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Antibacterial Activity and Phytochemical Screening of Aqueous and Methanolic Extracts of Pomegranate (*Punica granatum* Linn.) Peel Against Bacterial Wilt of Tomato

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***Abstract* – *Ralstonia solanacearum* is a major constraint for tomato production causes the bacterial wilt and affects large varieties of *solanaceous* plants worldwide. Management of bacterial wilt is very complicated as there are no effective curative chemicals. The aim of this study to find out pomegranate fruit peel extract with antimicrobial property that could be potentially used as natural preservative in foods. Plants are most important source of medicine and drugs and they have been used for treating different ailments in humans worldwide from the beginning of the civilization. Natural inhibitors for pathogenic microorganisms have been explored in numerous plants. Pomegranate extract was evaluated their antimicrobial activity by three different methods namely, agar dilution, agar well diffusion, and disk inhibition zone methods on Tryptone Soya agar (TSA) medium against *R. solanacearum*. Antibacterial effect of the extracts was studied and compared with commercial antibiotic. The results revealed that the average zone of inhibition of the extracts were in the aqueous and methanolic extracts were showed against ten *R. solanacearum* in the range of 11 mm to 22, 11 mm to 23 and 10mm to 22mm inhibition zone of different three methods respectively. The means and standard error of triplicate tests were recorded. The methanolic extract was found to be more effective than aqueous one against *R. solanacearum*. The minimum inhibitory concentration (MIC) was determined by two-fold micro broth dilution method for the tested *R. solanacearum*. The MIC of the Pomegranate methanolic extract and aqueous extracts were 512µg ml^{-1} and 2048µg ml^{-1} respectively. Phytochemical analysis of Pomegranate peel show presence of Phenols Tannins, Flavonoids, Alkaloids, Saponins, Steroides, terpenes and Cardiac glycosides using methanolic extraction of bioactive compounds are solvent dependent.**

***Keywords* – Bacterial Wilt, Minimum Inhibitory Concentration, Phytochemicals, Pomegranate, *Ralstonia Solanacearum*, Tomato.**

I. INTRODUCTION

Bacterial wilt of tomato (*Lycopersicon esculentum*) incited by *Ralstonia solanacearum* is one of the devastating bacterial diseases affecting vascular bundles of plants (Sood and Singh, 1993). The pathogen has a very wide host range and almost all the solanaceous vegetables are susceptible. Bacterial wilt is a serious disease in the production of tomatoes and many other crops in tropical, subtropical and warm temperature regions of the world (Ji *et al.,* 2005). It attacks over 450 plant species including ornamentals such as geranium, and limits the production of such economically important crops as tomatoes, tobacco, potatoes and bananas (Kelman *et al.,* 1994). The yield loss may vary between 10.8 and 90.6 percent depending on the environmental circumstances and the stage at which infection occurs (Kishun, 1987). Bacterial Wilt poses a constant threat to tomato in Karnataka, Madhya Pradesh, Marathwada region of Maharashtra and West Bengal in India. The pathogen infects susceptible plants in roots, usually through wounds (Pradhanang *et al.,* 2005) and colonizes within the xylem preventing the water movement into upper portion of the plant tissue (Kelman, 1998)

The concentrated and indiscriminate use of pesticides in agriculture has caused many problems to the environment such as water, soil, animals and food contamination; poisoning of farmers; elimination of non-target organisms and selection of phytopathogens, pest and weed insensitive to certain active ingredients (Stangarlin *et al.,* 1999). Aiming to minimize the negative effects of pesticides are been development the alternative control of plant disease, which includes the biological control, the induction of resistance and the use of natural products with induction of resistance and/or with direct antimicrobial activities. Control of bacterial wilt in diseased soils is very difficult. It is generally considered that crop rotation with a non host crop is of minimal value because of the wide range of crop and weed hosts of the pathogen (Hayward, 1991).

About 20% of the plants establish in the world have been submitted to pharmaceutical or biological test and a sustainable number of new antibiotics introduced on the market are obtained from natural resources. The use of plant extracts and phytochemicals both with known antimicrobial characteristics is of great significance, in the past few years a number of investigations have been conducted worldwide to prove antimicrobial activities from medicinal plants (Alonso-Paz *et al.,* 1995). Green plants represent a pool of effective chemotherapeutants and can supply valuable sources of natural pesticides (Mahajan and Das, 2003). Biopesticides has been suggested as an effective substitute for chemicals (Kapoor, 2001). Reports are available on the use of several plant by-products, which posses antimicrobial properties, on several pathogenic bacteria and fungi (Kilani, 2006). The use of plant extracts which are natural sources of

antimicrobial substances, regarded as safe and degraded by natural soil microbes; they do not pose any health residual or ecological problems at any concentration which they are used (Yang *et al.,* 2010). However, there is no single mean that would totally eliminate the disease, provide a complete cure or fully protect host plants against infection. *In vitro* and *in vivo* investigations by some researchers have confirmed the antimicrobial potential of some plant species (El-Ariqi, 2005).

The pomegranate (*Punica granatum)*, an ancient, mystical, and highly distinctive fruit, is the predominant member of *Punicaceae* family. Pomegranate has been highlighted in some studies as having this property (Al-Zoreky, 2009). Even though these pieces of confirmation associate pomegranate as antimicrobial therapeutic, some questions still remained to be answered. Also, more studies are required to investigate the antimicrobial effects of other types of pomegranate extracts and other parts of this plant. Different part of pomegranate like bark, leaves, immature fruits, and fruit rind have some medicinal importance (Neelam, 2012). Different investigations were carried out to determine antioxidant, anticarcinogenic, and anti-inflammatory properties of pomegranate constituents (Hossain *et al.,* 2013). Here, we evaluate the potential of pomegranate extracts for antibacterial activity against *R. solanacearum.* The present investigation was carried out to determine the Antagonistic activity pomegranate plant extracts against *Ralstonia solanacearum.* The objective of this study was to determine the efficacy pomegranate plant extracts for controlling *R. solanacearum* causing bacterial wilt of tomato. Therefore, in the present study we tested two extracts from pomegranate fruit skin against *R. solanacearum* causing bacterial wilt of tomato. Moreover, the antimicrobial activities of pomegranate were compared with antibiotic Streptomycin.

II. Material and Methods

A. Isolation and identification of R. solanacearum

The suspected plant material and soil samples were collected from the field survey, brought to the laboratory and tested for the presence of pathogenic bacterium. The Collected plant materials were surface sterilized with 1% NaOCl solution for 1 to 2 min, followed by three repeated washings with distilled water and blot dried. Then the plant sections (0.5–1 cm) were placed on Kelman's TZC (2, 3, 5 Triphenyl tetrazolium chloride) medium (Kelman, 1954). Isolation from rhizosphere soil samples were done by dilution plate technique on modified semi selective medium, South Africa (SMSA) agar medium (Elphinstone *et al.,* 1996). The plates were incubated at 28 ± 2 ^{o}C for 24–48 h. The identification of the ten selected strains based on pathogenicity was further confirmed by molecular methods based on 16S rRNA sequencing for *R. solanacearum*. NCBI BLAST search was performed and the top hit sequences were multiple aligned and phylogenetic tree was constructed using CLUSTAL X2 2.1 (Windows version) software by Neighbor Joining (NJ) analysis with 1000 bootstrap replications based on the algorithm (Waterman, 1986). The sequences were deposited to NCBI database.

B. Plant extracts

Pomegranate fruits were collected from Bangalore, Karnataka. The peels were removed manually, dried for 72 h at 50-60 °C and then ground into fine powder using an electric blender and ground to fine powder and two types of extracts were prepared. The 50 g of pomegranate fruit peel powder was extracted with 600 ml distilled water in a Soxhlet apparatus for 12 h. The solution was then filtered using muslin cloth, filtrate was centrifuged at 5000 rpm for 15 min and supernatant was again filtered using Whatman filter no.1 under aseptic conditions and the filtrate was collected in fresh sterilized bottles and stored at 4 °C until further use.

In Methanolic extract, Air-dried 10g of pomegranate fruit peel powder was mixed with 100ml methanol, the mixtures were placed at room temperature for 24h on shaker with 150 rpm. The solution was filtered through Whatman filter no.1 and thus obtained was concentrated by complete evaporation of solvent at room temperature to yield the pure extract. Stock solution of methanolic crude extracts was prepared by mixing well the appropriate amounts of dried extracts and appropriate solvent. The solution was stored at 4 °C after collecting in sterilized screw cap tubes until further use. Both extracts were then passed through filter paper and dried in oven at 50 °C (Pankaj *et al.,* 2011; Sadeghian *et al.,* 2011).

Fig.1. Pomegranate plant and fruit peel.

C. Preparation of bacterial inoculums

Inoculum of the *R. solanacearum* was prepared by culturing it in Casamino acid Peptone Glucose (CPG) broth (1 g of Casamino acids; 10 g of peptone; 5g of glucose in 1000 ml of distilled water) (Kleman, 1954). Cultures were centrifuged at 12000 g for 10 min at 10 °C. The pellet was resuspended in distilled water and was adjusted spectrophotometrically to 1×10^{8} CFU ml^{-1} (colony forming unit) (Ran *et al.,* 2005).

D. Antagonistic activity against R. solanacearum

The antagonistic activity of the pomegranate extracts were evaluated using three different methods namely, agar dilution, agar well diffusion, and disk inhibition zone. Antagonistic activities against *R. solanacearum* of aqueous and methanolic extracts from pomegranate fruit peel powder was determined by agar dilution method, 15 ml of TSA medium containing either the pomegranate extracts were added to each of the Petri dishes (size 9cm diameter). Then the isolates of *R. solanacearum* were inoculated on the agar surface. After incubation at 28 ± 2 °C for 24h, the antimicrobial activity was measured as

diameter of the inhibition zone. Organic solvent (Methanol), in which extract was prepared, solvent was used as negative control while Streptomycin antibiotic of one unit strength was used as positive control. The experiment was performed in triplicate under aseptic conditions and the antagonistic activity of the extract evaluated was expressed in terms of the average diameter of zone of inhibition in mm.

Antagonistic activity against *R. solanacearum* of aqueous and solvent extracts from pomegranate fruit peel powder was determined by standard agar well diffusion assay (Perez *et al.,* 1990). Petri dish containing 20ml of cool TSA medium (at 40 °C) was seeded with 100µl inoculum of *R. solanacearum* (1×10^8 CFU ml^{-1}) and Media was allowed to solidify. Wells of 5 mm diameter were cut into solidified agar media with the help of sterilized cork borer. Aliquot 100µl of each pomegranate extract was added in the respective well and the plates were incubated at 28 ± 2 °C for 24h.

Antagonistic activity against *R. solanacearum* of aqueous and solvent extracts from pomegranate fruit peel powder was determined by disk inhibition zone method, the TSA medium was inoculated with freshly prepared cells of *R. solanacearum* to yield a lawn of growth. After solidification of the agar, a number of sterilized disks were dipped into the solvent (negative control) or extract solutions and placed on the plates. After incubation at 28 ± 2 °C for 24h, the antimicrobial activity was measured as diameter of the inhibition zone formed around the disk. The experiment was performed in triplicate under aseptic conditions.

E. Determination of Minimum Inhibitory Concentration

Minimum inhibitory concentrations (MIC) values were determined for extracts producing an inhibition zone of *R. solanacearum* that was found to be sensitive to the most active extract revealed by the previous screening tests (NCCLS, 1997). A stock solution of extract was serially diluted in 96-wells micro titer plate with CPG broth to obtain a concentration ranging from 8µg/ml to 4096 µg/ml. A standardized inoculum for each bacterial strain was prepared so as to give inoculum size of approximately 1x 10^8 CFU ml^{-1} in each well. Micro titer plates were then kept at 28 ± 2 °C for 24h incubation. Following incubation, the MIC values are recorded as the lowest concentration of the extract that completely inhibited bacterial growth, giving a clear well.

F. Phytochemical screening

Phytochemical screening was carried out on the pomegranate fruit peel powder for the presence of bioactive components such as Phenols Tannins, Flavonoids, Alkaloids, Saponins, Steroides, terpenes and Cardiac glycosides according to the methods described by Raman (2006) and each of the tests was qualitatively as negative (-) or positive (+).

III. RESULTS

A. Isolation and identification of R. solanacearum

After incubation pink centers with white fluid colonies were selected and a total of 50 isolates of *R. solanacearum* were isolated and identified. Microscopic studies revealed that bacterial isolates were Gram negative, rod shaped, non spore forming, strictly aerobic bacteria and it was confirmed by standard biochemical tests. The identification of the *R. solanacearum* isolates was confirmed by molecular analysis. The BLAST analysis of the sequences showed 98% to 99% identity to several isolates of *R. solanacearum* strains. Among 50 isolates, ten highly virulent strains were characterized and they were identified as *R. solanacearum* - RS1, RS2, RS3, RS4, RS5 RS6, RS7, RS8, RS9 and RS10 with Gen bank Accession numbers KF924739, KF924740, KF924741, KF924742, KF924743, KF924744, KF924745, KF924746, KF924747and KF924748 respectively (Narasimha Murthy *et al.,* 2012).

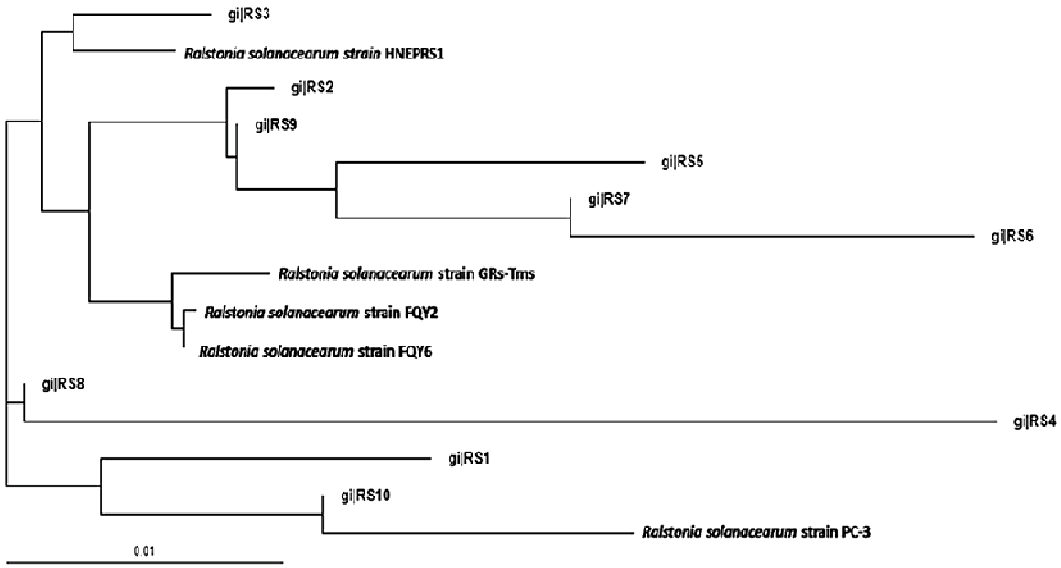

Fig.2. Phylogenetic relationships of *R. solanacearum* isolates inferred by Neighbor-Joining (NJ) bootstrap tree analysis of 16S rRNA sequences.

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B. Antagonistic activity against R. solanacearum

Antibacterial activity of pomegranate fruit peel powder extracts against ten *R. solanacearum* pathogens, were studied. Results of the antagonistic activity were shown in the Table 1. According to the results, pomegranate extracts showed the antagonistic activity against all tested *R. solanacearum* isolates. Aqueous and methanolic extracts were showed against ten *R. solanacearum* in the range of 11 mm to 22, 11 mm to 23 and 10mm to 22mm inhibition zone of different methods, agar dilution, agar well diffusion, and disk inhibition zone respectively.

Table 1: Antagonistic activity of aqueous and methanolic extracts of pomegranate fruit peel powder against *R. solanacearum* by agar dilution, agar well diffusion and disk inhibition methods.

	RS1	**RS2**	**RS3**	**RS4**	**RS5**	**RS6**	**RS7**	**RS8**	**RS9**	**RS10**
	Diameter of inhibition zone (mm) by agar dilution									
Aqueous extract	11.33±0.5	13.06±0.4	12.46±0.4	11.57±0.8	12.46±0.4	10.30±0.4	13.34±0.4	11.30±1.0	12.50±0.2	11.0±1.1
Methanolic extract	20.67±0.8	21.66±0.4	19.67±0.8	20.95±0.7	21.94±0.2	21.95±0.7	19.3 ± 0.3	19.7 ± 06	22.94±0.2	20.32±0.7
	Diameter of inhibition zone (mm) by agar well diffusion									
Aqueous extract	11.06±0.4	12.47±0.6	11.34±0.4	12.46±0.2	10.30±1.0	12.56±0.5	11.78±0.8	12.50±0.7	10.70±0.8	11.73±0.8
Methanolic extract	22.48±0.5	21.41±0.6	19.67±0.7	20.95±0.7	20.57±0.4	19.42±0.8	20.66±0.4	23.70±0.4	19.9±0.2	18.0± 0.5
	Diameter of inhibition zone (mm) by disk inhibition									
Aqueous extract	10.10±0.3	12.73±0.3	10.48±0.5	10.35±0.6	12.32±0.7	11.67±0.8	13.20±0.4	12.67±0.8	12.53±0.4	11.28±0.8
Methanolic extract	20.3± 0.3	21.3± 0.3	19.7 ± 06	21.3± 0.3	19.30±0.4	20.97±0.4	20.32±0.7	19.73±0.8	19.70±0.8	21.70±0.5
Methanol	4.21±0.4	5.72±0.7	3.0±0.03	7.5±0.2	5.32±0.1	4.21±0.5	3.92±0.2	4.56±0.3	3.66±0.1	5.33±0.4
Streptomycin	22.65±1.2	23.33±1.6	23.56±1.9	22.5±1.3	24.17±1.7	22.54±1.1	21.46±1.6	23.62±1.9	21.56±1.5	23.21±1.8

Data given are mean of three replicates ± standard error. $P < 0.05$

C. Minimum Inhibitory Concentration

Minimum inhibitory concentrations of extracts from pomegranate fruit peel powder extracts had been demonstrated, *R. solanacearum* were inhibited at 512µg ml^{-1} by methanol extract. It showed varying broad-spectrum antibacterial activity at different MIC against the *R. solanacearum* and aqueous extracts exhibited good antibacterial activity at the highest concentration 2048µg ml^{-1} and antibiotic while *R. solanacearum* was inhibited at 8µg ml^{-1} concentration (Table 2-4). Although it is ideal to test plant extracts against a wide range of target microorganisms, taxonomically representative bacterial species were used in this test to avoid handling numerous pathogenic microorganisms.

Table 2: Minimum inhibitory concentration of active crude Methanol extracts of pomegranate fruit peel powder against *R. solanacearum.*

Type of Extract	***R. solanacearum***	**Concentration of Extracts (in µg ml^{-1})**										**MIC (in µg ml^{-1})**
		4096	2048	1024	512	256	128	64	32	16	8	
Methanol	RS1	-	-	-	+	+	+	+	+	+	+	2048
	RS2	-	-	-	+	+	+	+	+	+	+	2048
	RS3	-	-	-	+	+	+	+	+	+	+	2048
	RS4	-	-	-	+	+	+	+	+	+	+	4096
	RS5	-	-	-	-	+	+	+	+	+	+	512
	RS6	-	-	-	+	+	+	+	+	+	+	2048
	RS7	-	-	-	+	+	+	+	+	+	+	1024
	RS8	-	-	-	+	+	+	+	+	+	+	2048
	RS9	-	-	-	+	+	+	+	+	+	+	1024
	RS10	-	-	-	-	+	+	+	+	+	+	2048

(-) represents 'No Growth Observed'; (+) represents 'Growth Observed

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Table 3: Minimum inhibitory concentration of Aqueous extracts of pomegranate fruit peel powder against *R. solanacearum.*

Type of Extract	*R. solanacearum*	Concentration of Extracts (in µg ml^{-1})										MIC (in µg ml^{-1})
		4096	2048	1024	512	256	128	64	32	16	8	
Aqueous	RS1	-	+	+	+	+	+	+	+	+	+	1024
	RS2	-	+	+	+	+	+	+	+	+	+	4096
	RS3	-	-	+	+	+	+	+	+	+	+	2048
	RS4	-	+	+	+	+	+	+	+	+	+	4096
	RS5	-	+	+	+	+	+	+	+	+	+	4096
	RS6	-	-	+	+	+	+	+	+	+	+	2048
	RS7	-	-	+	+	+	+	+	+	+	+	1024
	RS8	-	+	+	+	+	+	+	+	+	+	4096
	RS9	-	+	+	+	+	+	+	+	+	+	2048
	RS10	-	+	+	+	+	+	+	+	+	+	4096

(-) represents 'No Growth Observed'; (+) represents 'Growth Observed

Table 4: Minimum inhibitory concentration of antibiotic against *R. solanacearum.*

Type of Active Crude Extracts	*R. solanacearum*	Concentration of Extracts (in µg ml^{-1})										MIC (in µg ml^{-1})
		4096	2048	1024	512	256	128	64	32	16	8	
Streptomycin	RS1	-	-	-	-	-	-	-	-	-	-	<8
	RS2	-	-	-	-	-	-	-	-	-	-	<8
	RS3	-	-	-	-	-	-	-	-	-	-	<8
	RS4	-	-	-	-	-	-	-	-	-	-	<8
	RS5	-	-	-	-	-	-	-	-	-	-	<8
	RS6	-	-	-	-	-	-	-	-	-	-	<8
	RS7	-	-	-	-	-	-	-	-	-	-	<8
	RS8	-	-	-	-	-	-	-	-	-	-	<8
	RS9	-	-	-	-	-	-	-	-	-	-	<8
	RS10	-	-	-	-	-	-	-	-	-	-	<8

(-) represents 'No Growth Observed'; (+) represents 'Growth Observed

D. Phytochemical screening

The Phytochemical screening of methanolic pomegranate fruit peel powder extract showed the presence of Phenols Tannins, Flavonoids, Alkaloids, Saponins, Steroides, terpenes and Cardiac glycosides (Table 5).

Table 5: Phytochemical screening of pomegranate peels Assay

Assay	Result
Phenols Tannins	+
Flavonoids	+
Alkaloids	+
Saponins	+
Steroides	+
Terpenes	+
Cardiac Glycosides	+
Mucilages	+

+: Present; -: Absent.

IV. Discussion

Natural products with pesticidal activity are being explored in order to make available the pesticides, which are eco-friendly, selective and can be locally produced, particularly for the farmers who cannot pay for expensive synthetic pesticides (Rhouma *et al.,* 2009). Excessive and random application of the chemicals has created several environmental and health hazards and also some phytopathogens have been developed resistance. Green plants have been shown to represents a reservoir of effective chematherapeutants and can provide valuable sources of natural pesticides (Dorman *et al.,* 2000). Antibacterial properties of plant extracts against bacterial human pathogens were reported by several studies but only a few studies have been done on plant pathogens using plant extracts.

The control of bacterial disease in plants is mainly achieved by the use of synthetic pesticides which can result in toxicity to animals and humans as well as accumulation in living systems (Ansari and Malik, 2008). In addition, plant pathogens commonly acquire resistance to chemical pesticides as a result of their repeated uses (McManus *et al.,* 2002). New molecules for the control of plant pathogens are mandatory. Recently, plant extracts have been tested against plant bacteria with a certain degree of success (Slusarenko *et al.,* 2008). The use of naturally available chemicals from plants, which retards the reproduction of undesirable microorganisms, would be a more sensible and ecologically sound method for plant protection and will have an important role in the development of future commercial pesticides for crop

protection strategies, with special reference to the management of plant diseases (Gottlieb *et al.,* 2002).

Several authors have linked the presence of these bioactive compounds to the antimicrobial properties of crude plant extracts (Reddy *et al.,* 2007). This activity of pomegranate aqueous extracts, suggest that the active principles are soluble in water. The result revealed that both the pomegranate peels extracts have produced inhibitory activity against the *R. solanacearum* and the it was found much better than most of the antibiotic and the same may be useful against the *R. solanacearum.* In the present study the methanolic pomegranate peels extract produced better antibacterial activity. This indicates that the active constituents of the fruit parts have more ability to dissolve in methanol than the water used in this study. According to the results, the antagonistic activity of pomegranate peels extract against *R. solanacearum*. Aqueous and methanolic extracts were showed against ten *R. solanacearum* in the range of 11 mm to 22, 11 mm to 23 and 10mm to 22mm inhibition zone of different methods, agar dilution, agar well diffusion, and disk inhibition zone respectively. The methanolic extract of pomegranate fruit has been shown the antibacterial activity against *Listeria monocytogenes*, *S. aureus*, *Escherichia coli* (*E. coli*) and *Yersinia enterocolitica* (Al-Zoreky, 2009). The same activity has been established for pomegranate against *Klebsiella pneumoniae, Proteus vulgaris* and *Bacillus subtilis* (Prashanth *et al.,* 2001).

Antibacterial activity was found in high from pomegranate fruit peel extracts against the *R. solanacearum* were inhibited minimum inhibitory concentration (MIC) at 512µg ml^{-1} by methanol extract and the aqueous extracts was inhibited MIC at 2048µg ml^{-1} concentration. It was evident that the use of pomegranate solvent extract has a potential to substitute the antibiotics to control the infection. This kind of biological come up to would be economical, safe, environmental friendly. These plants are also available in plenty and farmers can use it for control of wilt in the solanaceous crops. However, the chemical compounds are yet to be isolated from this plants which requires further detail study. Phytochemical screening from pomegranate fruit peel extracts were used to study the presence of contained Phenols Tannins, Flavonoids, Alkaloids, Saponins, Steroides, terpenes, Cardiac glycosides and Mucilages. Different phytochemicals have been found to possess a wide range of activities, which may help in protection against phytopathogens. The antibacterial activity of *R. solanacearum* with plant extracts have been reported earlier (Larkin *et al.,* 2007). The finding of the result is encouraging and could be used as a source of antimicrobial compounds for reduce of bacterial wilt caused by *R. solanacearum.* It was evident that the use of pomegranate fruit peel solvent extracts has a potential to substitute the antibiotics to control the bacterial wilt. This kind of biological come up to would be safe and environmental friendly.

V. Conclusion

This study revealed the antibacterial activity of methanolic extract of pomegranate fruit peel against *R. solanacearum*, the causal agent of bacterial wilt in plants. The antibacterial activity of fruit extracts are found much better than the broad spectrum antibiotic. Further isolation and purification of the extracts are required to determine the bioactive components responsible for their activity. This is the first report of pomegranate fruit extract on the *in-vitro* antibacterial activity of *Ralstonia solanacearum*. The results are very encouraging and the identification of the novel antibacterial compounds could be useful in the control of bacterial wilt infection in plant caused by *R.solanacearum.* Although our results support the idea that pomegranate extracts are candidate for treatment of plant diseases.

References

[1] Alonso-Paz, E, Cerdeiras, M.P., Fernandez, J., Ferreira, F., Moyna, P., Soubes, M., Vazquez, A., Veros, S., Zunno, L. 1995. Screening of Uruguayan medicinal plants for antimicrobial activity. *J. Ethanopharm.* 45: 67–70

[2] Al-Zoreky, N.S. 2009. Antimicrobial activity of pomegranate (*Punica granatum* L.) fruit peels. *Int J Food Microbiol.* 134:244–8.

[3] Ansari, M.I., Malik, A., 2008. Genotoxicity of wastewaters used for irrigation of food crops. *Environ. Toxicol.* 24, 103–115.

[4] Dorman, H.J., Deans, S.G. 2000. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. Journal of *Applied Microbiology*. 88:308–316.

[5] El-Ariqi, S.N.S., El-Moflehi, M., El-Arbara, K., El-Kobati, A., El-Shaari, A. 2005. Antibacterial activity of extracts from *Withania somnifera* and *Aloe vera* aginst *Ralstonia solanacearum* in potato. *Arab J Plant Protect.* 23:95 – 99.

[6] Elphinstone, J. G., Hennessey, J., Wilson, J. K. and Stead, D. E. 1996. Sensitivity of different methods for the detection of *Ralstonia solanacearum* in potato tuber extracts. EPPO Bulletin. 26:663–678.

[7] Gottlieb, O.R., Borin, M.R. and Brito, N.R. 2002. Integration of ethnobotany and phytochemistry: dream or reality? *Phytochemistry.* 60: 145–152

[8] Hayward, A.C. 1991. Biology and epidemiology of bacterial wilt caused by *Pseudomonas solanacearum. Annual Review of Phytopathology.* 29: 65–87.

[9] Hossain, H., Ahmed, T., Md. Howlader, S.I., Dey, S.K, Hira, A., Ahmed, A., Sen R. 2013. *In vitro* Antioxidant Potential from the Leaves of *Punica granatum* Linn. Grown in Bangladesh. *Int J Pharm Phytopharmacol Res.,* 2(3):160-166.

[10] Ji, P., Momol, M.T., Olson, S.M., Pradhanang, P.M., Jones, J.B. 2005. Evolution of Thymol as Biofunmigant for control of Bacterial wilt of tomato under field conditions. *Plant Disease.* 89:497 – 500.

[11] Kapoor, A. 2001. Neem: The wonder plant. *Pesticides Inform*. 27: 33–34.

[12] Kelman, A. 1954. The relationship of pathogenicity in Pseudomonas solanacearum to colony appearance on a tetrazolium medium. *Phytopathology.* 44: 693–695.

[13] Kelman, A. 1998. One hundred and one years of research on bacterial wilt. In bacterial wilt disease: molecular and ecological aspects, edited by P. Prior, C. Allen and J. Elphinstone. Verlag Berlin Heidelberg: Springer.

[14] Kelman, A., Hartman, G.L., Hayward, A.C., 1994. Introduction. In: Hayward, A.C., Hartman, G.L. (Eds.), Bacterial wilt. *CAB International*, Oxon, UK, pp. 1–7.

[15] Kilani, A.M. 2006. Antibacterial assessment of whole stem bark of Vitex doniana against some Enterobactriaceae. *Afr. J. Biotechnol.* 5: 958–959.

[16] Kishun, R. 1987. Loss in yield of tomato due to bacterial wilt caused by *Pseudomonas solanacearum*. *Indian Phytopathology*. 40 152-155.
[17] Larkin, R.P., Griffins, T.S. 2007. Control of soilborne potato diseases using Brassica green manures. *Crop Protection*, 26, 1067–1077.
[18] Mahajan, A., Das, S. 2003. Plants and microbes- Potential source of pesticide for future use. *Pesticides Inform*. 28(4): 33–38.
[19] McManus, P.S., Stockwell, V.O., Sundin, G.W., Jones, A.L. 2002. Antibiotic use in plant agriculture. *Annu. Rev. Phytopathol.* 40:443–465.
[20] Narasimha Murthy, K. and Srinivas, C. 2012. *In vitro* screening of bioantagonistic agents and plant extracts to control bacterial wilt (*Ralstonia solanacearum*) of tomato (*Lycopersicon esculentum*). *Journal of Agricultural Technology*. 8(3): 999 – 1015.
[21] NCCLS-National Committee for Clinical Laboratory Standards. 1997. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved Standards M7-A4, Wayne, Pa.
[22] Neelam, A., Singh, D.P. 2012. *Punica granatum*: A review on pharmacological and therapeutic properties. *IJPSR*, 3(5):1240–1245.
[23] Pankaj, G., Purshotam, K. 2011. *In vitro* evaluation of antibacterial activity of various crude leaf extracts of Indian Sacred plant, Ocimum sanctum L. *Br. Microbiol. Res. .,* 1(3): 70–78.
[24] Perez, C., Pauli, M., Bazerque, P. 1990. An antibiotic assay by the agar-well diffusion method. *Acta Biol. Med. Exp.* 15: 113–115.
[25] Pradhanang, P.M., Ji, P., Momol, M.T., Olson, S.M., Mayfield, J.L., Jones, J.B. 2005. Application of acibenzolar-S-methyl enhances host resistance in tomato against *Ralstonia solanacearum. Plant disease.* 89: 989–993.
[26] Prashanth, D, Asha, M.K., Amit, A. 2001. Antibacterial activity of *Punica granatum*. Fitoterapia, 72:171-3.
[27] Raman, N .2006. Phytochemical Techniques. New India Publishing Agency, New Delhi pp. 19–24.
[28] Ran, L.X., Liu, C.Y., Wu, G.J., van Loon, L.C., Bakker, P.A.H.M. 2005. Suppression of bacterial wilt in Eucalyptus europhylla by Fluorescent Pseudomonas spp. *Chin. J. Biol. Control,* 32: 111–120.
[29] Reddy, M., Gupta, S., Jacob, M., Khan, S., Ferreir, D. 2007. Antioxidant, antimalarial and antimicrobial activities of tannin-rich fractions, ellagitannins and phenolic acids from *Punica granatum L. Planta Med.* 73:461–467. doi: 10.1055/s-2007-967167.
[30] Rhouma, A., Ben Daoud, H., Ghanmi, S., Ben Salah, H., Romdhane, M., Demak, M. 2009. Antimicrobial activities of leaf extracts of Pistacia and Schinus species against some plant pathogenic fungi and bacteria. J Plant Pathol. 91:339–345.
[31] Sadeghian, A., Ghorbani A., Mohamadi, N.A., Rakhshandeh, H. 2011. Antimicrobial activity of aqueous and methanolic extracts of pomegranate fruit skin. *Avicenna Journal of Phytomedicine*. Autumn 1(2):67–73.
[32] Slusarenko, A.J., Patel, A., Portz, D. 2008. Control of plant diseases by natural products: allicin from garlic as a case study. *Eur. J. Plant Pathol*. 121: 313–322.
[33] Sood, A.K. and Singh, B.M. 1993. Prevalence of bacterial wilt of solanaceous vegetables in the mid-hill sub humid zone of Himachal Pradesh (India). In: Bacterial wilt (Hartman GL and Hayward AC Eds).Proceedings on International Conference, Koashiung, Taiwan, 28-31 Oct 1992, *ACIAR Proceedings*. 45: 358–361.
[34] Stangarlin, J.R., Schwan Estrada, K.R.F., Cruz, M.E.S., Nozaki, M.H. 1999. Medicinal plants and alternative control of phytopathogens. Biotecnologia Ciência & Desenvolvimento. 11:16–21.
[35] Waterman, M.S. 1986. Multiple sequence alignment by consensus. *Nucleic Acids Res.* 14:9095–9102.
[36] Yang, X., Ma, X., Yang, L., Yu, D., Qian, Y., Ni, H. 2010. Efficacy of *Rheum officinale* liquid formulation on cucumber powdery mildew. *Biol. Cont*. 522:167–173.

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