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In-vitro evaluation of antimicrobial potential of some plant parts extracts targeting Ralstonia solanacearum, isolated from seeds of soybean

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Abstract - *Ralstonia solanacearum is a pathogen which infects many vegetables and some bean crops. It has been an enormously threatening hazard from previous times. It is primarily a soil borne bacterium now becoming versatile in infectivity due to its genetic diversity to persist in the environment and virulence factors which can evade the host immune system. Chemical pesticides are used since ages which led their precipitation in soil making it less fertile for crops. Use of botanicals for the control of either human or plant pathogens became a prioritized field of research to make the sustainable environment friendly pesticides. Different parts of 12 plants namely, Citrus limetta, Rosa indica,* Foeniculum vulgare*, Leptadenia pyrotechnica, Tamarindus indica,* Bougainvillea spectabilis*, Mangifera indica, Citrus sinensis, Punica granatum, Cordia dichotoma and Curcuma longa were tested for their activity against R. solanacearum. In aqueous extracts, P. granatum followed by R. indica, M. charantia, M. indica and B. spectabilis were effective while methanolic extract of C. longa was most effective followed by T. indica, P. granatum, F. vulgare, R. indica, M. charantia, Mangifera indica, B. spectabilis. Extracts of these plants significantly improved the seed germination and control the pathogen on seed treatment.*

Key Words: aqueous, disc diffusion method, infectivity, methanolic extract, pesticides, seed treatment, Soil-borne

1. INTRODUCTION

Ralstonia solanacearum Smith and Yabuuchi et al., generally a soil borne bacteria is one of the most life-threatening plant pathogens affecting variety of vegetable, pulse and other crops with a survival capacity extended upto several hundred years resisting different environmental conditions (1). Severity and yield loss is variable for countries but it has been found that potato producing countries are affected more due to the Ralstonia species complex (RSSC). It is a complex of pathogenic strains that can spread via soil, infected potato seeds, run-off water, plant debris, or contaminated tools used for farming and propagation (2). It has emerged as a notable study model for phytopathogenic bacteria due to its enormous genetic variability, persisting diverse environmental conditions, wide variety of hosts and most importantly its significant effect on agriculture. The infection is initiated through wounds (root) or openings followed by rapid colonization of xylem vessels which leads to biofilm formation that causes vascular impairment. The pathogen employs several factors such as extracellular pathogenic factors and intracellular type III effectors (T3Es) to invade and multiply in the host tissues (cell wall degradation by lytic enzymes and effector proteins for spread). Evasion and disruption of effector triggered immunity (3) is driven through type III secretion system leads to the systemic spread. Activation of stress related hormones and defence pathways are manipulated by the pathogen followed by progression of disease and low productivity (4).

Plants, in the ancient times were being used for the cure of different diseases and now a days, are preferred over chemically synthesized drugs due to their minimum side effect and also serve as the sources of alkaloids to formulate medicines in for deadly diseases also (5-8). Herbal extracts had been proved to be beneficial for control of phytopathogenic bacteria viz. X. campestris, P. syringae, R. solanacearum, C. michiganesis, E. caratovora etc. (9,10). Plants under the present study (Sweet lime, fennel, bougainvillea, turmeric, bitter gourd, mango, rose, khimp, orange, tamarind, pomegranate and indian cherry) are known for their antimicrobial, anti-inflammatory, cytotoxic and antiseptic activities (11-13). Due to the harmful effects and beneficial effects of plants, 12 selected plant extracts are used for the evaluation of their efficacy against the pathogen (2 isolates from seeds of soybean in the fields of Rajasthan, India) to combat bacterial wilt with enhancing sustainable agriculture.

MATERIALS AND METHODS

The bacterial cultures were isolated from the seeds of soybean using semi-selective TZC agar media (14) and identified by chemical as well as 16S rDNA sequencing (Genbank Accn. No. KX954589). Plant extracts (crude) were prepared using deionized water (aqueous) and methanol (methanolic) as solvents and diluted to various concentrations for measuring MIC (Minimum Inhibitory Concentration) of extracts (15-16). Bio-efficacy was evaluated using disc diffusion method (17,18) followed by the determination of seed germination efficiency of the

extracts was also checked by using seed treatment method (19).

Formulation of Plant Extracts

For the present study, various parts of plants having medicinal value, evaluated for their antagonistic properties are listed in the table.

Table.1. Detailed list of plant parts used for evaluation.

S. no.	Common name	Botanical name	Plant part used	Family
1	Sweet lime	Citrus limetta Risso	Fruit peel	Rutaceae
2	Rose	Rosa indica L.	Red petals	Rosaceae
3	Pomegranate	Punica granatum L.	Fruit peel	Punicaceae
4	Bougainvillea	Bougainvillea Spectabilis Willd	Flower with Bract	Nyctaginaceae
5	Khimp	Leptadenia pyrotechnica Forssk. Decne	Stem with leaves	Asclepiadaceae
6	Mango	Mangifera indica Wall.	Seed kernel	Anacardiaceae
7	Orange	Citrus sinensis Pers.	Fruit peel	Rutaceae
8	Indian cherry	Cordia dichotoma G.Forst.	Fruit	Boraginaceae
9	Tamarind	Tamarindus indica L.	Leaves	Fabaceae
10	Bitter gourd	Momordica charantia L.	Fruit Peel	Cucurbitaceae
11	Turmeric	Curcuma longa L.	Rhizome	Zingiberaceae
12	Fennel	Foeniculum vulgare Mill.	Inflorescence	Apiaceae

A. Aqueous Extract preparation (cold extraction):

Thoroughly washed parts of plants were taken to grind or crush, mixed with sterile distilled water (1:1 w/v) and filtered through sterilized double layered cheese cloth. The filtrate was taken and used as the extract of 100% conc. Further, dilutions of 20, 40, 60 and 80% conc. were made using sterile distilled water. The cold extraction was preferred to avoid the degradation of alkaloids due to high heat.

B. Methanolic Extracts: Cold extraction method was used for the preparation of methanolic extract. In 100ml of 95% methanol 10g of dried leaves powder was dissolved and incubated for 24-48 hrs in shaker incubator at 190-220 rpm. The suspension was then filtered using Whatman No.1 filter paper. After that methanol was evaporated and this dried extract was then stored at 4ºC under dry conditions (15). The used concentrations of methanolic extract were 10, 20, 30, 40 and 50 mg/ml. These concentrations were prepared by dissolving the extract in sterilized distilled water.

Evaluation of efficacy: Bacterial cultures were prepared by inoculating loopfull of 24 hr. old bacterial culture from identified isolate accn. No. KX954589 into nutrient broth and incubated at 28±2°C. After 24 hrs, the culture (100µl) was inoculated from the broth and spread onto nutrient agar plates. Discs of 5mm diameter were prepared with Whatmann no. 1 filter paper for disc diffusion method. Discs were soaked for an hour into various concentrations of extracts (Aqueous and methanolic), air dried in aseptic conditions and placed on inoculated nutrient agar plates. Discs of autoclaved distilled water and methanol (95%) were taken as control for aqueous and methanolic extracts respectively. The plates were further incubated at 28±2°C for 24-48h and inhibition zone was measured after evenly scattered colonization of the bacteria. Inhibition annulus was calculated using following formula:

$$\text{Inhibition Annulus (IA)} = \pi (R_1\text{-}R_2) (R_1+R_2)$$

Where R_1 = Radius of inhibition zone + radius of filter paper disc

R_2 = Radius of filter paper disc and
$\pi = 3.14$

Minimum Inhibitory Concentration (MIC): For the determination of minimal concentration of the extract which can inhibit microbial growth, 5 concentrations of each extract were taken. In aqueous extracts, concentrations taken were 5, 10, 15, 20 and 25mg/ml while in methanolic extracts 2.5, 5, 7.5, 10 and 12.5mg/ml concentrations were used for evaluation.

Seed Treatment Method: Seed samples (Accn. No. KARJ72 and KARJ90) which were naturally infected with the pathogen were taken for the seed treatment method. Seeds (100 seeds into 3 replicas) were soaked into selected extracts solutions (water as solvent in both extracts) for a period of 24h, seeds soaked into distilled water were used as control. Later the seeds were subjected to standard blotter test (19) for evaluation of germination effect. After 7 days, germination of seeds, percent seed germination and percent control of the pathogen was observed. Percent control of the pathogen was calculated by the following formula:

%c= Percent control; C= Incidence in check; T= Incidence in treated

$$\%c = \frac{(C) - (T)}{(C)} X100$$

RESULTS AND DISCUSSION

In aqueous extracts, P. granatum (IA 405.33 and 388.45mm 2) was found to be the most effective extract against the pathogen (ac.nos. KX954589 and MG489969) followed by R. indica (IA 183.01 and 194.96mm 2), M. charantia (IA 184.70 and 176.34mm 2), M. indica (IA 176.34 and 171.41mm 2), B. spectabilis (IA 166.55 and 161.41mm 2), T. indica (IA 36.65 and 60.98mm 2), L. pyrotechnica (IA 36.65 and 47.78mm 2) and C. sinensis (IA 24.92 and 36.65mm 2) at 100% concentration. All extracts were significant at 1% (P≥0.01) (Fig. 1). minimum inhibitory concentration of P. granatum (2% for accn. nos. KX954589 and MG489969) was found to be the least amongst all followed by R. indica (3%) and B. spectabilis (5%) for both 20 isolates (Table 2).

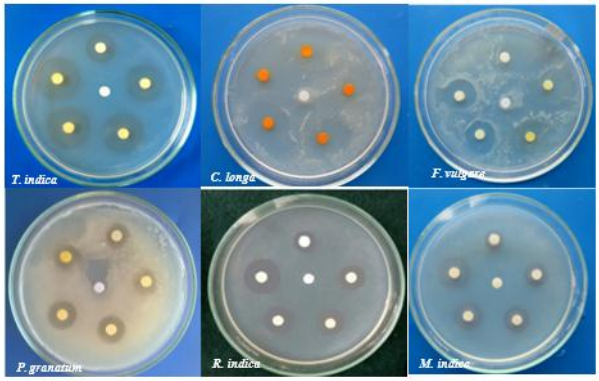


Fig. 1. Inhibitory activity by aqueous extracts of plant parts

Table 2: Evaluation of bio-efficacy of selected aqueous plant extracts against *R.* solanaceraum through disc diffusion method.

S. No.		Sample Ac. No. KX954589									
		20mg/ml**		40mg/ml**		60mg/ml**		80mg/ml**		100mg/ml**	
		R(mm)	IA(mm²)	R(mm)	IA(mm²)	R(mm)	IA(mm²)	R(mm)	IA(mm²)	R(mm)	IA(mm²)
1	*P. granat*	9.77	279.89	10.30	313.50	10.70	339.87	11.20	374.26	11.40	388.45
2	*M. charen*	5.17	64.20	5.70	82.39	6.37	107.65	7.37	150.78	8.07	184.70
3	*R. indica*	5.23	66.37	6.27	103.69	6.87	128.43	7.40	152.32	8.03	183.01
4	*M. indica*	5.30	68.58	5.93	90.92	6.30	105.00	6.80	125.57	7.90	176.34
5	*B. spectabil*	4.83	53.73	5.33	69.69	6.17	99.78	6.90	129.87	7.70	166.55
6	*L. pyrrotech*	1.97	-7.48^{NS}	2.70	3.27	3.23	13.20	3.67	22.59	4.23	36.65
7	*C. sinensi*	2.30	-3.01 ^{NS}	2.87	6.18	3.20	12.53	3.60	21.07	4.23	36.65
8	*T. indic*	2.47	-0.52 ^{NS}	3.23	13.20	3.37	15.97	3.23	13.20	3.77	24.92
	SEM		0.10		0.08		0.07	0.08			0.07
	CD 5%		0.31		0.23		0.22	0.24			0.21
	CD 1%		0.43		0.32		0.31	0.34			0.30

Methanolic extract of C. longa (IA 410.21 and 374.26mm²) was found to be most effective followed by T. indica (IA 369.58 and 346.62mm²), P. granatum (IA 216.22 and 230.96mm²), F. vulgare (IA 214.41and 223.54mm²), R. indica (IA 209.02 and 212.61mm²), M. charantia (IA 205.46 mm² for both isolates), M. indica (IA 129.87 and 140.14mm²), B. spectabilis (IA 99.78 and 86.00mm²), C. sinensis (IA 64.20 and 67.47mm²), C. limetta (IA 50.72 and 47.78mm²) and L. pyrotechnica (IA 49.74 and 40.25mm²) at 50mg/ml concentration. All extracts were significant at 1% (P≥0.01) (Fig.2). In methanolic extract, C. longa and P. granatum were found to be effective at the least conc. of 1mg/ml followed by T. indica (1.5mg/ml), R. indica, F. vulgare (2mg/ml), M. indica and M. charantia (2.5mg/ml) for both isolates (Table 3).

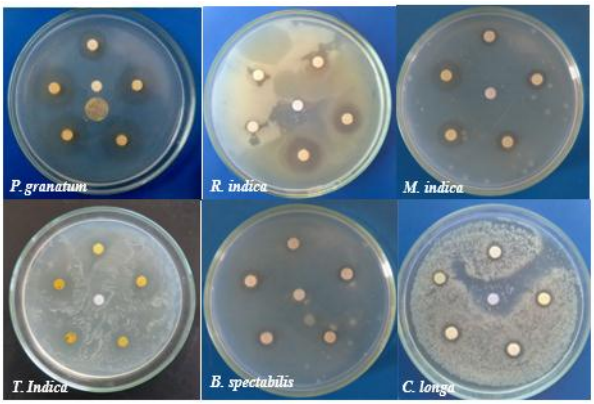


Fig. 2. Inhibitory activity by methanolic extracts of plant parts

Table 3: Evaluation of bio-efficacy of selected methanolic plant extracts against *R.* solanacearum through disc diffusion method.

S. No.	Plant Name	Sample Ac. no. KX954589									
		10mg/ml**		20mg/ml**		30mg/ml**		40mg/ml**		50mg/ml**	
		R (mm)	IA (mm^2)	R (mm)	IA (mm^2)	R (mm)	IA	R (mm)	IA (mm^2)	R (mm)	IA (mm^2)
1	C. longa	6.67	119.93	7.80	171.41	8.67	216.2	10.43	322.18	11.70	410.21
2	T. indic	7.30	147.71	8.77	221.70	9.03	236.6	10.23	309.20	11.13	369.58
3	P. granat	6.43	110.33	7.27	146.18	7.83	173.05	8.30	196.69	8.67	216.22
4	F. vulga	4.70	49.74	6.00	93.42	7.40	152.3	8.00	181.34	8.63	214.41
5	R. indic	4.93	56.80	6.03	94.67	6.73	122.7	7.63	163.34	8.53	209.02
6	M. charen	5.73	83.59	6.67	119.93	7.40	152.32	7.80	171.41	8.47	205.46
7	M. indic	4.83	53.73	5.90	89.68	6.17	99.78	6.27	103.69	6.90	129.87
8	B. spectabil	3.27	13.88	3.90	28.13	4.80	52.72	5.60	78.85	6.17	99.78
9	C. sinens	2.83	5.58	3.20	12.53	3.90	28.13	4.70	49.74	5.17	64.20
10	C. limet	2.50	0.00	3.43	17.39	3.97	29.78	4.20	35.76	4.73	50.72
11	L. pyrrotech	3.10	10.55	3.57	20.32	3.67	22.59	4.17	34.89	4.70	49.74
	SEM	0.07		0.09		0.09		0.10		0.10	
CD at 5%			0.20		0.27	0.26			0.30		0.29
CD at 1%			0.27		0.37	0.36			0.42		0.39

Values are the mean of three replicates; R – radius of inhibition zone; IA- inhibition annulus. ** Significant at 1% (P≥0.01)

In aqueous extracts, minimum inhibitory concentration of P. granatum (2% for ac.nos. KX954589 and MG489969) was found to be the least amongst all followed by R. indica (3%) and B. spectabilis (5%) for both isolates. In methanolic extract, C. longa and P. granatum were found to be effective at the least conc. of 1mg/ml followed by T. indica (1.5mg/ml), R. indica, F. vulgare (2mg/ml), M. indica and M. charantia (2.5mg/ml) for both isolates (Table 4 & 5).

Table 4: Evaluation of MIC of aqueous plant extracts against R. solanacearum.

S.no.	Plant name	Sample Ac. No. KX954589 Concentrations in %									
		1	2	3	4	5	5	10	15	20	25
1	P. granatum	-	+	+	+	+	+	+	+	+	+
2	M. charantia	-	-	-	-	-	-	-	+	+	+
3	R. indica	-	-	+	+	+	+	+	+	+	+
4	M. indica	-	-	-	-	-	-	-	+	+	+
5	B. spectabilis	-	-	-	-	+	+	+	+	+	+
6	C. sinensis	-	-	-	-	-	-	-	-	+	+

Table 5: Evaluation of MIC of methanolic plant extracts against *R.* solanacearum.

S. no.	Plant name	Sample Ac. No. KX954589 Concentrations in mg/ml								
		.5	1	1.5	2	2.5	5	7.5	10	12.5
1	P. granatum	-	+	+	+	+	+	+	+	+
2	M. charantia	-	-	-	-	-	-	+	+	+
3	R. indica	-	-	+	+	+	+	+	+	+
4	M. indica	-	-	-	-	-	-	+	+	+
5	B. spectabilis	-	-	-	-	+	+	+	+	+
6	C. sinensis	-	-	-	-	-	-	-	+	+

+ = presence of zone of inhibition; - = For no zone of inhibition

Seed treatment method: For percent seed germination and percent control of pathogen, five plant aqueous extracts were evaluated for their effect on seed germination using standard blotter method. All extracts had increased germination in seed samples (Ac. nos. KARJ72 and KARJ90 respectively) as compared to the check (50.0 and 55.0%). *R. indica* extract showed maximum percent seed germination (97.7 and 95.3%) at 100% concentration which was found significant at 1% (P≥0.01). In methanolic extracts, all plants increased the seed germination as compared to check (56.3 and 52.7%). *C. longa* showed maximum seed germination (97 and 94.7%) at 50mg/ml (P≥0.01) (Table 6&7: Fig.3,4).

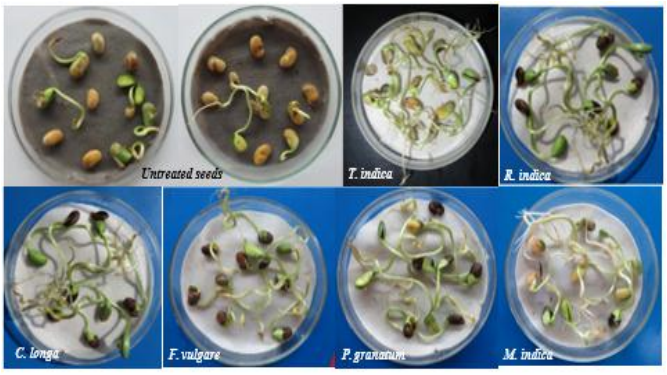


Fig.S3. Effect of aqueous extracts on visually discolored seeds of soybean

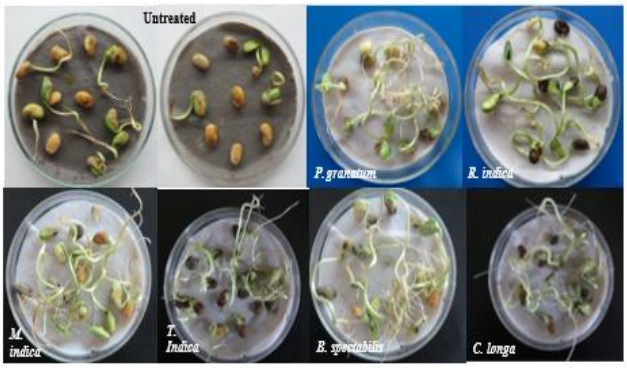


Fig.S4. Effect of methanolic extracts on visually discolored seeds of soybean

Table 6: Evaluation of effect of selected aqueous plant extracts on soybean seed germination through seed treatment method.

S. No.	Plant extracts used	Conc. Mg/ml (w/v)	Seed Samples Sample Ac. no. KARJ 72		
			Seed germination ** (%)	Incidence of pathogen (%)	Control of pathogen ** (%)
	Check	-	50.0 (45.00)	94.1 (75.94)	0 (0.00)
1	P. granatum	20	62.0 (51.94)	84.7 (66.97)	9.4 (17.85)
		60	76.7 (61.14)	66.0 (54.33)	28.1 (32.01)
		100	94.7 (76.69)	27.7 (31.76)	66.4 (54.57)
2	M. indica	20	62.7 (52.36)	77.7 (61.82)	16.4 (23.89)
		60	77.0 (61.34)	67.6 (55.30)	26.5 (30.98)
		100	89.3 (70.91)	36.3 (37.05)	57.8 (49.49)
3	R. indica	20	69.0 (56.17)	72.3 (58.24)	21.8 (27.83)
		60	81.3 (64.38)	53.3 (46.89)	40.8 (39.70)
		100	97.7 (81.28)	25.5 (30.33)	68.6 (55.92)
4	B. spectabilis	20	57.7 (49.43)	92.0 (73.57)	2.1 (8.33)
		60	63.3 (52.71)	78.2 (62.17)	15.9 (23.50)
		100	73.0 (58.69)	46.4 (42.94)	47.7 (43.68)
5	M. charentia	20	67.0 (54.94)	81.3 (64.38)	12.8 (20.96)
		60	73.3 (58.89)	65.6 (54.09)	28.5 (32.27)
		100	85.0 (67.21)	32.9	61.2

S. No.	Plant extracts used	Conc. Mg/ml (w/v)	Seed Samples		
			Sample Ac. no. KARJ 72		
			Seed germination ** (%)	Incidence of pathogen (%)	Control of pathogen ** (%)
				(35.00)	(51.47)
	S. Em.		0.38	0.44	0.39
	CD at 5%		1.10	1.27	1.13
	CD at 1%		1.47	1.71	1.53

Table 7: Evaluation of effect of selected methanolic plant extracts on soybean seed germination through seed treatment method.

S. No.	Plant extracts used	Conc. (mg/ml)	Seed Samples		
			Ac. No. KARJ 72		
			Seed germination**(%)	Incidence of pathogen (%)	Control of pathogen**(%)
	Check	-	56.3 (48.62)	96.0 (78.46)	0 (0.00)
1	**P. granatum**	10	68.3 (55.73)	81.1 (64.23)	14.9 (22.71)
		30	77.7 (61.82)	65.0 (53.73)	31.0 (33.83)
		50	95.0 (77.08)	22.2 (28.11)	73.8 (59.21)
2	**M. indica**	10	65.3 (52.91)	87.8 (69.56)	8.2 (16.64)
		30	74.3 (59.54)	69.4 (56.42)	26.6 (31.05)
		50	87.0 (68.87)	33.1 (35.12)	62.9 (52.48)
3	**Rosa indica**	10	69.0 (56.17)	84.1 (66.50)	11.9 (20.18)
		30	80.7 (63.94)	62.9 (52.48)	33.1 (35.12)
		50	93.7 (75.46)	24.1 (29.40)	71.9 (57.99)
4	**T. indica**	10	62.0 (51.94)	86.0 (68.03)	10.0 (18.44)
		30	75.0 (60.00)	72.4 (58.31)	23.6 (29.06)
		50	87.3 (69.12)	41.1 (39.87)	54.9 (47.81)
5	**B. spectabilis**	10	67.0 (54.94)	91.7 (73.26)	4.3 (11.97)
		30	73.3 (58.89)	75.0 (60.00)	21.0 (27.28)
		50	81.7 (64.67)	58.3 (49.78)	37.7 (37.88)
6	**C. longa**	10	75.3 (60.27)	77.0 (61.34)	19.0 (25.84)
		30	88.0 (69.73)	62.4 (52.18)	33.6 (35.43)
		50	97.0 (80.02)	22.1(28.04)	73.9 (59.28)
7	**M. charentia**	10	71.7 (57.86)	86.7 (68.61)	9.3 (17.76)
		30	83.7 (66.19)	66.3 (54.51)	29.7 (33.02)
		50	90.0 (71.56)	34.4 (35.91)	61.6 (51.71)
8	**F. vulgare**	10	73.0 (58.69)	79.7 (63.22)	16.3 (23.81)
		30	86.3 (68.28)	57.0 (49.02)	39.0 (38.65)
		50	93.7 (75.46)	26.3 (30.85)	69.7 (56.60)
9	**C. limetta**	10	63.0 (52.53)	91.7 (73.26)	4.3 (11.97)
		30	71.0 (57.42)	58.0 (49.50)	38.0 (38.06)
		50	84.3 (66.66)	47.3 (43.45)	48.7 (44.25)
	S. Em.		0.42	0.26	0.46
	CD at 5%		1.18	0.73	1.31
	CD at 1%		1.58	0.97	1.75

It was found the rich phytoconstituent amount in tamarind which is responsible for various bioactivities (20). Studies had shown significant antibacterial activity of T. indica ethanolic extract against clinically important bacterial species (21-24). The rose petal extract was examined against Staphylococcus aureus, Bacillus cereus (gram-positive) and E. coli, Salmonella typhi (gram-negative) bacteria (25,26). Rose petal extract has antifungal properties, zone of inhibition was found to be less than that of the antifungal ketoconazole but more efficient against Penicillium spp. when compared to Aspergillus niger and Candida albicans which makes rose a potent agent to be used to treat fungal infections (27).

significant antibacterial activity of pomegranate fruit peel and anthocyanin-rich baugainvillia flowers extracts against some human pathogenic gram positive and negative strains was observed (28). Researchers obtained pomegranate effective against Streptococcus mutans and R. dentocariosa (29). Researcher observed effectiveness of P. granatum fruit peel against some plant pathogenic bacteria viz. Erwinia carotovorum subsp. Carotovorum, Ralstonia solanacearum, and Xanthomonas gardneri (30). Antimicrobial activity of P. granatum peel against some gram positive and negative

bacteria has been described earlier (31,32). According to researchers, ethyl acetate extract of fruit peel of the plant is considerably effective for antimicrobial purposes (33). Effective antibacterial activity of P. granatum peel against clinically important microbes has been reported (34).

Significant antimicrobial activity of C. longa against clinically important bacteria such as E. coli and S. aureus was found (35). Demonstration of Ethyl acetate extract showed antifungal activity against phytopathogens such as Botrytis cinerea, Phytophthora infestans, Puccinia recondita and Rhizoctonia solani. Whereas hexane extract has shown efficacy against Erysiphe graminis, P. infestans and R. solani (36). Alkaloid extract was found to be efficient against Fusarium and Penicillium species when compared with ltraconazole (37,38). Observation of modulating activity of antibiotic resistance in MDR strains of Pseudomonas aeruginosa and Acinetobacter baumanni by mango seed extract has been done (39,40). Methanolic crude extracts of mango sed kernel shown impressive activity against S. aureus, E. coli and C. albicans (41). Studies on antimicrobial activity of mango seed kernel against Aeromonas caviae resulted into effective antibacterial agent with methanol as solvent (42). Other parts were also found to be effective against clinical pathogens in various studies (43).

The suppressive effect of fennel oil on various bacteria, fungi and foodborne pathogens was observed (44). Fennel seeds can be used for drug formulation as it has significant activity against some gram positive and negative bacteria and fungi (45). The seeds and essential oil of seeds are effective for the control of various bacteria and fungi (46-49). Essential oil of fennel against zucchini yellow mosaic virus and its vector in Cucurbita pepo plant was applied (50). Synergistic antimicrobial effect of fennel essential oil when incorporated with nanoparticles against clinical microbes were observed (51).

Staphylococcus aureus was found to be highly sensitive to Bougainvillea spectabilis leaves extract (52). B. spectabilis extract significantly found effective for inhibition of Salmonella typhi, Shigella flexneri, Bacillus thuringiensis and Tobamovirus infection in bell pepper and tomato plant (53,54). Botanicals were found inducers of germination parameters (55,56). Demonstration of antibacterial activity of M.charantia and found it significant against clinically important pathogens was carried out (57). Potent anti-infective activity of M. charantia extract were found against clinical bacteria, fungi as well as HIV-1 reverse transcriptase (58). Some also mentioned antibacterial activity of the fruit extract (59).

The fresh peel extract of Citrus sinensis (FPE) was proved highly effective against E. faecalis followed by S. aureus and P. aeruginosa followed by E. coli, S. typhimurium (60). Demonstration of antimicrobial capabilities of C. sinensis peel oil showed that it could be used as a natural preservative for food as well as antimicrobial agent against pathogenic organisms such as S. aureus, E. coli and Streptococcus agalactiae, A. flavus, A. niger, Alternaria alternata) (61). It is also found effective against carbapenem-resistant Acinetobacter baumannii, XDR strains of E. coli and P. aeruginosa followed by S. aureus and MRSA (62).

Antibacterial and antifungal activity of L. pyrotechnica were mentioned (63). Whole plant is employed in Yemeni traditional medicine for wound healing which is a proof of its antimicrobial efficacy against B. subtilis as well as S. aureus. Plant sap is found to have ability to treat conditions like eczema (64). Test was carried of root and fruit extracts (aqueous, n-hexane, methanol, chloroform, ethanol, acetone, butane and ethyle acetate) against S. aureus and S. epidermidis (65) which showed significant results for both the pathogens (66). C. limetta peel extracts were found effective against E. coli and P. aeruginosa (67), S. aureus (68), Chromobacterium violaceum (69), E. coli faecalis (70), Salmonella enterica, Klebsiella pneumoniae, Bacillus cereus, Yersinia pestis, Yersinia enterocolitis, and Listeri monocytogenes (71), M. smegmatis and S. sonnei and MDR Shigella (72). Ralstonia species exist as endophyte in C. dichotoma plant (73) as the results of the present study indicated (No zone) although methanolic extraction of C. dichotoma leaf, fruit and bark extract showed inhibitory activity for Pseudomonas (74).

Key findings indicate that plant-derived compounds and microbial metabolites effectively suppress R. solanacearum through growth inhibition and virulence disruption. Some compounds also enhance host resistance. However, field efficacy is influenced by varying environmental conditions. Natural compounds offer a sustainable, low-risk alternative for managing bacterial wilt (75).

CONCLUSION

Out of twelve plants, P. granatum (fruit peel), M. indica (seed kernel), R. indica (red petals), M. charantia (Fruit peel), B. spectabilis (flower with bract), T. indica (leaves), L. pyrotechnica (leaves with stem) and C. sinensis (fruit peel) were able to produce inhibition zone in both aqueous and methanolic extracts while C. longa (Rhizome), F. vulgare (fruits) and C. limetta (fruit peel) produced inhibition zone only in methanolic extract. Extract (aqueous and methanolic) of C. dichotoma (fruit) was not found effective against the pathogen. From the undergone study it was concluded that amongst them fruit peel extract of Punica granatum in aqueous and methanolic extracts and rhizome of Curcuma longa in methanolic extract proved to be most effective against the pathogen with least MIC value followed by red petals of Rosa indica and fruits of Foeniculum vulgare.

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CONFLICT OF INTEREST:

There is no conflict of interest of the other person or institute for the research work done.

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