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BACTERICIDAL ACTION OF CRUDE LEAF EXTRACTS OF COMMON WEEDS

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ABSTRACT

Leaf compounds extracted from *Oxalis corniculata, Chenopodium album, Solanum nigrum, Amaranthus viridis* and *Convolvulus arvensis* were evaluated for their potential against phytopathogenic bacteria namely; *Xanthomonas campestris, Pseudomonas syringae, Morganella morganii, Acinetobacter baumannii* and *Xylophilus* sp. Aqueous, methanol and ethanol soluble fractions from the leaves of selected weeds were extracted and employed against bacterial strains using well diffusion method. Results indicated that aqueous crude extracts of all test plants were least effective against bacteria as compared to both organic solvent extracts. In case of methanol, maximum zone of inhibition (42 mm) was recorded against *P. syringae* by *O. corniculata* leaf extract followed by *S. nigrum* leaf extract that restricted the growth of *X. campestris* in 40 mm diameter around the well. By ethanol crude extract, best results were obtained from *A. viridis* leaves extracts that produced inhibition zone of 44 mm diameter against *Xylophilus* sp. growth around well. *C. album* leaves extract that did not allow *P. syringae* to grow more than 40 mm diameter around the well. Present study suggested the need of phytochemical profiling of antibacterial compounds in the crude extracts of selected weeds.

Key words: Antibacterial activity, *A. viridis, C. album, C. arvensis, O. corniculata, S. nigrum,* methanol and ethanol.

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INTRODUCTION

Extensive use of pesticides is toxic to all types of life therefore eco-friendly methods are recommended to control the pests (Sribanditmongkol *et al.,* 2012; Wiwanitkit, 2013). Weeds are well adapted in all types of agricultural lands because they have unique

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type of bioactive phytochemicals. These phytochemicals have antioxidant, antimicrobial and anticancer properties (Chah *et al.,* 2006; Dhankhar *et al.,* 2013). It has been also proved that weeds are more resistant to microbial attack than the other plants especially crop plants (Sharma *et al.,* 2009; Udayaprakash *et al.,* 2011). Therefore weeds can be used as an inexpensive material for the management of pests being widely available and easy to collect. The use of weeds in pest management can also reduce the problems caused by the weeds to economical important crops (Afridi and Khan, 2014; Afridi et al., 2014). Another aspect of using weeds and other biological sources against microbes is due to the current knowledge of genetic and metabolic changes by continuous use of synthetic chemicals that make the microbes resistant against a drug or chemical (Raghunath, 2008).

Oxalis corniculata, Chenopodium album, Solanum nigrum, Amaranthus viridis and *Convolvulus arvensis* are common and notorious weeds in Pakistan either growing in wild or associated with the economically important crops. However these weeds are also known to possess useful biologically active compounds (Pal *et al.,* 2013; Singh and Prakash, 2014). Therefore these plants could serve as an alternate material to synthetic pesticides to control the pathogens.

Present study aimed at to explore the antibacterial efficacy of commonly found weeds. For this purpose aqueous, methanolic and ethanolic crude extracts of leaves of these weeds were tested against phytopathogenic bacteria.

MATERIALS AND METHODS

Selection of bacterial strains

A total of five bacterial strains isolated from the postharvest decayed fruits were selected for present study. All five selected bacterial strains were obtained from First Fungal Culture Bank of Pakistan (FCBP), Institute of Agricultural Sciences (IAGS), University of the Punjab, Lahore. Detail information about these bacterial strains is given in Table 1. Bacterial cultures were revived on nutrient agar medium at 37 ± 2 ºC and maintained at 4 ºC.

Selection of weeds

Five commonly found weeds with known antibacterial characteristics; *Oxalis corniculata, Chenopodium album, Solanum nigrum, Amaranthus viridis* and *Convolvulus arvensis* (Nasir, 1971) were selected to evaluate their efficacy to control the pathogenic bacteria. Weeds were collected from different fields of Quaid-e-Azam Campus, University of the Punjab, Lahore. Leaves were separated from the plants, washed thoroughly under running tap water to remove soil or plant debris and dried at room temperature for three

weeks. Dried leaves were crushed and then ground to a fine powder. Powdered leaf materials were used in extract preparation (Vaidya and Bhattarai, 2009).

Preparation of leaf extracts

Water-soluble fractions were collected from leaves powder of each plant separately. For this, 5 g of leaf powder was soaked in 100 ml sterilized distilled water for 48 h and then filtered using muslin cloth. Water from of the filtrate was evaporated in a drying oven at 40 °C until soluble compounds left in the form of paste. This crude extract was then kept in a desiccator with silica gel for residual water absorption. The extracted materials were stored in sterilized brown screw caped bottles at 4 ºC until further use.

Methanol and ethanol soluble fractions of weeds leaves were also tested for their antibacterial capacity. Five grams leaf powder of each weed was added to 100 ml of either solvent separately and incubated at constant shaking at room temperature for 7 days. After one week, these solutions were filtered through filter paper. The solvents were evaporated from the filtrate in rotary vacuum evaporator and finally kept in desiccator for complete removal of solvents. Each of these solvent extracts was preserved at 4^{o}C in bottles until further use (Vaidya *et al*., 2008).

To determine the bioactivity, homogenous mixtures of different working concentrations i.e 100, 75, 50 and 25% (w/v) of aqueous and solvent extracts were prepared by dilution in water and Dimethlysulfoxide (DMSO) respectively.

Bacterial growth assays

Well-diffusion method was used to evaluate the antibacterial activity of aqueous and organic solvents leaf extracts of selected weeds. Bacterial cell suspension was prepared in Saline Tween 80 and the number of cells per ml of this inoculum was determined by haemocytometer. Each pathogenic bacteria (Table 1) concentration @ 10^4 were spread uniformly on LB agar medium petriplates, separately. In the bacteria inoculated plates, wells of 8 mm size were made with the help of sterile cork borer. Then 60 µl of different concentrations of each treatment were poured into the three wells of each inoculated plate separately. Control plates received similar amount of solvent i.e sterilized water or DMSO. Each treatment was made in triplicate with each selected pathogen. Such petriplates were incubated overnight at 37 ºC. Antibacterial activity was evaluated by measuring the inhibition zones of bacterial growth (if present) around the wells in mm and expressed as the mean of three triplicates ± SE (standard error). Each set of experiment was repeated twice.

RESULTS AND DISCUSSION

In this present study, aqueous and organic solvents (methanolic and ethanolic) extracts of commonly found five weeds, *O. corniculata, C. album, S. nigrum, A. viridis* and *C. arvensis* were probed as an alternate of chemical bactericides against the different bacterial plants pathogens i.e., *X. campestris, P. syringae, M. morganii, A. baumannii* and *Xylophilus* sp (Table 1). All the selected weeds are found on cultivated and non-cultivated agricultural lands therefore easy to collect for assay.

Results showed variable effects of different leaf extracts on test bacterial strains. In general least effect was observed in case of aqueous extracts of all the tested weeds. Although growth of all bacterial strains was restricted by aqueous extracts of weeds to some extend but the effect was insignificant among all concentrations. Therefore almost similar diameter of inhibition zone was recorded providing any of the four concentrations of leaf extract. Such results show the low antibacterial efficacy of water extracts of test weeds. As compare to the aqueous extracts, bacterial pathogens were more sensitive to organic solvent extracts. In the present of increasing concentrations of both organic extracts of all weeds, an increase in the inhibition of bacterial growth was observed (Figure 1).

Oxalis corniculata (Oxalidaceae*)* leaves are known to possess different groups of chemicals such as niacin, phytosterols, flavonoids and phenols. In previous studies, the organic leaf solvent extracts of this weed plant has been proved effective against *X. compestris* (Chah *et al.,* 2006). Their results revealed that ethanolic and methanolic extracts were most effective against *X. compestris* and that may because presence of phenolic compounds in crude extracts (Raghavendra *et al.,* 2006). In this study, most significant inhibitory effect was observed for leaf methanolic extract of *O. corniculata* against *P. syringae,* where the growth diameter inhibition zone increased from 20 to 42 mm when the extract concentration was raised from 25% to 100 percent. Similarly ethanolic extract of *O. corniculata* also inhibited growth significantly of all pathogens tested at 100 % extract concentration. The inhibition zone 35, 33, 32, 32, 30 mm diameter was recorded for *X. campestris, M. morganii, P. syringae, Xylophilus* sp. and *A. baumannii* respectively (Table-2).

C. arvensis which is a salt tolerant weed has well established antibacterial potential. Recently Khan and colleagues (2015) evaluated the eight different organic solvents extracts of this plant for their antimicrobial ability. This study also confirmed the presence of bactericidal compounds such as coumarins, saponins, flavonoids, steroids and tannins in the leaf extract of *C. arvensis*. In a similar study, Ali *et al.* (2013) confirmed the high antibacterial potential of

organic solvent extracts of *C. arvensis* leaves. Results of present study indicates that the methanolic leaf extracts of *C. arvensis* exhibited almost similar control of selected pathogens however the ethanolic leaf extract of same plant was highly toxic to *Xylophilus* sp. while other bacterial strains showed more or less similar pattern of growth inhibition (Table-3).

S. nigrum is a traditional medicinal herb and has been investigated previously by many researchers with positive bacterial control (Rani and Khullar, 2004; Zubair *et al.,* 2011). The antibacterial capacity of aqueous, methanolic and ethanolic extracts was evaluated and results were presented in Table 4. The best control by *S. nigrum* leaf extract was recorded for *X. compestris* by its methanolic extract where diameter of inhibition zone increased from 19 to 40 mm. The 100 % concentration of same extract induced zone of inhibition of 34, 30, 29 and 27 mm for *P. syringae, M. morganii, A. baumannii,* and *Xylophilus* sp. respectively. The ethanolic extracts of same plant were equally effective against all test bacteria (except *P. syringae* where insignificant difference among all tested concentrations were observed) and increased the zone of inhibition to 8-9 mm from initial concentration to final concentration.

In this study, although the organic solvents leaf extracts of *C. album* were effective against all tested bacterial pathogens however *A. baumannii* and *P. syringae* were found to be most sensitive to methanolic and ethanolic extracts respectively (Table 5). *C. album* is an edible plant and traditionally used in curing many microbial diseases. Its leaves contain high percentage of phenolic compounds for example flavonoids and phenolic diterpenes (Shahidi *et al.,* 1992; Pietta, 1998; Kumar and Kumar, 2009). Singh *et al.* (2011) found that the methanol leaf extract of this plant displayed high antibacterial action against *P. aeruginosa, S. typhimurium*, *E. coli* and *P. vulgaris*). Finally *Xylophilus* sp. was highly sensitive to ethanolic extract of *A. viridis* leaves that forms the zone of inhibition of 44 mm when provided pure (100 %) leaf extract. The methanolic extract of same plant controlled the *P. syringae* most and produced 34 mm inhibition zone (Table-6).

CONCLUSION

Present study concludes that the leaves of selected weeds have a rich source of methanol and ethanol soluble bactericidal compounds. These valuable compounds possess high antibacterial potential therefore can be further explored for the isolation and identification of bioactive compound(s).

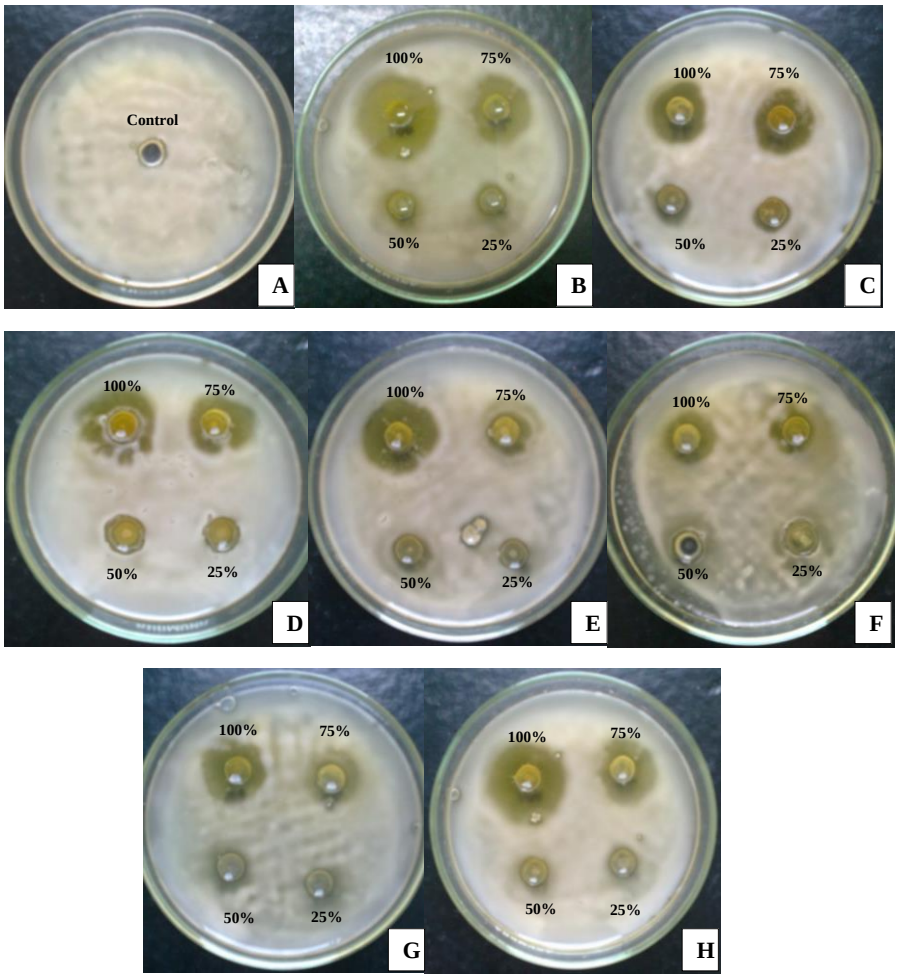

Figure 1. Some of the representative results of antibacterial potential of weeds. (A): Control; Effect of methanolic extract of *O. corniculata* on growth of *P. syringae* (B) and *X. campestris* (C); Effect of ethanolic extract of *C. arvensis* on growth of *Xylophilus* sp. (D); Effect of methanolic extract of *S. nigrum* on growth of *X. campestris* (E); Effect of methanolic extract of *C. album* on growth *A. baumannii* (F) and *P. syringae* (G); effect of ethanolic extract of *A. viridis* on growth of *Xyllophilus* sp. (H).

Table-1. List of Bacterial species procured from FCBP.

Sr. No.	FCBP Accession No.	Bacterial species	Source
1	FCBP0003	*X. campestris*	*Lycopersicon esculantum,* fruit
2	FCBP0009	*P. syringae*	*Prunus avium*, fruit
3	FCBP0122	*M. morganii*	*Pyrus malus,* fruit
4	FCBP0124	*A. baumannii*	*Psidium guajava*, fruit
5	FCBP0281	*Xylophilus* sp.	*Lycopersicon esculantum,* fruit

Table-2. Growth inhibition of bacteria by aqueous and solvent extracts of *O. corniculata.*

Bacterial species	Zone of Inhibition (mm)											
	Aqueous extract conc. (%)				Methanolic extract conc. (%)				Ethanolic extract conc. (%)			
	25	50	75	100	25	50	75	100	25	50	75	100
X. campestris	11± 1.54	13± 1.99	13±1 .68	14±1 .99	19±1 .99	26±1 .24	29±1 .67	34±1 .99	23±1 .67	24±1 .68	33±1 .54	35±1 .68
P. syringae	12± 1.99	14± 1.68	17±1 .99	18±1 .68	20±1 .45	26±2 .34	35±1 .99	42±1 .67	19±1 .54	24±1 .45	29±2 .24	32±1 .54
M. morganii	12± 2.88	13± 1.45	14±1 .99	14±2 .44	21±1 .87	26±2 .44	30±1 .68	33±1 .99	20±1 .68	27±1 .66	30±2 .34	33±1 .67
A. baumannii	11± 2.88	12± 1.99	12±0 .98	13±1 .87	19±1 .99	22±1 .67	28±1 .68	30±1 .54	20±0 .98	26±1 .87	30±1 .68	30±1 .99
Xylophilus sp.	11± 1.68	12± 2.44	13±0 .66	13±1 .54	20±2 .44	27±1 .68	30±1 .99	32±1 .68	19±1 .99	25±1 .68	32±1 .66	32±1 .68

Table-3: Growth inhibition of bacteria by aqueous and solvent extracts of *C. arvensis.*

Bacterial species	Zone of Inhibition (mm)											
	Aqueous extract conc. (%)				Methanolic extract conc. (%)				Ethanolic extract conc. (%)			
	25	50	75	100	25	50	75	100	25	50	75	100
X. campestris	11±1.99	12±1.54	12±1.99	13±1.68	20±1.24	25±1.99	27±1.99	30±1.67	20±1.68	24±1.67	24±1.68	25±1.54
P. syringae	12±1.68	12±1.99	13±1.68	13±1.99	20±2.34	20±1.45	25±1.67	28±1.99	18±1.54	19±1.54	19±1.54	20±2.24
M. morganii	11±1.45	12±2.88	12±2.44	14±1.99	18±2.44	24±1.87	24±1.99	25±1.68	22±1.68	22±1.68	23±1.67	24±2.34
A. baumannii	12±1.98	12±2.88	13±1.87	14±0.98	20±1.67	20±1.96	24±1.54	28±1.68	20±1.98	25±1.98	26±1.99	28±1.68
Xylophilus sp.	11±2.44	11±1.68	12±1.99	13±0.66	21±1.68	24±2.44	28±1.68	28±1.99	20±1.99	23±1.99	31±1.68	32±1.66

Table-4: Growth inhibition of bacteria by aqueous and solvent extracts of *S. nigrum.*

Bacterial species	Zone of Inhibition (mm)											
	Aqueous extract conc. (%)				Methanolic extract conc. (%)				Ethanolic extract conc. (%)			
	25	50	75	100	25	50	75	100	25	50	75	100
X. campestris	11±1.66	12±1.68	13±1.99	14±1.99	19±1.99	23±1.68	24±2.44	40±1.68	20±0.66	24±1.99	25±1.68	29±2.44
P. syringae	11±1.68	13±1.99	13±1.98	14±1.98	20±1.68	28±1.54	32±1.96	34±1.67	27±0.98	27±1.87	28±2.88	28±1.98
M. morganii	12±2.34	12±2.34	12±1.68	13±1.68	20±1.68	21±1.99	26±1.87	30±2.44	20±1.99	24±2.44	25±2.88	28±1.45
A. baumannii	11±2.24	11±2.24	12±1.54	13±1.54	24±1.99	28±1.67	28±1.45	29±2.34	20±1.99	24±1.68	24±1.99	29±1.68
Xylophilus sp.	11±1.54	11±1.54	12±1.67	14±1.68	24±1.67	26±1.99	26±1.99	27±2.34	21±1.68	21±1.99	28±1.54	30±1.99

Table-5: Growth inhibition of bacteria by aqueous and solvent extracts of *C. album*.

Bacterial species	Zone of Inhibition (mm)											
	Aqueous extract conc. (%)				Methanolic extract conc. (%)				Ethanolic extract conc. (%)			
	25	50	75	100	25	50	75	100	25	50	75	100
X. campestris	12±1.96	12±1.45	13±1.68	13±2.45	19±1.99	25±1.24	25±1.67	28±0.98	21±1.67	28±0.98	28±1.68	30±1.99
P. syringae	11±2.34	11±1.66	12±1.54	12±1.99	20±2.44	23±1.68	27±1.99	30±1.54	21±2.34	26±1.68	33±1.99	40±1.87
M. morganii	11±1.67	11±2.44	12±1.99	13±1.54	20±2.34	22±1.99	28±1.87	28±1.45	20±1.68	24±1.99	27±2.44	27±1.54
A. baumannii	11±1.99	12±2.34	14±2.44	14±1.87	18±1.99	22±1.99	30±1.67	34±1.68	20±1.99	20±2.88	24±1.68	28±0.96
Xylophilus sp.	11±1.68	11±1.68	12±1.96	13±1.67	18±1.54	26±1.45	26±1.68	29±1.99	20±1.99	27±1.54	27±1.99	29±1.45

Table-6: Growth inhibition of bacteria by aqueous and solvent extracts of *A. viridis.*

Bacterial species	Zone of Inhibition (mm)											
	Concentration of aqueous extract (%)				Concentration of methanolic extract (%)				Concentration of ethanolic extract (%)			
	25	50	75	100	25	50	75	100	25	50	75	100
X. campestris	11±1.68	12±1.68	12±1.45	13±1.28	21±1.99	21±1.24	25±0.96	28±1.54	18±1.66	22±1.67	22±1.54	29±1.68
P. syringae	11±1.99	11±1.99	12±1.68	13±1.95	30±1.68	33±1.99	34±1.67	34±1.26	24±1.98	28±1.66	28±1.98	29±2.34
M. morganii	12±1.99	12±1.87	13±2.34	13±1.99	24±1.26	25±1.54	28±1.99	28±1.26	17±1.54	19±2.44	20±1.99	20±1.66
A. baumannii	10±1.98	11±2.28	12±1.96	12±1.67	20±2.34	24±1.88	24±2.44	28±1.98	18±1.54	22±1.99	24±2.34	27±1.68
Xylophilus sp.	12±0.98	13±2.34	14±1.45	14±1.99	21±2.44	26±1.68	29±1.45	30±0.98	20±1.99	24±1.67	36±1.23	44±1.45

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