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Antibacterial Activity of *Punica granatum* against *Ralstonia solanacearum* from Nakuru, Kenya

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ABSTRACT

Potato wilt caused by *Ralstonia solanacearum* causes great potato yield reduction all over the world. Currently, there are no reliable chemicals for the control of the disease. This study aimed at testing the sensitivity of *R. solanacearum* isolates to crude extracts from *Punica granatum*. Symptomatic Irish potato plants samples were collected from the field and *R. solanacearum* isolated using Kelman's TZC. *P. granatum* samples were purchased from Njokerio market and crude extracts obtained. The chemical constituents of the crude extracts were established using GC-MS. Sensitivity test of *R. solanacearum* was carried out using Kirby Bauer disk diffusion technique and MICs and MBCs values determined. There was a significant difference in the weights of crude extracts obtained from the seeds of *P. granatum* using hexane, ethyl acetate and methanol (F=140.7 P=0.00468). The diameter of the zones of inhibition presented by the extracts from the fruit peels extracted using hexane, ethyl acetate and methanol varied significantly (F= 85.58 P=0.007). Similarly, there was a significant difference in the diameter of zones of inhibition from hexane, ethyl acetate and methanol crude extracts from the seeds (F=85.14 P=0.008). The MICs of extracts from the fruit peels, seed extracts and the tetracycline control varied significantly (F= 1484.53 P=0.00043). In addition, there was a significant difference in MBCs presented by the peel extracts, seed extracts and the tetracycline control (F=17934.03 P=0.00014). There is need for mass production of extracts from *P. granatum.*

INTRODUCTION

R. solanacearum causes bacterial wilt in Irish potatoes (Mutimawurugo *et al*., 2020). The disease ranks second as the most detrimental disease of potato in sub-tropical and tropical regions after late blight caused by *Phytophthora infestan*s (Ismail *et al*., 2012). Bacterial wilt is also observed in some cool temperate regions of the world (Bereika *et al*., 2020). In Africa, it causes great yield losses in central and southern regions mainly in Uganda, Rwanda, Ethiopia, Kenya, Burundi, Nigeria, Madagascar, and Cameroon (Chamedjeu *et al*., 2018). The bacteria infect tubers limiting their exports to world markets (Phondekar *et al.*, 2020).

The management of bacterial wilt using chemicals is cumbersome. Currently, there are no known chemicals that can successfully control growth and spread of *R. solanacearum* (Singh, 2017). In addition, the drug resistance, natural enemies and the ill effect of chemicals on consumers is another menace (Abo-Elvousr and Khalil-Bagy, 2018).

At the moment, farmers are using cultural practices such as growing resistant varieties, planting in non-infected pieces of land, crop rotation and use of disease free planting materials (Kataky *et al*., 2017). However, the methods have not been very successful mainly because of practical, technological or economic limitations (Salvi *et al*., 2020). Crop rotation is limited by the long survival of the pathogen in the soil (Shweta *et al.*, 2018). Moreover, increase in human population has led to reduced land sizes (Kansal, 2023). On the other hand, quarantines are difficult to apply and may limit production and commercialization of the produce (Ruzgar *et al*., 2022).

Vegetative propagation of Irish potatoes is another hindrance to control of bacterial wilt (Karim and Hossain, 2018). Although the seed potato may look healthy, it may have *R. solanacearum* infection leading to an infected crop after germination (Aguk *et al*., 2018). Plant breeders have also not managed to come up with potato seeds that have high resistant to the infection (Vu *et al*., 2017). In addition, the new potato varieties have not been received well by farmers due to high glycoalkaloid content and sensitivity to temperature conditions (Din *et al*., 2016).

Studies carried out elsewhere have shown that some plants produce secondary metabolites with antibiotic property (Ruzgar *et al*., 2022). These plants control plant pathogens by either inducing systemic resistance or antibacterial activity (Kupnik *et al*., 2021). This indicates that use of locally available, economically and environmentally friendly plant extracts with antibiotic properties could control bacterial wilt of potato (Gosset-Erard *et al*., 2021). Gigliobianco *et al.* (2022) suggested that plant secondary metabolites inhibits growth of pathogens either by their natural bioactive compounds (phytoanticipins) or compounds synthesized *de novo* as a reaction to pathogen attack or other stress conditions (phytoalexins) (Ge *et al*., 2021).

Majority of the previous studies have been confined to assessment of the antifungal and antibacterial activities of plant extracts in medical microbiology (Elshafie *et al*., 2021). However, studies on use of plant extracts in plant bacteriology are still scanty (Valdés *et al*., 2020). Moreover, use of plant extracts in the control of *R. solanacearum* has not been extensively studied (Guerrero-Solano *et al.*, 2020). Hanafy *et al.* (2021) asserted that yield and composition of bioactive compounds of each species is affected by factors such as environmental conditions, genetics (species, varieties), plant organs, stage of growth, extraction techniques and even the extraction solvents (Avinam *et al*., 2000).

P. granatum is an ancient, mystical, and highly distinctive fruit, belonging to *Punicaceae* family. The plant has been highlighted in some studies as having many medicinal properties (Singh *et al*., 2018). Many of the studies however have been skewed towards human diseases. Previous studies have investigated the antibiotic properties of different parts of the plant such as bark, leaves, immature fruits, and fruit rind (Bassiri-Jahromi and Doostkam, 2018). In addition, some studies have been carried out to investigate antioxidant, anti-carcinogenic, and anti-inflammatory properties of the plant (Khan *et al*., 2017). The current study was carried out to investigate the antibacterial property of *P. granatum* extracts against *R. solanacearum.*

MATERIALS AND METHODS

Isolation of *Ralstonia solanacearum:*

Symptomatic Irish potato plants samples were collected from the field survey, and transported to the laboratory. Preliminary test for infection by *R. solanacearum* was carried out. The stems of the potatoes were cut at a slanting position and placed in a beaker with distilled water. (Yuliar *et al*., 2015). The symptomatic plant tissue was surface sterilized with 70% ethanol for 15 min. The tissues were washed with distilled water 3 times and blot dried. Sections measuring 0.5cm were cut and placed on sterile Kelman's TZC (2, 3, 5 Triphenyl tetrazolium chloride) medium. The plates were incubated at 28 ± 2^{o}C for 24h. Colonies showing typical characteristics of *R. solanacearum* were sub cultured on TZC media to obtain pure cultures. The isolates were characterized using morphological and biochemical means.

Extraction of *Punica granatum* Crude Extracts:

P. granatum fruit samples were purchased from Njokerio market and transported to the Department of Biological Sciences in Egerton University. The fruits were cleaned using running tap water to remove dirt and insects. The fruits were sliced using a sterile scalpel and the seeds separated from the fruit peel. Separately, the seeds and peel samples were dried in a hot air oven at 50˚C for three days. The samples were crushed into fine powder using a cross beater mill (SK100, Retsch) machine with sieving size 0.50 mm. Two hundred grams of each *P. granatum* powder were placed into three separate (2 L) volume conical flask with 1L ethyl acetate, methanol and n-hexane. The flasks were shaken using an orbital shaker at 200rpm. The mixture was placed in the fume chamber for 2d with occasional shaking. The extracts were filtered using Whatman No.1 filter paper with an aid of aspirator (A-3S, Eyela) (Rosas-Burgos *et al*., 2017). The filtrates were evaporated using a vacuum evaporator and dried in a hot air oven at 50^{o}C and the weights determined. The extracts were placed in refrigerators at 4˚C awaiting further processing.

Thin Layer Chromatography (TLC) Analysis of The Crude Extracts:

The TLC plate silica gels 60 F254 (Marck) measuring 20 x 20 cm were cut into small pieces measuring 2cmx 7cm. A line 1cm from the bottom and top of each plate was drawn using a pencil. A microliters borosilicate glass pipette was used to spot an extract as a small dot at the bottom line. Briefly 10mL of hexane, ethyl acetate, and methanol in the ratio of 5:3:2 respectively was placed in the development tank. The plates were placed in the development tank for separation and development of the spots. When the solvent movement reached the top line, the TLC plate was removed from the tank. The plates were observed under low (245 nm) and high (365 nm) UV light. The Retention Factor (R_f) was calculated using the formula given by Malviva *et al*. (2014);

$$R_f = \frac{Distance\ travelled\ by\ the\ substance}{Distance\ travelled\ by\ the\ solvent\ front}$$

Direct TLC Bioautography Assay:

Three Petri dishes were dried and sterilized. Briefly, 100µL standardized *R. solanacearum* was placed on sterilized molten TZC growth media. The TLC plates having the crude extracts were separately placed on sterilized Petri dishes. The TZC growth media was dispensed in the Petri dishes and allowed to solidify. The plates were incubated at 28^{o}C for 24h and were observed for growth inhibition. The R_f values were calculated and determined as the active compounds with antibacterial property (Narasimha and Srinivas, 2012).

Determining the Chemical Constituents by Using GC-MS:

The peel and seed crude extracts were each injected into the GC-MS on a 30-m silica capillary column with internal diameter and film thickness of 0.25 mm and 0.25 µm, respectively. The GC temperature was adjusted to increase from 60°C to 290°C at a rate of 15°C/min and held isothermal for 1 min (split ratio 1:100) (Derakhshan *et al*., 2018).

Antibacterial Activity of *P. granatum* Peels and Seed Extracts:

Briefly 50 µl of standardized *R. solanacearum* suspension with O.D. = 0.1 was dispensed on sterile Mueller Hinton Agar (MHA) and spread with an L-shaped glass rod. Four wells of 0.6 mm diameter were made using a cork borer. Using a micropipette, the wells were filled with 50 µl of 200 mg/mL hexane, ethyl acetate and methanol peel extract. The remaining empty well was filled with 50 µl of tetracycline (30 µg/mL) as a positive control. This was repeated with the seed extracts in three replicates. The plates were incubated at 37˚C for 24h. The zones of inhibition were measured in mm using a ruler.

Determination of Minimal Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC):

MICs and MBCs values were determined using broth microdilution bioassay. The microplate wells were filled with a total 100 µL Muller-Hinton broth (MHB). The fruit peel and seed extracts were each serially diluted in MHB. Briefly, 100 µL of *R. solanacearum* was added to each well. The negative control contained non-inoculated medium with extract samples while positive control wells were prepared with inoculated culture medium with no extracts. Incubation was carried out at 37°C for 24 h. The MIC was given by the lowest concentration of extract which inhibited growth of the pathogen. To determine the MBC, 20 µL of the suspension of the well before MIC of the extract were cultured on TZC agar using the spread plate technique. MBC was evaluated by counting the number of bacterial colonies after 24 h of incubation at 37°C and IC_{50} and IC_{90} determined using probit analysis

Statistical Analysis:

Data was analyzed using analysis of variance (ANOVA) based on completely randomized design using Statistical Package of Social Science Software version 25.0 software. The weights of crude extracts between the fruit peels and seeds extracts were compared using t-test. The mean comparison was carried out using Duncan Multiple Range Test (DMRT). The significant differences were considered significant at $P < 0.05$. The results of IC_{50} and IC_{90} values were calculated using probit analysis using Polo Plus Ver 2.

RESULTS

***Ralstonia solanacearum* Isolates:**

When the stems of the symptomatic potatoes were cut at a slanting position and placed in a test tube having distilled water, viscous white slime stream of bacterial cells exuded from the cut surface into the water. The infected potato showed signs of wilting even with adequate moisture (Fig. 1). The cut stems of the infected plant produced a white slime when placed in a beaker with water. The *R. solanacearum* isolates presented pink colonies on growth media.

Fig. 1: A; Healthy potato plants, B; Potato plants infected by *R. solanacearum*, C; *R. solanacearum* exuding from eye of potato tuber, D; *R. solanacearum* oozing from cut potato tuber, E; White slime oozing out of the infected stem and F; Pure culture of *R. solanacearum* isolates.

Crude Extracts from *Punica granatum*:

The amounts of crude extracts obtained from the fruit peel of *P. granatum* using hexane, ethyl acetate and methanol varied significantly (F=149.6 P=0.0033). In addition, there was a significant difference in the weights of crude extracts obtained from the fruit seeds using hexane, ethyl acetate and methanol (F=140.7 P=0.00468). There was also a significant difference between the weights of crude extracts obtained from the fruit peel and seeds (P=0.0011). The mean crude extract from fruit peel extracts using hexane was 138.9±0.2mg, ethyl acetate (257.4±0.2mg) and methanol (439.2±0.1mg) (Table 1). However, the mean crude extract extracted from the seeds using hexane was 127.8±0.2mg, ethyl acetate (209.4±0.3mg) and methanol (243.9±0.2mg).

Table 1: Weight (mg) of crude extracts from *Punica granatum* peel and seed extracts

Replicate	Peel			Seed		
	Hexane	Ethyl acetate	Methanol	Hexane	Ethyl acetate	Methanol
1	153.0±0.2	249.0±0.3	452.0±0.2	149.2±0.3	203.2±0.3	249.2±0.3
2	126.0±0.3	220.0±0.2	424.0±0.2	112.0±0.2	205.0±0.2	238.0±0.2
3	100.0±0.2	299.0±0.3	400.0±0.1	120.6±0.1	213.6±0.1	243.6±0.1
4	180.0±0.1	294.0±0.1	490.0±0.2	141.4±0.2	211.4±0.2	233.4±0.2
5	135.0±0.1	225.0±0.2	430.0±0.1	115.6±0.2	215.6±0.2	255.6±0.2
Mean	138.9±0.2	257.4±0.2	439.2±0.1	127.8±0.2	209.4±0.3	243.9±0.2

Retention Factors of Crude Extracts from *P. granatum:*

The R_f of the crude extracts from the peels of *P. granatum* didn't vary significantly (F=1.70 P=0.216). Likewise, there was no significant difference in the R_f of the crude extracts from seeds (F=1.54 P=0.25). The R_f of crude extracts from the fruit peels extracted using hexane varied from 0.65 to 0.95, ethyl acetate (0.26-0.93)

and (0.46-0.92) (Table 2). In addition, the R_f of crude extracts from the seeds extracted using hexane ranged from 0.64 to 0.94, ethyl acetate (0.25-0.92) and methanol (0.47-0.91).

Phytochemical Constituents of *Punica granatum*:

The crude extracts from *P. granatum* fruit peels and seeds gave the same phytochemical constituents (Table 3 and Fig. 2).

Table 2: The retention factor (R_f) for *Punica granatum* crude extracts

Spot Number	Peel			Seed		
	Hexane	Ethyl acetate	Methanol	Hexane	Ethyl acetate	Methanol
1	0.95	0.93	0.92	0.94	0.92	0.91
2	0.93	0.85	0.83	0.93	0.86	0.84
3	0.87	0.77	0.75	0.85	0.76	0.77
4	0.80	0.52	0.65	0.81	0.53	0.67
5	0.75	0.37	0.55	0.73	0.39	0.54
6	0.65	0.26	0.46	0.64	0.25	0.47

Table 3. Phytochemicals identified in extracts of the *Punica granatum* fruit peel and seed

No	Name	Formula	Retention time	Percent	
				Seed	Peel
1	Furfural	$C_5H_4O_2$	3.62	6.55	2.95
2	Heptacosane	$C_{27}H_{56}$	19.12	22.37	38.96
3	5-Hydroxymethylfurfural	$C_6H_6O_3$	9.75	12.17	9.23
4	Ellagic acid	$C_{14}H_6O_8$	10.35	7.25	8.60
5	Ellagic acid, 3,3′-di-O-methyl	$C_{16}H_{10}O_8$	7.82	2.61	3.02
6	Ellagic acid, 3,3′, 4′-tri-Omethyl	$C_{17}H_{12}O_8$	7.12	0.70	2.71
7	Punicalagin	$C_{48}H_{28}O_{30}$	6.40	0.50	2.11
8	Pyrogallol	$C_6H_6O_3$	10.13	2.06	5.29
9	Gallic acid	$C_7H_6O_5$	12.92	11.93	8.28
10	Catechin	$C_{15}H_{14}O_6$	6.78	0.51	1.84
11	Epicatechin	$C_{15}H_{14}O_6$	7.10	2.47	4.28
12	Gallocatechin	$C_{15}H_{14}O_7$	6.05	1.07	0.12
13	Gallocatechin-(4,8)-catechin	$C_{30}H_{26}O_{13}$	7.60	0.80	0.04
14	Ascorbic acid	$C_6H_8O_6$	15.79	5.90	3.11
15	Linoleic acid	$C_{18}H_{32}O_2$	18.43	9.68	2.14
16	Stigmasterol	$C_{29}H_{48}O$	28.20	0.80	0.04
17	Gamma-sitosterol	$C_{29}H_{50}O$	28.61	5.23	0.10

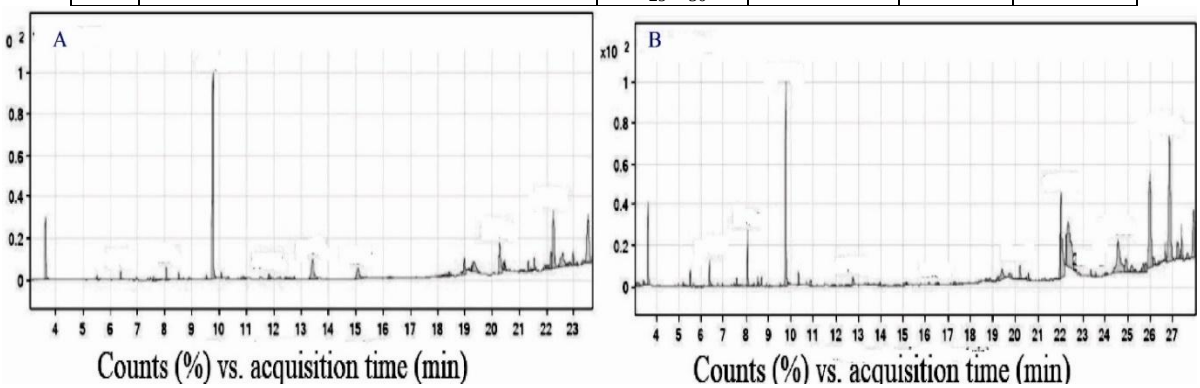

Fig. 2: GC-MS Chromatogram of *Punica granatum* peel (A) and seed (B) extracts

Diameter of Zones of Growth Inhibition of *R. solanacearum* by *P. granatum* Crude Extracts:

The diameter of the zones of inhibition presented by the extracts from the fruit peels extracted using hexane, ethyl acetate and methanol varied significantly (F= 85.58 P=0.007). Similarly, there was a significant difference in the diameter of zones of inhibition from hexane, ethyl acetate and methanol extracted crude extracts from the seeds (F=85.14 P=0.008). The mean zone of inhibition among the fruit peel extracts for hexane was 12.9±0.2mm, Ethyl acetate (16.5±0.2mm) and methanol (23.7±0.1mm) (Table 4 and Fig. 3). However, the mean diameter of zones of inhibition from the seed extracts obtained using hexane was 12.28±0.1mm, ethyl acetate (14.52±0.1mm) and methanol (18.4±0.2mm)**.**

Table 4: Diameter of zones of growth inhibition of *R. solanacearum* by *P. granatum* crude extracts.

S.No	Peel extracts			Seed extracts			Tetracycline
	Hexane	Ethyl acetate	Methanol	Hexane	Ethyl acetate	Methanol	
1	11.3 ± 0.1	17.3 ± 0.2	25.3 ± 0.2	12.1 ± 0.3	14.3 ± 0.3	19.2 ± 0.3	15.2 ± 0.2
2	12.3 ± 0.2	15.3 ± 0.2	23.3 ± 0.2	11.4 ± 0.2	15.1 ± 0.3	18.1 ± 0.3	15.4 ± 0.1
3	15.3 ± 0.3	18.3 ± 0.4	24.3 ± 0.3	12.3 ± 0.1	13.9 ± 0.1	17.2 ± 0.2	15.2 ± 0.2
4	12.3 ± 0.1	16.3 ± 0.3	22.3 ± 0.1	13.5 ± 0.1	14.1 ± 0.2	19.3 ± 0.3	15.1 ± 0.1
5	13.3 ± 0.2	15.3 ± 0.3	23.3 ± 0.2	12.1 ± 0.1	15.2 ± 0.1	18.2 ± 0.1	15.2 ± 0.1
Mean	12.9±0.2	16.5±0.2	23.7±0.1	12.28±0.1	14.52±0.1	18.4±0.2	15.2± 0.1

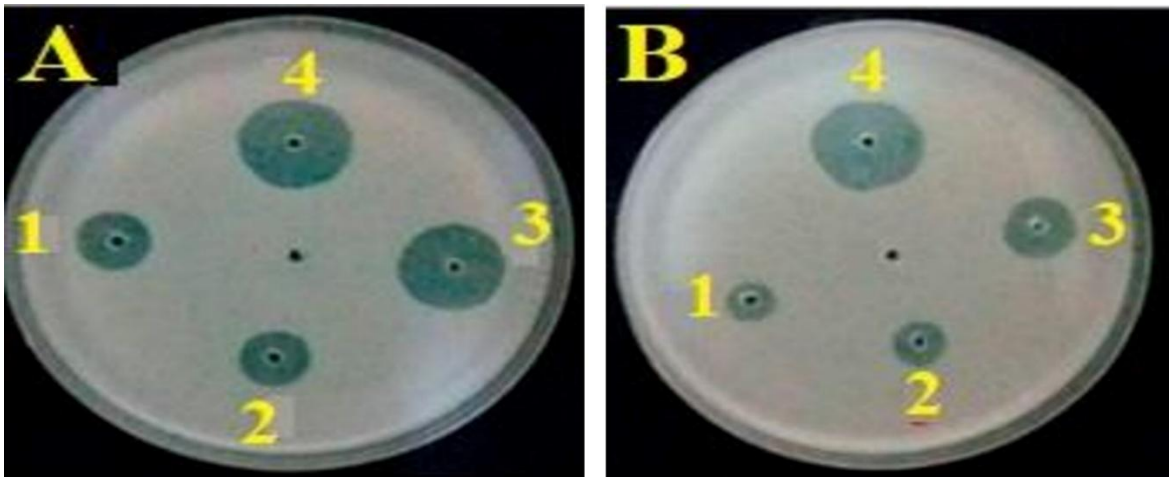

Fig. 3: A; Peel extract zone of inhibition for hexane (1), ethyl acetate (2), tetracycline (3) and methanol (4): B; Seed extract zone of inhibition for hexane (1), ethyl acetate (2), tetracycline (3) and methanol (4).

Minimal Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC):

The MICs of extracts from the fruit peels, seed extracts and the tetracycline control varied significantly (F= 1484.53 P=0.00043). In addition, there was a significant difference in MBCs presented by the peel extracts, seed extracts and the tetracycline control (F=17934.03 P=0.00014). The mean MIC in the peel extracts was 36.66±2.1, seed extracts (54.4±1.2) and tetracycline (24.3±2.5) (Table 5). The mean MBC for peel extracts was 69.8±2.2, seed extracts (74.2±2.3) and tetracycline (1.238±0.5).

The results of IC_{50} and IC_{90} values were calculated using probit analysis using

Polo plus Ver 2. The fruit seed extracts presented IC_{50} of 59.90 and peel extracts 31.50 (**Table 6**). Similar results were obtained for IC_{90} with fruit seed giving a value of 727.10 and peel extracts 482.21.

Table 5: MICs and MBCs values of *Punica granatum* extracts tested using *Ralstonia solanacearum*

Replicate	MIC			MBC		
	Peel extracts	Seed extracts	Tetracycline	Peel extracts	Seed extracts	Tetracycline
1	37.5± 1.7	55.0± 1.2	23.3±2.9	69.0± 2.7	74.0± 2.3	1.240± 0.5
2	36.3± 2.5	54.0± 1.2	25.3±1.9	70.0± 1.9	75.0± 2.4	1.230± 0.6
3	35.7± 3.0	55.0± 1.1	24.3±2.9	70.0± 2.6	73.0± 2.2	1.250± 0.4
4	36.6± 2.6	53.0± 1.2	25.3±1.8	71.0± 1.2	75.0± 2.2	1.240± 0.3
5	37.2± 0.5	55.0± 1.1	23.3±3.0	69.0± 2.5	74.0± 2.3	1.230± 0.5
Mean	36.66±2.1	54.4±1.2	24.3±2.5	69.8±2.2	74.2±2.3	1.238±0.5

Table 6: Probit regression line parameter and inhibition concentration (IC).

Extract	Regression Equation	IC_{50}	IC_{90}
Peel	Y = 1.086 - 1.644 X	31.50	482.21
Seed	Y = 1.207 - 2.181 X	59.90	727.10

DISCUSSION

The flow of viscous white slime stream of bacterial cells from the cut surface into the water indicated the presence of the bacterium in the infected stem tissues This study established that methanol was the best solvent for extracting crude antibiotics from *P. granatum* when compared to ethyl acetate and hexane. This could be attributed to differences in the polarity of the solvents (Raniha *et al*., 2021). Rummun *et al.* (2013) attributed variation in the weights of antibiotics extracted from *P. granatum* using assorted solvents to the nature of the antibiotics accumulated by the plant. The findings of the current study concurred with a previous study carried out by Karimi *et al*. (2017) which may have been caused by accumulation of the same phytochemicals by the plants.

The mobile phase hexane, ethyl acetate, and methanol in the ratio of 5:3:2 separated the different fractions of phytochemicals present in *P. granatum*. This mobile phase gave 6 spots when observed under UV light. Hexane, ethyl acetate, and methanol mobile phase was used in a different study which produced 11 spots when observed under UV (Nozohour *et al*., 2018). This may be attributed to differences in the phytochemicals present in the plant extracts. Jalili *et al*. (2020) observed that the solvents used in obtaining the crude extracts greatly contribute to the number of spots observed after TLC.

The developed TLC plates presented various inhibition zones on the TLC plates with different retention factor (Rf) values in direct TLC bioautography assay. The experiment was performed using the *R. solanacearum* isolates. The inhibition zone on extracts obtained using hexane appeared at R_f (0.93), Ethyl acetate (0.85) and methanol (0.83). On the other hand, the zones of inhibition for fruit seed extracts extracted using hexane appeared of R_f (0.73), ethyl acetate (0.86) and methanol (0.84) These findings agreed with a previous study carried out elsewhere (Ding *et al*., 2019). The extraction of the same phytochemicals could have contributed to the similarity in the results.

Previous studies show that, *P. grantum* contain ellagitannins, phenols, tannins, punicic acid, flavonoids, anthocyanins, estrogenic flavonoids and

flavones (Orak *et al.,* 2012; Young *et al*., 2017). These compounds have antibiotic activity. Tannins having ellagitannins and phenolic acids of *Punica granatum* peel have antibiotic activity (Akhayan *et al*., 2015). Kalaycıoˇ glu, and Erim (2017) suggested that gallic acid in phenolic compounds had the highest antibacterial property. *P. grantum* contains contain 25% tannins. In addition, Carvacrol methyl ether present in pomegranate have antimicrobial effects. However, Thymol is an isomer of carvacrol, which accords it antimicrobial activity (Mohammed *et al*., 2016). The antibiotic property of the extracts could be attributed to adsorption to cell membranes, interaction with enzymes, substrate and metal ion deprivation (Almiahy and Jum'a, 2017). Further, pomegranate extracts have been observed to enhance the activity of some antibiotics such as chloramphenicol, gentamicin, ampicillin, tetracycline, and oxacillin against bacterial pathogens (Al-Tai and Al-Mayyahi, 2021).

In the current study both *P. grantum* peel and seed extracts showed antibacterial activity against *R. solanacearum.* Previous studies produced smaller diameter of zones of inhibition from those obtained in the present study. In a study carried out by Narasimha *et al.* (2015) on antibacterial activity and phytochemical screening of aqueous and methanolic extracts of *P. granatum* against bacterial wilt of tomato, *P. grantum* methanolic extracts gave a zone of inhibition of 19.42±0.8mm. In addition, Khaleel *et al.* (2016) reported that peel extracts of pomegranate caused a mean zone of inhibition of 15.75 ± 0.48mm against *R. solanacearum*. The differences in the zones of inhibition may be attributed to variation in the phytochemicals accumulated by the plants (Mutimawurugo *et al*., 2023). The physico-chemical properties of the soil in which *P. grantum* grow influence the phytochemicals they accumulate which has an impact on the antibiotic properties of the extracts (Hamedo and Makhlouf, 2016).

The MIC and MBC values showed the antibacterial activity of *P. grantum* extract against *R. solanacearum*. However, the seed extracts produced higher MIC and MBC than peel extracts. The findings agreed with a previous study (Malviva *et al*., 14). Hanafy *et al.* (2021) reported that the pomegranate peel extract has the greatest antimicrobial activity. In addition, Khan *et al.* (2017) maintained that methanolic pomegranate peel extracts are more effective on gram-positive bacteria than gram-negative bacteria.

DECLARATIONS:

Ethical Approval: Ethical clearance for this study was obtained from the ethical and protocol review committee of the biosafety, animal use and ethics committee, faculty of veterinary medicine, University of Nairobi with reference number FVM BAUEC/ 2020/252.

Authors' Contributions: BGM, PNW and EMG conceptualized, designed the research protocol, participated in the entire research work and wrote the first and final draft of the manuscript. In addition, all authors read and approved the final draft of the manuscript.

Declaration of Competing Interest: The authors declare no competing interests.

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