Acta Scientiarum
http://periodicos.uem.br/ojs/acta
ISSN on-line: 1807-863X
Doi: 10.4025/actascibiolsci.v41i1.45402

Antibacterial activity of medicinal plant extracts against *Ralstonia solanacearum* (Smith) that causes bacterial wilt in hot pepper (*Capsicum annuum* L.)

Endalamaw Yihune[1] and Getahun Yemata[2]*

[1]Department of Chemistry, College of Science, Bahir Dar University, Bahir Dar, Ethiopia. [2]Department of Biology, College of Science, Bahir Dar University, P.O. Box 79, Bahir Dar, Ethiopia, *Author for correspondence. E-mail: gyemata12@gmail.com

ABSTRACT. This study was conducted to evaluate the *in vitro* antibacterial activity of some medicinal plants against *Ralstonia solanacearum*. Bioactive chemicals were extracted from *Burcea antidysenterica, Eucalyptus citriodora, Justicia schimperiana, Lantana camara, Melia azedarach* and *Ricinus communis* leaves using maceration method. The bioassay was evaluated by disc diffusion method. The pathogen was isolated from infected *Capsicum annuum* plants using Casamino acid-Peptone-Glucose agar (CPG) medium. The isolate was identified using cultural, biochemical characteristics, pathogenicity test and found to be *R. solanacearum*. The methanol extracts had different composition, percentage extract yield, antibacterial activity and relative percentage inhibition. Unlike others, extracts of *E. citriodora* and *R. communis* consisted of all the tested secondary metabolites. All species showed antibacterial activity except *M. azedarach*. Significant differences were recorded in antibacterial activity among species and test concentrations. The highest antibacterial activity and the lowest bacteriostatic and bactericidal concentrations were found from *E. citriodora* and *R. communis* extracts. The higher potency of *E. citriodora* and *R. communis* extracts suggested the potential of the two species as a biocide to control bacterial wilt. However, further *in vivo* studies on these species extracts are compulsory.

Keywords: crude extract; *Eucalyptus citriodora* (Hook); inhibition zone, phytochemicals; *Ricinus communis* L.

Received on November 16, 2018.
Accepted on January 1, 2019.

Introduction

Hot pepper (*Capsicum annuum L.*) is one of the cultivated species of the genus *Capsicum* in the family *Solanaceae*. The genus originated in Central and South America (Grubben & Denton, 2004). The combination of four lines of evidence indicated that domestication of *C. annuum* could have occurred in Mexico (Kraft et al., 2014). The Spanish and Portuguese have brought capsicum pepper to Europe, from where especially hot pepper was widely spread to all tropical and sub-tropical areas of the world (Grubben & Denton, 2004). *C. annuum* has been grown as a popular vegetable and spice ubiquitous in the tropics and many very distinct types and landraces had been developed at the end of the 17th C (Grubben & Denton, 2004).

Hot pepper is cultivated so widely in Africa that African people consider it as a traditional African vegetable or spice (Grubben & Denton, 2004). Similarly, the history of hot pepper in Ethiopia is perhaps the most ancient than the history of any other vegetable product and established as a national spice in the country. It has been grown extensively under wide range of environmental conditions, from lowlands to 3000 meters above sea levels (Grubben & Denton, 2004; Yadeta, Belew, Gebreselasie, & Marame, 2011). The crop is a warm season crop requiring an optimum day and night temperatures of 20-30 and 15-20°C, respectively. Hot pepper grows better on loam or sandy loam soil with a pH of 6-7. It is grown as a rainfed crop in areas receiving 850 - 1200 mm of annual rainfall (Zeleke & Derso, 2015). Evidences showed that the world's average yield of hot pepper is 1.7 tons ha^{-1} for dry pod and 16.3 tons/ha for green pod (Food and Agriculture Organization of the United Nations Statistic [FAOSTAT], 2012). In the same cropping season, the average yield of hot pepper dry and green pod was found to be 2.3 and 8.1 tons/ha, respectively (Central Statistical Agency of Ethiopia [CSA], 2007). The average green pepper yield decreased from 6.5 to 6.2

tons/ha from 2016 to 2017. Similarly, the average red pepper yield has been reduced from 1.8 tons/ha in 2016 to 1.7 tons/ha in 2017 (Central Statistical Agency of Ethiopia [CSA], 2010).

In Ethiopia, hot pepper has nutritional, medicinal and economic values. Such importance of the vegetable is entrenched in the chemical composition of the fruits (capsaicin and oleoresin). Nutritionally, hot pepper is produced as spice flavoring and color to food while providing essential vitamins and minerals (Shumeta, 2012; Zeleke & Derso, 2015). It is one component of the daily diet of Ethiopian people. The average daily consumption of hot pepper by an Ethiopian adult is estimated to be 15 g, which is higher than tomatoes and most other vegetables (Marcelis, Heuvelink, Hofman-Eijer, Bakker, & Xue, 2004). It is used in different ways and forms. The fine powdered pungent product is a necessary flavoring and coloring ingredient in the common traditional sauce 'Wot' while mature green pods ('karia') are eaten as salads (Shumeta, 2012). Moreover, hot pepper is rich source of soluble and insoluble fibers, vitamins (C, niacin, A, E, K and folic acid) and minerals (calcium, sodium, magnesium, phosphorus and copper) (Grubben & Denton, 2004; Emmanuel-Ikpeme, Henry, & Okiri, 2014). Ethiopians believe that a person who frequently consumes hot pepper has resistance to various diseases (Shumeta, 2012). This is attributed to the chemical composition of hot pepper fruit in that it contains higher amount of tannins, flavonoid, saponin, terpenoid and carotenoids as compared to other species of capsicum (Emmanuel-Ikpeme et al., 2014). Besides, capsaicin has shown anti-oxidant, antimutagenic, anticarcinogenic and immunosuppressive activities. It also inhibits bacterial growth and platelet aggregation (Grubben & Denton, 2004). It is an important cash crop for small holder farmers that generate income both in domestic and export markets (Lin et al., 2013). It also creates employment to urban and rural workers (Shumeta, 2012).

Despite its importance, the production of hot pepper in Ethiopia has shown a progressive decrease through years. This is caused by several abiotic and biotic factors (Welderufael, 2016). One of the major constraints that contribute to the low productivity of hot pepper is bacterial wilt disease, caused by a soil borne bacterium *R. solanacearum* (Smith) (Yabuuchi, Kosako, Yano, Hotta, & Nishiuchi, 1995). The pathogen is the most destructive disease of the crop. It is xylem inhabiting that clogs the tissue and blocks water and mineral transport in the plant. This results in rapid wilting especially young and succulent plants. The first visible symptom appears on the youngest leaves followed by stunting, downward curling of leaflets and petioles (Singh, Singh, Kumar, & Birah, 2014). Currently, the common management strategies include mechanical removal of infected plant materials and the application of bactericidal chemicals. However, the continuous use of chemicals results in indiscriminate damage and accumulation of harmful residues in the soil, water and grains (Grubben & Denton, 2004). This shows the need to investigate alternative, ecofriendly, biodegradable and easily accessible disease controlling strategies. Thus, this study was aimed to evaluate the *in vitro* antibacterial activity of methanol leaf extracts from *Burcea antidysenterica, Eucalyptus citriodora, Justicia schimperiana, Lantana camara, Melia azedarach* and *Ricinus communis* and characterize the chemical composition of the extracts.

Material and methods

Plant materials

Medicinal plants were selected on the basis of their traditional use in treating human ailments and antimicrobial activity reports in the literature. Accordingly, fresh leaf samples of *B. antidysenterica* (JF. Mill), *E. citriodora* (Hook), *J. schimperiana* (Hochst. ex Nees) T. Anderson, *L. camara* L., *M. azedarach* L. and *R. communis* L. were collected from Bahir Dar city, Ethiopia and the surrounding areas and brought to the laboratory. The leaf samples were washed with running tap water to remove dust and other debris. The sample was dried to constant weight under shade at room temperature. The dried leaf samples were milled to fine powder using an electrical grinder. The powder was sieved through 0.5 mm mesh and stored in polythene (plastic) bags. The bags were tightly closed and preserved at 4°C until needed for extraction.

Extraction and phytochemical of screening of methanol extracts

The bioactive ingredients of medicinal plant leaf samples were extracted using maceration method as described in Rai, Pai, Kedilaya, and Hegde (2013). Methanol was used for extraction. The leaf powder of each species and solvent were added into separate conical flasks in the ratio of 1:10 (for every gram of leaf powder, 10 mL of solvent). The flasks were tightly closed and placed on an orbital shaker at a speed of 170

rpm. The content of each flask was shaken for 72 hours under room temperature. The extract of each species was decanted to new flasks in the presence of four layers of cheese cloth and cotton. The residue was squeezed to the flasks and discarded. The crude extract was further passed through Whatman no. 1 filter paper. The solvent was evaporated using rotary evaporator. Dried extracts of each species were stored at 4ºC until used for antibacterial testing. Extract yield (%) was determined gravimetrically using the dry weight of the extract and the initial weight of the leaf powder as follows:

$$\text{Extract yield (\%)} = \frac{\text{Weight of crude extract}}{\text{Initial weight of powder}} * 100$$

Qualitative analysis was performed to confirm the presence or absence of major secondary metabolites in the methanol leaf extracts. Phytochemical screening was carried out for phenolic compounds, terpenoids, tannins, flavonoids, alkaloids and saponins using standard chemical methods as described in Mendonça-Filho (2006) and Ayoola et al. (2008).

Isolation and identification of *R. solanacearum*

Infected plant samples were collected from one of the major hot pepper growing areas, in Mankusa, Amhara Regional State. Sampling sites were selected based on number of hot pepper plants showing wilting symptoms. Accordingly, a site with wider prevalence and higher severity was used for sample collection. Infected plants were uprooted from these sites and separately put in polythene bags and thoroughly washed with water to remove soil particles and other debris. The collected samples were preserved in an ice box and transported to Bahir Dar University for further processing.

The collected infected hot pepper plants were surface sterilized by soaking in 5% sodium hypochlorite solution. These materials were rinsed three times in sterilized distilled water. Infected plant parts were cut into small pieces and macerated with mortar and pestle in the presence of 0.85% NaCl sterile saline solution. Serial dilutions were prepared from the bacterial suspension and inoculated to separate Petri plates poured with CPG growth medium. The Petri plates were incubated at 28 ± 1°C for 72 hours. After this period, suspension was prepared from isolated white creamy colonies and stored in screw caped vials at 4ºC. This suspension was inoculated to separate Petri plates filled with CPG growth medium. Sub culturing was made until pure culture of *R. solanacearum* is found. The isolate was regularly renewed by plating on CPG medium. The pathogen was identified based on colony characteristics, biochemical and pathogenicity tests.

Different biochemical tests were performed to characterize *R. solanacearum*. The Gram staining test was performed as described by Schaad (1980) and confirmed that the bacterium isolated was Gram negative. *R. solanacearum* colonies were aseptically removed from Petri plates with an inoculating wire loop and put on glass slide in a drop of 3% KOH solution. This was stirred for 10 seconds and observed for the formation of slime threads (Suslow, Schroth, & Isaka, 1982).

Catalase test was carried out by mixing a loop full of a fresh bacterial culture with two drops of solution of 3% H_2O_2 on a microscope slide according to the method described by He, Sequeria, and Kelman (1983). Bubble formation indicated a positive test for catalase.

Nutrient agar plates containing 0.2% soluble starch (w v^{-1}) were streaked with *R. solanacearum* suspension and incubated at 28 ± 1ºC until heavy growth occurred. Then the plates were flooded with KI solution. A clear zone around a colony was record as positive reaction for starch hydrolysis (Sands, 1990).

Pathogenicity test

The pathogenicity test was carried out according to Mimura, Yoshikawa, and Hirai (2009). Seeds of hot pepper were sown and grown in plastic buckets for three months and half under glasshouse conditions. The bucket soil was infested by pouring 10 mL of *R. solanacearum* suspension containing 1.5 x 10^8 CFU mL^{-1} around the root. Plants in the control were irrigated with equal volume of sterilized water. The development of disease symptoms was monitored for 6 weeks for every other day in the greenhouse. Plants with at least one wilted leaf were classified as wilted (Figure 1). The pathogen was re-isolated from infected plants using CPG agar medium. The colonies were compared with the initial colonies and found to be similar.

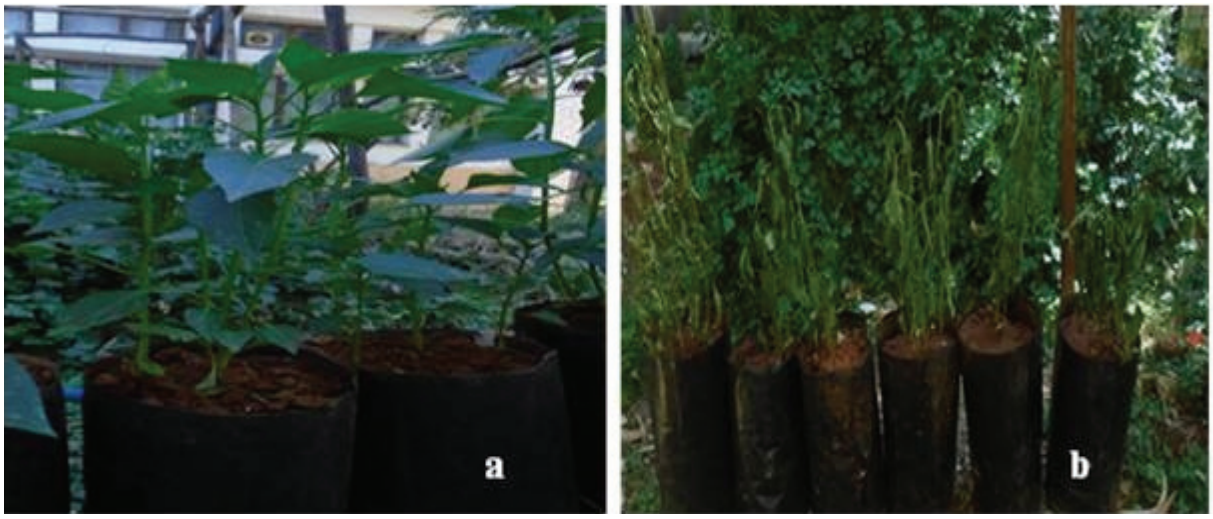

Figure 1. Pathogenicity test for *Ralstonia solanacearum* on *Capsicum annuum*: (a) plants treated with sterilized distilled water; and (b) plants inoculated with *R. solanacearum* suspension (Photo by Endalamaw Yihune).

Antibacterial activity assay

The antibacterial activity of methanol leaf extract of selected medicinal plants against *R. solanacearum* was tested *in vitro* using disc diffusion method. Serial test concentrations (100, 50, 25, 12.5, and 6.25 mg mL^{-1}) of each species extract were prepared first by dissolving the dry extract in 2 mL of methanol and then adding one mL sterilized distilled water to reach to the required test concentration. Discs of 5 mm in diameter were prepared from Whatman no. 1 filter paper using paper borer. These discs were impregnated to each test concentration for 24 hours. Then after, the discs were taken out from the extract and dried under aseptic condition.

Ralstonia solanacearum suspension was prepared from 72 hours old pure culture of *R. solanacearum*. Sterilized distilled water was used to prepare the suspension. The optical density of the suspension was adjusted to 0.132 at 600 nm using a spectrophotometer (Jenway, Models 7310 & 7315; United Kingdom). This is equivalent to 0.5 McFarland turbidity standards. The number of bacterial population at this turbidity standard is 1.5 x 10^8 CFU mL^{-1}. Approximately 0.5 mL of the bacterial suspension was aseptically inoculated to CPG medium and uniformly distributed using cotton swab. The dried discs were placed on the inoculated Petri plates within 15 minutes. Sensitive discs of tetracycline at one concentration (30 µg mL^{-1}) were used as standard controls. Discs soaked in methanol were used as negative controls. All the Petri plates were incubated at 28 ± 1ºC. Each test concentration was replicated thrice, and the test was performed three times. The antibacterial activities of extracts were evaluated in terms of diameter of zone of inhibition. The inhibition zone was measured using a transparent ruler including the disc.

The minimum inhibitory concentration (MIC) of leaf extracts was determined using agar dilution method as described in European Committee for Antimicrobial Susceptibility Testing EUCAST (2000). One mL of each test concentration of each methanol extract was thoroughly mixed with 19 mL of CPG molten growth medium and poured to Petri plates. The medium was allowed to solidify at room temperature and inoculated with 0.1 mL of *R. solanacearum* suspension adjusted to 0.5 McFarland turbidity standards and evenly spread. The Petri plates were incubated at 28 ± 1°C for 72 hours. Petri plates without extracts and the results were compared against these controls.

Minimum bactericidal concentration (MBC) of leaf extracts was determined as described in Njinga et al. (2014). Serial test concentrations of each species extract were prepared. CPG growth medium was prepared and autoclaved at 121°C for 15 minutes. One milliliter of each test concentration was mixed with 19 mL of molten CPG medium and poured to sterile Petri plates. This was allowed to cool and solidify. *R. solanacearum* suspension was prepared from the MIC Petri plates that did not show growth or showed growth less than 80% of the controls and sub-cultured onto the medium. The Petri plates with their contents were incubated at 28 ± 1°C for 72 hours. Test concentrations that didn't show bacterial growth were considered as minimum bactericidal concentrations.

The relative percentage inhibition of the leaf extracts with respect to the positive control was calculated as described by Kumar & Arya (2006).

$$\text{Relative percentage inhibition of the test extract} = \frac{100(X-Y)}{(Z-Y)}$$

where:
X: total area of inhibition of the test extract
Y: total area of inhibition of the solvent
Z: total area of inhibition of the standard antibiotic.
The total area of inhibitions was calculated by using $A = \pi r^2$; where, r = radius of zone of inhibition.

Statistical analysis

All data were subjected to analysis of variance (ANOVA). This was done after carrying out test of homogeneity and normal distribution of each measured parameter. Mean values were compared with Tukey's Honestly Significant Difference (HSD) test at $p < 0.05$. All the statistical analyses were computed with SPSS Software for Windows version 20, IBM, NY, USA.

Results and discussion

Identification of *R. solanacearum*

The bacterium isolated from infected *C. annum* plants was identified using cultural, biochemical characters and pathogenicity test. White creamy colored, irregularly round and non-transparent colonies were formed on CPG medium which matches with the reports of Alemu, Lemessa, Wakjira, and Berecha (2013), Popoola et al. (2015) and Din et al. (2016). According to the Gram staining test, the isolate was found to be Gram negative. This was corroborated by the positive KOH solubility test result, whereby drops of KOH mixed with the isolate suspension produced viscous solution that causes a thin strand of slime when lifted with a wire loop. Moreover, the bacterium showed catalase activity and hydrolyzed starch. The result of the present study agrees with Murthy and Srinivas (2012), Alemu et al. (2013), Popoola et al. (2015) and Kataky, Tamuli, Teron, and Sarma (2017), who reported Gram-negativity, KOH solubility, catalase activity and starch hydrolysis of *R. solanacearum*.

Extract yield

Plants produce plethora of secondary metabolites with biological activities and thus can be used as source for the development of pesticides (Jeyaseelan, Pathmanathan, & Jeyadevan, 2010). The search for new biocides from plant materials is dependent on the productivity of extraction and the type of solvent. There were variations in percentage extract yield between medicinal plant species (Figure 2). It ranged from 23.75-34%. The highest was obtained from the leaves of *E. citriodora* followed by *R. communis*, while the lowest percentage extract yield was found from *J. schimperiana*. The remaining species had an intermediate percentage extract yield (Figure 2). Maximum extract yield has been reported when methanol is used as a solvent since methanol is the most potent and all-purpose solvent that dissolves most secondary metabolites in plants and enhances the release of these chemicals from cellular matrix (Moteriya, Padalia, Rathod, Menpara, & Chanda, 2014; Gaitonde & Ramesh, 2016). Moreover, the differences in extract yield are also due to the genes that dictate the types of phytochemicals present, while environment such as biotic and abiotic factors influence the expression and relative abundance of secondary metabolites (Moore, Andrew, Kulheim, & Foley, 2014).

The phytochemical analysis showed the presence of different secondary metabolites in the methanol leaf extracts of the studied species. Extracts of *E. citriodora* and *R. communis* found to have alkaloids, flavonoids, phenols, saponins, tannins and terpenoids (Table 1). Javed, Shoaib, Mohmood, Mushtaq, and Iftikhar (2012) and Suurbaar, Mosobil and Donkor (2017) have found out similar constituents of *E. citriodora* (except tannins) and *R. communis* leaf extracts, respectively. Unlike the present study, alkaloids, tannins and terpenoids are absent from the methanol leaf extract of *E. citriodora* (Pathmanathan, Uthayarasa, Jeyadevan, & Jeyaseelan, 2010). Alkaloids are also absent in the extract of *R. communis* (Minakshi et al., 2016). Moreover, extracts of *J. schimperiana* and *L. camara* had alkaloids, flavonoids, phenols, tannins and terpenoids (Table 1). Similar compositions of these

species extracts have been reported by Abebe, Kibret, and Haile (2014) and Sousa, Rodrigues, Campos, and Costa (2015). Contrary to this study, Feyera, Deressa, Deyou, and Abdisa (2018) have found alkaloids, flavonoids, phenols, saponins and terpenoids in the methanol leaf extract of *M. azedarach*. The deviations in the composition of these species extracts might be ascribed to the difference in site of sample collection, analysis methodology and reagent used.

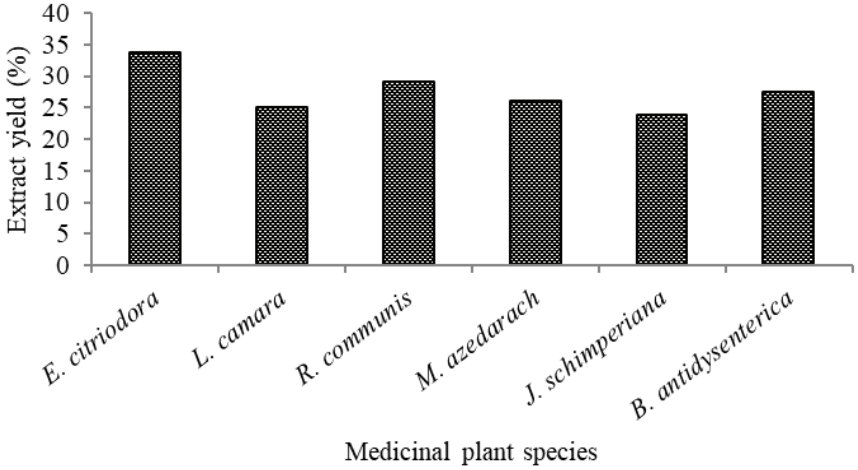

Figure 2. Percentage extract yield of the medicinal plant leaf samples tested.

Phytochemical screening of methanol extracts

Table 1. Phytochemical screening of methanol leaf extracts of medicinal plants used in the bioassay.

Medicinal plant Species	Phytochemicals					
	Alkaloids	Flavonoids	Phenols	Saponins	Tannins	Terpenoids
B. antidysenterica	+	+	+	+	+	+
E. citriodora	+	+	+	+	+	+
J. schimperiana	+	+	+	-	+	+
L. camara	+	+	+	-	+	+
M. azedarach	-	-	-	+	-	+
R. communis	+	+	+	+	+	+

Key: + = detected; - = not detected

Antibacterial activity assay

The *in vitro* bioassay studies demonstrated a significant growth inhibition of *R. solanacearum*. Statistically significant ($p < 0.05$) variations were observed among test concentrations of each species and between species extract (Table 2). Antibacterial activity was not detected by the negative control. Most species extracts showed a progressive increase in growth inhibition zone in a dose dependent manner. Significantly greater growth inhibition zone was produced by the leaf extracts of *E. citriodora* at the higher two test concentrations than the remaining concentrations. In *R. communis* extract, the highest test concentration had remarkably greater growth inhibition than the lower concentrations (Table 2). Similar antibacterial activity of *E. citriodora* (Pathmanathan et al., 2010; Ghaffar et al., 2015) and *R. communis* (Suurbaar et al., 2017) leaf extracts has been reported against both Gram negative and positive bacterial pathogens of humans such as *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Aspergillus niger* and *Rhizopus solani*. Moreover, the two upper test concentrations (100 and 50 mg mL^{-1}) of *L. camara* and *J. schimperiana* revealed significantly higher growth inhibition within a species than the remaining lower test concentrations (Table 2). Alemu et al. (2013) and Revathi, Narayanaswamy, Patil, Naik, and Shindhe (2017) have described moderate growth inhibition of *L. camara* leaf extract against *R. solanacearum*.

Furthermore, significant differences were also documented between species at the different test concentrations (Table 2). Accordingly, wider growth inhibition zones were formed by the extracts of *E. citriodora* and *R. communis* at all test concentrations as compared to other species (Table 2). The differences among the remaining species extracts were nonsignificant. The methanol leaf extract of *M. azedarach* did not show growth inhibition against *R. solanacearum* at all test concentrations, which agrees with the result of Din et al. (2016) against the same pathogen.

Table 2. Antibacterial activity of methanol leaf extracts of medicinal plants against *Ralstonia salanacearum* evaluated in terms of bacterial growth inhibition zone (mm).

Medicinal plant species	Test concentrations (mg mL^{-1})				
	100	50	25	12.5	6.25
B. antidysenterica	10.61 ± 0.05^{aBC}	10.41 ± 0.07^{aBC}	10.29 ± 0.15^{aBC}	10.09 ± 0.24^{aB}	10.09 ± 0.21^{aBC}
E. citriodora	12.89 ± 0.11^{aA}	12.36 ± 0.18^{aA}	11.71 ± 0.14^{bA}	11.42 ± 0.13^{bA}	11.08 ± 0.08^{bAC}
J. schimperiana	10.50 ± 0.09^{aC}	9.82 ± 0.21^{bB}	9.67 ± 0.16^{bB}	9.42 ± 0.03^{bB}	9.29 ± 0.03^{bB}
L. camara	10.50 ± 0.09^{aC}	9.89 ± 0.11^{bB}	9.59 ± 0.07^{bcB}	9.51 ± 0.08^{bcB}	9.36 ± 0.06^{cB}
R. communis	11.54 ± 0.10^{aB}	11.36 ± 0.07^{abAC}	11.18 ± 0.09^{bAC}	11.29 ± 0.03^{bA}	11.22 ± 0.11^{bA}

The same lower-case letters in a row and upper case letters in a column indicate the absence of statistical differences between means at $p < 0.05$.

The potency of the extracts was also evaluated through bacteriostatic and bactericidal concentrations and found to give different results. Comparatively lower bacteriostatic (MIC) and bactericidal (MBC) concentrations were recorded by the extracts of *E. citriodora* and *R. communis* (Figure 3). Opposite to the present study, Lekganyane, Matsebatlela, Howard, Shai and Masoko (2012) and Sousa et al. (2015) have discovered low MIC values of *R. communis* and *L. camara* extracts, respectively, against human pathogens. Similarly, MIC values ranging from 3.125-6.25 mg mL^{-1} and MBC from 25-50 mg mL^{-1} have been reported from methanol leaf extracts of *L. camara* against different human pathogens (Dubey & Padhy, 2013). The stronger antibacterial activity of *E. citriodora* and *R. communis* extracts might be explained by the presence of diverse and abundant number of secondary metabolites. Crude extracts are complex blends of phytochemicals where some have specific role on single target and most interfere with several targets on the pathogen in a pleiotropic manner (Wink, 2008). The constituents of crude extracts have both additive and synergistic bioactivity against bacterial pathogens and employ their antibacterial activity into two different ways; by killing the bacteria and by impairing the key events in the pathogenic process (Wink, 2008). It is well-known that multiple constituents in crude extract act at different sites thus contributing to the overall activity of the extract. Accordingly, alkaloids intercalate with DNA causing impaired cell division and consequently death (Savoia, 2012). Flavonoids are diverse and have ability to stick to and produce complex with bacterial cell walls and soluble proteins; inactivating enzymes of the pathogen (Ghosh et al., 2011). Tannins on the other hand involved in deactivation of cell envelope transport proteins and microbial adhesins (Sher, 2004). Moreover, terpenoids are ascribed to disruption of membranes in microbes (Termentzi, Fokialakis, & Skaltsounis, 2011). In general, the diverse phenolic compounds adversely affect microbes by reducing pH, increasing membrane permeability and changing efflux pumping (Emeka, Badger-Emeka, & Fateru, 2012).

The relative percentage inhibition (RPI) indicates the growth inhibition capacity of extracts with respect to the standard antibiotic, tetracycline. According to Gaitonde & Ramesh (2016) RPI of 50% or more is considered as active product for further study. In this regard, methanol extracts of all the studied species showed above 50% RPI indicating high potency of the extracts (Table 3). The highest RPI was produced by the extract of *E. citriodora* followed by extracts of *R. communis* and *B. abyssinica* as compared to other species (Table 3).

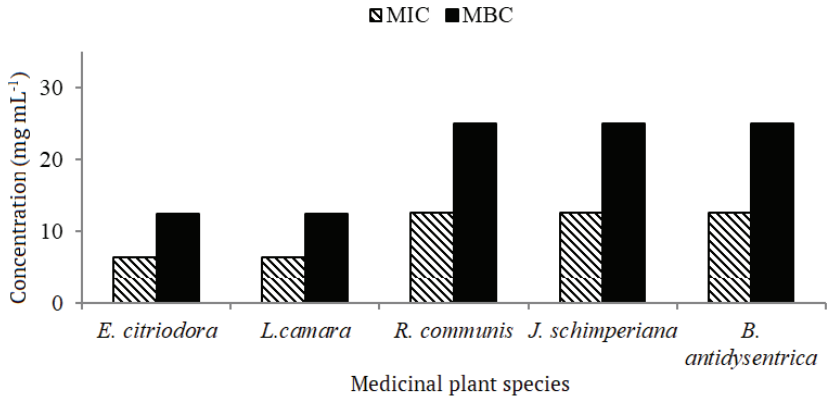

Figure 3. Minimum inhibitory and bactericidal concentrations of methanol leaf extracts of medicinal plants against *Ralstonia solanacearum*.

Table 3. Relative percentage inhibitions of medicinal plant methanol leaf extracts against *Ralstonia solanacearum* as compared to the standard antibiotic, tetracycline.

Medicinal plant species	Test concentrations (mg mL^{-1})				
	100	50	25	12.5	6.25
E. citriodora	101.67 ± 1.73^{aA}	93.40 ± 2.78^{aA}	83.80 ± 2.14^{bA}	79.77 ± 1.94^{bA}	75.03 ± 1.13^{bA}
L. camara	68.60 ± 0.67^{aC}	59.70 ± 1.1^{bC}	56.16 ± 0.92^{bcC}	55.27 ± 1.01^{bcB}	53.57 ± 0.77^{cBC}
R. communis	84.53 ± 1.69^{aB}	78.87 ± 0.99^{abB}	76.43 ± 1.32^{bA}	77.80 ± 0.40^{bA}	76.90 ± 1.50^{bA}
J. schimperiana	67.30 ± 1.27^{aC}	58.93 ± 2.61^{bC}	57.17 ± 1.92^{bCB}	54.20 ± 0.40^{bB}	52.73 ± 0.67^{bB}
B. antidysenterica	68.77 ± 0.73^{aC}	66.23 ± 0.99^{aC}	64.73 ± 1.99^{aB}	61.80 ± 2.97^{aB}	62.20 ± 2.56^{aC}

The same lower-case letters in a row and upper case letters in a column indicate the absence of statistical differences between means at $p < 0.05$.

Conclusion

The methanol leaf extracts of the studied medicinal plant species showed different antibacterial activity attributed to the concentration and diversity of secondary metabolites. Although not quantified, extracts with abundant amount and large number of secondary metabolites had higher antibacterial activity. Accordingly, extracts of *E. citriodora* and *R. communis* had higher antibacterial activity and the potential as biocide formulations in controlling hot pepper bacterial wilt. However, the antibacterial activity of the extracts should be evaluated under greenhouse and field conditions to draw a solid conclusion.

References

Abebe, H., Kibret, B., & Haile, A. (2014). Phytochemical investigation on leaf extract of *Adhatoda schimperiana,* Ethiopia. *Journal of Medicinal Plants Studies, 2*(2), 23-31.

Alemu, D., Lemessa, F., Wakjira, M., & Berecha, G. (2013). Antibacterial activity of some invasive alien species extracts against tomato (*Lycopersicom esculentum* Mill) bacterial wilt caused by *Ralstonia solanacearum* (Smith). *Plant Pathology Journal, 12*(2), 61-70. doi: 10.3923/ppj.2013.61.70

Ayoola, G. A., Coker, H. A. B., Adesegun, S. A., Adepoju-Bello, A. A., Obaweya, K., Ezennia, E. C., & Atangbayila, T. O. (2008). Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. *Tropical Journal of Pharmaceutical Research, 7*(3), 1019-1024. doi: 10.4314/tjpr.v7i3.14686

Central Statistical Agency of Ethiopia [CSA]. (2007). *Agricultural sample survey of 2006/2007: Volume I - Report on area and production of major crops - Private peasant holdings, meher season* (Statistical Bulletin 388). Addis Ababa, ET: Central Statistic Agency.

Central Statistical Agency of Ethiopia [CSA]. (2010). A*gricultural sample survey of 2017/2018 - Report on area and production of major crops* (Statistical Bulletin 586). Addis, Ababa, ET: Central Statistic Agency.

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) Yabunchi. *Spanish Journal of Agricultural Research, 14*(3), e1006. doi: 10.5424/sjar/2016143-9012

Dubey, D., & Padhy, R. N. (2013). Antibacterial activity of *Lantana camara* L. against multidrug resistant pathogens from ICU patients of a teaching hospital. *Journal of Herbal Medicine, 3*(2), 65-75. doi: 10.1016/j.hermed.2012.12.002

Emeka, P. M., Badger-Emeka, L. I., & Fateru, F. (2012). *In vitro* antimicrobial activities of *Acalypha ornate* leaf extracts on bacterial and fungal clinical isolates. *Journal of Herbal Medicine, 2*(4), 136-142. doi: 10.1016/j.hermed.2012.09.001

Emmanuel-Ikpeme, C., Henry, P., & Okiri, O. A. (2014). Comparative evaluation of the nutritional, phytochemical and microbiological quality of three pepper varieties. *Journal of Food and Nutrition Sciences, 2*(3), 74-80. doi: 10.11648/j.jfns.20140203.15

European Committee for Antimicrobial Susceptibility Testing [EUCAST]. (2000). Determination of minimum inhibitory concentration (MIC) of antibacterial agents of by agar dilution. *European Society of Clinical Microbiology and Infectious Diseases, 6*(9), 509-515. DOI: 10.1046/j.1469-0691.2000.00142.x

Food and Agriculture Organization of the United Nations Statistic [FAOSTAT]. (2012). *FAO statistics division*. Rome, Italy: FAO.

Feyera, M., Deressa, F., Deyou, T., & Abdisa, N. (2018). Isolation and characterization of compounds from the leaves of *Melia azedarach* and stem bark of *Albizia schimperiana* and evaluation for antimicrobial activities. *Medicinal Chemistry, 8*(6), 154. doi: 10.4172/2161-0444.1000507

Gaitonde, S. S., & Ramesh, R. (2016). Screening plant products for *Ralstonia solanacearum* inhibition and characterization of antibacterial compounds in *Garcinia indica* and *Tamarindus indica*. *Proceedings of the National Academy of Sciences India Section B: (Biological Sciences), 88*(1), 265-276. doi: 10.1007/s40011-016-0755-6

Ghaffar, A., Yameen, M., Kiran, S., Kamal, S., Munir, B., Saleem, S., ... Jabbar, A. (2015). Chemical composition and *in vitro* evaluation of the antimicrobial and antioxidant activities of essential oils extracted from seven *Eucalyptus* species. *Molecules, 20*(11), 20487-20498. doi: 10.3390/molecules201119706

Ghosh, G., Subudhi, B. B., Badajena, L. D., Ray, J., Mishra, M. K., & Mishra, S. K. (2011). Antibacterial activity of *Polyalthia longifolia var. angustifolia* stem bark extract. *International Journal of PharmTech Research, 3*(1), 256-260.

Grubben, G. J. H., & Denton, O. A. (2004). *Plant resources of tropical Africa 2*: vegetables. Wageningen, NL: Backhuys Publishers.

He, L. Y., Sequeria, L., & Kelman, A. (1983). Characteristics of strains of *Pseudomonas solanacearum* from China. *Plant Disease, 67*(1), 1357-1361. doi: 10.1094/PD-67-1357

Javed, S., Shoaib, A., Mohmood, Z., Mushtaq, S., & Iftikhar, S. (2012). Analysis of phytochemical constituents of *Eucalyptus citriodora* L. responsible for antifungal activity against post-harvest fungi. *Natural Product Research, 26*(18), 1732-1636. doi: 10.1080/14786419.2011.607451

Jeyaseelan, E. C., Pathmanathan, M. K., & Jeyadevan, J. P. (2010). Inhibitory effect of different solvent extracts of *Vitex negundo* L. and *Allium sativum* L. on phytopathogenic bacteria. *Archives of Applied Science Research, 2*(6), 325-331.

Kataky, M., Tamuli, A. K., Teron, R., & Sarma, R. K. (2017). Biochemical characterization of *Ralstonia solanacerum* causing bacterial wilt of Brinjal in the Hilly district of Assam. *International Journal of Pure and Applied Bioscience, 5*(4), 2147-2157. doi: 10.18782/2320-7051.5307

Kraft, K. H., Brown, C. H., Nabhan, G. P., Luedeling, E., Ruiz, J. D. J. L., D'Eeckenbrugge, G. C., ... Gepts, P. (2014). Multiple lines of evidence for the origin of domesticated chili pepper, *Capsicum annuum*, in Mexico. *Proceedings of the National Academy of Sciences of the United States of America, 111*(17), 6165-6170. doi: 10.1073/pnas.1308933111

Kumar, V. I., & Arya, S. (2006). Medicinal uses and pharmacological properties of *Calotropis procera*. In J. N. Govil, & V. K. Singh (Ed.), *Recent progress in medicinal plants 11*: drug development from molecules (p. 373-388). Houston, TX: Stadium Press.

Lekganyane, M. A., Matsebatlela, T. M., Howard, R. L., Shai, L. J., & Masoko, P. (2012). The phytochemical, antibacterial and antioxidant activity of five medicinal plants against the wound infecting bacteria. *African Journal of Biotechnology, 11*(68), 13210-13219. doi: 10.5897/AJB12.885

Lin, S. W., Chou, Y. Y., Shieh, H. C., Ebert, A. W., Kumar, S., Mavlyanova, R., ... Gniffke, P. A. (2013). Pepper (*Capsicum* spp.) germplasm dissemination by AVRDC –the world vegetable center: an overview and introspection. *Chronica Horticulture, 53* (3), 21-27.

Marcelis, L. F. M., Heuvelink, E., Hofman-Eijer, L. R., Bakker, J. D., & Xue, L. B. (2004). Flower and fruit abortion in sweet pepper in relation to source and sink strength. *Journal of Experimental Botany, 55*(406), 2261-2268. doi: 10.1093/jxb/erh245

Mendonça-Filho, R. R. (2006). Bioactive phytocompouds: new approaches in the phytosciences. In I. Ahmad, F. Aqil, & M. Owais (Ed.), *Modern phytomedicine turning plants into drugs* (p. 1-25). Heinheim, DE: Willey-VCH.

Mimura, Y., Yoshikawa, M., & Hirai, M. (2009). Pepper accession LS2341 is highly resistant to *Ralstonia solanacearum* strains from Japan. *HortScience, 44*(7), 2038-2040. doi: 10.21273/HORTSCI.44.7.2038

Minakshi, B., Jharna, D., Devi, E. C., Nayan, T., Partha, P. K., Kundal, N. & Manash, P. S. (2016). Phytochemical analysis of traditional medicinal plants and their antibacterial activity. An experience from East India. *Journal Pharmaceutical Research, 1*(1), 1-7.

Moore, B. D., Andrew, R. L., Kulheim, C., & Foley, W. J. (2014). Explaining intraspecific diversity in plant secondary metabolites in an ecological context. *New Phytologist, 201*(3), 733-750. doi: 10.1111/nph.12526

Moteriya, P., Padalia, H., Rathod, T., Menpara, D., & Chanda, S. (2014). Phytochemical analysis and antibacterial activity of *Maytenus emarginata* leaf and stem. *Journal of Pharmacognosy and Phytochemistry, 3*(4), 202-208.

Murthy, N. K., & Srinivas, C. (2012). *In vitro* screening of bioantagonistic agents and plant extracts to control bacterial wilt (*Ralstonia solanacearum*) of tomato (*Lycopersicon esculentum*). *Journal Agricultural Technology, 8*(3), 999-1015.

Njinga, N. S., Sule, M. I., Pateh, U. U., Hassan, H. S., Usman, M. A., Bilkisu, A., ... Ache, R. N. (2014). Phytochemical and antimicrobial activity of the stem-barks of *Gardenia aqualla* Stapf & Hutch (Rubeacea). *Journal of Medicinal Plant Research, 8*(27), 942-946. doi: 10.5897/JMPR2013.5207

Pathmanathan, M. K., Uthayarasa, K., Jeyadevan, J. P., & Jeyaseel.an, E.C. (2010). *In vitro* antibacterial activity and phytochemical analysis of some selected medicinal plants. *International Journal of Pharmaceutical and Biological Archives, 1*(3), 291-299.

Popoola, A. R., Ganiyu, S. A., Enikuomehin, O. A., Bodunde, J. G., Adedibu, O. B., Durosomo, H. A., & Karunwi, O. A. (2015). Isolation and characterization of *Ralstonia solanacearum* causing bacterial wilt of tomato in Nigeria. *Nigerian Journal of Biotechnology, 29*(1), 1-10. doi: 10.4314/njb.v29i1.1

Rai, M. V., Pai, V. R., Kedilaya, H. P., & Hegde, S. (2013). Preliminary phytochemical screening of members of *Lamiaceae* Family: *Leucas linifolia, Coleus aromaticus* and *Pogestemon patchouli. International Journal of Pharmaceutical Sciences Review and Research, 21*(1), 131-137.

Revathi, R. M., Narayanaswamy, H., Patil, B., Naik, S. N., & Shindhe, M. (2017). *In vitro* evaluation of botanicals, bio agents and anti-bacterial chemicals against *Ralstonia solanacearum. International Journal Chemical Studies, 5*(6), 1894-1898.

Sands, D. C. (1990). *Methods in phytobacteriology*. Budapest, HU: Academia Kiado.

Savoia, D. (2012). Plant-derived antimicrobial compounds: alternatives to antibiotics. *Future Microbiology, 7*(8), 979-990. doi: 10.2217/FMB.12.68

Schaad, N. W. (1980). *Laboratory guide for the identification of plant pathogenic bacteria*. Staint Paul, MN: American Phytopathology Society Press.

Sher, A. (2004). Antimicrobial activity of natural products from medicinal plants. *Gomal Journal Medical Science, 7*(1), 65-67.

Shumeta, Z. (2012). Hot pepper production and marketing in southwest Ethiopia: An alternative enterprise for small scale farmers. *Trends in Agricultural Economics, 5*(3), 83-95. doi: 10.3923/tae.2012.83.95

Singh, S., Singh, D. R., Kumar, K., & Birah, A. (2014). Eco-friendly management modules for bacterial wilt (*Ralstonia solanacearum*) of tomato for protected cultivation in a tropical island ecosystem. *Biological Agriculture and Horticulture, 30*(4), 219-227. doi: 10.1080/01448765.2014.913263

Sousa, E. O., Rodrigues, F. F. G., Campos, A. R., & Costa, J. G. M. (2015). Phytochemical analysis and modulation in aminoglycosides antibiotics activity by *Lantana camara* L. *Acta Scientiarum*. *Biological Sciences, 37*(2), 213-218. doi: 10.4025/actascibiolsci.v37i2.22877

Suslow, T. V., Schroth, M. N., & Isaka, M. (1982). Application of a rapid method for gram differentiation of plant pathogenic and saprophytic bacteria without staining. *Phytopathology, 72*(7), 917-918. doi: 10.1094/Phyto-72-917

Suurbaar, J., Mosobil, R., & Donkor, A. M. (2017). Antibacterial and antifungal activities and phytochemical profile of leaf extract from different extractants of *Ricinus communis* against selected pathogens. *BMC Research Notes*, *10*(1), 660. doi: 10.1186/s13104-017-3001-2

Termentzi, A., Fokialakis, N., & Skaltsounis, A. L. (2011). Natural resins and bioactive natural products thereof as potential antimicrobial agents. *Current Pharmaceutical Design, 17*(13), 1267-1290. doi: 10.2174/138161211795703807

Welderufael, H. A. (2016). Assessment of horticultural crops (vegetables, tubers and fruits) production constraints and opportunities in west and southwest Shewa zones of Oromia Region, Ethiopia. *International Journal of Agricultural Economics, 1*(3), 84-90. doi: 10.11648/j.ijae.20160103.16

Wink, M. (2008). Evolutionary advantage and molecular modes of action of multi-component mixtures used in phytomedicine. *Current Drug Metabolism, 9*(10), 996-1009. doi: 10.2174/138920008786927794

Yabuuchi, E., Kosako, Y., Yano, I., Hotta, H., & Nishiuchi, Y. (1995). Transfer of two *Burkholderia* and an *Alcaligenes* species to *Ralstonia* genus nov.: proposal of *Ralstonia pickettii* (Ralston, Palleroni and Douderoff 1973) comb.nov., *Ralstonia solanacearum* (Smith 1896) comb. nov. and *Ralstonia eutropha* (Davis 1969) comb. nov. *Microbiology and Immunology, 39*(11), 897-904. doi: 10.1111/j.1348-0421.1995.tb03275.x

Yadeta, B., Belew, D., Gebreselasie, W., & Marame, F. (2011). Genetic associations among some attribute of hot pepper (*Capsicum annuum* L) genotypes in west Shewa, Ethiopia. *Middle East Journal of Scientific Research, 7*(4), 567-573.

Zeleke, A., & Derso, E. (2015). *Production and management of major vegetable crops in Ethiopia*. Addis Ababa, ET: Eth-Cana Printing Press.