Journal of Agricultural Science; Vol. 14, No. 11; 2022
ISSN 1916-9752 E-ISSN 1916-9760
Published by Canadian Center of Science and Education

Determination of Bioactive Compounds Against Bacterial Wilt of Potato (*Ralstonia pseudosolanacearum* sp. nov.) in *Psidium guajava* and *Pelargonium zonale* Leaf Extracts

Gidraf Onduru Okeyo[1], Miriam Karwitha Charimbu[1], Jane Nyaanga[1] & Thiago Mendes[2]

[1] Department of Crops, Horticulture and Soil Sciences, Egerton University, Njoro, Kenya

[2] The CGIAR Research Program on Roots, Tubers and Bananas (RTB), International Potato Center, Sub-Saharan Africa Regional Office, ILRI Campus, Nairobi, Kenya

Correspondence: Gidraf Onduru Okeyo, Department of Crops, Horticulture and Soil Sciences, Egerton University, Njoro, Kenya. E-mail: ogidraf@gmail.com

Received: August 6, 2022 Accepted: September 14, 2022 Online Published: October 15, 2022

doi:10.5539/jas.v14n11p64 URL: https://doi.org/10.5539/jas.v14n11p64

Abstract

Common guava (*Psidium guajava*) and Pelargonium (*Pelargonium zonale*) have shown *in-vitro* antibacterial activity against *Ralstonia pseudosolanacearum* sp. nov. in previous studies. However, their phytochemical constituents and bioactive compounds against the pathogen have not been identified. The present study investigated the phytochemical components of *P. guajava* and *P. zonale* leaf extracts by phytochemical screening and gas chromatography-mass spectrometry (GC-MS). Phytochemical screening was done using different solvents while 100 mg of the dried ethanolic extract pastes from each plant sample was subjected to GC-MS analysis. Automated mass spectral deconvolution and identification system software (AMDIS, US) was used to analyze chromatograms and spectra representing individual compounds. Compound identification was performed by comparing each of the mass spectra with the database of NIST 11 (Gaithersburg, MD, USA), Wiley 7N (John Wiley, NY, USA) and by comparing the calculated Kovats linear retention indices using retention times of n-alkane series against the values in the NIST webbook. Flavonoids, phenols, alkaloids, saponins, terpenoids and tannins were detected in both plant samples. GC-MS analysis revealed presence of 35 and 26 compounds from *P. zonale* and *P. guajava* respectively. Both *P. zonale* and *P. guajava* had 7 similar compounds with antibacterial properties; Fumaric acid, Phytol, Pyrogallol, 4-Hydroxybenzoic acid, Shikimic acid, Protocatechuic acid and 3, 4, 5-Trihydroxybenzoic acid ethyl ester but *P. zonale* had one additional antibacterial compound; Lactic acid. In both cases, Shikimic acid had the highest percent peak areas of 3.2% for *P. zonale* and 6.8% *P. guajava* respectively. Therefore, *P. zonale* and *P. guajava* can serve as alternative sources of active ingredients for formulation of commercial botanicals for the management of bacterial wilt of potato.

Keywords: botanicals, bacterial wilt, *Psidium guajava*, *Pelargonium zonale*, phytochemical

1. Introduction

Bacterial wilt of potato caused by different species of gram-negative bacterium *Ralstonia solanacearum* mainly *Ralstonia pseudosolanacearum* sp. nov. [*R. solanacearum* (phylotypes I and III)] is one of the major biotic constraints to potato production worldwide (Safni et al., 2014; Boschi et al., 2017). To date there is no satisfactory management option available for complete eradication of the disease hence affected farmers have relied on integrated disease management options. Conversely, efficiency of integrated diseases management has been challenging due to its site-specific nature (Priou et al., 1999). This limitation has propelled adoption and extensive use of conventional pesticides for its management in potato fields (Sarkar & Chaudhuri, 2016; Biswal & Dhal, 2018). However, improper use of these chemical pesticides poses human and environmental health risks especially in developing countries where most farmers use poor quality personal protective equipment (PPE) and deploy limited good agricultural practices (GAPs) (Mulugeta et al., 2020). Additionally, these pesticides can infiltrate into the soil as well as spill into water bodies causing both terrestrial and aquatic health hazards (Rahman et al., 2012; Mulugeta et al., 2020).

Efforts have consequently been focused on developing botanicals (plant extracts) as eco-friendly management options against bacterial wilt pathogen (Rahman et al., 2012). Plant extracts contain numerous bioactive

compounds with bioactivity against various plant pathogens (fungi, bacteria and nematodes). For instance, various researchers have used raw plant extracts and oils for the management of fungi, bacteria, and nematodes (Borges et al., 2018). Some of the bioactive compounds in plant extracts include alkaloids, cyanogenic glycosides, glucosinolates, lipids, phenolics, terpenes, polyacetylenes and polythienyls. These compounds have shown good antimicrobial efficacy both *in-vitro* and *in-vivo* (under greenhouse) condition (Isman, 2000; Zaker, 2016). However, with few exceptions, these efficacy results have not been reproduced in the field and this phenomenon has been attributed to rapid degradation and volatilization of their bioactive compounds under field conditions due to varied abiotic factors (Borges et al., 2018).

Several studies have reported phytobiocidal effect of various plant extracts against bacterial wilt disease both *in-vitro* and *in-vivo* (Hassan et al., 2009; Oboo et al., 2014; Din et al., 2016; Mutimawurugo et al., 2020; Wamani 2020). Examples of reported plant extracts with phytobiocidal effect against bacterial wilt pathogen include; onion (*Allium cepa* L.), garlic (*Allium sativum* L.), lemongrass (*Cymbopogon citratus* Stapf), castor bean (*Ricinus communis* L.), rosemary (*Rosmarinus officinalis* L.), lion's ear (*Leonotis nepetifolia* R.Br.), African basil (*Ocimum gratissimum* L.), tobacco (*Nicotiana tabacum* L.), wild marigold (*Tagetes minuta* L.), stinging nettle (*Urtica massaica* Mildbr), moringa (*Moringa oloifera*), guava (*Psidium guajava*), geranium (*Bauhinia recimosa*), camphor brush (*Tarchonanthus camphoratus*) and French marigold (*Tagetes patula*) among others (Terblanche & Villiers, 1998; Oboo et al., 2014; Biswal, 2015; Mutimawurugo et al., 2020; Okeyo et al., 2021).

In-vitro study by Okeyo et al. (2022) revealed antibacterial activity of *Pelargonium zonale* and *Psidium guajava* against bacterial wilt of potato [*R. pseudosolanacearum* sp. nov. (*R. solanacearum* (phylotype I)]. However, they did not conduct phytochemical profiling nor identified the specific bioactive compounds with antibacterial effect against the target pathogen. Therefore, the present study investigated the phytochemical components in ethanolic leaf extracts of *P. zonale* and *P. guajava* by quantitative phytochemical screening and gas chromatography-mass spectrometry (GC-MS).

2. Material and Methods

2.1 Sample Collection and Identification

Fresh leaves of pelargonium (*Pelargonium zonale*) and guava (*Psidium guajava*) were collected from Taita Taveta and Mau Narok, Kenya, respectively. The identities of the test plants were confirmed by a taxonomist and sample specimens kept at the Department of Crops, Horticulture and Soil Sciences (CHS), Egerton University, Kenya.

2.2 Preparation of the Plant Materials

Five kilograms of *Pelargonium zonale* and *Psidium guajava* leaf samples were washed and rinsed under running tap water followed by shade drying at room temperature for 21 days. For complete drying, the dried plant materials were transferred into an oven at 40 °C for two days and dried materials ground into fine powders using sterile mortars and pestles. The ground powders were passed through 1 mm sieves to remove coarse particles. Twenty grams (20 g) of each powdered sample was weighed and stored separately for phytochemical screening. For Gas Chromatography spectrometry (GC-MS) analysis, 20 g of fine powder of each plant material was soaked in 200 mL of extraction solvent (98% ethanol) with regular stirring for 48 hours after which 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 minutes and the supernatants filtered through Whatman filter papers grade 1 (11 µm) to remove coarse particles. The filtrates were then concentrated to pastes at 60 °C slightly below the boiling point of ethanol (which is 78.37 °C). The resultant pastes were air-dried overnight, weighed, and stored at 4 °C.

2.3 Phytochemical Screening

Phytochemical screening of *Pelargonium zonale* and *Psidium guajava* leaf extracts was conducted at the animal nutrition laboratory, department of animal production at the University of Nairobi. Quantitative phytochemical profiling of *P. zonale* and *P. guajava* powdered leaf samples was carried out to determine the presence of alkaloids, flavonoids, saponins, phenols, terpenoids and tannins (Harborne, 1973; Quettier et al., 2000; Obdoni & Ochuko 2002; Padma et al., 2013; Indumathi et al., 2014; Shah & Yadav, 2015).

2.4 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

Gas chromatography-mass spectrometry (GC-MS) analysis of *P. zonale* and *P. guajava* leaf extracts was conducted at the Mycotoxin and Nutrition Platform laboratory of the International Livestock Research Institute (ILRI), Nairobi Kenya. One hundred milligrams of the dried paste per plant sample was transferred into 2 ml eppendorf tubes containing 2000 µl of absolute methanol and each tube vortexed for 2 minutes for total

dissolution. After complete dissolution, 150 µl aliquot per sample was trasfered into a 1.5 ml eppendorf tube and vacuum dried in a vacuum concentrator at room temperature. Vacuum dried samples were derivatized according to Lisec et al. (2006). Briefly, vacuum dried samples were transferred into dried sample vials followed by addition of 100 µl of pyridine and 75 µl of methoxyamination reagents respectively and the sample vials were tightly capped. The samples were heated at 37 °C on a heating block for 2 hours with regular vortexing after every 15 minutes. After 2 hour, 75 µl of silylation reagent was added in each sample and heated at 70 °C on a heating block for 1 hour with regular vortexing after every 15 minutes. The derivatized samples were cooled and transferred into 250 µl glass inserts loaded in GC vials and the lids capped.

The derivatized samples were analyzed by GC-MS. A portion (1 µl) of the derivatized sample solution was injected in to a 7890A GC system (Agilent Technologies, USA) coupled with a 240-ion trap mass spectrometer detector (Agilent Technologies) using the Agilent 7693A automatic liquid sampler at a split ratio of 10:1. A VF5-MS (5% phenyl methylpolysiloxane, 30.0 m × 0.25 mm, 0.25 µm) film capillary column was used with the injector port set at 280 °C. Helium was used as carrier gas at a flow rate of 1 mL/min. The oven temperature was held at 50 °C followed by an increase of 4 °C/min to 180 °C and finally followed by an increase to 250 °C at 3 °C/min. The ion trap mass spectrometer parameters were as follows: scan range 50-450 (m/z), ionization mode EI, filament delay time 8 min. The transfer line temperature, manifold temperature and trap temperature of 250 °C, 100 °C and 150 °C, respectively. The total run time was 56 minutes.

2.5 Compound Identification

A homologous n-alkane series was analyzed alongside the derivatized sample and used to compute the Kovats Linear retention index. Chromatograms and spectra representing individual compounds were analyzed using the automated mass spectral deconvolution and identification system software (AMDIS, US). The identification of the individual compounds was performed by comparing each of the mass spectra with the database of NIST 11 (Gaithersburg, MD, USA) and Wiley 7N (John Wiley, NY, USA) consisting of more than 62,000 patterns of known compounds and also by comparing the calculated Kovats linear retention indices using retention times of n-alkane series against the values obtained in the NIST webbook (https://webbook.nist.gov/chemistry/) for the same capillary column stationary phase (Strehmel et al., 2008). The compounds were identified as their corresponding Silyl and or Oxime derivatives. Absolute compound identity was assigned for matches within +/- 5 of the database Kovats linear retention index. The quantification of individual compounds was performed by the peak area percentage method. The identified compound concentrations were expressed as percentage of each individual compound to the total of all compounds detected in the derivatized sample.

2.6 Data Analysis

Data obtained from quantitative phytochemical profiling was first tested for normality using the Wilk's Shapiro test and the difference in the mean compositions compared using Mann-Whitney U test at 5% probability level (Wilcoxon, 1945; Mann & Whitney, 1947; Wilcoxon, 1992) in R software, version 4.1.0 (R Studio Team, 2020).

3. Results

3.1 Quantitative Phytochemical Screening

Quantitative phytochemical profiling of *Pelargonium zonale* and *Psidium guajava* leaves revealed the presence of all the six tested phytochemicals; flavonoids, phenols, alkaloids, saponins, terpenoids and tannins in both *P. zonale* and *P. guajava* leaves as illustrated in Table 1. A comparison between *P. guajava* and *P. zonale* leaf extracts showed that there was no significant difference at $p \leq 0.05$ in their phytochemical composition.

Table 1. Phytochemical components of *Psidium guajava* and *Pelargonium zonale* leaf extracts

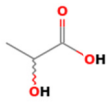
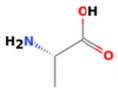
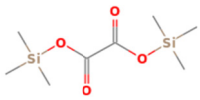
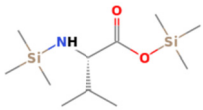
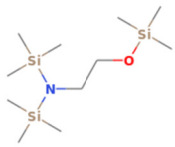
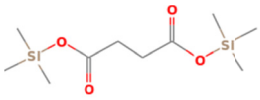
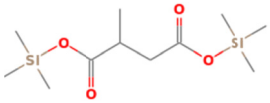
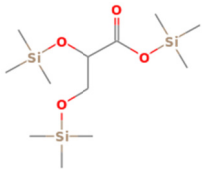
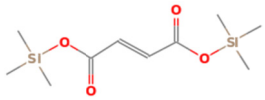
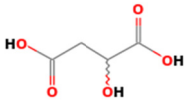
Plant species	Relative abundance					
	Flavonoids (mg Q.E./g)	Phenols (mg G.A.E./g)	Alkaloids (%)	Saponins (%)	Terpenoids (%)	Tannins (mg T.A.E./g)
Psidium guajava	34.44±0.01[a]	46.22±0.02[a]	1.26±0.02[a]	6.08±0.02[a]	0.67±0.01[a]	106.40±0.02[a]
Pelargonium zonale	16.24±0.01[a]	47.62±0.02[a]	1.93±0.01[a]	9.41±0.02[a]	2.27±0.01[a]	190.30±0.02[a]
p-value (α = 0.05)	0.08	0.10	0.08	0.10	0.08	0.10

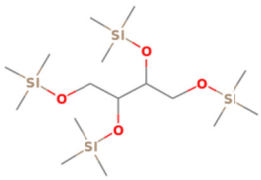
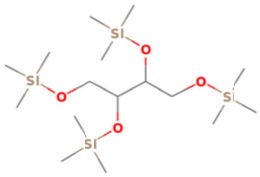
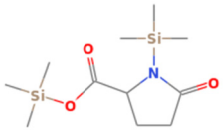
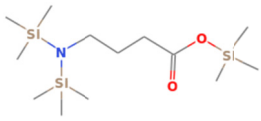
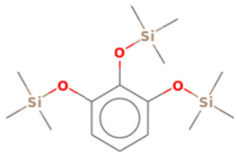
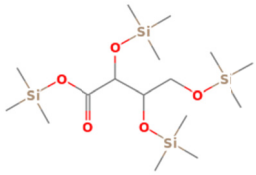
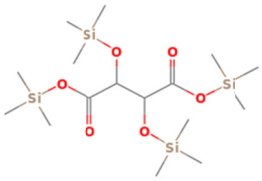
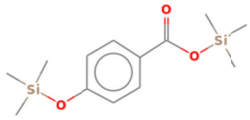
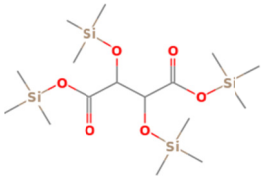
Note. The values are presented as average means±standard deviation. Q.E. = Quercetin Equivalent, G.A.E. = Gallic Acid Equivalent, T.A.E. = Tannic Acid Equivalent.

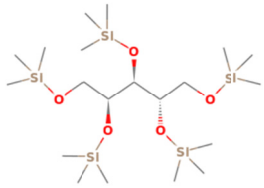
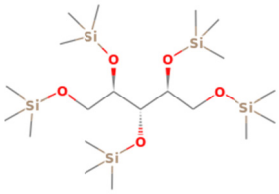
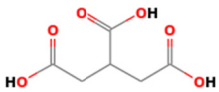
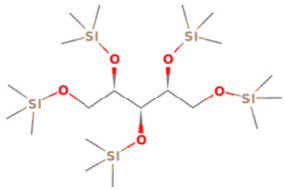
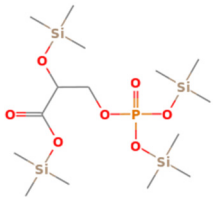
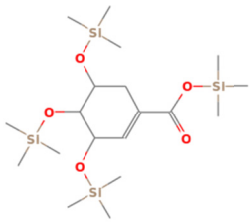
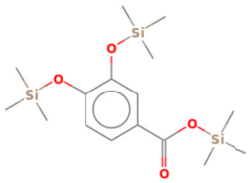
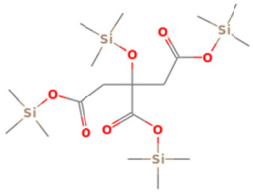
3.2 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

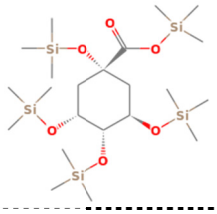
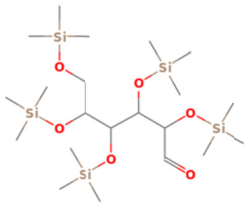
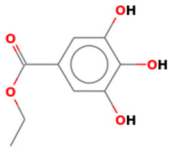
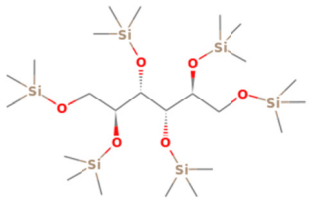
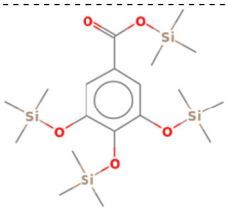
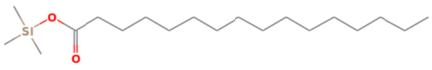
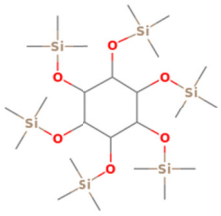
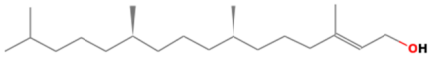
A total of 35 and 26 compounds were identified from GC-MS analysis of ethanolic leaf extracts of *Pelargonium zonale* and *Psidium guajava* respectively. These phytochemical constituents, their retention time (RT), molecular formula, molecular weight (g/mol), percent peak areas and structures of detected compounds are presented in Tables 2 and 3.

Table 2. Bioactive compounds in aqueous ethanolic fraction of *Pelargonium zonale*

Peak No.	RT (min)	Compound ID	Molecular formula	MW (g/mol)	Peak area (%)	Structure of detected compounds
2	15.45	Propane-1,3-diol	$C_3H_8O_2$	76.09	0.04	
3	15.63	Lactic acid	$C_3H_6O_3$	90.08	0.08	
6	17.18	Alanine	$C_3H_7NO_2$	89.09	0.06	
8	18.53	Oxalic acid	$C_8H_{18}O_4Si_2$	234.40	0.47	
11	21.31	Valine	$C_{11}H_{27}NO_2Si_2$	261.51	0.09	
13	23.13	Ethanolamine	$C_{11}H_{31}NOSi_3$	277.63	0.07	
16	24.54	Succinic acid	$C_{10}H_{22}O_4Si_2$	262.45	0.16	
17	24.98	Methylsuccinic acid	$C_{11}H_{24}O_4Si_2$	276.48	0.03	
18	25.34	Glyceric acid	$C_{12}H_{30}O_4Si_3$	322.62	0.11	
21	25.68	Fumaric acid	$C_{10}H_{20}O_4Si_2$	260.43	0.03	
23	30.49	Malic acid	$C_4H_6O_5$	134.09	2.47	

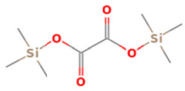
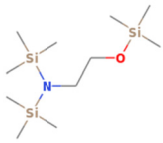
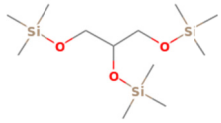
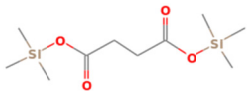
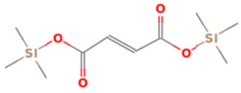
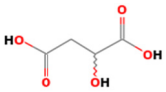
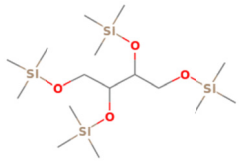
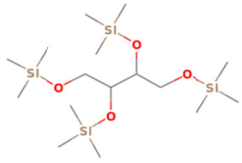
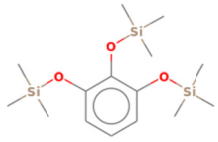
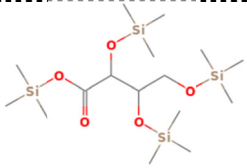
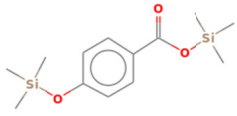
26	31.03	Theitol	$C_{16}H_{42}O_4Si_4$	410.84	0.12	
27	31.31	Erythritol	$C_{16}H_{42}O_4Si_4$	410.84	2.24	
29	31.57	Oxoproline	$C_{11}H_{23}NO_3Si_2$	273.48	0.4	
30	31.81	Aminobutyric acid,	$C_{13}H_{33}NO_2Si_3$	319.66	0.25	
34	32.35	Pyrogallol	$C_{15}H_{30}O_3Si_3$	342.65	0.12	
38	32.96	Threonic acid	$C_{16}H_{40}O_5Si_4$	424.83	0.08	
46	34.23	Tartaric acid	$C_{16}H_{38}O_6Si_4$	438.81	0.16	
47	34.84	4-Hydroxybenzoic acid	$C_{13}H_{22}O_3Si_2$	282.48	0.04	
50	35.46	Tartaric acid	$C_{16}H_{38}O_6Si_4$	438.81	1.81	

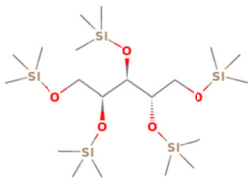
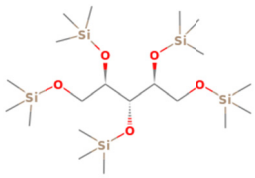
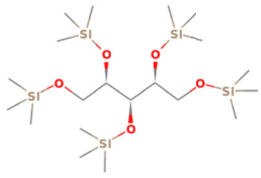
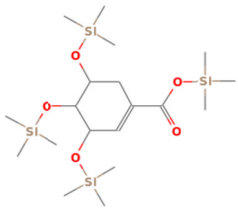
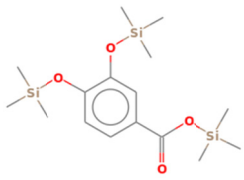
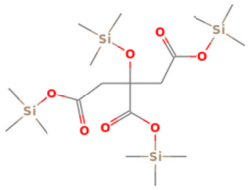
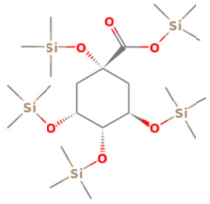
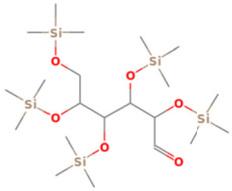
55	37.53	Arabitol	$C_{20}H_{52}O_5Si_5$	513.05	0.31	
58	37.87	Ribitol	$C_{20}H_{52}O_5Si_5$	513.05	0.35	
59	37.9	Tricarballylic acid	$C_6H_8O_6$	176.12	0.36	
64	38.27	Xylitol	$C_{20}H_{52}O_5Si_5$	513.05	9.94	
69	39.42	Glycerol-3-phosphate	$C_{15}H_{39}O_7PSi_4$	474.78	0.35	
74	40.73	Shikimic acid	$C_{19}H_{42}O_5Si_4$	462.88	3.15	
76	41.03	Protocatechuic acid	$C_{16}H_{30}O_4Si_3$	370.66	0.21	
78	41.21	Citric acid	$C_{18}H_{40}O_7Si_4$	480.85	0.33	

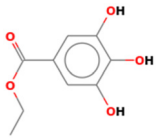
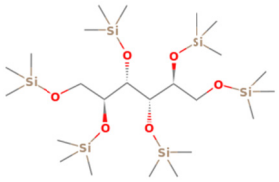
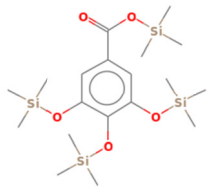
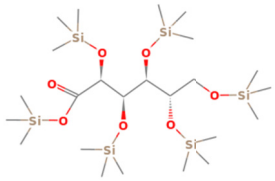
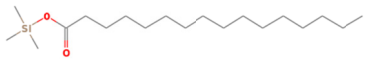
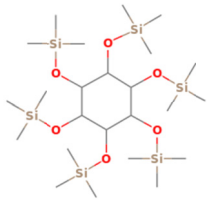
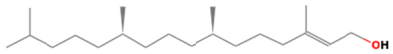
83	42.77	Quinic acid	$C_{22}H_{52}O_6Si_5$	553.07	1.81	
99	44.05	Glucose	$C_{21}H_{52}O_6Si_5$	541.06	2.32	
102	44.84	3,4,5-Trihydroxybenzoic acid ethyl ester	$C_9H_{10}O_5$	198.17	0.34	
104	45.13	Mannitol	$C_{24}H_{62}O_6Si_6$	615.26	10.59	
112	45.49	Gallic acid	$C_{19}H_{38}O_5Si_4$	458.84	48.96	
121	47.65	Palmitic acid	$C_{19}H_{40}O_2Si$	328.61	0.76	
129	49.99	Myo-Inositol	$C_{24}H_{60}O_6Si_6$	613.24	0.43	
134	51.58	Phytol	$C_{20}H_{40}O$	296.53	0.59	

Note. RT = Retention time. Structures, molecular formulas and molecular weights were sourced from NIST webbook.

Table 3. Bioactive compounds in aqueous ethanolic fraction of *Psidium guajava*

Peak No.	RT (min)	Compound ID	Molecular formula	MW (g/mol)	Peak area (%)	Structure of detected compounds
1	18.52	Oxalic acid	$C_8H_{18}O_4Si_2$	234.40	0.40	
3	23.13	Ethanolamine	$C_{11}H_{31}NOSi_3$	277.63	0.02	
4	23.39	Glycerol	$C_{12}H_{32}O_3Si_3$	308.64	6.00	
6	24.56	Succinic acid	$C_{10}H_{22}O_4Si_2$	262.45	0.03	
7	25.68	Fumaric acid	$C_{10}H_{20}O_4Si_2$	260.43	0.09	
10	30.50	Malic acid	$C_4H_6O_5$	134.09	0.06	
11	31.05	Theitol,	$C_{16}H_{42}O_4Si_4$	410.84	0.09	
12	31.31	Erythritol	$C_{16}H_{42}O_4Si_4$	410.84	0.73	
14	32.37	Pyrogallol	$C_{15}H_{30}O_3Si_3$	342.65	0.09	
16	32.97	Threonic acid	$C_{16}H_{40}O_5Si_4$	424.83	0.08	
18	34.85	4-Hydroxybenzoic acid	$C_{13}H_{22}O_3Si_2$	282.48	0.02	

23	37.53	Arabitol	$C_{20}H_{52}O_5Si_5$	513.05	0.14	
24	37.88	Ribitol	$C_{20}H_{52}O_5Si_5$	513.05	0.04	
25	38.27	Xylitol	$C_{20}H_{52}O_5Si_5$	513.05	2.01	
31	40.75	Shikimic acid	$C_{19}H_{42}O_5Si_4$	462.88	6.77	
32	41.05	Protocatechuic acid	$C_{16}H_{30}O_4Si_3$	370.66	0.14	
33	41.18	Citric acid	$C_{18}H_{40}O_7Si_4$	480.85	0.03	
37	42.79	Quinic acid	$C_{22}H_{52}O_6Si_5$	553.07	10.04	
45	44.06	Glucose	$C_{21}H_{52}O_6Si_5$	541.06	11.15	

48	44.86	3,4,5-Trihydroxybenzoic acid ethyl ester	$C_9H_{10}O_5$	198.17	0.04	
49	45.11	Mannitol	$C_{24}H_{62}O_6Si_6$	615.26	1.66	
52	45.46	Gallic acid	$C_{19}H_{38}O_5Si_4$	458.84	21.78	
60	47.36	Gluconic acid	$C_{24}H_{60}O_7Si_6$	629.24	0.10	
61	47.65	Palmitic acid	$C_{19}H_{40}O_2Si$	328.61	0.77	
63	50.00	Myo-Inositol	$C_{24}H_{60}O_6Si_6$	613.24	7.24	
68	51.60	Phytol	$C_{20}H_{40}O$	296.53	0.54	

Note. RT = Retention time. Structures, molecular formulas and molecular weights were sourced from NIST webbook.

The chromatograms are presented in Figures 1 and 2. *P. zonale* had 8 compounds with antibacterial properties; Shikimic acid (3.15%), Phytol (0.59%), 3, 4, 5-Trihydroxybenzoic acid ethyl ester (0.34%), Protocatechuic acid (0.21%), Pyrogallol (0.12%), Lactic acid (0.08%), 4-Hydroxybenzoic acid (0.04%) and Fumaric acid (0.03%). *P. guajava* had 7 compounds with antibacterial properties; Shikimic acid (6.77%), Phytol (0.54%), Protocatechuic acid (0.14%), Pyrogallol (0.09%), Fumaric acid (0.09%), 3,4,5-Trihydroxybenzoic acid ethyl ester (0.04%) and 4-Hydroxybenzoic acid (0.02%).

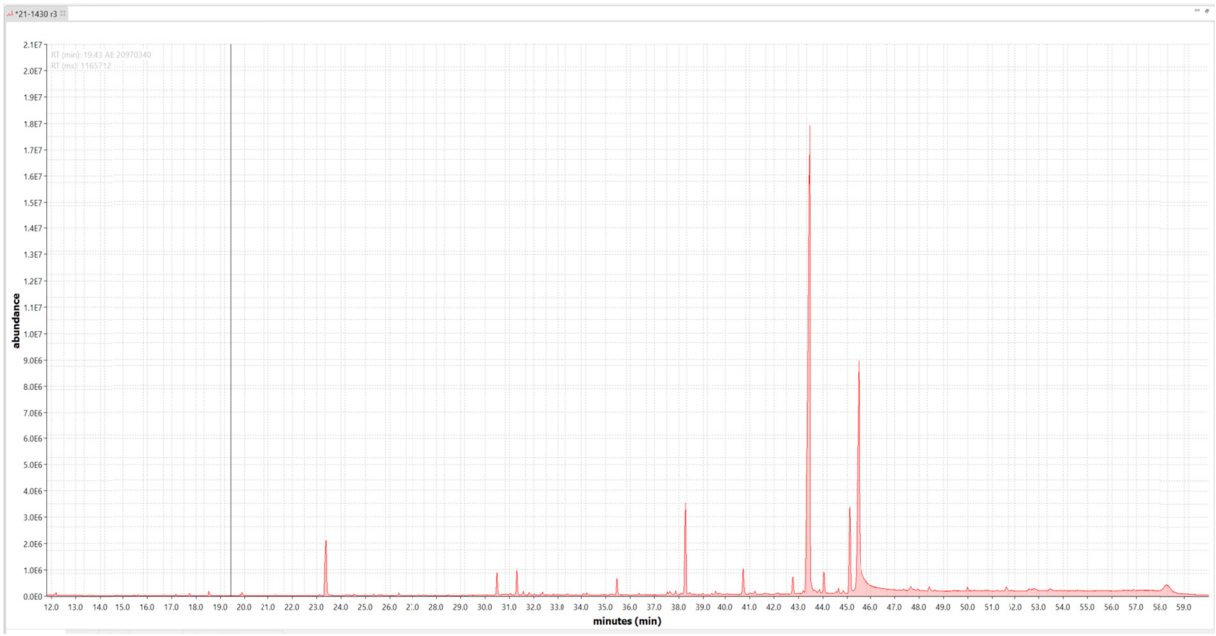


Figure 1. GC-MS chromatogram of ethanolic extracts of *Pelargonium cucullatum*

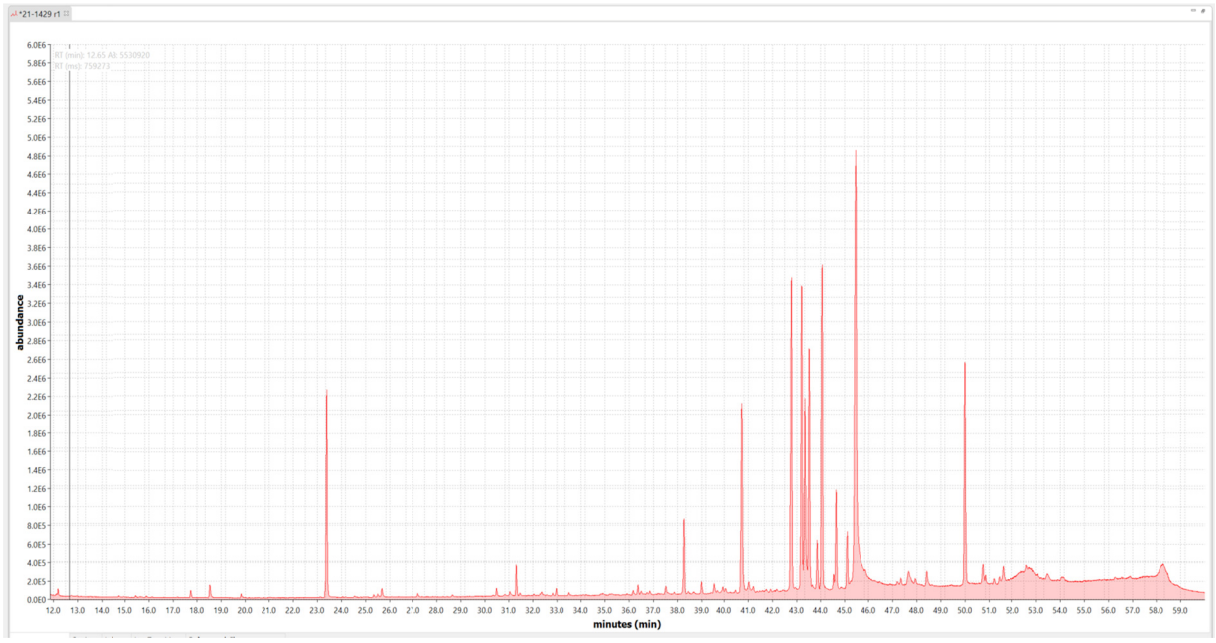


Figure 2. GC-MS chromatogram of ethanolic extracts of *Psidium guajava*

4. Discussion

Results from phytochemical profiling of *Pelargonium zonale* and *Psidium guajava* leaf extracts revealed the presence of six phytochemicals namely flavonoids, phenols, alkaloids, saponins, terpenoids and tannins. Flavonoids are polyphenolic compounds (secondary antioxidants) synthesized by plant in response to infections from various abiotic and biotic stresses (Kumar & Pandey, 2013; Karak, 2019). They are reported to function as antibacterial agents against both gram-negative and gram-positive bacteria (Karim & Hossain, 2018; Adamczak et al., 2020). Karim and Hossain (2018) and Mutimawurugo et al. (2020), reported efficacy of flavonoids against bacterial wilt of potato. Flavonoids controls plant pathogenic bacterial through coagulation of bacterial cell proteins as well as affecting different amino acid synthesis enzymes (Al-Obaidi, 2014; Din et al., 2s016).

Alkaloids have antibacterial and antifungal properties which have propelled their adoption and use in plant protection field (Adamski et al., 2020). *In-vitro* and *in-vivo* studies have revealed their antibacterial properties against bacterial wilt of solanaceous plants (Din et al., 2016; Mutimawurugo et al., 2020; Abd-Elrahim et al., 2021). Some alkaloids act through inhibition of topoisomerase enzyme while others such as bisindole monoterpenoid act as DNA intercalating agents and hence poisoning the target bacteria (Tanaka et al., 2006).

Saponins protect plant against infection by different microbes as well as infestation by insect pests (Desai et al., 2009). Steroidal saponins react with bacterial membrane sterols inhibiting cell growth and hence overall bacterial growth (Wang et al., 2000). Some groups of terpenoids are used as pesticides and fungicides due to their insecticidal and antimicrobial properties (Martin-Smith & Khatoon, 1963). They exhibit antibacterial activity by acting on the phospholipid bilayers of bacterial cells affecting electron transport, phosphorylation process, protein translocation and other enzyme dependent reactions leading to cell mortality (Dorman & Deans, 2000). Phenols aids adaptation of plant species to abiotic and biotic stresses (Cosme et al., 2020). Some phenols such as thymol and carvacrol are documented to have antibacterial activity against bacterial wilt of solanaceous plants (Abd-Elrahim et al., 2021). Tannins also have anti-microbial properties (Din et al., 2016). They can directly kill the target bacteria by damaging the cell membrane and or can bind to adhesins in the host tissue preventing attachment of bacterial inoculum, disease establishment and spread (Mainasara et al., 2012; Wang, 2014).

From the GC-MS analysis results, *P. zonale* had 8 compounds documented to have antibacterial activity while *P. guajava* had 7. The 7 compounds were similar for the two plant extracts but their percent concentration varied per plant. *P. zonale* had one additional antibacterial compound. These compounds comprised of Fumaric acid, Pyrogallol, 4-Hydroxybenzoic acid, Shikimic acid, Protocatechuic acid, 3, 4,5-Trihydroxybenzoic acid ethyl ester (Gallic acid ethyl ester) and Phytol for both the two plant extracts and Lactic acid for *P. zonale* (He et al., 2011; M. Estevez & J. Estevez, 2012; Khan et al., 2015; Cynthia et al., 2018; Islam et al., 2018; Miret-Casals et al., 2018; Aldulaimi et al., 2019; Imade et al., 2021). In both plants, Shikimic acid had the highest peak area while the other compounds had less than 1% concentration and this can be an indication that Shikimic acid was the main bioactive component against *Ralstonia pseudosolanacearum* sp. nov. in the two plant extracts. However, the antibacterial effect might have also resulted from any of the detected compounds and or from the synergistic effect between either or all the identified antibacterial compounds per plant extract.

Shikimic acid has shown *in-vitro* antibacterial activity against different bacteria but with higher inhibition activity against gram-negative bacteria as opposed to gram-positive bacteria (Tripathi et al., 2015; Bai et al., 2022). The high inhibitory activity against gram-negative bacteria can be attributed to thinner peptidoglycan layer in gram-negative bacteria as opposed to gram-positive bacteria (Tripathi et al., 2015). Shikimic acid is assumed to exhibit different modes of action against pathogenic bacteria; disrupts oxidative phosphorylation pathway, inhibits membrane fluidity by changing glycerophospholipid and fatty acid levels, disturbs the normal functions of potassium and calcium channels, dishevels protein synthesis through influenced ribosome function and aminoacyl-tRNA synthesis upon penetration of the bacteria cell membrane and finally, it interferes with the pyruvate metabolic pathway (Bai et al., 2022). The un-dissociated form of Fumaric acid and Lactic acid passes freely through the bacterial cell membrane into cytoplasm. Upon entry, the acid dissociates to release protons which acidify the cytoplasm leading to cell mortality (Lu et al., 2011; Tango et al., 2015). Even though pyrogallol has shown antibacterial activity against different bacterial pathogens, its mechanism of action and toxicity have not been studied (Tinh et al., 2016; Kharouf et al., 2022).

Protocatechuic acid exhibits antibacterial activity through depolarization of the cell membrane, reduction of intracellular pH and adenosine triphosphate (ATP) as well as leakage of cell content and destruction of cell morphology. Additionally, Protocatechuic acid affects energy metabolism and amino acid biosynthesis of the target bacteria (Wu et al., 2022). The 4-Hydroxybenzoic acid affects the fluidity of the bacterial cell membrane (Patra, 2012). Gallic acid ethyl ester induces permanent changes in the cell membrane such as increased hydrophobicity, alters the surface charge as well as increased pore formation in the cell membrane resulting to leakage of essential intracellular constituents and hence mortality of the target bacteria (Borges et al., 2013; Aldulaimi et al., 2019). Phytol induces intracellular reactive oxygen species (ROS) accumulation in the bacterial cell leading to imbalance between intracellular ROS and the antioxidant defense system hence reducing glutathione (GSH) cell content. The low GSH exposes the cell to detrimental effects from the action of low pH, chlorine compounds, as well as oxidative and osmotic stresses. Phytol also causes DNA damage of affected bacteria (Lee et al., 2016).

5. Conclusion

The study revealed that *Pelargonium zonale* and *Psidium guajava* leaf extracts have various secondary metabolites with different bioactivities. GC-MS analysis showed a total of 8 and 7 antibacterial compounds from ethanolic leaf extracts of *P. zonale* and *P. guajava* respectively. In both plants, Shikimic acid had the highest percent peak area among the detected antibacterial compounds and hence could be the main bioactive component against *Ralstonia pseudosolanacearum* sp. nov. in the two plant extracts. Further screening should be done with each compound to confirm their singular and or synergistic antibacterial activity as well as their mode of action against *R. pseudosolanacearum* sp. nov.

Acknowledgements

The authors appreciate 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.

Reference

Abd-Elrahim, R., Tohamy, M. R., Atia, M. M., Elashtokhy, M. M., & Ali, M. A. (2021). Bactericidal activity of some plant essential oils against *Ralstonia solanacearum* infection. *Saudi Journal of Biological Sciences*. https://doi.org/10.1016/j.sjbs.2021.11.045

Adamczak, A., Ożarowski, M., & Karpiński, T. M. (2020). Antibacterial activity of some flavonoids and organic acids widely distributed in plants. *Journal of Clinical Medicine, 9*(1), 109. https://doi.org/10.3390/jcm9010109

Adamski, Z., Blythe, L. L., Milella, L., & Bufo, S. A. (2020). Biological activities of alkaloids: From toxicology to pharmacology. *Toxins, 12*(4), 210. https://doi.org/10.3390/toxins12040210

Aldulaimi, O., Drijfhout, F., Uche, F. I., Horrocks, P., & Li, W. W. (2019). Discovery, synthesis and antibacterial evaluation of phenolic compounds from Cylicodiscus gabunensis. *BMC Complementary and Alternative Medicine, 19*(1), 1-11. https://doi.org/10.1186/s12906-019-2589-2

Al-Obaidi, O. (2014). Studies on antibacterial and anticancer activity of Nerium oleander extracts. *Eur Chem Bull, 3*(3), 259-262.

Bai, J., Wu, Y., Bu, Q., Zhong, K., & Gao, H. (2022). Comparative study on antibacterial mechanism of shikimic acid and quinic acid against Staphylococcus aureus through transcriptomic and metabolomic approaches. *LWT-Food Science and Technology, 153*, 112441. https://doi.org/10.1016/j.lwt.2021.112441

Biswal, G. (2015). Studies on antibacterial activity of some aqueous plant extracts against *Ralstonia solanacearum* causing bacterial wilt and brown rot of potato. *International Journal of Plant Sciences (Muzaffarnagar), 10*(1), 1-6. https://doi.org/10.15740/HAS/IJPS/10.1/1-6

Biswal, G., & Dhal, N. K. (2018). Management of bacterial wilt disease of potato in coastal plains of Odisha. *African Journal of Microbiology Research, 12*(12), 284-289. https://doi.org/10.5897/AJMR2017.8714

Borges, A., Ferreira, C., Saavedra, M. J., & Simões, M. (2013). Antibacterial activity and mode of action of ferulic and gallic acids against pathogenic bacteria. *Microbial Drug Resistance, 19*(4), 256-265. https://doi.org/10.1089/mdr.2012.0244

Borges, D. F., Lopes, E. A., Moraes, A. R. F., Soares, M. S., Visôtto, L. E., Oliveira, C. R., & Valente, V. M. M. (2018). Formulation of botanicals for the control of plant-pathogens: A review. *Crop Protection, 110*, 135-140. https://doi.org/10.1016/j.cropro.2018.04.003

Boschi, F., Schvartzman, C., Murchio, S., Ferreira, V., Siri, M. I., Galván, G. A., & Dalla-Rizza, M. (2017). Enhanced bacterial wilt resistance in potato through expression of Arabidopsis EFR and introgression of quantitative resistance from *Solanum commersonii*. *Frontiers in Plant Science, 8*, 1642. https://doi.org/10.3389/fpls.2017.01642

Cosme, P., Rodríguez, A. B., Espino, J., & Garrido, M. (2020). Plant phenolics: Bioavailability as a key determinant of their potential health-promoting applications. *Antioxidants, 9*(12), 1263. https://doi.org/10.3390/antiox9121263

Cynthia, I. F., Hery, S., & Akhmad, D. (2018). Antibacterial and antioxidant activities of pyrogallol and synthetic pyrogallol dimer. *Research Journal of Chemistry and Environment, 22*, 39-48.

Desai, S. D., Desai, D. G., & Kaur, H. (2009). Saponins and their biological activities. *Pharma Times, 41*(3), 13-16.

Din, N., Ahmad, M., Siddique, M., Ali, A., Naz, I., Ullah, N., & Ahmad, F. (2016). Phytobiocidal management of bacterial wilt of tomato caused by *Ralstonia solanacearum* (Smith) Yabuuchi. *Spanish Journal of Agricultural Research, 14*(3), 1-13. https://doi.org/10.5424/sjar/2016143-9012

Dorman, H. D., & Deans, S. G. (2000). Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *Journal of Applied Microbiology, 88*(2), 308-316. https://doi.org/10.1046/j.1365-2672.2000.00969.x

Harbone, J. B. (1973). *Phytochemical methods chapman and hall* (3rd ed., pp. 21-29). London, New York.

Hassan, M. A. E., Bereika, M. F. F., Abo-Elnaga, H. I. G., & Sallam, M. A. A. (2009). Direct antimicrobial activity and induction of systemic resistance in potato plants against bacterial wilt disease by plant extracts. *The Plant Pathology Journal, 25*(4), 352-360. https://doi.org/10.5423/PPJ.2009.25.4.352

He, C. L., Fu, B. D., Shen, H. Q., Jiang, X. L., & Wei, X. B. (2011). Fumaric acid, an antibacterial component of Aloe vera L. *African Journal of Biotechnology, 10*(15), 2973-2977. https://doi.org/10.5897/AJB10.1497

Imade, E. E., Omonigho, S. E., Babalola, O. O., & Enagbonma, B. J. (2021). Lactic acid bacterial bacteriocins and their bioactive properties against food-associated antibiotic-resistant bacteria. *Annals of Microbiology, 71*(1), 1-14. https://doi.org/10.1186/s13213-021-01652-6

Indumathi, C., Durgadevi, G., Nithyavani, S., & Gayathri, P. K. (2014). Estimation of terpenoid content and its antimicrobial property in Enicostemma litorrale. *International Journal of ChemTech Research, 6*(9), 4264-4267.

Islam, M. T., Ali, E. S., Uddin, S. J., Shaw, S., Islam, M. A., Ahmed, M. I., & Atanasov, A. G. (2018). Phytol: A review of biomedical activities. *Food and Chemical Toxicology, 121*, 82-94. https://doi.org/10.1016/j.fct.2018.08.032

Isman, M. B. (2000). Plant essential oils for pest and disease management. *Crop Protection, 19*(8), 603-608. https://doi.org/10.1016/S0261-2194(00)00079-X

Karak, P. (2019). Biological activities of flavonoids: an overview. *International Journal of Pharmaceutical Sciences and Research, 10*(4), 1567-1574.

Karim, Z., & Hossain, M. S. (2018). Management of bacterial wilt (*Ralstonia solanacearum*) of potato: Focus on natural bioactive compounds. *Journal of Biodiversity Conservation and Bioresource Management, 4*(1), 73-92. https://doi.org/10.3329/jbcbm.v4i1.37879

Khan, A. K., Rashid, R., Fatima, N., Mahmood, S., Mir, S., Khan, S., & Murtaza, G. (2015). Pharmacological activities of protocatechuic acid. *Acta Polonise Pharmaceutica, 72*(4), 643-650.

Kharouf, N., Sauro, S., Hardan, L., Fawzi, A., Suhanda, I. E., Zghal, J., & Mancino, D. (2022). Impacts of Resveratrol and Pyrogallol on Physicochemical, Mechanical and Biological Properties of Epoxy-Resin Sealers. *Bioengineering, 9*(3), 85. https://doi.org/10.3390/bioengineering9030085

Kumar, S., & Pandey, A. K. (2013). Chemistry and biological activities of flavonoids: an overview. *The Scientific World Journal, 2013*. https://doi.org/10.1155/2013/162750

Lee, W., Woo, E. R., & Lee, D. G. (2016). Phytol has antibacterial property by inducing oxidative stress response in Pseudomonas aeruginosa. *Free Radical Research, 50*(12), 1309-1318. https://doi.org/10.1080/10715762.2016.1241395

Lisec, J., Schauer, N., Kopka, J., Willmitzer, L., & Fernie, A. R. (2006). Gas chromatography mass spectrometry-based metabolite profiling in plants. *Nature Protocols, 1*(1), 387-396. https://doi.org/10.1038/nprot.2006.59

Lu, H. J., Breidt Jr, F., Pérez-Díaz, I. M., & Osborne, J. A. (2011). Antimicrobial effects of weak acids on the survival of Escherichia coli O157: H7 under anaerobic conditions. *Journal of Food Protection, 74*(6), 893-898. https://doi.org/10.4315/0362-028X.JFP-10-404

M Estevez, A., & J Estevez, R. (2012). A short overview on the medicinal chemistry of (—)-shikimic acid. *Mini Reviews in Medicinal Chemistry, 12*(14), 1443-1454. https://doi.org/10.2174/138955712803832735

Mainasara, M. M., Aliero, B. L., Aliero, A. A., & Yakubu, M. (2012). Phytochemical and antibacterial properties of root and leaf extracts of Calotropis procera. *Nigerian Journal of Basic and Applied Sciences, 20*(1), 1-6. https://doi.org/10.5923/j.ijmb.20110101.03

Mann, H. B., & Whitney, D. R. (1947). On a test of whether one of two random variables is stochastically larger than the other. *The Annals of Mathematical Statistics, 18*, 50-60. https://doi.org/10.1214/aoms/1177730491

Martin-Smith, M., & Khatoon, T. (1963). Biological activity of the terpenoids and their derivatives. *Progress in Drug Research/Fortschritte der Arzneimittelforschung/Progrès des Recherches Pharmaceutiques*, 279-346. https://doi.org/10.1007/978-3-0348-7050-4_4

Mulugeta, T., Muhinyuza, J. B., Gouws-Meyer, R., Matsaunyane, L., Andreasson, E., & Alexandersson, E. (2020). Botanicals and plant strengtheners for potato and tomato cultivation in Africa. *Journal of Integrative Agriculture, 19*(2), 406-427. https://doi.org/10.1016/S2095-3119(19)62703-6

Mutimawurugo, M. C., Ogweno, J. O., Muhinyuza, J. B., & Wagara, I. N. (2020). In vitro antibacterial activity of selected plant extracts against potato bacterial wilt (*Ralstonia solanacearum* Smith) in Rwanda. *Journal of Applied Horticulture, 22*(3). 202-208.

National Center for Biotechnology Information. (2022). *PubChem Compound Summary for CID 617748, Methyl bis(trimethylsilyl) phosphate*. Retrieved March 28, 2022, from https://pubchem.ncbi.nlm.nih.gov/compound/Methyl-bis_trimethylsilyl_-phosphate

Obadoni, B. O., & Ochuko, P. O. (2002). Phytochemical studies and comparative efficacy of the crude extracts of some haemostatic plants in Edo and Delta States of Nigeria. *Global Journal of Pure and Applied Sciences, 8*(2), 203-208. https://doi.org/10.4314/gjpas.v8i2.16033

Oboo, H., Muia, A. W., & Kinyua, Z. M. (2014). Effect of selected essential oil plants on bacterial wilt disease development in potatoes. *Journal of Applied Biosciences, 78*, 6666-6674. https://doi.org/10.4314/jab.v78i1.2

Okeyo, G. O., Charimbu, M. K., Nyaanga, J., & Mendes, T. (2022). Antibacterial activity of guava, moringa, camphor bush and pelargonium extracts on bacterial wilt (*Ralstonia pseudosolanacearum* sp. nov.) of potato. *Saudi Journal of Biological Sciences*, 103438. https://doi.org/10.1016/j.sjbs.2022.103438

Padma, R., Parvathy, N. G., Renjith, V., Kalpana, P. R., & Rahate, P. (2013). Quantitative estimation of tannins, phenols, and antioxidant activity of methanolic extract of Imperata cylindrica. *International Journal of Research in Pharmaceutical Sciences, 4*(1), 73-77.

Patra, A. K. (2012). An overview of antimicrobial properties of different classes of phytochemicals. *Dietary Phytochemicals and Microbes*, 1-32. https://doi.org/10.1007/978-94-007-3926-0_1

Priou, S., Aley, P., Chujoy, E., Lemaga, B. A., French, E. R., & French, E. (1999). Integrated control of bacterial wilt of potato. *CIP Slide Training Series IV-3*. *International Potato*. Retrieved January 25, from http://cipotato.org/wp-content/uploads/2014/10/guiaing.pdf

Quettier-Deleu, C., Gressier, B., Vasseur, J., Dine, T., Brunet, C., Luyckx, M., & Trotin, F. (2000). Phenolic compounds and antioxidant activities of buckwheat (*Fagopyrum esculentum* Moench) hulls and flour. *Journal of Ethnopharmacology, 72*(1-2), 35-42. https://doi.org/10.1016/S0378-8741(00)00196-3

Rahman, M. M., Khan, A. A., Ali, M. E., Mian, I. H., Akanda, A. M., & Abd Hamid, S. B. (2012). Botanicals to control soft rot bacteria of potato. *The Scientific World Journal, 2012*(2), 796472. https://doi.org/10.1100/2012/796472

RStudio Team (2020). *RStudio: Integrated Development for R*. RStudio, PBC, Boston, MA. Retrieved from http://www.rstudio.com

Safni, I., Cleenwerck, I., De Vos, P., Fegan, M., Sly, L., & Kappler, U. (2014). Polyphasic taxonomic revision of the *Ralstonia solanacearum* species complex: Proposal to emend the descriptions of *Ralstonia solanacearum* and *Ralstonia syzygii* and reclassify current *R. syzygii* strains as *Ralstonia syzygii* subsp. syzygii subsp. nov., *R. solanacearum* phylotype IV strains as *Ralstonia syzygii* subsp. indonesiensis subsp. nov., banana blood disease bacterium strains as *Ralstonia syzygii* subsp. celebesensis subsp. nov. and *R. solanacearum phylotype* I and III strains as. *International Journal of Systematic and Evolutionary Microbiology, 64*(9), 3087-3103. https://doi.org/10.1099/ijs.0.066712-0

Sarkar, S., & Chaudhuri, S. (2016). Bacterial wilt and its management. *Current Science, 110*(8), 1439-1445.

Shah, R. K., & Yadav, R. N. S. (2015). Qualitative phytochemical analysis and estimation of total phenols and flavonoids in leaf extract of sarcochlamys pulcherrima wedd. *Global Journal of Bio-science and Biotechnology, 4*(1), 81-4.

Shapiro, S. S., & Wilk, M. B. (1965). An analysis of variance test for normality (complete samples). *Biometrika, 52*(3/4), 591-611. https://doi.org/10.1093/biomet/52.3-4.591

Strehmel, N., Hummel, J., Erban, A., Strassburg, K., & Kopka, J. (2008). Retention index thresholds for compound matching in GC–MS metabolite profiling. *Journal of Chromatography B, 871*(2), 182-190.

Tanaka, J. C. A., Da Silva, C. C., De Oliveira, A. J. B., Nakamura, C. V., & Dias Filho, B. P. (2006). Antibacterial activity of indole alkaloids from *Aspidosperma ramiflorum*. *Brazilian Journal of Medical and Biological Research, 39*, 387-391. https://doi.org/10.1590/S0100-879X2006000300009

Tango, C. N., Mansur, A. R., & Oh, D. H. (2015). Fumaric acid and slightly acidic electrolyzed water inactivate gram positive and gram negative foodborne pathogens. *Microorganisms, 3*(1), 34-46. https://doi.org/10.3390/microorganisms3010034

Terblanche, J., & De Villiers, D. A. (1998). The suppression of *Ralstonia solanacearum* by marigolds. *Bacterial Wilt Disease* (pp. 325-331). Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-662-03592-4_49

Tinh, T. H., Nuidate, T., Vuddhakul, V., & Rodkhum, C. (2016). Antibacterial activity of pyrogallol, a polyphenol compound against Vibrio parahaemolyticus isolated from the central region of Thailand. *Procedia Chemistry, 18*, 162-168. https://doi.org/10.1016/j.proche.2016.01.025

Tripathi, P., Rawat, G., Yadav, S., & Saxena, R. K. (2015). Shikimic acid, a base compound for the formulation of swine/avian flu drug: statistical optimization, fed-batch and scale up studies along with its application as an antibacterial agent. *Antonie Van Leeuwenhoek, 107*(2), 419-431. https://doi.org/10.1007/s10482-014-0340-z

Wamani, A. O. (2020). *Screening of Microbial Antagonists and Plant Extracts Against Selected Tomato Pathogens and Their Potential in the Management of Bacterial Wilt* (Doctoral dissertation, University of Nairobi, Kenya). Retrieved from http://erepository.uonbi.ac.ke/bitstream/handle/11295/154678/Wamani_Screening%20of%20Microbial%20Antagonists%20and%20Plant%20Extracts%20Against%20Selected%20Tomato%20Pathogens%20and%20Their%20Potential%20in%20the%20Management%20of%20Bacterial%20Wilt.pdf?sequence=1&isAllowed=y

Wang, L. (2014). *The antibacterial effect of secondary compounds in plants* (pp. 6-7). 50th Annual Southeastern Michigan Junior Science and Humanities Symposium. https://doi.org/10.3923/rjmp.2014.204.213

Wang, Y., McAllister, T. A., Yanke, L. J., & Cheeke, P. R. (2000). Effect of steroidal saponin from Yucca schidigera extract on ruminal microbes. *Journal of Applied Microbiology, 88*(5), 887-896. https://doi.org/10.1046/j.1365-2672.2000.01054.x

Wilcoxon, F. (1945). Individual comparisons by ranking methods. *Biometrics Bulletin, 1*, 80-83. https://doi.org/10.2307/3001968

Wilcoxon, F. (1992). Individual comparisons by ranking methods. *Breakthroughs in statistics* (pp. 196-202). Springer, New York, NY. https://doi.org/10.1007/978-1-4612-4380-9_16

Wu, M., Tian, L., Fu, J., Liao, S., Li, H., Gai, Z., & Gong, G. (2022). Antibacterial mechanism of Protocatechuic acid against Yersinia enterocolitica and its application in pork. *Food Control, 133*, 108573. https://doi.org/10.1016/j.foodcont.2021.108573

Zaker, M. (2016). Natural plant products as eco-friendly fungicides for plant diseases control-A review. *The Agriculturists, 14*(1), 134-141. https://doi.org/10.3329/agric.v14i1.29111

Copyrights