



## Original article

Antibacterial activity of guava, moringa, camphor bush and pelargonium extracts against bacterial wilt (*Ralstonia pseudosolanacearum* sp. nov.) of potatoGidraf Onduru Okeyo<sup>a,\*</sup>, Miriam K. Charimbu<sup>a</sup>, Jane Nyaanga<sup>a</sup>, Thiago Mendes<sup>b</sup><sup>a</sup> Department of Crops, Horticulture and Soil Sciences, Egerton University, Njoro, Kenya<sup>b</sup> The CGIAR Research Program on Roots, Tubers and Bananas (RTB), International Potato Center, Regional Office Sub-Saharan Africa, ILRI Campus, Nairobi, Kenya

## ARTICLE INFO

## Article history:

Received 4 January 2022

Revised 7 August 2022

Accepted 1 September 2022

Available online 6 September 2022

## Keywords:

Antibacterial activity

Bacterial wilt

Bio-control

Plant extracts

## ABSTRACT

Bacterial wilt (*Ralstonia pseudosolanacearum* sp. nov.) is a major disease devastating global potato production. Proposed management options are mostly expensive and ineffective. This has necessitated efforts to develop cheaper and eco-friendly management options such as use of botanicals. Antibacterial activity of ethanol and acetone plant extracts from guava (*Psidium guajava*), drumstick (*Moringa oleifera*), camphor bush (*Tarchonanthus camphoratus*) and pelargonium (*Pelargonium zonale*) against *R. pseudosolanacearum* sp. nov. was evaluated in-vitro at a concentration of 100 mg/mL of 1 % Dimethylsulfoxide (DMSO) using disk diffusion technique. The *R. pseudosolanacearum* sp. nov. was isolated from infected haulms collected from potato growing field at the University of Nairobi. The most effective extracts were subjected to further screening at different concentrations to determine their minimum inhibitory concentrations (MICs). All the four plant extracts showed varied antibacterial efficacy. *P. zonale* and *P. guajava* leaf extracts were the most effective with growth inhibition zone of 18.73 mm and 18.60 mm for ethanol and acetone solvents respectively. The average of growth inhibition zones for each plant extract was not significantly different at  $p \leq 0.05$  among extraction solvents. The minimum inhibitory concentration (MIC) results showed that antibacterial activity of *P. zonale* and *P. guajava* leaf started at 6.25 mg/mL with growth inhibition zones of 7.67 and 8.0 mm for ethanol and acetone solvents respectively. *P. zonale* and *P. guajava* leaf extracts exhibited significantly higher antibacterial activity at  $p \leq 0.05$  compared to other extracts. Thus, further research should be conducted to assess their antibacterial potency against *R. pseudosolanacearum* sp. nov. both in-vivo and under field condition.

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## 1. Background

*Ralstonia solanacearum* is a heterogeneous species of plant pathogenic bacteria causing vascular wilt to more than 200 plant species from over 50 families globally (Safni et al., 2014). Within its species complex, *R. solanacearum* (phyloptype II), *Ralstonia pseudosolanacearum* sp. nov. (*R. solanacearum* (phylotypes I and III), and *Ralstonia syzygii* subsp. *indonesiensis* subsp. nov. (*R. solanacearum* (phyloptype IV) are known to affect global production of solana-

ceous crops mainly in the tropical and temperate regions (Rahman et al., 2010; Safni et al., 2014; Boschi et al., 2017). *R. pseudosolanacearum* sp. nov. is ranked second after late blight caused by *Phytophthora infestans* among plant pathogenic diseases affecting potato production in tropical, sub-tropical and cool temperate regions (Muthoni et al., 2012; Karim and Hossain 2018; Mutimawurugo et al., 2020). Globally, *R. pseudosolanacearum* sp. nov. is estimated to affect more than 1.7 M hectares of land under potato production with annual losses of more than USD 950 million (Muthoni et al., 2012; Mwankemwa 2015). In Kenya, for example, the pathogen affects more than 70 % of potato farms resulting in high yield losses ranging from 50 to 100 % (Kaguongo et al., 2010).

Because of its soil-borne nature and ability to persist in the soil over a long period of time, bacterial wilt management in potato fields has proved difficult (Gutarra et al., 2017; Sharma et al., 2017). Various management options such as crop rotation and field

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Peer review under responsibility of King Saud University.



hygiene (Katafiire et al., 2005; Kassa 2016; Sharma et al., 2017; Choudhary et al., 2018), use of resistant varieties (Fock et al., 2000), positive and negative selection (Gildemacher et al., 2011; Sharma et al., 2017), biological control (Whipps 2001; Karim and Hossain 2018) and chemical control (Sarkar and Chaudhuri 2016; Biswal and Dhal 2018) have been suggested for adoption and implementation. However, most of the proposed management options have various limitations such as high phytosanitary standards and negative environmental effects associated with chemical control (Mwankemwa 2015; Muthoni et al., 2014), increased labor requirements associated with cultural control, limited commercialized biological control agents and lack of complete immunity to *R. pseudosolanacearum* sp. nov. from resistant varieties due to genetic variability of its species (Patil et al., 2012).

Due to these limitations especially high phytosanitary standards and negative environmental effects associated with few commercialized chemical bactericides as well as inadequate number of commercialized botanicals (plant extracts) for management of bacterial wilt of potatoes, efforts have been focused on development of botanicals as eco-friendly management options against bacterial wilt pathogen (Oboo et al., 2014; Karim and Hossain 2018). The phytobiocidal effect of various plant extracts against *R. pseudosolanacearum* sp. nov. has been demonstrated by several researchers both in-vitro and in-vivo (Hassan et al., 2009; Oboo et al., 2014; Din et al., 2016; Mutimawurugo et al., 2020; Wamani 2020). However, the phytochemical composition of these bioactive compounds varies from one plant to another and their effectiveness is also affected by variation in agro-climatic conditions coupled with varied abiotic factors during plant growth (Liu et al., 2016a, 2016b; Kumar et al., 2017; Gololo 2018).

Despite the documented positive efficacy results of antibacterial activity of various plant extracts against *R. pseudosolanacearum* sp. nov. of potatoes, this research area has not been fully explored (Borges et al., 2018; Mutimawurugo et al., 2020). Therefore, the purpose of this work was to test the antibacterial activity of acetone and ethanol extracts of various plant species in vitro against *Ralstonia pseudosolanacearum* sp. nov. [*R. solanacearum* (phylo type I)] isolated from infected potato haulms collected from potato growing field at the University of Nairobi, Kenya.

## 2. Materials and methods

### 2.1. Sample collection

Infected plants showing typical bacterial wilt symptoms were collected from potato growing field at the University of Nairobi, Upper Kabete Campus. The station is located at a mean altitude of 1980 m, latitude 1° 15' S and longitude 36° 41' E, in Lower Highland Zone II (LH2) of the Agro-ecological zone (AEZs) of Kenya (Jaetzold et al., 2007). Ten infected plant samples were collected in khaki paper bags, placed in a cool box and taken to the food microbiology laboratory at the University of Nairobi.

### 2.2. Isolation and purification of *Ralstonia pseudosolanacearum* sp. Nov pathogen

To confirm their infection, the samples were tested for bacterial ooze production and bacterial wilt pathogen isolated from samples which produced bacterial ooze. The bacterial wilt pathogen was isolated on a selective medium; Triphenyl tetrazolium chloride (Kelman's TZC agar) as described by Kelman (1954) and Karim and Hossain (2018).

### 2.3. Bacterial wilt pathogen identification and confirmatory tests

The isolated bacterium was identified based on morphological, physiological, cultural, biochemical and pathogenicity tests according to She et al., (2017). The virulent and non-virulent colonies were differentiated using colony characteristic on Kelman's TZC medium. A loopful of the test bacterium was smeared on a clean glass slide with a drop of sterile water to determine Gram staining. The smeared bacterium was air-dried and then heat-fixed over a Bunsen flame. The smear was then stained with crystal violet, Lugol's iodine and safranin with 3 rinses using water according to Rahman et al., (2010). After counterstaining, the slide was blot-dried and examined under a light microscope at x100 magnification with a drop of immersion oil. The Potassium hydroxide (KOH) solubility test was carried out according to Priou et al., (1999) but with slight modification. As opposed to Priou et al., (1999) who carried out KOH directly on bacterial ooze from potato tuber, in this study, KOH test was conducted using isolated bacteria on a sterile glass slide. Biovar identification through carbohydrate fermentation test was carried out according to Rahman et al., (2010) but with slight modifications. In their study, they used microtiter plates and bromothymol blue as an indicator as opposed to this study in which the experiment was carried out using universal bottles and phenol red as an indicator.

### 2.4. Pathogenicity test

Pathogenicity test was performed using one week old seedlings from certified seed potato tubers (Shangi variety) as described by Priou et al., (1999). Ten certified seed potato tubers were planted in pots (polythene sleeves) containing sterile soil media in a greenhouse and allowed to emerge. From the emerged seedlings, 5 seedlings were inoculated while the remaining 5 non-inoculated seedlings were used as checks. Bacterial cultures of  $4.5 \times 10^8$  CFU/mL were prepared by culturing the isolated bacteria on casein peptone glucose (CPG) media without triphenyl tetrazolium chloride (TZC) at  $28 \pm 1$  °C for 48 h. The bacterial cells were harvested by washing the cultures in sterile distilled water. Two days prior to inoculation, the test plants were starved without irrigation and wounds created around the root zone using sterile scalpels. 10 mL of  $4.5 \times 10^8$  CFU/mL bacterial suspension was inoculated around the root zone of 5 emerged seedlings using a syringe while the other 5 seedlings were inoculated with 10 mL of sterile distilled water. The plants were maintained and monitored for symptom development for a period of 2–5 weeks. The temperatures inside the glass house ranged from 25 to 32 °C while the relative humidity ranged from 80 to 90 %. Bacterial pathogen was re-isolated from the symptomatic seedlings using TZC media.

### 2.5. Preparation of plant extracts

Four plants namely guava (*Psidium guajava*), drumstick (*Moringa oleifera*), camphor bush (*Tarhchonanthus camphoratus*) and pelargonium (*Pelargonium zonale*) were used in this study Table 1. The identities of the test plants were confirmed by a taxonomist and voucher samples kept at the Department of Crops, Horticulture and Soil Sciences (CHS), Egerton University, Kenya. The crude extracts were extracted from leaves in all the selected plants except for *Moringa oleifera* in which the extracts were taken both from leaves and seeds as described by (Biswal 2015) but with slight modifications. As opposed to Biswal (2015) who used only water (a polar solvent) as an extraction solvent, in this study—two solvents; ethanol (polar solvent) and acetone (non-polar solvent) were used as extractants. Additionally, the extracted compounds were concentrated to pastes through solvent evaporation to estimate extract yields. Healthy plant parts were collected and washed

**Table 1**  
Plant species and plant parts used in the study.

Plant species	Family	Local name	Common name	Plant part
<i>Moringa oleifera</i>	Moringaceae	Moringa	Drumstick tree	leaves and seeds
<i>Tarchonanthus camphoratus</i>	Asteraceae	Leleshwa	Camphor bush	leaves
<i>Psidium guajava</i>	Myrtaceae	Mapera	Common guava	Leaves
<i>Pelargonium zonale</i>	Geraniaceae	Geraniums	Pelargoniums	Leaves

under a running tap water followed by shade drying at room temperature for three weeks. After complete drying, the plant materials were ground into fine powders. Before extraction, the pH of each plant extract was determined according to Silas et al., (2012) with slight modifications. As opposed to Silas et al., (2012) who soaked 100 g of each plant sample in 5 L of distilled water for a period of 90 days with continuous pH testing on a daily basis, in this study 5 g of each ground plant material was dissolved in 50 mL of distilled water in the ratio of 1:10. The mixtures were shaken on a mechanical shaker (end to end reciprocating shaker) at 600 revolutions per minute for 1 h and the pH of each solution measured using a pH meter. After pH determination, 20 g of fine powder of each plant material was soaked in 200 mL of each extraction solvent (ethanol and acetone) with regular stirring for 48 h. After 48 h, the solutions were filtered through double layers of muslin cloth and the filtrates collected in different sterile bottles. The filtrates were centrifuged at 9000 rpm for 10 min and the supernatants filtered through Whatman filter papers to remove the remaining particles. The filtrates were concentrated to pastes at temperatures slightly below the boiling points of each solvent (50 °C for acetone extracts and 60 °C for ethanol extracts) in two different water baths. The boiling point of acetone is 56 °C while that of ethanol is 78 °C. The beakers were stirred regularly to prevent the pastes from sticking on their walls. The pastes were air-dried overnight, weighed and stored at 4 °C. Percent extract yields were calculated using Eq. (1) as outline by Mostafa et al., (2018).

$$\text{Yield}(\%) = \frac{\text{Weight of extracted plant residue (paste)}}{\text{Weight of raw plant sample}} * 100 \quad (1)$$

## 2.6. Antibacterial bioassays

The antibacterial activity experiment was laid out in a completely randomized design (CRD) with 5 replicates (5 disks per plate) and 9 treatments Table 2. The antibacterial activity of extracts from the four plants was tested using disk diffusion tech-

**Table 2**  
Treatment description of antibacterial assay experiment.

Treatments	Treatment type
Treatment 1	Plant extracts Conc. 100 mg/mL <i>Pelargonium zonale</i> leaves
Treatment 2	<i>Psidium guajava</i> leaves
Treatment 3	<i>Tarchonanthus camphoratus</i> leaves
Treatment 4	<i>Moringa oleifera</i> leaves
Treatment 5	<i>Moringa oleifera</i> seeds
Treatment 6	Positive controls at commercial rates ENRICH BM (Bronopol 27 %w/w)
Treatment 7	KOBE 1.2 SL (Chrysophanol 12 g/l)
Treatment 8	Negative controls Distilled water
Treatment 9	1 % DMSO

Positive control comprised registered conventional bactericide (ENRICH BM (Bronopol 27 %w/w)) and botanical (KOBE 1.2 SL (Chrysophanol 12 g/l)) form management bacterial wilt of potatoes at commercial rates. BM (Bronopol 27 %w/w) was sourced from Osho Chemical Industries Limited while KOBE 1.2 SL (Chrysophanol 12 g/l) was sourced from Amiran Kenya limited. Negative control comprised distilled water and 1 % Dimethylsulfoxide (DMSO).

nique according to Mostafa et al., (2018). 100 mg of each plant extract (paste) was reconstituted in 1 mL of 1 % Dimethylsulfoxide (DMSO). Twenty milliliters of molten TZC medium were poured on each sterile petri dish and allowed to cool and solidify. About 100 µL of bacterial suspension at a concentration of  $4.2 \times 10^5$  CFU/mL was added on the surface of each petri dish and spread gently and uniformly using a sterile L-shaped glass rod. Sterile disks of 6 mm diameter prepared through punching and sterilization of Whatman filter papers were impregnated with 15 µL of each plant extract and allowed to drain for 30 min. For negative control, the disks were impregnated with 15 µL of sterile distilled water and 1 % DMSO respectively while those of positive control were impregnated with 15 µL of KOBE 1.2 SL (Chrysophanol 12 g/l) (registered plant extract in management of bacterial wilt of potatoes) and ENRICH BM (Bronopol 27 %w/w) registered bactericide for management of bacterial wilt of potatoes. The disks were placed on the inoculated TZC media and plates refrigerated at 4 °C for 2 h for optimal diffusion of applied treatments and then incubated at  $28 \pm 1$  °C for 48 h. Presence of growth inhibition zones were observed, the diameters measured using a calibrated ruler and considered as a sign of antibacterial activity.

## 2.7. Determination of minimum inhibitory concentration (MIC) of effective plant extracts

The minimum inhibitory concentration (MIC) experiment of most effective plant extracts was laid out in a complete randomized design (CRD) with 5 replicates (5 disks per plate) and 5 treatments (different concentrations) Table 3. The most effective extracts (*Pelargonium zonale* and *Psidium guajava*) which exhibited high antimicrobial activity at 100 mg/mL were subjected to further screening to determine the minimum inhibitory concentration (MIC) using disk diffusion method as described by Mostafa et al., (2018). Different concentrations of these two plant extracts were prepared by further dilution of 100 mg/mL concentrations to attain 50 mg/mL, 25 mg/mL, 12.50 mg/mL, 6.25 mg/mL and 3.13 mg/mL. Twenty milliliters of molten TZC medium per petri dish was poured on sterile petri dishes and allowed to cool and solidify. 100 µL of bacterial suspension at a concentration of  $4.2 \times 10^5$  CFU/mL was added on the surface of each petri dish and spread gently and uniformly using a sterile glass rod. Different sterile disks (6 mm diameter) were impregnated with 15 µL of each con-

**Table 3**  
Treatment description of minimum inhibitory concentration (MIC) experiment.

Treatments	Treatment type
	Conc. Mg/mL
Treatment 1	50.00
Treatment 2	25.00
Treatment 3	12.50
Treatment 4	6.25
Treatment 5	3.13

*Pelargonium zonale* and *Psidium guajava* extracts were used for MIC experiment.

centration and allowed to drain for 30 min. The disks were then placed on the inoculated TZC media and plates were placed in the refrigerator at 4 °C for 2 h for optimal diffusion of applied treatments and then incubated at 28 ± 1 °C for 48 h. Presence of growth inhibition zones was observed and their diameters were measured and recorded.

### 2.8. Data analysis

The antibacterial activity data were subjected to analysis of variance (ANOVA) using R software, version 4.1.0 (R Studio Team, 2020). The treatment means were separated using Tukey's Honest significant difference (HSD) at  $p \leq 0.05$  with the agricolae package.

## 3. Results

### 3.1. Confirmatory and biovar identification through carbohydrate fermentation tests

The bacterial isolate colonies had an irregular, spherical, white fluidal appearance with pink cores Fig. 1A. A mucoid (elastic and viscous) thread was observed when a wire loop was raised from the bacterial solution a few centimeters from the glass slide used for potassium hydroxide (KOH) test Fig. 1B. The microscopic result indicated that the bacterial isolate cells did not preserve the crystal violet color in the Gram stain test, but they did retain the pink color of the counter strain, and their form was rod shaped. This indicates that the bacterial isolate was a Gram-negative bacterium Fig. 1C. The isolated bacterial pathogen oxidized all the disaccharide sugars and hexose alcohols. This was indicated by color change from red to yellow in inoculated universal bottles compared to the checks (non-inoculated bottles) Fig. 2.

### 3.2. Pathogenicity test

The inoculated seedlings began to exhibit wilt symptoms at 7 days after inoculation. The initial symptoms appeared as wilted apical leaves during the day but the affected seedlings recovered at night. As the symptoms progressed, the wilted leaves failed to recover and the whole infected plants withered two weeks later. No symptoms were observed on non-inoculated plants Fig. 3. The isolated bacteria from the symptomatic plants displayed same morphological characteristics with that of the original pathogen on TZC media. The isolated bacterium was identified as *R. pseudosolanacearum* sp. nov. based on morphological and biochemical traits as well as pathogenicity tests.

### 3.3. Plant extract yields

The pH and yield percentage of plant extracts extracted by both acetone and ethanol are shown in Table 4. Extracts from 20 g of dried and ground powder of plant material produced varied yields ranging from 0.22 to 3.02 g for those extracted using acetone solvents and 1.39–4.59 g for those extracted using ethanol solvents. Except for Moringa seed powder, Ethanol solvent recorded high percent yields compared to acetone.

### 3.4. Antibacterial bioassays

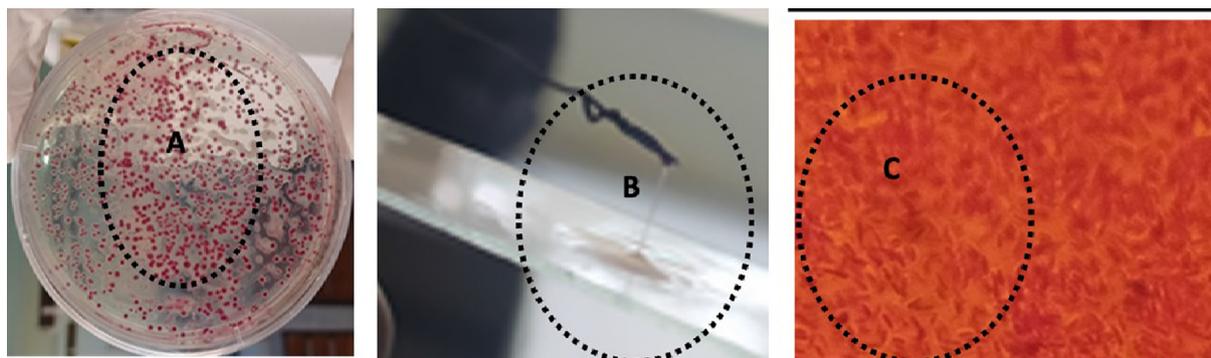
Antibacterial activity of plant extracts from four different plant species and the controls are presented in Table 5. All the tested extracts displayed varied antibacterial potency against *R. pseudosolanacearum* sp. nov. The antibacterial activity of the four plant extracts differed significantly at  $p \leq 0.05$  compared to negative and positive controls. From both solvents, extracts from *Pelargonium zonale* leaves were the most effective at 100 mg/mL of 1 % DMSO based on average of growth inhibition zones followed by *Psidium guajava*. The antibacterial activity of *P. zonale* was significantly different at  $p \leq 0.05$  to those of negative controls and positive controls except ENRICH BM (Bronopol 27 % w/w). The antibacterial activity of *P. guajava* was significantly different at  $p \leq 0.05$  to those of negative and positive controls respectively. Antibacterial activity of each plant extract and controls were depicted by clear zones around the impregnated disks Fig. 4.

### 3.5. Minimum inhibitory concentration (MIC) of effective plant extracts

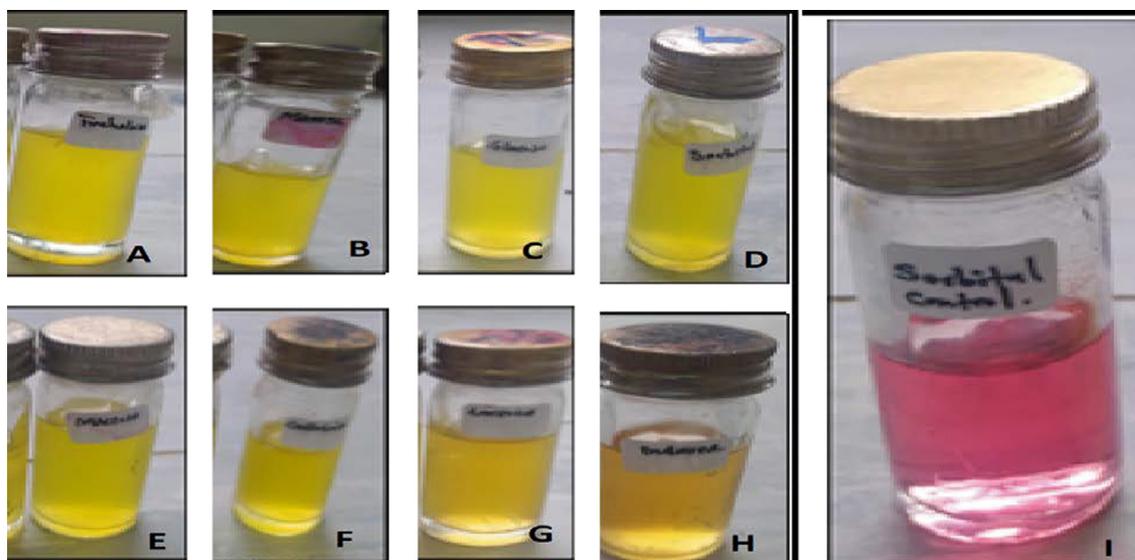
The minimum inhibitory concentration (MIC) of *Pelargonium zonale* and *Psidium guajava* leave extracts are illustrated in Table 6. The inhibitory effect of *P. zonale* against *Ralstonia pseudosolanacearum* sp. nov. started at 6.25 mg/mL of 1 % DMSO with inhibition zones of 7.67 and 8.0 mm for ethanol and acetone extracts while *P. guajava* exhibited inhibitory effect against the same pathogen at 6.25 mg/mL of 1 % DMSO with inhibition zones of 7.67 and 8.0 mm for ethanol and acetone extracts respectively.

## 4. Discussion

The isolated bacteria exhibited irregular, round, and white fluidal colonies with pink centers on triphenyl tetrazolium chloride (TZC) media which was consistent with *R. pseudosolanacearum* sp. nov. characteristics on this medium as described by Kelman (1954). Both the Gram stain and KOH solubility tests confirmed that the isolated bacterium was gram negative and this was in



**Fig. 1.** A) Colony characteristics of *R. pseudosolanacearum* sp. nov. on TZC medium, B) The formation of *R. pseudosolanacearum* sp. nov. mucoid threads as a result of a KOH solubility test and C) Rod-shaped Gram-Negative *R. pseudosolanacearum* sp. nov. bacterium under a light microscope.



**Fig. 2.** Biovar identification of the isolated bacterium based on oxidation of disaccharides sugars and hexose alcohols. A) Trehalose, B) Mannitol, C) Dextrose, D) Sorbitol, E) Maltose, F) Cellulose, G) Lactose, H) Dulcitol I) negative check.



**Fig. 3.** Symptom expression in test plants used for pathogenicity test.

**Table 4**  
The pH of plant extracts and their extract yields per extraction solvent.

Plant species	pH	Extract yield (g)		Percent extract yield (%)	
		Acetone	Ethanol	Acetone	Ethanol
<i>Moringa oleifera</i> leaves	5.75	2.41	4.59	12.05	22.95
<i>Moringa oleifera</i> seeds	5.33	3.02	2.93	15.10	14.65
<i>Tarchonanthus camphoratus</i>	5.51	0.50	1.39	2.50	6.95
<i>Psidium guajava</i>	5.26	1.62	3.40	8.10	17.00
<i>Pelargonium zonale</i>	4.78	0.22	3.81	1.10	19.05

agreement with the research findings by [Rahman et al., \(2010\)](#), [Khasabulli et al., \(2017\)](#) and [She et al., \(2017\)](#). The isolated bacterium oxidized all the disaccharide sugars and hexose alcohols. Similar results were reported by various scientists who classified the isolated *R. pseudosolanacearum* sp. nov. with similar characteristics as biovar III race 1 (*Ralstonia solanacearum* (phylo type I)) ([Rahman et al., 2010](#); [Popoola et al., 2015](#); [Boschi et al., 2017](#); [Khasabulli et al., 2017](#)). Pathogenicity test of the isolated *R. pseudosolanacearum* sp. nov. to susceptible potato seedlings in the greenhouse produced similar wilt symptoms to those diagnosed in the field. Similarly, morphological characteristics of the re-isolated bacterium from these test plants were identical to those

of the original pathogen on TZC media. Similar results were reported in other studies ([Rahman et al., 2010](#); [Popoola et al., 2015](#); [Khasabulli et al., 2017](#); [She et al., 2017](#)).

Ethanol as an extraction solvent recorded significantly higher percent extract yields from all the leaves compared to acetone but this was different with the *M. oleifera* seed extract in which acetone recorded slightly higher yield. Ethanol is a polar solvent thus extracts more diverse secondary metabolites from various plant parts as opposed to acetone which is non-polar ([Yusnawan 2013](#); [Snehlata et al., 2018](#)). Even though polar solvents are documented for high extract yields, the yielded extracts are always low in phenolic and flavonoid content in comparison to extracts from

**Table 5**  
Antibacterial activity of plant extracts against *Ralstonia pseudosolanacearum* sp. nov.

Plant species	Extraction solvent	
	Ethanol Inhibition zone (mm)	Acetone Inhibition zone (mm)
Plant extracts Conc. 100 mg/mL		
<i>Pelargonium zonale</i> leaves	18.73 ± 0.31 a	18.60 ± 0.20 a
<i>Psidium guajava</i> leaves	14.27 ± 0.12b	14.13 ± 0.12b
<i>Tarzonanthus camphoratus</i> leaves	8.40 ± 0.00c	8.73 ± 0.30c
<i>Moringa oleifera</i> leaves	7.37 ± 0.15 d	7.47 ± 0.12 d
<i>Moringa oleifera</i> seeds	7.33 ± 0.12 d	7.33 ± 0.06 d
Positive controls at commercial rates		
ENRICH BM (Bronopol 27 %w/w)	18.13 ± 0.46 a	18.13 ± 0.46 a
KOBE 1.2 SL (Chrysophanol 12 g/l)	8.67 ± 0.12c	8.67 ± 0.12c
Negative controls		
Distilled water	0.00 ± 0.00 e	0.00 ± 0.00 e
1 % DMSO	0.00 ± 0.00 e	0.00 ± 0.00 e
Mean	10.20	9.24
MSD	0.62	0.60
CV	2.11	2.25

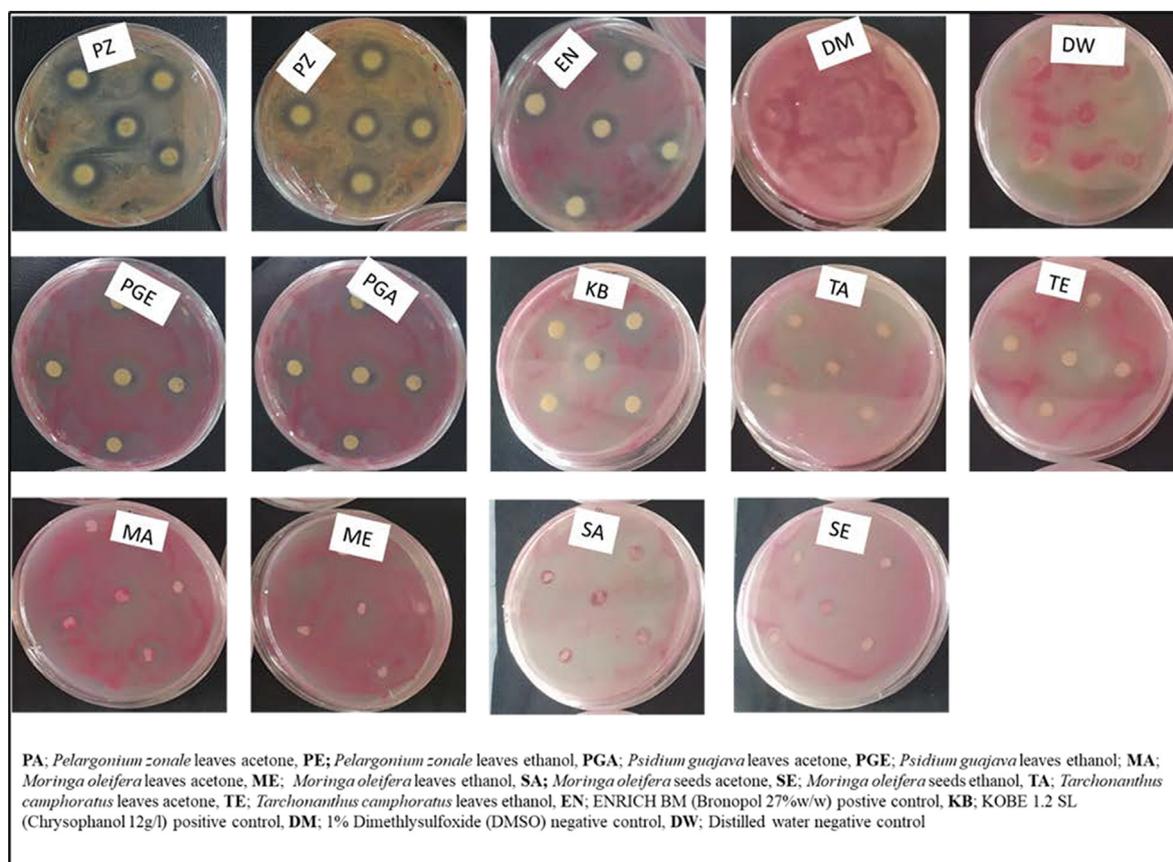
The values are average growth inhibition zones (mm) ± standard deviation from triplicates of ethanol and acetone extracts of each of the four plant materials and controls. Means within the same column having same letter(s) do not differ significantly at  $p \leq 0.05$ , DMSO = Dimethylsulfoxide, MSD = mean square displacement, CV = coefficient of variation.

non-polar solvents (Nawaz et al., 2020). High yields of *M. oleifera* seed extracts from acetone solvent can be attributed to high oil content observed after extract concentration. It is argued that oils are easily extracted by non-polar solvents such as acetone as

opposed to polar ones (Nwabueze and Okocha 2008). Similarly, *M. oleifera* seed extracts might have contained high proportions of phenolic and flavonoid compounds in addition to oils which are highly soluble in non-polar solvents as opposed to polar solvents (Nawaz et al., 2020).

Results from in vitro screening of antibacterial activity of the four plant extracts against *R. pseudosolanacearum* sp. nov. revealed that *P. zonale* leaves were the most effective extract followed by *P. guajava* leaves while *Moringa oleifera* seeds was the least. The varied antibacterial activity between the plant extracts can be attributed to diversity and or difference in concentrations of secondary metabolites in each plant extract (Yihune and Yemata 2019). These in-vitro results were in accordance with those of Oboo et al., (2014) and Biswal (2015) who reported antibacterial activity of these plant extracts against *R. pseudosolanacearum* sp. nov. in-vitro. However, *P. zonale* and *P. guajava* leaf extracts exhibited high growth inhibition zones which contrasted the findings of Biswal (2015) and this can be attributed to difference in plant species, plant parts used in the study, adopted extraction method and varied agro-climatic conditions coupled with diverse abiotic factors during the plant growth (Liu et al., 2016a, 2016b; Kumar et al., 2017; Gololo 2018; Mutimawurugo et al., 2020). Oboo et al., (2014) reported high antibacterial activity of *T. camphoratus* against *R. solanacearum* both in-vitro and in-vivo. In this study, *T. camphoratus* extract was the third best extract and this contrasted their findings. Its dismal performance can be attributed to failure of the extracted paste to dissolve in the reconstituting solvent.

KOBE 1.2 SL (Chrysophanol 12 g/l) and ENRICH BM (Bronopol 27 %w/w) are documented to control bacterial wilt pathogen through induction of host plant resistance (<https://agroduka.com/enrich-bm>; Liu et al., 2016a, 2016b). Stretton and Manson (1973)



**Fig. 4.** Growth inhibition zones of various plant extracts and controls against *Ralstonia pseudosolanacearum* sp. nov.

**Table 6**

Minimum inhibitory concentration (MIC) of effective plant extracts against *Ralstonia pseudosolanacearum* sp. nov.

Plant extract	Conc. Mg/mL	Inhibition zones (mm)	
		Ethanol	Acetone
<i>Pelargonium zonale</i> leaves	50.00	16.17 ± 0.76	16.00 ± 0.00
	25.00	12.83 ± 0.29	12.17 ± 0.29
	12.50	10.00 ± 0.87	10.33 ± 0.29
	6.25	7.67 ± 0.29	8.00 ± 0.50
	3.13	0.00 ± 0.00	0.00 ± 0.00
<i>Psidium indicum</i> leaves	50.00	11.17 ± 0.29	11.83 ± 0.29
	25.00	8.67 ± 0.29	8.67 ± 0.58
	12.50	8.00 ± 0.00	8.17 ± 0.29
	6.25	7.67 ± 0.58	8.00 ± 0.00
	3.13	0.00 ± 0.00	0.00 ± 0.00

The values are average growth inhibition zones (mm) ± standard deviation from triplicates of ethanol and acetone extracts of each concentration of the two effective plant extracts.

reported in-vitro efficacy of bronopol against different strains of bacteria and this was confirmed by the findings of this study which demonstrated its in-vitro antibacterial activity against *R. pseudosolanacearum* sp. nov. The study results also revealed in-vitro antibacterial potency of Chrysophanol against *R. pseudosolanacearum* sp. nov.

The minimum inhibitory concentration (MIC) results revealed that the antibacterial activity of the *P. zonale* and *P. guajava* leaf extracts decreased with decreasing extract concentration. For both extraction solvents, the antibacterial activity of *P. zonale* and *P. guajava* leaf extracts against *R. pseudosolanacearum* sp. nov. started at 6.25 mg/mL of 1 % DMSO. These results were in accordance with the findings of Mutimawurugo et al. (2020), who reported varied MICs ranging from 6.25 to 12.5 mg/mL for different plant extracts against *R. pseudosolanacearum* sp. nov. Similarly, Mutimawurugo et al. (2020) also reported decreased antibacterial activity of different plant extracts against *Ralstonia solanacearum* with decreased extract concentrations and this can be attributed to reduced toxicity levels of bioactive compounds due to dilution effect.

Numerous researchers have investigated the antibacterial efficacy of different plant extracts and their respective bioactive compounds against *R. pseudosolanacearum* sp. nov. both in-vitro and in-vivo (Hassan et al., 2009; Oboo et al., 2014; Biswal 2015; Din et al., 2016; Mutimawurugo et al., 2020; Wamani 2020). Some of the documented bioactive compounds against *R. pseudosolanacearum* sp. nov. include flavonoids and alkaloids (Mutimawurugo et al., 2020), 5-(3-buten-1-ynyl)-2, 2'-bithienyl and 5-(4-acetoxy-1-butynyl)-2, 2'-bithienyl from *Tagetes patula* (Terblanche and de Villiers, 1998). These bioactive compounds are reported to demonstrate bactericidal effect through interaction with enzymes and proteins of the target bacterial cell membrane causing disruption. Additionally, the bioactive compounds with hydrophobic characteristics can react with proteins of the target cell membrane and mitochondria of the target bacteria thereby changing its membrane permeability (Sánchez et al., 2010; Gonelimali et al., 2018; Mostafa et al., 2018).

## 5. Conclusion

These results revealed varied antibacterial potency of the tested plant extracts against *R. pseudosolanacearum* sp. nov. in-vitro. *Pelargonium zonale* and *Psidium guajava* leaf extracts displayed significantly high antibacterial activity compared to other extracts. Further research studies should be conducted to assess the antibacterial potency of these two plant extracts against *R. pseudosolanacearum* sp. nov. both in-vivo and under field condition. Similarly, phytochemical analysis studies should be carried out

on these two extracts to identify the bioactive compounds against *R. pseudosolanacearum* sp. nov.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgments

The Authors appreciates The Centre of Excellence in Sustainable Agriculture and Agribusiness Management (CESAAM), Egerton University for funding the work through World Bank Funded project IDA CREDIT NO-5798.

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