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Bio-control of bacterial species isolated from diseased citrus fruits by methanolic extracts of weeds *in vitro*

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ABSTRACT

The present study evaluated the aqueous and methanol extract of selected weeds (Sonchus asper, Convolvulus arvensis and Solanum nigrum) for their antibacterial activity against different bacterial species isolated from diseased citrus fruits. The frequency of bacterial population was also estimated in diseased fruit samples of kinnow mandarin (Citrus reticulata) plants. Fourteen bacterial species were isolated on Luria Bertani *(L.B.) medium and identified on the basis of morphological and biochemical features. The high frequency of bacteria was observed from Lahore, Jhang and Sargodha than that of Faisalabad, Jhelum and Bahawalnagar. Burkholderia pseudomallai has high frequency upto 14.8% as compare to other bacterial species. Aqueous and methanol extract of selected weeds were prepared and observed their antibacterial efficacy by using the well diffusion method in vitro. The significant results were obtained show that the both extracts of selected weeds tested inhibited the bacterial growth. Methanol extract of S. nigrum and C. arvensis were most effective against K. gibsonii with percentage of inhibition zone 59% and 61% respectively. Besides, X. luminescens (53%), Syntrophospora sp. (66%) in case of methanol extract of S. nigrum and Aerococcus sp. (56%), Acidovorax temperans (51%) in methanol extract of C. arvensis were also showed maximum inhibition zone percentage. However, aqueous and methanol extracts of S. asper were showed minimum range of percentage of inhibition upto 41%-48%. The present investigation strongly indicates antibacterial potential of aqueous and methanol extracts of weeds against* bacterial pathogen of citrus *in sustainable disease management.*

Key Words: Antibacterial activity, citrus, methanolic extract, *S. asper*, *C. arvensis*, *S. nigrum.*

INTRODUCTION

Citrus is a prized fruit of Pakistan and holds number one position among all fruits both in area and production in the country. Citrus occupies a prominent position in fruit industry of the world. Pakistan is among the leading citrus growing countries of the world and earns substantial amount of foreign exchange annually. The national average yield is 10.6 tons/hectare which is very low as compared to other citrus growing countries. Diseases are one of the major factors which impede the fruit yield and quality. Among diseases i.e: bacterial spot, black pit fruit, canker, die back, variegated chlorosis and greening are most devastating and occurs throughout citrus growing countries of the world including Pakistan. The disease causes extensive damage to citrus and severity of infection varies with different species and varieties [4]. The management option of citrus diseases is the application of preventive sprays

of copper-based bactericides, although use of these toxic materials increases the risk of environmental pollution. Similarly use of natural products for the control of diseases in plants is considered as an alternative to synthetic pesticide due to their lower negative impacts on the environment. Besides being harmless and non-phytotoxic it has been proved that plant extracts exhibit inhibitory effect on pathogens. Several higher plants and their constituents have been successful in plant disease control and have proved to be harmless and nonphytotoxic, unlike chemical bactericides [12, 14].

The antimicrobial property resides in weed that will be an added advantage. This concept of weed was also used by man since prehistoric times and many of them used in the past for food, drug and fiber. Many of weeds would still be useful, but they have been superseded by plants of greater productivity and superior flavor [20]. Some of the antimicrobial compounds produced by weeds are affective against plant and human pathogenic microorganisms. Antimicrobial agents, including food preservatives, have been used to inhibit food borne bacteria and increase the processed food shelf life [17]. Many naturally occurring extracts from weeds, medicinal plants, and as well as from various spices have been shown to possess antimicrobial functions and could serve as a source for antimicrobial agents against food spoilage and pathogens [9].

Convolvulus arvensis, *Sonchus asper* and *Solanum nigrum* are the common weeds in Pakistan. *Convolvulus arvensis* L is a species of family Convolvulaceae, native to Europe and Asia. It is a climbing or creeping herbaceous perennial plant with glabrous or pubescent stems. It is use as a medicine for spider bites and an intestinal stimulant. Although it's antimicrobial activity has not been investigated for plant pathogens whereas its antimicrobial activity against human pathogens has been reported [19]. *Sonchus asper* L. is an annual plant with spiny leaves and yellow flowers resembling those of the dandelion. Its edible leaves make a palatable and nutritious leaf vegetable. It is used in various human disorders and contains flavonoids, glycosides, ascorbic acid and carotenoids possess antioxidant, anticancer, anti-inflammatory properties. The traditional use of *S. asper* fractions as bacteriocidal, fungicidal and phytotoxic activities are also reported earlier [9]. *Solanum nigrum* L. is a species in the *Solanum* genus and has a long history of medicinal usage such as a strong sudorific, analgesic and sedative with powerful narcotic properties. It is an important ingredient in traditional medicines against human diseases. It is used plant in oriental medicine where it is considered to be antitumorigenic, antioxidant, anti-inflammatory, hepatoprotective, diuretic, and antipyretic and is reported to possess a number of useful biological activities [7]. The present study is therefore, undertaken to test the efficacy of methanolic extract these common weed against the bacterial pathogens isolated from diseased citrus fruits *in vitro*.

MATERIALS AND METHODS

Collection of Diseased Citrus Fruits

The diversity of bacterial population was estimated in diseased fruit samples of kinnow mandarin (*Citrus reticulata*) plants. Infected citrus fruits were collected in polythene bags from different areas of Punjab, Pakistan.

Surface sterilization of Diseased Citrus Fruits

Diseased citrus fruits were washed in running tap water and graded by surface appearance in order to exclude samples that showed superficial damage. Surface sterilization was done by stepwise washing in 70% ethanol for 5 min, sodium hypochlorite solution for 5 min, and 70% ethanol for 30 s, followed by three rinses in sterile distilled water [1].

Isolation and identification of bacteria

The surface of diseased fruit was removed with a sterilized razor blade, and the inner infected tissue was cut into pieces 4 to 6 mm long, which were placed on Luria Bertani (L.B.) agar medium (g/L) plates. Incubation was carried out at 37°C for 24 hours to allow growth of endophytic bacteria from the cut pieces. In a further experiment, fragments of diseased citrus fruits were homogenized in 5 ml of sterile autoclave saline solution with a blender and serial dilutions (1ml of 10^5) were spread with sterilized spreader onto L.B. agar medium [1]. The plates were placed in an incubator at 37°C for 24 hours and distinct individual colonies purified by streaking on a new agar plate. Identification of bacterial species was done by recording phenotypic characteristics, e.g., colony morphology, colony color, cell shape, motility and growth rate. The purified colonies were subjected to gram staining and characterized using biochemical tests and consulting the pertinent literature [6, 11 and 2]. The relative abundance/frequency (%) of each bacterial isolated by dilution plating was also calculated as: (Number of colonies of a bacterial species/ total number of bacterial colonies) × 100 [13].

Weeds collection

Three common weeds were collected from of various places University of the Punjab, Lahore, Pakistan and are easily identified. Table 1 shows the list of common weeds used in this study. The leaves of all weeds stems were washed under running tap water and shade dried for three weeks. The dried leaves were then homogenized by using a grinder to make fine powder and were then extracted.

Table 1: List of weeds Selected for Anti-Microbial Activity

S. No	Botanical Name	Family	Local or Vern. name	Habitat	Flowering / Fruiting period	Parts Used
1	*Sonchus asper* (L.) Hill.	Asteraceae	Bhattar, Pili Dodak	Annual herb	Feb.-Sept.	Leaves
2	*Convolvulus arvensis* L.	Convolvulaceae	Hiran padi	Annual herb	Round the year.	Leaves
3	*Solanum nigrum* L.	Solanaceae	Kanwal. Mako.	Annual herb	July-Sept.	Leaves

Extract preparation

Thirty grams (30g) of dried weed material were exhaustively extracted for 6 hours with 300ml of solvent methanol in soxhlet extractor. Extraction was allowed to proceed for 6h. The resulting extract was concentrated over a rotary vacuum for complete solvent removal until a crude solid extract was obtained. The resulting solid masses were preserved in refrigerator at 4°C. Simultaneously, for aqueous extract preparation 30g each of the dried specific weed was soaked in 300ml distilled water for 48h at room temperature. The aqueous extract water was filtered with muslin cloth and filter and resulting crude extract was freeze-dried. The dried powder extract was kept in refrigerator until use.

Preparation of the Methanolic extracts of weeds

The final concentration of weed extracts (aqueous and methanol extract) were prepared in sterile 100% dimethylsulfoxide (DMSO) by dissolving 0.2g/ml of each extract. This crude extract was stored at 4ºC for further use. Sterile 100% DMSO served as negative control.

Determination of Antibacterial activity

The methanol leaf extracts of three weeds were screened for antibacterial activity by agar well diffusion method (Okeke *et al*., 2001) with cork borer of size 0.8 cm. For all bacterial strains, overnight cultures grown in nutrient broth were used for inoculation of the nutrient agar plates. An aliquot (0.02 ml) of inoculums was introduced to molten and cooled to 45°C nutrient agar and placed on petri plate by using sterile cotton swab technique. The appropriate wells were made on respective agar plate by using cork borer. In agar well diffusion method 60µL of methanol extracts were introduced to their respective wells following an incubation period of 24 to 48 hours at 37°C. Antibacterial activity was evaluated by qualifying inhibition zones (IZ) of bacterial growth surrounding the plant extracts. The entire antibacterial assay was carried out under strict aseptic conditions. Penicillin (5µg/disc) was used as positive control and DMSO as a negative control. Triplicates were carried out for each extract against each of the test bacterium. Antibacterial activity Index was calculated as:

Activity Index (AI) = Da/ Db – 1

Where: Da is the diameter (cm) of the growth zone in the experimental dish and Db is the diameter of the growth zone in the control dish.

Statistical evaluation

The antimicrobial activity was determined by measuring the diameter of zone of inhibition that is the mean of triplicates ± SE of three replicates.

RESULTS

Determination of bacterial species in diseased citrus fruits

The diversity of bacterial species from citrus was assessed in diseased samples of fruits collected from six different citrus-growing areas of the Punjab. To avoid contamination and to isolate endophytic bacteria only from inner fruit tissues, the fruits were peeled after surface disinfection. All samples were inoculated on sterilized plates of L.B. medium and incubated at 37°C for 24 hours. All bacterial species were identified by recording morphological and biochemical characters and consulting the pertinent literature of Bergey's Manual of Determinative Bacteriology (9th

Edition). The endophytic bacterial community that was isolated from citrus fruits included: *Bordetella pertussis*, *Arthrobecter* sp., *Xanthobacter flavus*, *Acinetobacter* sp., *Burkholderia pseudomallai*, *Ensifer adhaerens*, *Oscillospira* sp., *Xanthomonas axonopodis*, *Kurthia gibsonii*, *Xenorhabdus luminescens*, *Syntrophospora* sp., *Enterobacter agglomerans*, *Aerococcus* sp. and *Acidovorax temperans*. Out of fourteen bacterial species nine were gram negative and five were gram positive while cell shapes of three bacterial species were cocci and rest of other were rods (Table: 3). However, the frequency of endophytic bacteria recovered from diseased fruits on L.B. medium was different within species and location (Table. 3).. The frequency of species of bacteria from diseased citrus fruits from Lahore, Jhang and Sargodha was higher than that of Faisalabad, Jhelum and Bahawalnagar. Most species occurred at low frequencies throughout the study, regardless of the fruit or sampling time. *Burkholderia pseudomallai* has high frequency upto 14.8 while other bacterial species have i.e; *Ensifer adhaerens*, *Enterobacter agglomerans* and *Acidovorax temperans* (F=11.1), *Bordetella* pertussis, Xanthobacter *flavus*, *Kurthia gibsonii* and *Xenorhabdus luminescens* (F=7.40), *Arthrobecter* sp., *Acinetobacter* sp., *Oscillospira* sp., *Xanthomonas axonopodis* and *Syntrophospora* sp. (F=3.70).

Antibacterial Activity of Selected weeds

The present study with the aqueous and methanolic extracts of selected weeds gives varied results. Table 4-6 shows the results of antibacterial activity of selected weeds extracts. Fig. 1-3 shows the inhibition percentage of weed leaf extracts against different bacterial strains isolated from diseased citrus fruits.

Antibacterial activity against aqueous extract

The aqueous extracts of *S. asper* and *C. arvensis* show evidence of high antibacterial activity as compare to *S. nigrum*. *B. pertussis*, *Arthrobecter* sp., *X. flavus*, *Acinetobacter* sp., *E. adhaerens*, *X. axonopodis*, *K. gibsonii*, *X. luminescens* and *A. temperans* have least resistance against extract of *S. nigrum* ranging from 0.7-1.8cm as compare to *S. asper* and *C. arvensis* extracts with 1.2-1.6cm and 1.3 -2.5cm inhibition zone respectively. The growth of *K. gibsonii*, *E. agglomerans*, *X. luminescens Aerococcus* sp. and *A. temperans* were strongly inhibited by aqueous extract of all selected weeds ranging from 2.8-5.2cm. However, aqueous extract of *S. nigrum* was showed minimum range of percentage of inhibition as compare to other selected weeds.

Table 2: Endophytic Bacterial species isolated from Diseased Citrus Friuts Collected from Different Location of Punjab

Source	Medium Cultured on	Location	Bacterial species	No. of colonies	Frequency
Diseased fruit samples of kinnow mandarin (*Citrus reticulata*)	Luria Bertani (L.B.) agar medium (g/L)	Lahore	*Bordetella pertussis*	02	7.40
			Arthrobecter sp.	01	3.70
			Xanthobacter flavus	02	7.40
		Jhang	*Acinetobacter* sp.	01	3.70
			Burkholderia pseudomallai	04	14.8
		Sargodha	*Ensifer adhaerens*	03	11.1
			Oscillospira sp.	01	3.70
			Xanthomonas axonopodis	01	3.70
		Faisalabad	*Kurthia gibsonii*	02	7.40
			Xenorhabdus luminescens	02	7.40
		Jhelum	*Syntrophospora* sp.	01	3.70
			Enterobacter agglomerans	03	11.1
		Bahawalnagar	*Aerococcus* sp.	01	3.70
			Acidovorax temperans	03	11.1
		Total		**27**	**99.9**

Antibacterial activity against methanol extract

The methanol extract of all weeds of were found to be moderately active against all organisms tested. The range of inhibition zone diameter against *S. asper*, *C. arvensis* and *S. nigrum* extract were 1.3-4.9cm, 1.2-5.0cm and 2.0-5.9cm respectively. The *S. asper* and *S. nigrum* extracts showed high activity (5.9 cm) against *Syntrophospora* sp. in comparison of moderately active against *C. arvensis* with inhibition zone of 3.2cm. The *C. arvensis* extracts was more active against *K. gibsonii*, *Aerococcus* sp. and *A. temperans* (4.5cm, 5.0cm and 4.6cm) whereas the least activity was showed by *B. pertussis X. flavus*, *Acinetobacter* sp., *B. pseudomallai*, *E. adhaerens*, *Oscillospira* sp. and *X. luminescens* with ranging from 1.2-2.3cm of inhibition zone. The growth of *Xanthobacter flavus* (2.4cm), *Acinetobacter* sp. (2.3cm), *Ensifer adhaerens* (2.1cm) and *Xanthomonas axonopodis* (2.0cm) were moderately inhibited by the extracts of *S. asper* while *C. arvensis* extract was least active against these pathogens. *S. nigrum* and *C. arvensis* were most effective against *K. gibsonii* with percentage of inhibition zone 59 and 61 respectively. Although in case of *S. asper*, the inhibition zone percentage was exhibited moderately range from 41-48.

Table 3: An Outline of the Morphological and Biochemical Characteristic of Bacterial species Isolated from Diseased Citrus fruit

S. No	Bacterial species	M	GS	NRT	OT	UT	MRT	CUT	HSPT	CT	IT	LT	RT	GT	MT	XT	ST	AT	RFT
01	*Bordetella pertussis*	c	-	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-
02	*Arthrobecter* sp.	r	+	-	+	-	+	-	+	+	-	-	+	-	+	-	-	+	-
03	*Xanthobacter flavus*	r	-	+	-	-	-	-	+	-	+	-	-	+	-	-	-	-	-
04	*Acinetobacter* sp.	r	-	-	+	-	+	+	-	+	-	-	-	-	-	+	-	-	+
05	*Burkholderia pseudomallai*	c	-	-	-	+	-	+	+	-	-	+	-	+	-	-	-	-	+
06	*Ensifer adhaerens*	r	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
07	*Oscillospira* sp.	r	+	+	+		+	-	-	-	-	-	-	-	-	-	-	-	+
08	*Xanthomonas axonopodis*	r	-	-	-	-	-	+	+	-	-	-	+	-	+	-	-	+	-
09	*Kurthia gibsonii*	r	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
10	*Xenorhabdus luminescens*	c	-	-	-	-	-	+	-	+	-	-	+	-	-	-	-	-	-
11	*Syntrophospora* sp.	r	+	+	-	-	+	-	-	-	-	-	-	-	-	-	+	+	-
12	*Enterobacter agglomerans*	r	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-
13	*Aerococcus* sp.	c	+	+	-	-	-	-	-	-	-	-	+	+	+	-	-	+	-
14	*Acidovorax temperans*	r	-	-	-	-	+	-	+	+	+	-	-	-	-	-	-	-	+

***M:** Morphology; **G.S:** Gram stain; **S:** Spore; Mt: Motility; **P:** Pigment; **NRT:** Nitrate Reductase Test; **OT:** Oxidase Test; **UT**: Urease Test; **MRT:** Methyl Red Test; **CUT:** Citrate Utilization Test; **HSPT:** Hydrogen Sulfide Production Test; **CT:** Catalase Test; **IT:** Indole Test; **LT:** lysine Test; **RT:** Rhamnose Test; **GT:** Glucose Test; **MT:** Mannitol Test; **XT:** Xylose Test; **ST:** Sorbitol Test; **AT:** Arabinose Test; **RFT:** Raffinose Test **c:** cooci; **r:** rod; **(+):** positive reaction; **(-):** Negative reaction.*

Table 4: Antibacterial activity of *Sonchus asper* leaf extracts.

Bacterial species	Methanol extract				Aqueous Extract			
	Control (cm)	Inhibition zone (cm)	Experimental growth zone	Index	Control (cm)	Inhibition zone (cm)	Experimental growth zone	Index
Bordetella pertussis	9.0±0.0	3.0±0.02	5.6±0.04	0.37	9.0±0.0	2.1±0.01	6.8±0.05	0.24
Arthrobecter sp.	9.0±0.0	1.6±0.03	7.5±0.01	0.16	9.0±0.0	1.4±0.02	7.7±0.06	0.13
Xanthobacter flavus	9.0±0.0	2.4±0.05	6.4±0.05	0.28	9.0±0.0	2.3±0.11	6.6±0.04	0.25
Acinetobacter sp.	9.0±0.0	2.3±0.01	6.4±0.01	0.28	9.0±0.0	1.6±0.02	7.5±0.06	0.16
Burkholderia pseudomallai	9.0±0.0	1.3±0.19	5.5±0.13	0.26	9.0±0.0	2.2±0.10	5.0± 0.12	0.33
Ensifer adhaerens	9.0±0.0	2.1±0.02	3.0±0.05	0.40	9.0±0.0	1.5±0.03	3.7±0.02	0.26
Oscillospira sp.	9.0±0.0	1.9±0.02	3.7±0.21	0.32	9.0±0.0	2.2±0.10	3.2±0.30	0.41
Xanthomonas axonopodis	9.0±0.0	2.0±0.10	4.5±0.10	0.30	9.0±0.0	1.2±0.10	4.7±0.10	0.27
Kurthia gibsonii	9.0±0.0	3.0±0.01	6.0±0.02	0.34	9.0±0.0	3.0±0.01	6.0±0.03	0.33
Xenorhabdus luminescens	9.0±0.0	3.1±0.02	6.0±0.04	0.34	9.0±0.0	2.8±0.04	6.2±0.01	0.31
Syntrophospora sp.	9.0±0.0	4.9±0.01	4.7±0.01	0.48	9.0±0.0	2.2±0.03	6.8±0.01	0.24
Enterobacter agglomerans	9.0±0.0	4.1±0.03	5.1±0.02	0.43	9.0±0.0	2.5±0.02	6.5±0.01	0.27
Aerococcus sp.	9.0±0.0	3.6±0.01	4.9±0.01	0.45	9.0±0.0	3.2±0.02	5.8±0.03	0.35
Acidovorax temperans	9.0±0.0	3.6±0.01	5.4±0.01	0.40	9.0±0.0	3.5±0.01	5.5±0.05	0.38

Table 5: Antibacterial activity of *Convolvulus arvensis* leaf extracts.

Bacterial species	Methanol extract				Aqueous Extract			
	Control (cm)	Inhibition zone (cm)	Experimental growth zone	Index	Control (cm)	Inhibition zone (cm)	Experimental growth zone	Index
Bordetella pertussis	9.0±0.0	1.5±0.02	7.6±0.10	0.15	9.0±0.0	2.6±0.10	6.4±0.03	0.28
Arthrobecter sp.	9.0±0.0	2.5±0.01	6.5±0.03	0.27	9.0±0.0	3.2±0.10	5.8±0.03	0.35
Xanthobacter flavus	9.0±0.0	1.2±0.03	7.7±0.02	0.15	9.0±0.0	2.6±0.05	6.4±0.03	0.28
Acinetobacter sp.	9.0±0.0	2.1±0.05	6.9±0.03	0.23	9.0±0.0	2.1±0.10	6.9±0.03	0.23
Burkholderia pseudomallai	9.0±0.0	2.1±0.11	4.9± 0.11	0.30	9.0±0.0	2.6±0.10	4.4±0.10	0.37
Ensifer adhaerens	9.0±0.0	2.2±0.08	2.8±0.20	0.44	9.0±0.0	1.3±0.32	3.6±0.03	0.28
Oscillospira sp.	9.0±0.0	2.3±0.14	3.2±0.14	0.42	9.0±0.0	2.8±0.08	2.7±0.08	0.50
Xanthomonas axonopodis	9.0±0.0	2.7±0.06	3.7±0.03	0.43	9.0±0.0	2.1±0.16	4.4±0.12	0.32
Kurthia gibsonii	9.0±0.0	4.5±0.01	3.5±0.01	0.61	9.0±0.0	4.2±0.04	3.9±0.06	0.50
Xenorhabdus luminescens	9.0±0.0	1.8±0.02	7.2±0.01	0.20	9.0±0.0	2.5±0.03	6.5±0.05	0.27
Syntrophospora sp.	9.0±0.0	3.2±0.02	5.8±0.02	0.35	9.0±0.0	2.6±0.01	5.6±0.04	0.32
Enterobacter agglomerans	9.0±0.0	2.5±0.05	6.5±0.06	0.27	9.0±0.0	2.8±0.03	6.2±0.06	0.32
Aerococcus sp.	9.0±0.0	5.0±0.06	4.0±0.02	0.56	9.0±0.0	4.0±0.01	5.0±0.05	0.44
Acidovorax temperans	9.0±0.0	4.6±0.01	4.4±0.03	0.51	9.0±0.0	5.2±0.02	2.8±0.0.1	0.64

Table 6: Antibacterial activity of *Solanum nigrum* leaf extracts.

Bacterial species	Methanol extract				Aqueous Extract			
	Control (cm)	Inhibition zone (cm)	Experimental growth zone	Index	Control (cm)	Inhibition zone (cm)	Experimental growth zone	Index
Bordetella pertussis	9.0±0.0	3.3±0.10	5.6±0.03	0.37	9.0±0.0	0.7±0.05	8.2±0.03	0.08
Arthrobecter sp.	9.0±0.0	3.5±0.06	5.5±0.03	0.37	9.0±0.0	1.6±0.06	7.4±0.03	0.17
Xanthobacter flavus	9.0±0.0	3.1±0.05	5.9±0.03	0.34	9.0±0.0	1.4±0.05	7.6±0.03	0.14
Acinetobacter sp.	9.0±0.0	3.2±0.11	5.8±0.03	0.35	9.0±0.0	0.9±0.08	8.1±0.03	0.09
Burkholderia pseudomallai	9.0±0.0	2.9±0.08	4.1±0.08	0.41	9.0±0.0	2.7±0.11	4.3±0.11	0.38
Ensifer adhaerens	9.0±0.0	2.2±0.20	2.8±0.17	0.44	9.0±0.0	1.2±0.23	3.8±0.05	0.24
Oscillospira sp.	9.0±0.0	2.4±0.15	3.2±0.03	0.42	9.0±0.0	2.0±0.23	3.5±0.23	0.36
Xanthomonas axonopodis	9.0±0.0	2.0±0.02	4.5±0.05	0.30	9.0±0.0	1.5±0.26	5.0±0.26	0.23
Kurthia gibsonii	9.0±0.0	5.0±0.01	4.0±0.05	0.59	9.0±0.0	1.3±0.01	7.8±0.01	0.14
Xenorhabdus luminescens	9.0±0.0	4.8±0.02	4.2±0.06	0.53	9.0±0.0	1.3±0.02	7.8±0.04	0.14
Syntrophospora sp.	9.0±0.0	5.9±0.03	3.1±0.06	0.66	9.0±0.0	2.3±0.04	6.7±0.04	0.26
Enterobacter agglomerans	9.0±0.0	3.8±0.05	5.2±0.07	0.42	9.0±0.0	5.2±0.05	3.8±0.08	0.57
Aerococcus sp.	9.0±0.0	3.5±0.06	5.5±0.03	0.38	9.0±0.0	4.0±0.01	5.0±0.03	0.45
Acidovorax temperans	9.0±0.0	4.0±0.07	5.0±0.02	0.44	9.0±0.0	1.8±0.03	7.2±0.01	0.20

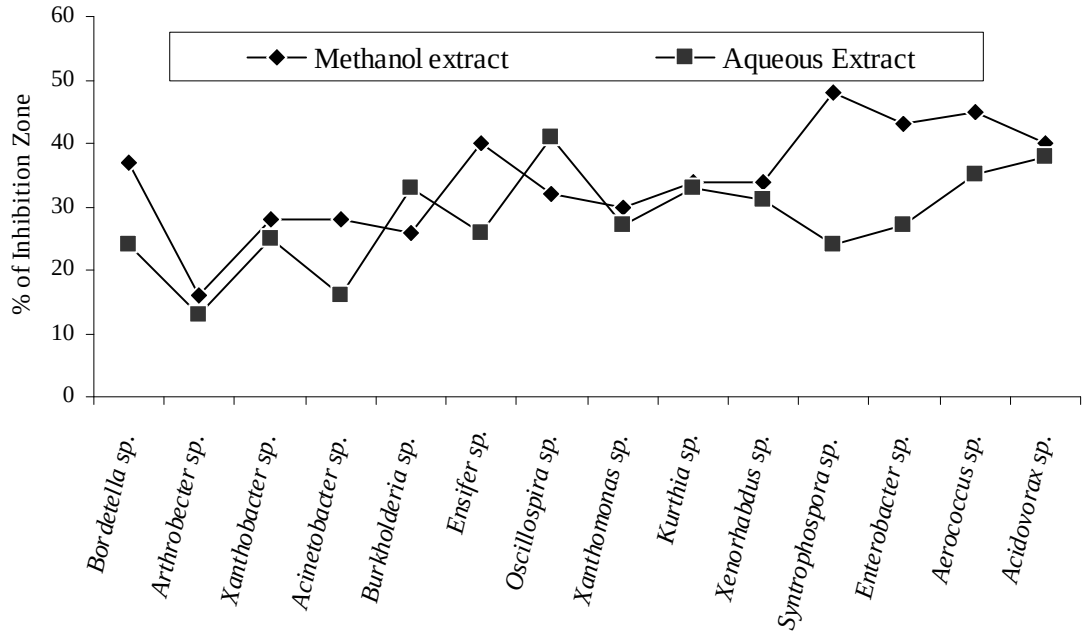

Figure. 1: % of inhibition Zone of *Sonchus asper* leaf extracts on Bacterial strains isolated from Diseased Citrus Fruits

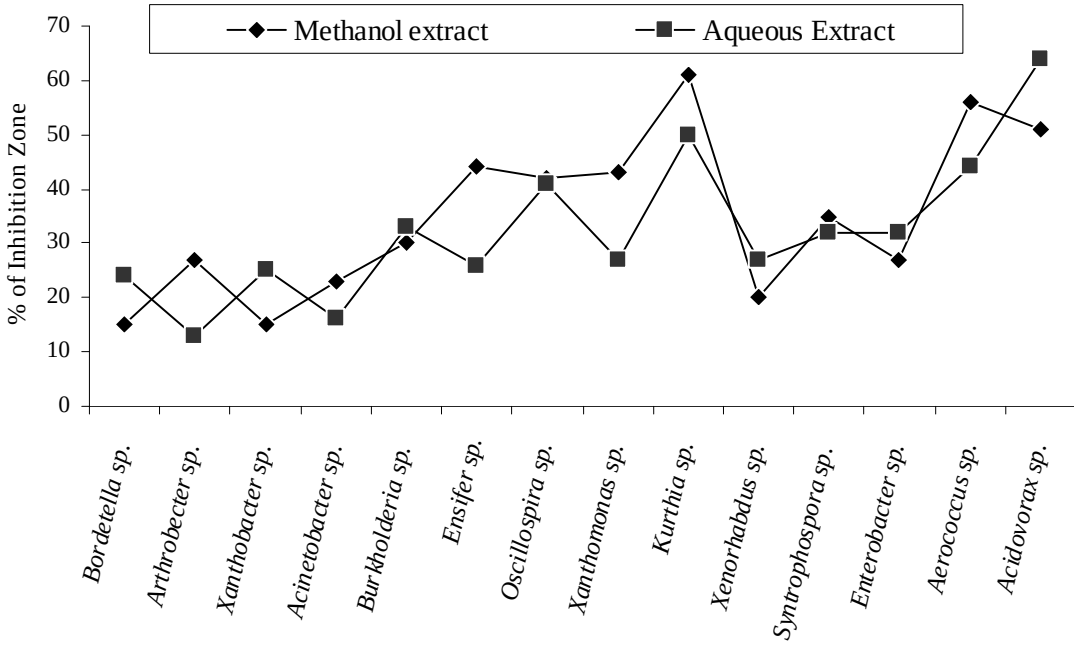

Figure. 2: % of inhibition Zone of *Convolvulus arvensis* leaf extracts on Bacterial strains isolated from Diseased Citrus Fruits

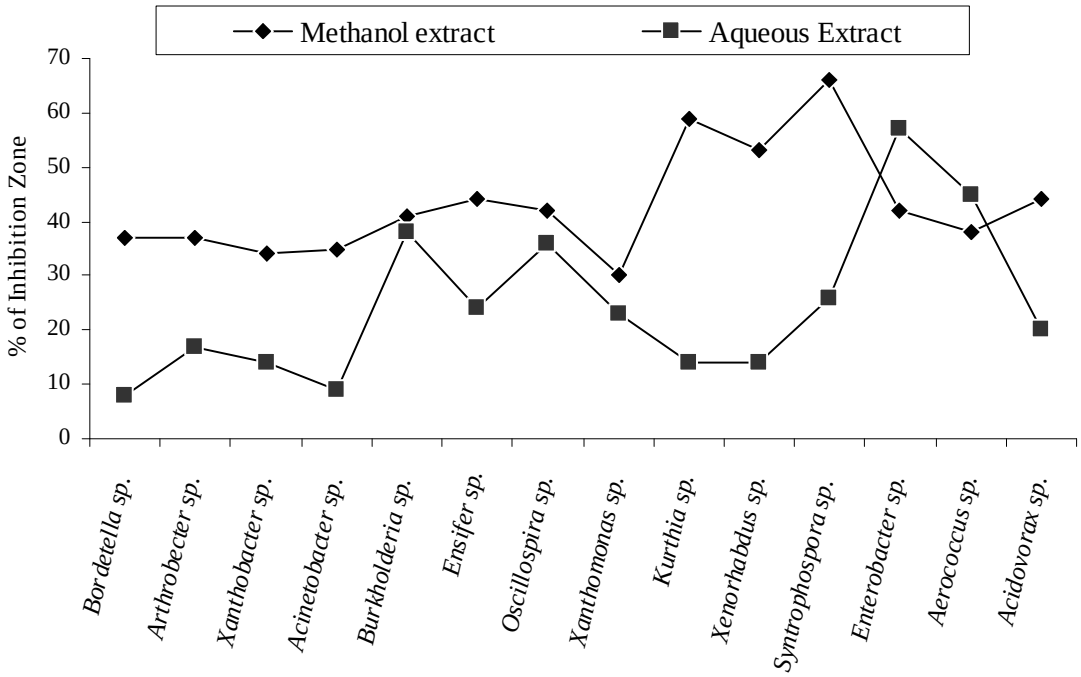

Figure. 3: % of inhibition Zone of *Solanum nigrum* leaf extracts on Bacterial strains isolated from Diseased Citrus Fruits

DISCUSSION

Microorganisms are associated, in a variety of ways with all the food we eat. They may influence the quality availability, and quantity of our food. Naturally occurring foods such as fruits and vegetables normally contain some microorganisms and may be contaminated with additional organisms during handling. Therefore different disease problems arise when crops are harvested, because seed, fruit or other storage organs are essentially dormant structures and their cells are physiologically unlike those of growing plant [3]. Fruits are infected easily with bacterial pathogens by the principle of spread of bacterial infection in fruits supports that a single infected citrus fruit can be the source of infection to other fruits during storage and on transit [8]. Thus recently scientist have focused to increase the crop production to meet the needed of world population, but unfortunately, crop yield losses every year due to plant diseases caused by various pathogens and slow biodegradation of herbicides. To control these shortcomings researchers have focused on allelo-chemicals and bio-herbicides produced by plants themselves. Consequently, testing the antimicrobial activity of plants remains an area of intense interest and few studies are available on the antiviral, antifungal, antibacterial, antihelmintic, antimolluscal, and anti-inflammatory properties of plant [14, 20]. However reports on the exploitation of antimicrobial property of weeds are scanty whereas search of natural herbicides from the plant sources would definitely be a better alternative to hazardous chemicals. The antibacterial activities of *C. arvensis*, *S. asper* and *S. nigrum* have been studied previously. Detailed studies of their leaf extracts are lacking, however, some have been individually sporadically tested among other afflictions. Despite the wide use of biocontrol potential of these weeds, detailed knowledge and studies are scarce except for some preliminary reports. In an antibacterial studies Parameswari et al., (2012); Manu and Manav (2011); Jimoh et al., (2011); Sridhar et al., (2011) and Sheeba (2010) they were reported that these weeds may be considered as a potential source of natural herbicides. Among weeds the leaf extract of *C. arvensis* was very active and inhibited the growth of all tested bacterial pathogens except *B. pertussis*, *X. flavus* and *X. luminescens*. Previous studies revealed that *C. arvensis* showed an allelopathic and antioxidant activity while it also contained tropane alkaloids and phenolic compounds [5]. The biochemical investigation reported that *S. asper* constitute of antioxidant compounds such as carotenoids, catechin, rutin, quercetin and other phenolics. Moreover, its activities against oxidative stress, antibacterial and antitumor were yet to be explored [10]. The antibacterial activities of *S. nigrum* further lend credence to the biological value thus; it can be accomplished that their leaf extract can contribute significantly to the biocontrol of plant diseases. Zubair et al., (2011) reported the antimicrobial activities against four types of bacteria

and three fungus of methanolic extract. The leaf extract of *S. nigrum* inhibited the growth of pathogenic microorganisms and showed varying degree of inhibitory effects. Khan et al., (2012) evaluated the antibacterial activity of various fractions of *S. asper* against six bacterial species i.e; *Escherichia coli*, *Bacillus subtilis*, *Micrococcus luteus*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Thus present experiment demonstrates that the leaf extracts of these weeds exhibit antibacterial effect, which offers a scientific basis for using these weeds as a good sources of biocontrol agent against plant diseases. Further work is however required to be done for its formulation.

CONCLUSION

It can be concluded that the methanolic extracts of the tested weeds exhibited varying degrees of antibacterial activity against bacterial species isolated from diseased citrus fruits. The diameter of inhibition zones of some extract showed superior activities although some values tended to be relatively low for some bacteria. Results validate the use of these weeds in the biological control of plant diseases, however further studies are needed to identify the active ingredients. This study is also needs further study to isolate and purification of bioactive compounds responsible for the antimicrobial activity.

REFERENCES

[1] Araujo, L., Joelma, M., Walter, M., Jan, D.E., Jim, W.L., Vuurde, K., Joao, L.A. *Appl. Env. Microbiol*., **2002,** 68(10): 4906–4914.
[2] Benson, H.J; Microbiological applications, laboratory manual in general microbiology. Dubuque, U.S.A: W. M. C. Brown Publishers. **1996**.
[3] Bukar, A., Mukhtar, M.D., Adamu, S. Bayero *J. Pure and Appl. Sci.*, **2009**, 2(1): 122 –124.
[4] Burhan, M., Sahi, S.T., Ahmad, S. *Pak. J. Bot*., **2007**, 39(5): 1867-1871.
[5] Elzaawely, A., Tawata, S. *Asian J. Crop Sci*., **2012**, 4(1): 32-40.
[6] Holt, J.G., Krieg, N.R., Sneath, P.H.A., Staley, J.T., Williams, S.T; Bergey's Manual of Determinative Bacteriology, 9th ed. Williams & Wilkins, Baltimore, **2000**.
[7] Jain, R., Sharma, A., Gupta, S., Sarethy, I.P., Gabrani, R. *Altern Med Rev.*, **2011**. 16(1): 78-85.
[8] Jay, J.M; Microbial Spoilage of Food. Modern Food Microbiology. 4th ed. Chapman and Hall Inc. New York, **2003**.
[9] Khan, R.A., Khan, M.R., Sahreen, S., Bokhari, J. *African J. Biotechnol*., **2010**, 9(25): 3883-3887.
[10] Khan, R.A., Muhammad, R., Sumaira, S., Mushtaq, A. *Chemistry Central Journal*, **2012**, 6: 1-7.
[11] Koneman, E.W., Allen, S.D., Janda, W.M., Schreckenberger, P.C., Winn, W.C; Atlas and textbook of diagnostic microbiology. 5th ed. Philadelphia, USA: Lippincott Williams and Wilkins, **1997**.
[12] Malan, R., Walia, A., Saini, V., Gupta S. *Eur. J. Exp. Bio*, **2011**, 1(2): 33-40.
[13] Manu, A., Manav, M. *Pharmacologyonline*, **2011**, 3: 1296-1305.
[14] Mukhtar, I., Khokhar, I.., Mushtaq, S., Ali, A. *Pak. J. Weed Sci. Res.*, **2010**, 16(3): 287-297.
[15] Mushatq, S., Haider, M.S., Ali, A., Javed, S., Khokhar, I., Mukhtar, I. *Pak. J. Weed Sci. Res.*, **2012**, 18(1): 15-25.
[16] Okeke, M.I., Iroegbu, C.U., Eze, E.N., Okoli, A.S., Esimone, C. O. *J Ethnopharmacol.*, **2001**, 78: 119-127.
[17] Parameswari, K., Sudheer, A., Kishori, B. *Indian Streams Res. J.*, **2012**, 2(7): 1-4.
[18] Peraman, M.K., Ramalingam, P., Bapatla S. *Eur. J. Exp. Bio*, **2011**, 1(2):172-177.
[19] Sheeba, E. *J. Sci. Eng. Technol.*, **2010**, 6(1): 1-4.
[20] Sridhar, T.M., Josthna, P. Naidu, C.V. Medicinal Plant. *J. Experimental Sci*., **2011**, 2(8): 24-29.
[21] Srivastav, S., Pradeep, S., Jha, K., Mishra, G., Shruti, S.M., Karchuli, S., Khosa, R.L. *Eur. J. Exp. Bio*, **2011**, 1(2): 97-102.
[22] Stone, A.E., Peeper, T.F. Kelley, J.P. *Weed Technol*., **2005**, 19: 148-153.
[23] Tahir, M., Tahir, S.S., Ahmed, B., Arain, M.A. *Pak. J. Bot*., **2008**, 40(1): 65-70.
[24] Zubair, M., Komal, R., Nasır, R., Nosheen, A., Muhammad, S., Viqar, A. *Int. J. Phytomedicine*, **2011**, 3: 63-67.