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In vitro antibacterial activity of traditionally used medicinal plants against *Xanthomonas campestris* pv. *musacearum* in Ethiopia

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Abstract. *Yemata G, Desta B, Fetene M. 2019. In vitro antibacterial activity of traditionally used medicinal plants against* Xanthomonas campestris *pv.* musacearum *in Ethiopia. Biodiversitas 20: 555-561.* In Ethiopia, traditional medicinal plants have long been used to treat human and livestock ailments. Nevertheless, studies about the use of these plant extracts to control crop diseases are scarce. Therefore, the aim of the present study was to evaluate the antibacterial activity of traditionally used medicinal plants against *Xanthomonas campestris* pv. *musacearum* (Xcm). The bioactive chemicals from leaf samples were drawn using methanol by maceration method. Total phenolic content of the extracts was determined by Folin Ciocalteau reagent. The antibacterial activity of leaf extracts was evaluated by disc diffusion method. The phytochemical analysis revealed the presence of alkaloids, flavonoids, phenolic compounds, terpenoids, tannins and saponins. Extracts with higher total phenolic content had greater antibacterial activity. The extract of each species showed antibacterial activity against Xcm on a dose dependent manner. Significant differences were recorded between species and test concentrations. The extract of *Bersama abyssinica* exhibited the strongest antibacterial activity at 200 mg/mL followed by *Ricinus communis*, *Eucalyptus citriodora* and *Acokanthera schimperi*. At lower test concentrations, extracts of *E. citriodora* and *R. communis* revealed higher antibacterial activity. Moreover, these species had lower bacteriostatic and bactericidal concentrations. The results showed the potential potency of *E. citriodora* and *R. communis* leaf extracts in controlling enset bacterial wilt. However, further studies on the identification of chemical compounds and *in vivo* evaluation of the extracts are recommended.

Keywords: Antibacterial, enset, *Ensete ventricosum*, leaf extract, *Xanthomonas campestris* pv. *musacearum*

INTRODUCTION

Plant diseases caused by phytopathogenic bacteria represent an emerging threat to global food security. Xanthomonas is a large genus of Gram-negative bacteria that cause disease in several host plants leading to considerable yield losses (Bajpai et al. 2011). Pathogenic species and pathovars within species show high degree of host plant specificity. Several of these exhibit tissue specificity, invading either the vascular system or the mesophyll tissue of the host (Ryan et al. 2011). *Xanthomonas campestris* pv. *musacearum* is a member of the genus that causes a destructive disease in enset (*Ensete ventricosum*) and banana (Blomme et al. 2017; Nakato et al. 2017).

Ensete ventricosum (Welw.) Cheesman) is a multi-purpose root crop that has been produced for thousands of years only in Ethiopia. It serves as a staple/co-staple food supporting the livelihood of approximately 20 million people in the south and southwestern part of the country (Magule et al. 2014). As a food crop, enset has several merits. It produces high yield per unit area as compared to other crops; tolerant to prolonged drought and can be harvested at any developmental stage if people face food shortage. Thus, the crop is considered as a field bank for food (Tsegaye and Struik 2002). However, enset production is threatened by enset bacterial wilt (EBW) disease, caused by Xcm. Recent survey studies showed that EBW is increasing both in severity and distribution in most of the enset growing agroecologies (Handoro 2017). Xcm is soil borne and infested fields should remain fallow for at least 6 months to avoid re-infestation (Tripathi et al. 2009). EBW causes up to 100 % yield loss (Handoro, 2017), which severely affects food security and livelihoods in enset based farming households (Blomme et al. 2017).

The pathogen is transmitted mechanically through contaminated farming tools, infected plant materials, water flash and insects (Tripathi et al. 2009; Addis et al. 2010). The management of Xcm has relied on cultural practices such as burying infected plants and sterilizing farming tools that keep the pathogen population at tolerable levels (Addis et al. 2010; Nakato et al. 2017). However, these methods are not effective as farmers are inconsistent and reluctant to employ labor-intensive disease controlling measures (Tripathi et al. 2009). Furthermore, no bactericide has been suggested against Xcm (Bajpai et al. 2011). In Ethiopia, traditional medicinal plants have long been used to treat human and livestock ailments. However, studies about the use of these medicinal plants extracts to control plant diseases are absent. Thus, the aim of this study was to evaluate the antibacterial activity of traditional medicinal plants in controlling EBW.

MATERIALS AND METHODS

Plant material collection and extraction

Leaf samples and specimens of traditionally used medicinal plants namely; *Acokanthera schimperi* (A. DC.) Schwein, *Albizia schimperiana* Oliv., *Bersama abyssinica* Fresen, *Brucea antidysenterica* JF. Mill, *Eucalyptus citriodora* (Hook), *Fagaropsis angolensis* (Engl.) Dale, *Indigofera arrecta* Hochst. ex A. Rich, *Laggera crispata* (Vahl) Hepper & Wood, *Olinia rochetiana* A. Juss., *Peponium vogelii* (Hook.f) Engl., *Ricinus communis* L., *Rumex abyssinicus* Jacq and *Solamu incanum* L. were collected from Gedeo, Sidama and Wolaita zones (Figure 1). Plant species most frequently used by the people were selected and used in this study. The pressed plant specimens were placed in a drier box with an in-built 60W light bulb. The drier has adequate ventilation. The dried specimens were trimmed to fit the mounting sheet, carefully put and attached with a few dots of glue on the under sides of plant parts. The specimens were identified at a species level by the taxonomist expert and compared visually with the authenticated plant specimens in the National Herbarium (ETH). The voucher specimens were deposited at the National Herbarium (ETH), Addis Ababa University. The leaf samples were washed with tap water to remove dust and other debris, and air dried under shade at room temperature to constant weight. The dried samples were ground to fine powder with a mechanical grinder. Powder of each species was passed through 0.6 mm mesh and put in a tightly closed bag. Powdered leaf sample of each species was macerated with 99.8% methanol in the ratio of 1:10 (w/v) and shaken for 72 h on an orbital shaker at a speed of 200 rpm. The extract was filtered first with four layers of cheese cloth and cotton followed by Whatman's filter paper. All the activities were performed under room temperature. Methanol was evaporated using rotary evaporator at 42°C and the dry extract was stored at 4°C. Percent extract yield was calculated gravimetrically by dividing the dry weight of the extract to the weight of the leaf powder multiplied by 100.

Phytochemical analysis of extracts

Qualitative analysis was performed to affirm the presence or absence of major secondary metabolites in the methanol crude leaf extract of each species. The presence of alkaloids, flavonoids, phenolic compounds, saponins, tannins and terpenoids was checked using Wagner test, Alkaline reagent, Foam test, Ferric Chloride test and Salkowski test, respectively as described by (Tiwari et al. 2011; Rai et al. 2013).

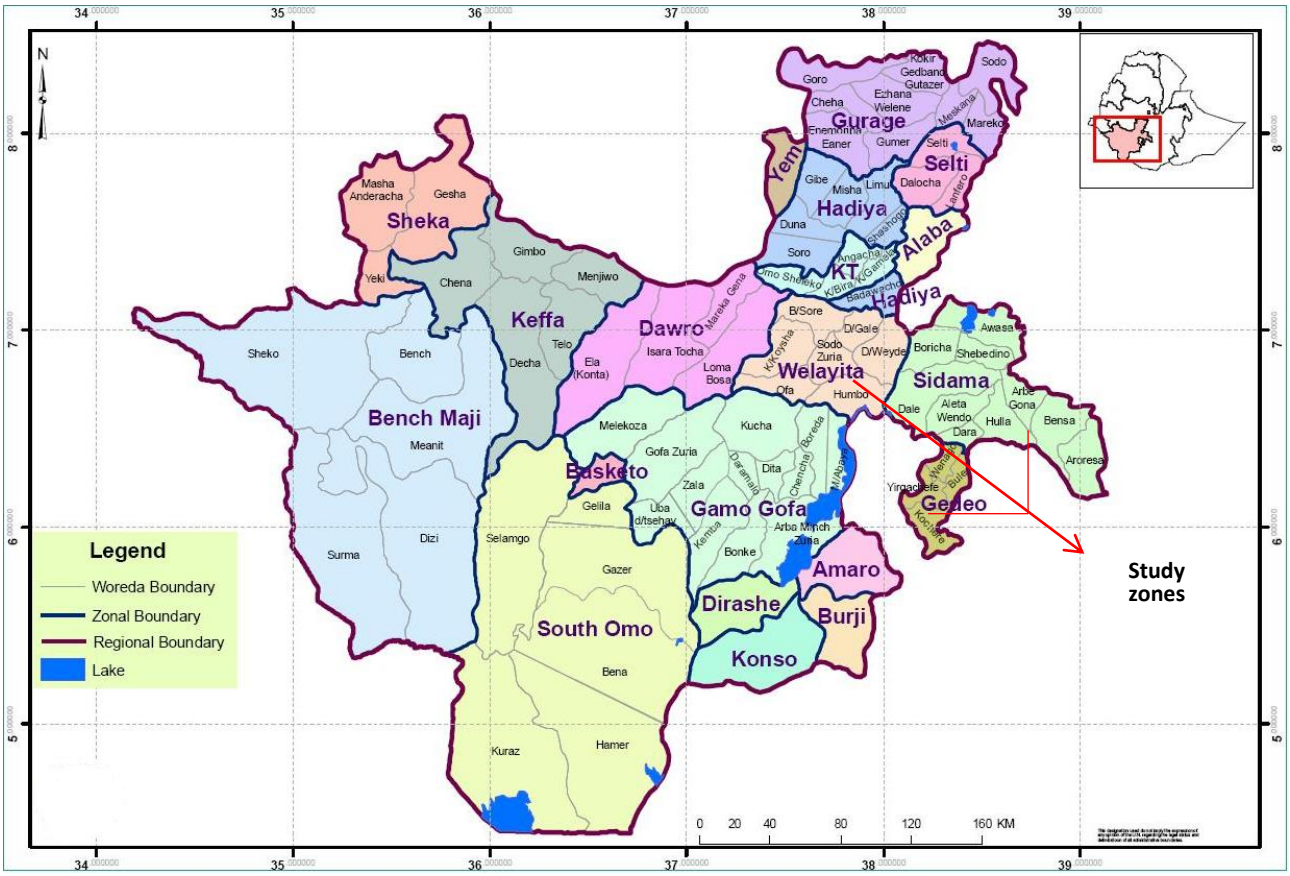

Figure 1. Map of the region where the study zones are Wolaita (Welayita), Sidama and Gedeo, Ethiopia

Estimation of total phenolic content of extracts

Extract of each species (0.5 g) and 10 ml of distilled water were added in separate test tubes, shaken and centrifuged. An aliquot of (0.1 ml) of the supernatant was taken and diluted to 3 ml with distilled water. Consecutively, 0.25 ml of Folin Ciocalteau reagent was added. After 3 minutes, 1 ml of 20 % (w/v) sodium carbonate was added and thoroughly mixed. The tubes were placed in boiling water for 1 min and cooled. The absorbance of the resulting solution was measured at 650 nm against a reagent blank using a spectrophotometer (NV202 Spectrophotometer, Sunny). The blank was composed of 3 ml of distilled water, 0.25 ml of Folin Ciocalteau and 1 ml of 20 % sodium carbonate. The absorbance of the blank was subtracted from each reading. Catechol was used to prepare the standard calibration curve from which the amount of total phenols in the sample was calculated. The amount of total phenols was expressed in mg of catechol equivalent of phenol/g of sample (Zieslin and Ben-Zaken 1993).

***Xanthomonas campestris* pv. *musacearum* isolation (Xcm)**

Xcm was isolated from infected enset leaf samples collected from Sidama zone. The midrib of the leaf samples was cut longitudinally and ooze of the bacterium was picked with cotton swab and suspended in 10 mL of sterilized distilled water. Serial dilutions of the bacterial suspension were prepared and loopful of the dilutions (10^{-2} and 10^{-3}) were streaked to sterilized semi-selective growth medium composed of yeast extract (10 g L^{-1}), peptone (10 g L^{-1}), sucrose (10 g L^{-1}), agar (15 g L^{-1}), cephalexin (50 mg L^{-1}) and amphotericin (150 mg L^{-1}) (Tripathi et al. 2007). The streaked Petri dishes were incubated in an inverted position at 28°C for 72 h. Sub-culturing was carried out by taking loopful of the bacterium directly from separate and uniform colonies in growth plates. Identification was made based on colony characteristics. Accordingly, yellowish, mucoid, circular and convex colonies were identified as Xcm. Pathogenicity test was carried out to confirm the identity of the bacterium. Suckers of Arkia (susceptible enset clone) were planted in plastic buckets filled with mixture of soil, manure and sand in the ratio of 3:2:1(v/v) and kept in a greenhouse. After establishment, suckers were inoculated at the base of the midrib with 10 mL of Xcm suspension (1.5 x 10^8 CFU/mL) in four replications. Negative controls were inoculated with equal volume of sterilized distilled water. The development of symptoms was monitored every day and yellowing of Xcm inoculated leaves was observed after three weeks, while leaves treated with sterilized distilled water remained green. Xcm was re-isolated from infected suckers, compared with the initial colony and found to be similar (Welde-Michael et al. 2008).

***Xanthomonas campestris* pv. *musacearum* inoculum preparation**

Xcm inoculum was prepared from72 h old bacteria grown in yeast extract (1%), peptone (1%), sucrose (1%) and agar (1.5%) medium (YPSA). The upper surfaces of several isolated (pure) colonies were swabbed with cotton swab and suspended in distilled water in a test tube. The content of the test tube was thoroughly shaken until a homogenous suspension was formed. The absorbance of the bacterial suspension was measured with a spectrophotometer (NV202, Sunny) at 600 nm and adjusted to 0.132 equivalent to the density of 0.5 McFarland standards (Sutton 2011). The bacterial population at 0.5 McFarland standards is approximately equal to 1.5 × 10^8 CFU/mL.

Antibacterial activity of leaf extracts

Extracts of each species were dissolved in methanol and serial test concentrations were prepared. Approximately 0.6, 0.3, 0.15 and 0.075 g of each species extract was separately dissolved in 3 mL of methanol producing 200, 100, 50 and 25 mg/mL test concentrations. Discs (5 mm in diameter) were prepared from Whatman's filter paper using paper borer. Discs were sterilized and impregnated in each test concentration of each species extract for 12 h. Similarly, discs soaked in 25 mg/mL of tetracycline and 3 mL of 99.8% methanol served as positive and negative controls, respectively. Subsequently, the discs were taken out and dried before application.

The biocide potential of crude leaf extracts against Xcm was evaluated in terms of antibacterial activity, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The antibacterial activity of extracts was evaluated using disc diffusion method (EUCAST, 2012). MIC and MBC of leaf extracts were determined by agar dilution method as described in EUCAST (2000) and Njinga et al. (2014), respectively. The bacterial suspension adjusted to 1.5 x 10^8 CFU/mL was inoculated to the Petri dishes containing YPSA growth medium using cotton swab. The sterilized swab was dipped into the bacterial suspension and the excess fluid was removed by turning the swab against the inside of the test tube. The inoculum was spread evenly over the entire surface of the Petri dishes by swabbing in three directions. Extract soaked and dried discs were applied to the inoculated Petri dishes within 15 minutes of inoculation. During application, the discs were pressed downward and the Petri dishes were kept in a normal position until the discs get wet. All tests were carried out in triplicates. Materials used in the assay were sterilized before usage. The Petri dishes were inverted and incubated at 28°C for 72 h. The Petri dishes were observed for the presence of inhibition of bacterial growth daily wise. Zones of complete inhibition were measured using transparent ruler at the widest possible diameter including the disc.

Determination of relative percentage inhibition

The relative percentage inhibition of the test extract at 25 mg/mL concentration was determined with respect to the same concentration of the positive control (tetracycline). Relative percentage inhibition was calculated by using the formula described in Naz and Bano (2012) as:

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

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

Statistical analysis

All the experiments were carried out in triplicates. The data were expressed as mean ± standard error. The data were statistically analyzed using one-way analysis of variance (ANOVA). The mean difference between samples was compared by Tukey's Honestly Significant Difference (HSD) using SPSS (version 20), values were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Yield extract and phytochemical analysis

The results of the present study showed variations in percent extract yield and phytochemical analysis. The highest percent extract yield was obtained from *O. rochetiana* leaf followed by *F. angolensis* (Table 1). *E. citriodora* and *R. communis* had moderate percent extract yield, while the lowest was obtained from *I. arrecta.* This might be caused by intrinsic (age of the leaf) and environmental factors. According to Chen et al. (2018), young leaves are found to have higher extract yield as compared to mature leaves. Moreover, higher yield has been reported in *Anoigeissus leiocarpus* leaves gathered from colder than warmer areas (Muraina et al. 2008), might be due to the fact that volatile compounds are retained within the extract in colder areas.

The phytochemical analysis of the leaf extracts proved the presence of alkaloids, flavonoids, phenolic compounds, terpenoids, tannins and saponins in most species extracts (Table 1). The medicinal value of each species could be attributed to the presence of these phytochemicals. The extracts of *B. abyssinica, E. Citriodora, R. Communis* and *F. Angolensis* had all the tested secondary metabolites in different proportions (Table 1). This is consistent with the results of Pathmanathan et al. (2010), Bhagat et al. (2012), Khursheed et al. (2012), Byadgi et al. 2017) and Nicholas et al. (2016).These authors reported comparably similar amount of alkaloids, phenols, tannins, saponins and terpenoids. Extracts of *I. arrecta* lacked flavonoids, terpenoids and tannins (Table 1). In contrast to this, Efuntoye et al. (2014) have found flavonoids, terpenoids and tannins in the methanol leaf extract of *I. arrecta*, which might be due to the variation in reagent used for terpenoids and place of leaf sample collection that differ in climatic and soil conditions for flavonoids and tannins. Although the extracts of *F. angolensis*, *Laggera crispata* and *S. incanum* had all the tested secondary metabolites in different proportions, their antibacterial activity was found to be low. The extract of *Peponium vogelii* lacked most of the secondary metabolites.

The quantitative chemical analysis also showed a significant difference ($P < 0.01$) in total phenolic content among species (Table 1), of which extracts *B. abyssinica, E. citriodora, L. crispata* and *O. rochetiana* found to have high content followed by *R. communis*. Equivalent content of total phenols has been reported by Tauchen et al. (2015) in the methanol leaf extract of *B. abyssinica*. However, Iqbal et al. (2012) have revealed greater total phenolic content in the extract of *R. communis* as compared to the result of the present study. The lowest total phenolic content was found in the extract of *P. vogelii*. Most extracts with high total phenolic content revealed greater antibacterial activity which might indicate the antimicrobial activity of phenolic compounds.

Table 1. Percent extract yield and phytochemical analysis results of methanol leaf crude extracts of medicinal plants

Plant name	Extract yield (%)	Alkaloids	Flavonoids	Phenols	Terpenoids	Tannins	Saponins	Total phenolic content (mg/g)
A. schimperi	22.6	+	++	++	++	++	-	0.11±0.0e
A. schimperiana	19.5	-	+	+	++	+	-	0.18±0.0d
B. abyssinica	12.4	+	++	++	+++	++	+	0.29±0.0a
B. antidysenterica	15.5	+	++	+	+	-	+++	0.04±0.0g
E. citriodora	22.7	+	++	++	+	++	++	0.28±0.0a
F. angolensis	23.1	+	+++	+	+	+	+++	0.06±0.0gf
I. arrecta	3.5	+	-	++	-	-	++	0.04±0.0g
L. crispata	16.5	+	+++	++	++	++	+	0.29±0.0a
O. rochetiana	29.4	+	+	++	++	++	-	0.30±0.0a
P. vogelii	14.9	+	-	-	-	-	+	0.02±0.0g
R. communis	18.1	+	++	++	+	++	+	0.23±0.0b
R. abyssinicus	9.9	+	+	++	+	-	++	0.08±0.0f
S. incanum	16.0	+	++	+	+	+	+	0.16±0.0c

Note: -= absent; + = trace; ++ = moderate; +++ = abundant. Mean total phenolic values in a column followed by the same letter are not significantly different at $P < 0.05$

Table 2. Antibacterial bioassay and total phenolic content of medicinal plant crude leaf extracts

Medicinal plant species	Inhibition zone (mm) Test concentrations (mg/mL)				MIC (mg/mL)	MBC (mg/mL)
	200	100	50	25		
A. schimperi	14.2 ± 0.4^{d}	-	-	-	100.0	100.0
A. schimperiana	9.8 ± 0.5^{eA}	8.5 ± 0.2^{cdB}	7.7 ± 0.3^{bC}	-	25.0	25.0
B. abyssinica	27.2 ± 0.4^{aA}	10.7 ± 0.4^{ceB}	-	-	25.0	25.0
B. antidysentrica	6.7 ± 0.4^{f}	-	-	-	100.0	100.0
E. citriodora	18.0 ± 0.3^{cA}	15.5 ± 0.2^{bB}	13.2 ± 0.5^{aC}	9.3 ± 0.6^{D}	12.5	12.5
F. angolensis	7.3 ± 0.3^{efA}	6.7 ± 0.3^{dA}	6.3 ± 0.3^{bA}	-	100.0	100.0
I. arrecta	7.3 ± 0.3^{efA}	7.0 ± 0.0^{dA}	-	-	100.0	100.0
L. crispata	8.0 ± 0.6^{efA}	7.0 ± 0.0^{dAB}	6.0 ± 0.0^{bB}	6.0 ± 0.0^{B}	25.0	25.0
O. rochetiana	9.3 ± 0.3^{eA}	8.8 ± 0.5^{edAB}	7.3 ± 0.5^{bB}	-	25.0	25.0
P. vogelii	7.0 ± 0.4^{efA}	6.7 ± 0.3^{dA}	6.0 ± 0.0^{bA}	5.7 ± 0.3^{A}	100.0	100.0
R. communis	21.3 ± 0.8^{bA}	19.7 ± 1.1^{aA}	15.5 ± 1.0^{aB}	9.7 ± 0.3^{C}	6.3	6.3
R. abyssinicus	8.7 ± 0.4^{efA}	6.8 ± 0.4^{dB}	6.0 ± 0.0^{bB}	-	25.0	25.0
S. incanum	7.4 ± 0.0^{ef}	-	-	-	100.0	100.0
Tetracycline	ND	ND	ND	35.3±2.4	0.02	0.049
Methanol	-	-	-	-	-	-

Note: ND = not determined; - = No activity. Mean values within a column and row that share the same lowercase and uppercase letters, respectively, are not significantly different at $P < 0.05$

Antibacterial activity

The leaf extracts of most medicinal plants species showed antibacterial activity at all test concentrations (Table 1), demonstrating the potential role of traditionally used medicinal plants in controlling enset bacterial wilt. However, consistent antibacterial activity against Xcm was exhibited at the highest test concentration (200 mg/mL). At this concentration, the extract of *B. abyssinica* revealed the strongest activity followed by *R. communis*, *E. citriodora* and *A. schimperi*, respectively, compared to other species extracts. The differences in antibacterial activity between the aforementioned species and each with others were statistically significant at $P < 0.05$. Similarly, extract of *R. communis* followed by *E. citriodora* showed significantly ($P < 0.05$) greater antibacterial activity at 100 mg/mL test concentration, while extract of *B. abyssinica* showed moderate activity against the test bacterium at this concentration (Table 2). At 50 mg/mL test concentration, the extract of *R. communis* showed the highest antibacterial activity followed by *E. citriodora*. Extracts of *A. schimperi*, *B. antidysentrica* and *S. incanum* exhibited no antibacterial activity at 100 and 50 mg/mL test concentrations. The extracts of most medicinal plant species showed no antibacterial activity at the lowest (25 mg/mL) test concentration (Table 2).

The findings of Teka et al. (2015) and Lulekal et al. (2013) corroborate the results of the present study. In this regard, extracts of *B. abyssinica*, *O. rochetiana* (Lulekal et al. 2013; Teka et al. (2015) and *S. incanum* (Teka et al. 2015) have shown low or no antibacterial activity, higher MIC and MBC values. Only extracts of *E. citriodora*, *L. crispata*, *P. vogelii* and *R. communis* had antibacterial activity at this test concentration, of which extract of *R. communis* showed the highest antibacterial activity followed by *E. citriodora*, indicating the higher potency of the two species against Xcm. Similar effect of *R.communis* extracts has been reported against *Xanthomonas axonopodis* pv. *punicea* by Alane and Swami (2016) causal agent of bacterial blight of *Punica granatum*. Likewise, Luqman et al. (2008) and Vyas et al. (2015) have found out higher antibacterial activity of *E. citriodora* essential oil and methanol leaf extract, respectively, against human pathogens.

Furthermore, statistically significant ($P < 0.05$) differences were noticed between test concentrations of each species extract (Table 2). Antibacterial activity was directly proportional to concentration. There were statistically significant ($P < 0.05$) variations between all test concentrations of *A. schimperiana*, *B. abyssinica* and *E. citriodora* (Table 2). In *L. crispata*, significant variation was recorded between the highest (200 mg/mL) and the two lower test concentrations (50 and 25 mg/mL). Similarly, significant differences were recorded among test concentrations of *R. communis* extract except between 200 and 100 mg/mL. Extracts of the remaining species showed inconsistent variations between test concentrations (Table 2).

The higher antibacterial activity of *E. citriodora* and *R. communis* extracts might be attributed to the presence of diverse secondary metabolites and a relatively higher total phenolic content (Table 1) that work individually and/or synergistically with different modes of actions. The low antibacterial activity of *B. Abyssinica* and *O. rochetiana* might be due to the presence of phenolic species with lower biological activity (Lulekal et al. 2013). Accordingly, phenols cause irreversible changes to the pathogen membrane properties through hydrophobicity changes and formation of pore in the cell membrane with consequent leakage of essential intracellular constituents (Borges et al. 2012). In addition, flavonoids inhibit bacterial growth and kill bacterial cells by impairing cell membrane integrity and cell adhesion (Babii et al. 2015). Flavonoids on the other hand are involved in inhibition of nucleic acid synthesis, cytoplasmic membrane function, and energy metabolism (Cushnie and Lamb 2005).

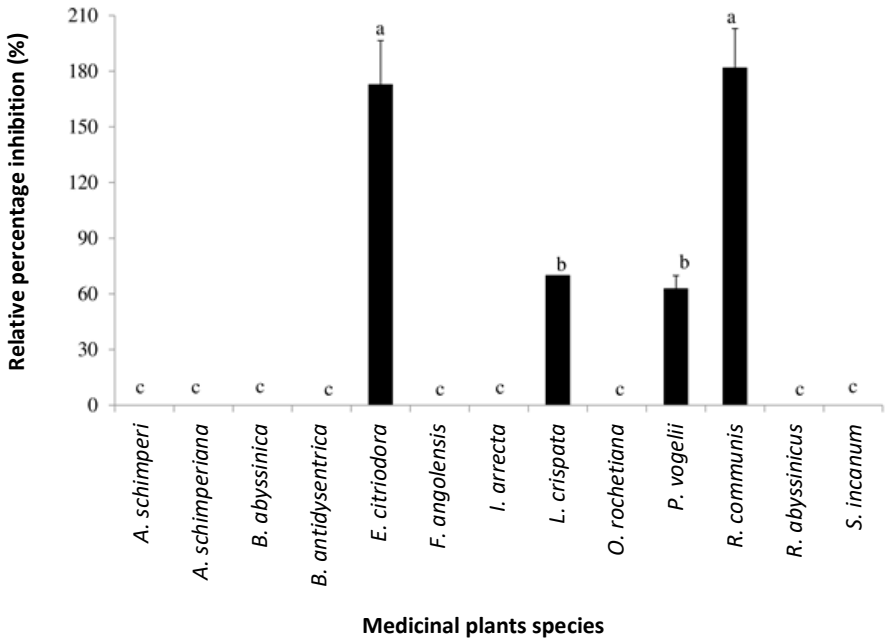

Figure 2. Relative percentage inhibition of methanol leaf extract of medicinal plants calculated against the standard antibiotic; tetracycline at 25 mg/mL test concentration

According to Rasoul et al. (2012), terpenoids show inhibitory effects on pathogens through interaction with membrane structure and function. The interactions result in membrane expansion, increased membrane fluidity and permeability, disturbance of membrane-embedded proteins and alteration of ion transport processes (Rasoul et al. 2012). Similarly, the antibacterial activity of alkaloids is related to the presence of OH group in the structure that reacts and changes the nature of bacterial cell, consequently increasing the permeability of the cell membrane (Straus and Hancock 2006).

Extracts of some medicinal plants showed remarkably higher relative percentage inhibition when calculated against the antibacterial activity of the standard antibiotic, tetracycline at the same concentration (Figure 2). The highest relative percentage inhibition was obtained from the extracts of *E. citriodora* and *R. communis* followed by extracts of *L. crispata* and *P. vogeli*. The remaining species had nil relative percentage inhibition (Figure 2). The result showed that extracts of *E. citriodora* and *R.communis* had higher potency as compared to the antibiotics and could be used as an alternative source of resistance modifying agents. This role of the crude extracts is attributed to the presence of complex mixture of secondary metabolites with multiplicity of targets including receptors, ion channels, cell wall, membranes, transport proteins etc. (Gupta and Birdi 2017). Most compounds in crude extracts have synergistic effect so that the development of bacterial resistance is very much slower than for antibiotics (Wagner and Ulrich-Merzenich 2009).

To conclude, the results in the present investigation revealed the potential role of traditionally used medicinal plants in the management of plant diseases. Most plant species extracts showed antibacterial activity against Xcm. Extracts with high antibacterial activity had comparatively greater total phenolic content and trace amounts of alkaloids and terpenoids. Among these, extracts of *E. citriodora* and *R. communis* demonstrated high potency and could be candidates for further development as biocides in the control of EBW. However, further studies about the identification of the active ingredients and evaluation of the crude extracts of these two species under glasshouse conditions are needed.

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