is one mechanism by which this extract are effective against the pathogens.

It was found that the petroleum ether extract of *Sida acuta* (1.8 cm) performed better against the *A. niger*. This was followed by *C. gigantea* (1.6 cm) (Table 1). Similar results were reported by Mangathayaru *et al*. (2005).

The methanol extract of *P. minima* (1.7 cm) exhibited the highest activity against the *P. aureginosa*. However, the extracts of *T. procumbens* were found to be ineffective or showed poor inhibition of bacterial growth. The results revealed that chloroform extract of *L. aspera* (1.9 cm) was most effective against *V. cholerae* (Table 2). Prajapati *et al*. (2010) reported that the essential oils from *L. aspera* possessed bacteriostatic activity against *Vibrio cholerae.*

The methanol extract of *P. niruri* (1.7cm) recorded more actvity against the *E. coli* (Table 2). These results are in line with the findings of Ekwenye and Njoku (2006). For *S. faecalis,* highest activity (2.1 cm) was observed by methanol extract of *M. coromandelianum.* Similarly chloroform extract of *L. aspera* (2.8 cm), petroleum ether extract of *P. minima* (2.1 cm) and methanol extract of *Sida acuta* (1.3 cm) showed highest inbition zone against the *S. aureus, S. typhi* and *Enterococcus.* sp*.,* respectively (Table 2). Karou *et al.* (2005) stated that the two major alkaloids such as cryptolepine and quindoline from *Sida acuta* displayed good antimicrobial activity against *Enterococcus*.

Methanol extract of *P. niruri* showed more activity against *B. subtilis* (2.1 cm) and *B. licheniformis* (1.2 cm), *B. amylovorus* (4.1 cm). The intensity of inhibition zone produced by *P. niruri* was higher when compared to other weed extracts (Table 2). Antimicrobial activities of *P. niruri* have been documented by several workers. Sumathi and Parvathi (2010) reported that lowest concentration of the leaf extracts of (50 g/ml) *P. niruri* was found to be very effective in inhibiting the growth of all the selected strains of *Bacillus*. It was highlighted in our study that the extracts of *L. aspera* and *P. niruri* showed more antimicrobial activity than other weeds. Further investigations are suggested to isolate the compounds responsible for these activities.

Table 1. Antimicrobial activity of weed extracts against plant pathogens

Name of plant pathogen	Inhibition zone (cm)																														
	1			2			3			4			5			6			7			8			9			10			11		
	Calotropis gigantea			*Lucas aspera*			*Phyllanthus niruri*			*Sida acuta*			*Tridex procumbens*			*Abelmoschus moschatus*			*Lantana camara*			*Emilia sonchifolia*			*Datura stramonium*			*Physalis minima*			*Malvastrum coromandelianum*		
	ME	CF	PE	ME	CF	PE	ME	CF	PE	ME	CF	PE	ME	CF	PE	ME	CF	PE	ME	CF	PE	ME	CF	PE	ME	CF	PE	ME	CF	PE	ME	CF	PE
F. oxysporum	1.0	1.7	1.6	0.8	1.8	0.2	0.8	0.2	1.4	0.9	1.5	1.4	-	-	0.1	0.7	0.2	1.2	-	0.1	-	0.4	-	0.8	1.1	1.5	0.6	0.6	-	0.8	0.6	0.2	0.4
C. capsici	0.3	0.4	-	1.0	1.5	1.2	0.9	1.1	-	0.9	1.0	0.9	-	-	0.2	-	-	-	0.3	0.7	-	-	-	-	0.3	0.4	-	-	-	-	0.4	0.5	0.3
C. falcatum	0.4	0.3	0.1	0.3	1.2	1.4	0.5	0.3	-	0.7	0.8	0.7	-	0.1	0.2	0.1	0.2	-	0.4	0.6	0.1	0.1	0.2	-	0.3	0.1	-	0.1	0.2	-	0.3	0.2	-
A. niger	0.8	1.2	1.6	0.7	1.2	0.1	0.9	0.3	0.2	0.8	1.2	1.8	-	0.3	0.1	0.6	0.4	0.8	0.3	0.2	0.1	0.4	0.1	0.3	0.7	1.3	0.9	0.4	0.1	0.3	0.5	0.3	0.1
LSD(P=0.05)	0.0	0.1	0.1	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

ME- Methanol extract; CE - Chloroform extract; PE Petroleum ether extract

Table 2. Antimicrobial activity of weed extracts against human pathogens

Name of human pathogen	Inhibition zone (cm.)																														
	1			2			3			4			5			6			7			8			9			10			11		
	Calotropis gigantea			*Lucas aspera*			*Phyllanthus niruri*			*Sida acuta*			*Tridex procumbens*			*Abelmoschus moschatus*			*Lantana camara*			*Emilia sonchifolia*			*Datura stramonium*			*Physalis minima*			*Malvastrum coromandelianum*		
	ME	CF	PE	ME	CF	PE	ME	CF	PE	ME	CF	PE	ME	CF	PE	ME	CF	PE	ME	CF	PE	ME	CF	PE	ME	CF	PE	ME	CF	PE	ME	CF	PE
P.aeruginosa	1.0	0.6	-	0.6	0.7	-	0.8	1.6	0.6	-	-	1.3	-	-	-	1.5	0.7	-	1.6	0.7	0.6	1.0	0.7	0.9	0.8	-	1.5	1.7	0.8	0.6	0.6	0.2	-
V. cholerae	1.4	0.9	0.4	0.2	1.9	0.9	0.7	0.4	-	-	0.6	-	-	0.8	-	-	-	-	1.4	0.9	1.2	0.4	0.6	0.3	-	-	-	0.4	0.7	-	0.4	-	0.2
E. coli	-	0.4	0.6	0.9	0.7	0.6	1.7	-	-	0.5	0.7	1.1	0.4	-	-	-	-	-	0.6	0.8	0.9	-	0.7	0.6	-	-	1.5	-	0.7	0.3	0.6	0.9	0.8
S. faecalis	1.2	-	1.3	1.1	1.1	0.8	1.5	1.2	1.1	-	0.1	0.8	-	-	0.6	1.2	-	2.3	0.5	0.9	1.5	1.6	1.0	1.1	-	-	-	1.2	1.5	1.1	2.1	1.0	1.9
S. aureus	-	0.6	0.7	0.9	2.8	2.4	0.9	0.5	0.7	0.7	0.7	1.4	-	-	1.2	1.1	0.8	1.2	1.5	1.3	0.7	0.7	0.6	0.4	-	0.7	1.7	0.5	0.9	0.7	-	-	-
S. typhi	0.4	0.5	0.7	1.7	1.1	1.5	1.6	1.0	-	-	-	1.6	-	0.1	0.4	2.0	-	-	1.0	0.2	0.5	0.6	-	0.8	-	-	-	1.0	0.6	2.1	-	0.6	0.8
Enterococcus sp.	0.9	0.6	0.7	1.1	0.9	0.7	1.0	-	-	1.3	-	0.4	0.1	-	0.2	0.8	0.3	-	1.2	0.9	0.1	-	-	0.7	-	-	-	-	-	-	0.6	-	0.5
B. subtilis	1.3	1.3	1.4	1.5	0.9	1.1	2.1	1.3	0.9	-	1.3	0.8	-	0.3	-	0.9	-	0.7	0.9	0.6	1.4	-	-	-	1.3	-	0.4	1.3	1.1	0.9	0.9	1.9	0.8
B. licheniformis	0.7	0.6	-	1.1	0.8	-	1.2	0.6	-	-	-	0.8	-	-	-	-	-	-	-	-	-	-	-	0.6	0.7	-	0.8	0.6	1.1	-	-	0.7	0.6
B. amylovorus	-	-	2.4	1.8	0.4	-	4.1	2.1	0.8	1.0	-	1.2	0.6	-	0.3	2.5	1.7	1.2	0.6	1.2	1.7	1.4	1.2	0.7	-	-	-	2.1	1.8	0.8	0.8	-	0.7
LSD(P=0.05)	0.1	0.1	0.1	0.1	0.1	0.0	0.2	0.1	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1

ME- Methanol extract; CE - Chloroform extract; PE Petroleum ether extract

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