In vitro evaluation of the antibacterial activity of some medicinal plant extracts against *Xanthomonas campestris pv. musacearum*

Getahun Yemata[1*] and Masresha Fetene[2]
[1]Bahir Dar University, Department of Biology, mail-79, Bahir Dar, Ethiopia;
[2]Addis Ababa University, Department of Plant Biology and Biodiversity Management, mail-1176, Addis Ababa, Ethiopia

ABSTRACT

Enset bacterial wilt caused by *Xanthomonas campestris pv. musacearum* (*Xcm*) is a destructive disease of *Ensete ventricosum* (Welw.) Cheesman) in Ethiopia. The antibacterial activities of methanol leaf extracts of *Achyranthes aspera*, *Agarista salicifolia, Datura stramonium, Melia azedarach, Pycnostachys abyssinica* and *Vernonia amygdalina* were evaluated *in vitro* against Xcm. Fresh leaves were collected, dried under shade and ground to fine powder. Extraction was carried out using maceration method. The antibacterial activity of extracts was evaluated by disc diffusion method. Total phenolics content was estimated using Folin Ciocalteau method. The result revealed that higher percent extract yield was obtained from *A. salicifolia* followed by *P. abyssinica*. Extracts of all species showed antibacterial activity except *M. azedarach*. Significant differences in inhibition zone diameter were recorded between species and among test concentrations. The widest inhibition zone was recorded by *A. salicifolia* followed by *P. abyssinica*. Unlike others, extract of *A. salicifolia* had abundant amount of alkaloids, flavonoids, phenols, terpenoids, saponnins, tannins and cardiac glycosides. The remaining species lacked one or more of these metabolites and the existing ones occurred either in small or moderate amount. Moreover, the extract of *A. salicifolia* was found to have the highest total phenolics content and this was positively correlated with inhibition zone diameter at all test concentrations suggesting its potential antibacterial activity. This shows that the extract of *A. salicifolia* has the potential for further bioformulation and commercialization as biocide with broad spectrum activity. However, further research should be conducted to identify the active compounds responsible for such antibacterial activity.

Keywords: *Ensete ventricosum; Agarista salicifolia, Pycnostachys abyssinica, Inhibition zone;Total phenolics*
DOI: http://dx.doi.org/10.4314/ejst.v10i1.2

INTRODUCTION

Enset *(Ensete ventricosum* (Welw.) Cheesman) is a perennial staple food crop widely cultivated in the South and Southwestern Ethiopia. It supports the lives of approximately 20 million people (Temesgen Addis, 2005). It is a multipurpose crop providing a range of services such as food, forage, medicine, and environment protection (Dereje Fekadu, 2009). As a food crop enset has several advantages. First, the plant can be harvested at any time during the year and be harvested at any stage over a period of several years. Second, enset foods can be stored for long periods (Dereje Fekadu, 2009). Third, compared to cereals, it gives high yield per unit area. Enset growing regions of Ethiopia are well known for their high population density and the land holding of each farmer is very small and yet the population rarely faces food shortage due to the high productivity of enset (Admasu Tsegaye and Struik, 2001). Fourth, enset grows in a wide range of environments extending

*Corresponding author: gyemata12@gmail.com

from about 1200 to 3100 meters above sea level (Temesgen Addis, 2005). This allows farmers to grow the crop in all parts of the country including areas not suitable for cereal cultivation. Fifth, it is considered tolerant to environmental conditions (Dereje Fekadu, 2009).

However, enset cultivation is impeded by a disease commonly called enset bacterial wilt (*Xanthomonas* wilt). It is caused by the Gram negative phytopathogenic bacterium *Xanthomonas campestris* pv *musacearum* (Dagnachew Yirgou and Bradbury, 1968; Dereje Ashagari, 1985). The disease is a major constraint of enset based agriculture, affecting the lives of millions of people in Ethiopia (Afza *et al*., 1996; Gizachew Welde-Michael *et al*., 2008). In addition to enset, the disease also affects other *Musa* species like banana which is the main staple food source in the whole of east Africa. The disease poses serious food insecurity in the region (Mwangi *et al*., 2007). Unlike other diseases, enset bacterial wilt is both extreme and rapid causing gradual increasing losses over years. The economic impact of bacterial wilt is due to death of the mother plant that would otherwise contribute to the continuation of enset production cycles. Fields infested with Xcm cannot be replanted for at least 6 months due to carryover of soil borne inoculum (Tripathi *et al*., 2009).

The major transmission means of the disease across or within fields are insects, contaminated tools and infected plant materials (Mwangi *et al*., 2007). The spread of the disease is prevented by cultural disease management practices such as burying infected plants, restricting movement of infected plant materials and sterilizing farming tools (Biruma *et al*., 2007; Mwangi *et al*., 2007; Gizachew Wolde-Michael *et al*., 2008; Tripathi *et al*., 2009; Temesgen Addis *et al*., 2010). However, these methods are not effective as farmers are inconsistent and reluctant to employ labor-intensive disease control measures (Tripathi *et al*., 2009). Moreover, the disease is systemic and hence surface application of chemicals has little or no use to control the disease (Smith *et al*., 2008). Thus, investigations for alternative disease controlling strategies that are effective, eco-friendly, long-lasting, low cost, and easy to prepare have prime importance.

In recent years, much interest has been developed in the antimicrobial effects of medicinal plants for plant disease control. Plant extracts are important sources of new agrochemicals for the control of plant diseases. Furthermore, biocides of plant origin are nonphytotoxic, systemic and easily biodegradable (Kagale *et al.,* 2004; Guleria and Tiku, 2009). It is now known that various plant extracts can reduce populations of foliar pathogens and control disease development. Consequently, they have a potential as environmentally safe alternatives and components in integrated disease management programs (Bowers and Locke, 2004). Therefore, the aim of the present study was to evaluate the antibacterial activity of medicinal plant extracts against Xcm and see the potential to control enset bacterial wilt disease.

MATERIALS AND METHODS

Medicinal plant material collection

Fresh leaves of medicinal plants viz. *Achyranthes aspera* L. *(Amaranthaceae*), *Agarista salicifolia* (*Com.ex Lamb.)* G. Don *(Ericaceae), Datura stramonium* L. *(Solanaceae), Melia azedarach* L. *(Meliaceae), Pycnostachys abyssinica (*Fresen*) (Lamiaceae)* and *Vernonia amygdalina* Del.

(Asteraceae) were collected from different areas. The study species were selected on the basis of follow-up of antimicrobial activity reports and ethnomedical or traditional uses against diseases (Fabricant and Farnsworth, 2001). Specimens of each species were freshly pressed, mounted and identified by experts and authenticated specimens in the National Herbarium, Department of Plant Biology and Biodiversity, Addis Ababa University. Voucher specimens of each species have been deposited in the National Herbarium. Collection number was designated using the first letters from the full name of the collector and order of collection time. Accordingly, voucher specimens of *A. aspera, D. stramonium, M. azedarach, A. salicifolia, P. abyssinica,* and *V. amygdalina* were given GY-01, GY-02, GY-03, GY-04, GY-05 and GY-06 collection numbers, respectively.

The leaves were washed with running tap water to remove dust and other debris, and air dried under shade at room temperature to constant weight. The dried leaves were ground to fine powder using mechanical grinder. The powder was sieved through 0.6 mm wide mesh, homogenized and used for extraction.

Preparation of crude leaf extract

Extraction was performed using maceration method (Rai *et al*., 2013). Leaf powder and the solvent (methanol) were added into conical flasks at the ratio of 1:10 (w/v). The flasks were tightly closed and the mixture was shaken for 72 h using orbital shaker at a speed of 250 rpm under room temperature. After 72 h, the extract was filtered first by four layer of cheese cloth and cotton followed by Whatman No.1 filter paper. The extract was dried and concentrated by evaporating methanol using rotary evaporator. The concentrated extract was stored at 4°C 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:

$$\text{Extract yield (\%)} = \frac{\text{dry weight of extract}}{\text{initial powder weight}} \times 100$$

Infected enset material collection

Newly infected enset pseudostem samples were collected from a homegarden at Wondo Genet College of Forestry and Natural Resources. The samples were cut into small pieces of approximately 2-5 mm^2 in size using sterilized knife. Each piece was placed in separate plastic bags and kept as cool as possible in an ice box to prevent drying, microbial degradation and avoid tissue decomposition. The specimens were then transported to the laboratory for Xcm isolation.

Isolation of the pathogen

Infected pseudostem specimens were further cut into smaller pieces using sterilized scalpel. The pieces were surface disinfected by dipping in 5 % sodium hypochlorite solution for one minute and immediately immersed in distilled water three times to remove the disinfectant. Then after, the cut pieces were immersed into a test tube containing 5 mL of sterilized water and allowed to stand for 5 minutes until the bacterial population diffuses out of the cut tissue into the sterilized water and form a suspension. A loopful of bacterial suspension was 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}) as developed by Tripathi *et al*.

(2007). Cephalexin and amphotericin were used to inhibit the growth of most saprophytes and fungal contaminants, respectively. The streaked Petri dishes were incubated in an inverted position at 28°C for 72 h. Isolated colonies were selected and streaked on a newly prepared yeast extract, peptone, sucrose and agar (YPSA) growth medium. The sub-culturing of the bacterium was carried out using streak plate method. In this method, loopful of bacteria was directly taken using wire loop from growth plates that contain uniform colonies of Xcm. Sub-culturing was done several times until pure colonies were produced. The pure culture grown on YPSA was stored at 4°C, and every time activated at 28°C before use (Schaad and Stall, 1988).

Pathogenicity test

Plastic buckets filled with soil, sand and manure in the ratio of 2:1:1 were prepared in the glasshouse and suckers of a susceptible enset clone were transplanted. After establishment, individual enset plants were inoculated with 10 ml of Xcm suspension adjusted to $1.5x10^8$ CFU/mL (0.5 McFarland standard) at the base of midrib in three replications. The negative control was inoculated with the same amount of distilled water using syringes with metal needle. A week after inoculation, symptom development was monitored for every other day. Yellowing of the inoculated leaf was seen after three weeks. Pseudostem of the infected plants was taken and the bacterium was re-isolated using the standard isolation procedure (Kidist Bobosha, 2003).

Antibacterial susceptibility testing of leaf extracts

The antibacterial activity of crude leaf extracts against Xcm was evaluated by disc diffusion method (EUCAST, 2012). Petri dishes, paper discs, cotton swabs, forceps, test tubes and other materials were autoclaved every time before use. All the tests were performed in three replications and repeated at least three times. Extracts of each species were dissolved in methanol and serial test concentrations were prepared. Approximately 1.2, 0.6, 0.3, 0.15, 0.075, 0.038 and 0.019 g of each species extract was separately dissolved in 3 mL of methanol producing 400, 200, 100, 50, 25, 12.5 and 6.25 mg/mL serial concentrations. Streptomycin sulphate was used as a standard antibiotic. Discs with a size of 5 mm in diameter were prepared from Whatman's No 1 filter paper using paper borer. The discs were sterilized and impregnated in each serial concentration of each species for 12 h. Discs immersed in methanol served as controls. Subsequently, the discs were taken out and dried before application.

Inoculum preparation and inoculation

The inoculum was prepared from 72 h old bacteria grown on YPSA medium. The upper surfaces of several pure culture colonies were swabbed with cotton swab and mixed with 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 inoculum was measured with a spectrophotometer (NV202, Sunny) at 600 nm and adjusted to 0.132. This value is equivalent to turbidity of a 0.5 McFarland standard (Sutton, 2011). The bacterial population equivalent to a 0.5 McFarland standard turbidity is approximately 1.5×10^8 CFU/mL.

Cotton swab was used for inoculation. 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.

This avoids over inoculation of the Petri dish. The inoculum was spread evenly over the entire surface of the Petri dish by swabbing in three directions. The dried discs were applied to the inoculated Petri dish within 15 minutes of inoculation. During application, the discs were pressed downward and the Petri dish was kept in normal position until the discs got wet. Discs rinsed in methanol were used as negative controls. Streptomycin sulphate was used as a positive control. All the Petri dishes were inverted and incubated at 28°C for 72 h. Inhibition zone was measured after 72 h of incubation. Zone of complete inhibition was measured using transparent ruler at the longest possible diameter including the disc (Ortez, 2005).

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of leaf extracts was determined using agar dilution method as described in EUCAST, (2000). One mL of each test concentration of each extract was thoroughly mixed with 19 mL of YPSA molten growth medium and poured to Petri dishes. The medium was then allowed to solidify at room temperature. The inoculum adjusted to turbidity of a 0.5 McFarland standard (0.3µl) was inoculated at four points on each Petri dish. The inoculated Petri dishes were incubated at 28°C for 72 h. Parallel to this, Petri dishes without extract were used as controls and the results were compared against these controls.

Minimum bactericidal concentration (MBC)

Minimum bactericidal concentration (MBC) of leaf extracts was determined as described in Njinga *et al*. (2014). YPSA growth medium was prepared and autoclaved at 121°C for 15 minutes. The medium was poured into sterile Petri-dishes and allowed to cool and solidify. The contents of the MIC Petri-dishes that did not show growth or showed growth less than 80 % of the control were sub-cultured onto the prepared Petri-dishes. The Petri-dishes were then incubated at 28°C for 72 h. Then after, the Petri-dishes were observed for growth. The Petri-dishes without growth represent the minimum bactericidal concentration (MBC). After 72 h, the results were recorded and taken as MBC.

Phytochemical screening of crude leaf extracts

Qualitative analysis was performed to confirm the presence or absence of major secondary metabolites in the crude leaf extracts. The screening was carried out following standard chemical methods (Harborne, 1998; Tiwari *et al*., 2011; Jones and Kinghorn, 2006; Wadood *et al*., 2013; Kumar *et al*., 2013; Rai *et al*., 2013).

Test for alkaloids: Wagner's test

Each extract (20 mg) was dissolved in 2% HCl and filtered with glass funnel. The filtrate was used for the detection of alkaloids. Wagner's reagent was prepared by dissolving 1.27 g of iodine and two gram of potassium iodide (PI) in 100 mL of distilled water. One mL of Wagner's reagent was added to two mL of the filtrate. The formation of redish or brown solution was an indication for the presence of alkaloids.

Test for flavonoids

Sample from each plant extract (0.5 g) was taken in separate test tubes and 10 mL of distilled water was added, shaken and filtered. Five mL of diluted ammonia solution (10%) was mixed with 3 mL of

the aqueous filtrate followed by the addition of 1 mL of concentrated sulphuric acid. Yellow color was formed showing a positive test for flavonoids.

Test for phenolic compounds: Ferric Chloride ($FeCl_3$)

To two mL of the extract filtrate, 3-4 drops of 5% Ferric Chloride solution were added. Bluish black color production indicated the presence of phenols.

Test for terpenoids

Half gram of each species leaf powder was added in separate test tubes and poured with 10 mL of methanol. The content was shaken well and centrifuged. Five mL of the supernatant was mixed with two mL of chloroform followed by the addition of three mL of sulphuric acid to the solution. Reddish brown layer production between chloroform and sulphuric acid was an indicator for the presence of terpenoids.

Test for saponins: Froth test

One gram of each extract was boiled in five mL of distilled water and filtered with glass funnel. Three mL of distilled water was added to one mL of the filtrate and vigorously shaken for about 5 minutes. Frothing that persisted for 15 minutes was taken as an evidence for the presence of saponins.

Test for tannins

Each extract (0.2 g) was dissolved in 5 mL of distilled water in separate test tubes, warmed in a water bath and filtered. Two mL of 5 % ferric chloride solution prepared in distilled water was added to one mL of the filtrate. The appearance of deep blue color affirmed the presence of tannins.

Test for cardiac glycosides

Approximately 20 mg of each extract was dissolved in 3 mL of 2% HCl and filtered. One mL of the filtrate was treated with five drops of glacial acetic acid and two drops of 5% ferric chloride solution and thoroughly mixed. Subsequently, two mL of concentrated sulfuric acid was added and two layers were formed; the lower white layer and upper acetic acid layer which turned bluish green indicating a positive test for glycosides.

Estimation of total phenolics content

Half gram of each extract and 10 mL of distilled water were added to a test tube, shaken and centrifuged. An aliquot of the supernatant (0.1 mL) was taken and diluted to three mL with distilled water. Consecutively, 0.25 mL of Folin Ciocalteau reagent was added. After three minutes, one mL of 20% (w/v) sodium carbonate was added and thoroughly mixed. The tube was warmed in boiling water for one 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 three mL of distilled water, 0.25 mL of Folin Ciocalteau reagent and one 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 catechol equivalent of phenol/g of sample (Zieslin and Ben-Zaken, 1993).

Statistical analysis

All data were subjected to analysis of variance using SPSS (Statistical Package for Social

Sciences, version 20). This was done after carrying out test of homogeneity and normal distribution for each measured parameter. Means were compared by Tukey's Honestly Significant Difference (HSD) test at 95 % confidence interval.

RESULTS

Extract yield (%)

The result of the present study showed a wide variation in extract yield between the studied plants (Figure 1). The highest extract yield was obtained from the leaves of *A. salicifolia* (54.3%) followed by *P. abyssinica* (45.2%), while the lowest was obtained from *A. aspera* (7.8%). The remaining species had an intermediate percent extract yield.

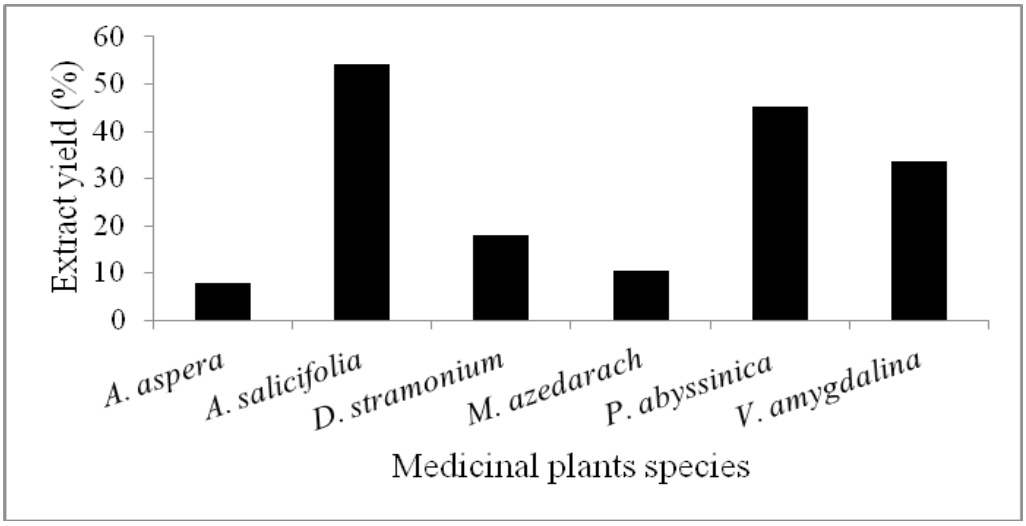

Figure 1: Extract yield (%) of leaf of medicinal plant species

Antibacterial activity of leaf extracts

The antibacterial activities of methanolic crude leaf extracts of *A. salicifolia*, *P. abyssinica*, *A. aspera*, *D. stramonium*, *M. azedarach* and *V. amygdalina* against Xcm were tested *in vitro*. The test was carried out at 50, 100, 200 and 400 mg/mL concentrations. The extracts showed a wide variation in mean inhibition zone diameter of the test organism (Figure 2). The inhibition zone between the test concentrations in extracts of *A. salicifolia* and *P. abyssinica* was significantly different at $p < 0.01$. Compared to others, extracts of these species produced the widest inhibition zone at all test concentrations. Extract of *A. salicifolia* produced a significantly wider inhibition zone at all test concentrations ($p < 0.01$). Likewise, the leaf extract of *P. abyssinica* produced significantly higher inhibition zone than extracts of *A. aspera, D. stramonium* and *V. amygdalina* at 200 and 50 mg/mL test concentrations. The antibacterial activity of *D. stramonium* and *V. amygdalina* leaf extracts was not significant among test concentrations. At the highest test concentration (400 mg/mL), extract of *A. aspera* produced significantly wider inhibition zone than extract of *V. amygdalina.* However, the difference in antibacterial activity between extracts of *A. aspera, D. stramonium* and *V. amygdalina* at 200, 100 and 50 mg/mL test concentrations was not

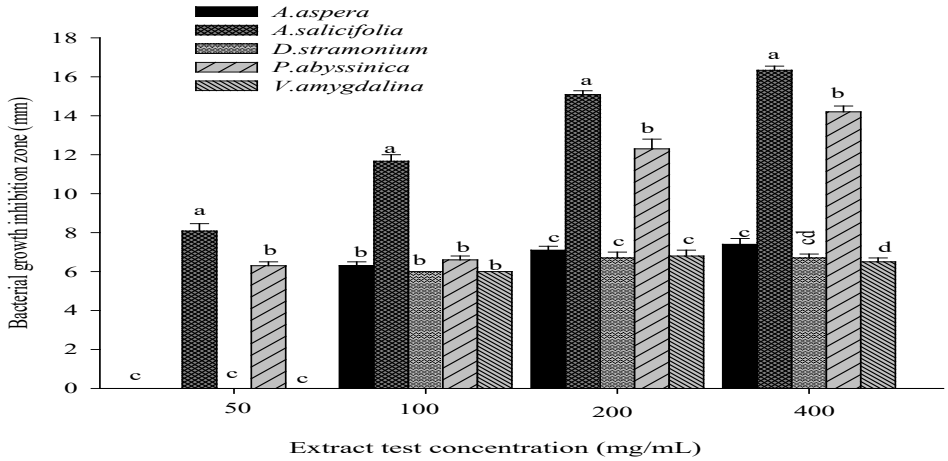

Figure 2: Growth inhibition zone of methanol crude leaf extracts at various test concentrations. Bars at each test concentration followed by different letters are significantly different at $p < 0.01$ ($n = 6$).

significant. The antibacterial susceptibility test of *M. azedarach* extract against Xcm did not show inhibition at all test concentrations.

Minimum inhibitory and bactericidal concentrations of extracts

Minimum inhibitory and bactericidal concentration values showed the potency of extracts against Xcm. Their effect on the growth of Xcm was variable among species (Figure 3). The highest MIC and MBC values were recorded by extracts of *A. aspera*, *D. stramonium* and *V. amygdalina* (Figure 3). Extracts of *A. salicifolia* and *P. abyssinica* were potent; they inhibited the growth of the test bacterium at relatively low concentration as compared to others. The MIC and MBC values of the other extracts were 87.5 mg/mL higher than *A. salicifolia* and *P. abyssinica*. The extract of *M. azedarach* didn't exhibit any growth inhibition at 400, 200, 100 and 50 mg/mL concentrations and thus, MIC and MBC were not determined (Figure 3).

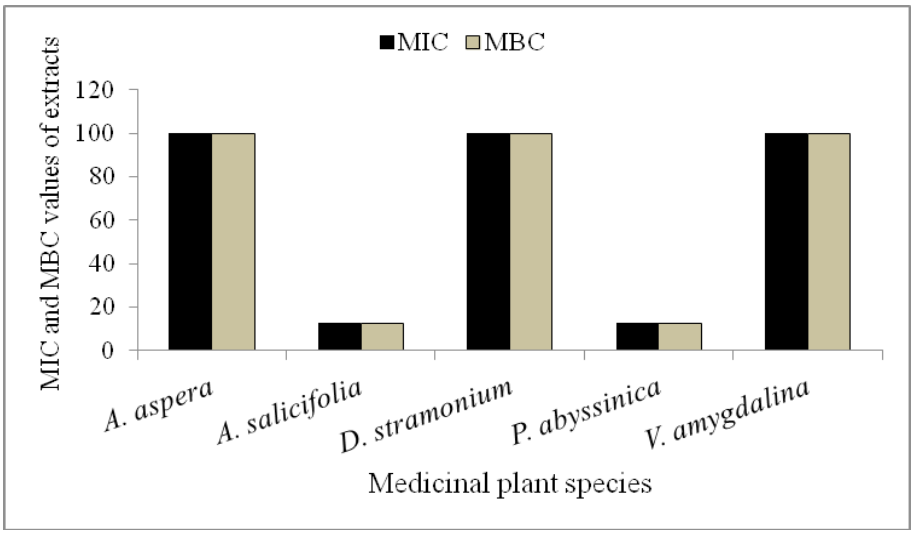

Figure 3: Minimum inhibitory and bactericidal concentrations of crude leaf extracts of medicinal plants

Phytochemical screening

The preliminary phytochemical screening results revealed that the chemical constituents of extracts vary between species (Table 1). Accordingly, extracts of *A. salicifolia* and *P. abyssinica* had abundant amount of the tested secondary metabolites. However, cardiac glycosides were absent in the extract of *P. abyssinica*. Extract of *V. amygdalina* on the other hand had abundant amount of phenols, terpenoids and tannins, and moderate amount of alkaloids, flavonoids and saponnins (Table 1).

Table 1. Classes of secondary compounds found in leaf extracts of medicinal plants in a preliminary screening

Medicinal plant species	Phytochemicals						
	Alkaloid	Flavonoid	Phenol	Terpenoid	Saponin	Tannin	Cardiac glycoside
A. aspera	-	+	-	-	+	-	-
A salicifolia	++	++	++	++	++	++	++
D. stramonium	-	+	+	+	+	++	-
M. azedarach	-	-	-	+	-	-	-
P. abyssinica	++	++	++	++	++	++	-
V. amygdalina	+	+	++	++	+	++	-

++ = abundant; + = moderate; - = absent

Total phenolics content

The result of the quantitative chemical analysis of extracts showed that total phenolics content was significantly different between the studied plant species (Figure 4). The highest total phenolics content was recorded by the leaf extract of *A. salicifolia* (Figure 4). It showed a 100, 85.7, 100, 61.2 and 12.3 % increment from *A. aspera, D. stramonium, M. azedarach, P. abyssinica,* and *V. amygdalina,* respectively. Extract of *V. amygdalina* ranked second and extract of *P. abyssinica* ranked third in terms of total phenolics content. The total phenolics content of the remaining extracts was negligible (lower than the minimum concentration used to construct the standard calibration curve).

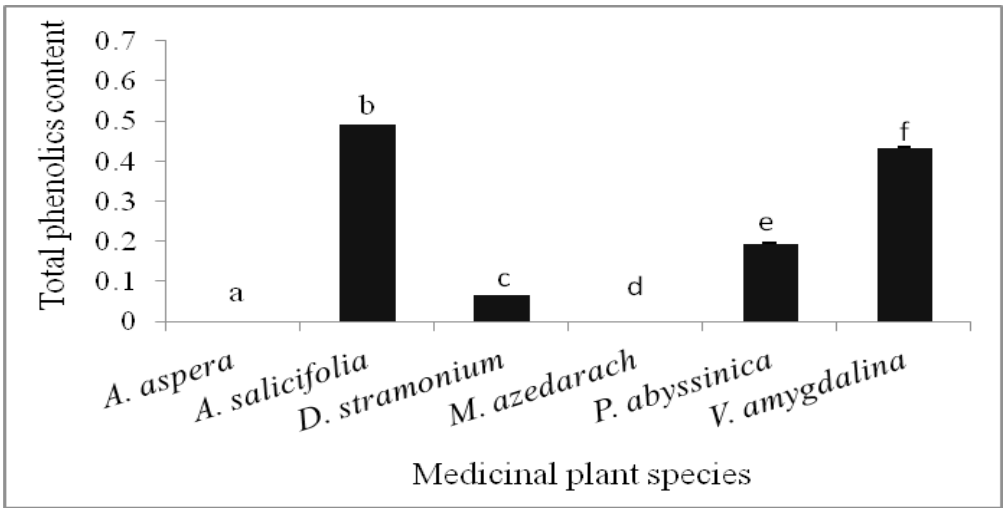

Figure 4: Total phenolics content (mg of catechol equivalent of phenol/g of fresh weight) of medicinal plant leaf extracts. Bars followed by different letters are significantly different at $P < 0.01$. Values represent means of six replicates

DISCUSSION

The exploitation of higher plant products as novel chemical treatments in plant protection has gained emphasis recently and some plant products are being used globally as ecofriendly biocontrol agents (Gurjar *et al*., 2012). The present study was conducted to test the *in vitro* antibacterial activity of some medicinal plants and antibiotics against enset pathogen, Xcm. The tested plants are used in traditional medicine for the treatment of many diseases either in unprocessed or extracted forms. Efficiency of extraction is an important step in the discovery of bioactive components from plant materials. In the present study, *A. salicifolia* produced the highest extract yield followed by *P. abyssinica*. Extract yield of *V. amygdalina* was moderate. Similarly, Odey *et al.* (2012) found out 13.6 and 12 % extract yields of root and stem of *V. amygdalina,* respectively, which is twofold lower than the leaf extract yield in the present study. The differences in the extract yields might be ascribed to the different availability of extractable components, resulting from the varied chemical composition of plants (Sultana *et al*., 2009). Moreover, Zongo *et al*. (2011) and Moteriya *et al*. (2014) reported maximum percent extract yield when methanol is used as a solvent. Methanol is an all purpose solvent that dissolves most secondary metabolites in plants and enhances the release of these chemicals from cellular matrix (Visht and Chaturvedi, 2012; Moteriya *et al*., 2014).

The preliminary phytochemical screening shows the presence of different secondary metabolites in the leaf extracts of the studied species. The variation is both in type and amount of chemical categories. The high antibacterial activity of methanolic extract of *A. salicifolia* and *P. abyssinica* may be due to the abundance of alkaloids, flavonoids, phenols, tannins, saponins and terpenoids. In addition, *A. salicifolia* extract contains glycosides. Each bioactive constituent exert antibacterial activity through different mechanisms (Kotzekidou *et al.,* 2008).

Accordingly, alkaloids intercalate into cell wall and hinder its formation (Gurjar *et al*., 2012). Flavonoids are antimicrobial substances against a wide array of microorganisms *in vitro* and have the ability to bind and complex with bacterial cell walls and soluble proteins. This inactivates enzymes (Marjorie, 1999; Ghosh *et al*., 2011; Gurjar *et al*., 2012). Terpenoids on the other hand are responsible for disbanding of the cell wall of microorganisms by deteriorating the membranous tissue (Marjorie, 1999; Ghosh *et al*., 2011; Jasmine *et al*., 2011; Gurjar *et al*., 2012). Similarly, saponins have the ability to cause leakage of proteins and certain enzymes from the cell (Marjorie, 1999). Moreover, Taguri *et al*. (2006), Rodriguez *et al*. (2009) and Boulekbache-Makhlouf *et al*. (2013) reported that hydrolysable tannins have potent antibacterial effects on various bacteria. They bind and form irreversible complexes with proline rich protein and cause inhibition of cell wall synthesis (Mamtha *et al*., 2004; Igbinosa *et al*., 2009; Ghosh *et al*., 2011; Gurjar *et al*., 2012). Overall, extracts that possess diversity of secondary metabolites in relatively large amount revealed high antibacterial activity against Xcm.

In the present study, extracts of most plant species inhibited the growth of the test bacterium *in vitro*, indicating the presence of antibacterial compounds in the extracts. These compounds are secondary metabolites that belong to various chemical categories in plant materials and work either individually or in combination to produce an antibacterial activity (Ghosh *et al*., 2011). The quantitative analysis revealed that the leaf extract of *A. salicifolia* contain the highest amount of total phenolics content as compared to others and thus the maximum potency against the test bacterium. This is evident from the strong positive correlation between total phenolics content and bacterial growth inhibition zone. Similar correlation was observed in extracts of *P. abyssinica* at all test concentrations. Therefore, the antibacterial activity of the two species may be explained by the total phenolics content. Many research reports reveal that phenolic compounds contribute to the antimicrobial properties of plant extracts whereby the extent of inhibition depends on the concentration of these compounds (Taguri *et al*., 2006; Rhouma *et al*., 2009; Rodriguez *et al*., 2009; Tian *et al*., 2009; Zongo *et al*., 2011; Moteriya *et al*., 2014). The biological activities of phenolic compounds are associated to the presence of hydroxyl groups and phenolic ring in their molecular structure. Membrane proteins of bacteria interact with hydroxyl groups of phenolics by hydrogen bonding. This interaction changes the membrane permeability that in turn causes loss of cellular constitutes and cell destruction (Marcucci *et al*., 2001; Kang *et al*., 2011). They also bind to adhesins, complex with cell wall and inactivate proteolytic macerating enzymes used by plant pathogens (Mohanta *et al*., 2007; Gurjar *et al*., 2012). From this it can be presumed that phenolic compounds act at two different points (cell membrane and cell wall), affecting the growth and metabolism of bacteria (Taguri *et al*., 2006; Stefanovic *et al*., 2012). Similar results have also been reported in other studies (Rajeshwar *et al*., 2005; Kuete *et al*., 2007). Extract of *V. amygdalina* contain high amount of total phenolics next to *A. salicifolia.* However, the antibacterial activity of the extract is low. This may be due to the moderate amount of flavonoids and saponins in the leaf extract of *V. amygdalina* and weak synergistic effect of all kinds of phenolic compounds on Xcm.

The minimum inhibitory concentration (MIC) is the lowest concentration value that inhibits the growth of microorganisms. It is the measure of assaying the effectiveness of antimicrobial plant

extracts and predictive of the inhibitive outcomes. Extracts with low antibacterial activity against Xcm give high MIC and MBC, Viz. *A. aspera, D. stramonium, M. azedarach and V. amygdalina.* To the contrary, extracts with high antibacterial activity yield low MIC and MBC, Viz. *A. salicifolia* and *P. abyssinica.* Similarly, various MIC and MBC concentrations of methanol extracts of medicinal plants against both Gram positive and negative bacteria have been reported by Kang *et al.* (2011). Unlike the present study, *M. azedarch* showed MIC against several human pathogens (Sen and Batra, 2012).

CONCLUSION

This study demonstrated that extracts of *A. salicifolia* and *P. abyssinica* with high antibacterial activity *in vitro* against *X. campestris pv. musacearum* could be used for biological control of *Enset* bacterial wilt. The maximum percent extract yield plus the highest anti- *Xanthomonas* activity of the two species could be designated as distinctive features of these medicinal plants for further development, formulation and commercialization as biocides with broad spectrum activity. Such developments in the use of natural products from plants in disease control will minimize or avoid environmental and health hazards caused by synthetic chemical pesticides. However, further researches into these extracts should be conducted to identify the active compounds responsible for their antibacterial activity and to evaluate the performance under field conditions.

ACKNOWLEDGEMENTS

The authors are grateful to Addis Ababa University, Office of Vice President for Research and Technology Transfer for providing the financial support for this research. Bahir Dar University is also acknowledged for financially supporting the research.

REFERENCES

Admasu Tsegaye and Struik, P.C. (2001). Enset (*Ensete ventricosum* (Welw) Cheesman) Kocho yield under different crop establishment methods are compared to yields of other carbohydrate rich foods crops. *Netherlands Journal of Agricultural Science* **49:** 81-94.

Afza, R., Duren, M. V and Morpurgo, R. (1996). Regeneration of *Ensete ventricosum* through somatic embryogenesis and adventitious buds. *Plant Cell Reports* **15:** 445-448.

Biruma, M., Pillay, M., Tripathi, L., Blomme, G., Abele, S., Mwangi, M., Bandyopadhyay, R., Muchunguzi, P., Kassim, S., Nyine, M., Turyagyenda, L and Eden-Green, S. (2007). Banana *Xanthomonas* wilt: of the disease, management strategies and future research directions. *African Journal Biotechnology* **6:** 953-962.

Boulekbache-Makhlouf, L., Slimani, S and Madani, K. (2013). Total phenolic content, antioxidant and antibacterial activities of fruits of *Eucalyptus globulus* cultivated in Algeria. *Industrial Crops and Products* **41:** 85–89.

Bowers, J. H and Locke J. C. (2004). Effect of formulated plant extracts on population density of *Phythophythora nicotianae* in soil and control of *Phythophythora* blight in the greenhouse. *Plant Disease* **88:**11-16.

Clinical and Laboratory Standard Institute (CLSI) (2012). Methods for dilution antimicrobial susceptibility tests f or bacteria that grow aerobically; approved standard—Ninth Edition. CLSI document M07-A9. Wayne, PA.

Dagnachew Yirgou and Bradbury J. F. (1968). Bacterial wilt of enset (*Ensete Ventricosum*) incited by *Xanthomonas musacearum* sp. n. *Phytopathology* **58:** 111–112.

De Britto, A. J., Gracelin, D. H. S and Sebastian, S. R. (2011). Antibacterial activity of a few medicinal plants against *Xanthomonas campestris* and *Aeromonas hydrophila. Journal Biopesticides* **4** (1): 57–60.

Dereje Ashagari (1985). Studies on the bacterial wilt of enset (*Ensete ventricosum*) and prospects for its control. *Ethiopian Journal Agricultural Sci*ence **7** (1): 1-14.

Dereje Fekadu (2009). Characterizing farming practices from three regions of Ethiopia on which enset (*Ensete ventricosum*) is widely profited as a multipurpose crop plant. Ethiopian Institute of Agricultural Research, Holetta Research Center, Ethiopia.

European Committee for Antimicrobial Susceptibility Testing (EUCAST) (2000). Determination of minimum inhibitory concentrations (MICs) of antibacterial agents by agar dilution. *European Society of Clinical Microbiology and Infectious Diseases* **6** (9)**:**509-515.

European Committee on Antimicrobial Susceptibility Testing (EUCAST). (2012). Antimicrobial Susceptibility testing. Version 2.1.

Fabricant, D.S and Farnsworth N. R. (2001). The value of plants used in traditional medicine for drug discovery. *Environmental Health Perspectives* **109:** 69–75.

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

Gizachew Welde-Michael, Kidist Bobosha, Blomme, G., Temesgen Addis, Tilahun Mengesha and Simeamelak Mekonnen (2008). Evaluation of enset clones against enset bacterial wilt**.** *African Crop Science Journal* **16**(1): 89-95.

Guleria, S and Tiku A.K. (2009). Botanicals in Pest Management: Current Status and Future Perspectives. In*:* **Integrated Pest Management: Innovation-Development Process**, pp. 317-330, (Peshin, R. and Dhawan, A. K. (eds.).Springer Science+Business Media B.V., North Carolina, USA.

Gurjar, M.S., Ali S., Akhtar, M and Singh, K. S. (2012). Efficacy of plant extracts in plant disease management. *Agricultural Sciences* **3**(3): 425-433.

Harborne, J.B. (1998). Phytochemical Methods: A guide to modern techniques of plant analysis. Chapman and Hall, London.

Igbinosa, O. O., Igbinosa, E.O and Aiyegoro, O. A. (2009). Antimicrobial activity and phytochemical screening of stem bark extracts from *Jatropha curcas* (Linn). *African Journal of Pharmacy and Pharmacology* **3**(2): 058-062.

Jasmine, R., Selvakumar, B. N and Daisy, P. (2011). Investigating the mechanism of action of terpenoids and the effect of interfering substances on an Indian medicinal plant extract demonstrating antibacterial activity. *International Journal of Pharmaceutical Studies and* Research **2**(2): 19-24.

Jones, W. P and Kinghorn A. D. (2006). Extraction of plant secondary metabolites. In: **Natural Products Isolation**, pp.323-353, (Sarker *et al*., eds.). Humana Press Inc. Totowa, New Jersey.

Kagale, S., Marimuthu, T., Thayumanavan, B., Nandakumar, R and Samiyappan R. (2004). Antibacterial activity and induction of systemic resistance in rice by leaf extract of

Datura metel against *Rhizoctonia solani* and *Xanthomonas oryzae pv. oryzae*. *Physiological Molecular Plant Pathology* **65:** 91-100.

Kang, C. G., Hah, D.S., Kim, C. H., Kim, Y. G., Kim, E and Kim, J. S. (2011). Evaluation of antimicrobial activity of the methanol extracts from 8 traditional medicinal plants. *Toxicology Research* **27**(1): 31-36.

Kidist Bobosha (2003). Characterization of *Xanthomonas campestris* pv. *musacearum* isolates: causal agent of enset bacterial wilt disease**.** M.Sc. Thesis. Addis Ababa University.

Kotzekidou, P., Giannakidis, P and Boulamatsis A. (2008). Antimicrobial activity of some plant extracts and essential oils against food-borne pathogens *in vitro* and on the fate of inoculated pathogens in chocolate. *LWT* **41:** 119-127.

Kuete, V., Nguemeving, J. R., Beng, V. P., Azebaze, A. G. B., Etoa, F. X., Meyer, M., Bodo, B and Nkengfack, A. E. (2007). Antimicrobial activity of the methanolic extracts and compounds from vismia laurentii De Wild (Guttiferae). *Journal of Ethnopharmacol*ogy **109:** 372-379.

Mamtha, B., Kavitha, K., Srinivasan, K. K and Shivananda, P. G. (2004). An *in vitro* study of the effect of *Centella asiatica* (Indian pennywort) on enteric pathogens. *Indian Journal of Pharmacology* **36**(1): 41-44.

Marcucci, M. C., Ferreres, F., Viguera, C., Bankova, V. S., Castro, S. L and Dantas, A. P., (2001). Phenolic compounds from Brazilian ropolis with pharmacological activities. *Journal of Ethnopharmacol*ogy **74:** 105-112.

Marjorie, C. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews* **12**(4): 564–582.

Mohanta, T. K., Patra, J. K., Rath, S. K., Pal, D. K and Thatoi, H. N. (2007). Evaluation of antimicrobial activity and phytochemical screening of oils and nuts of *Semicarpus anacardium* L. *Scientific Research and Essay* **2**(11): 486-490.

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

Mwangi, M., Valentine, N and Sarah, M. (2007). Methods for disinfecting tools in management of banana *Xanthomonas* wilt disease*. African Crop Science Conference Proceedings* **8:** 891–894.

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

Odey, M. O., Iwara I. A., Udiba, U.U., Johnson, J. T., Inekwe, U.V., Asenye, M. E and Victor, O. (2012). Preparation of plant extracts from indigenous medicinal plants. *International Journal of Science and Technology* **1**(12): 688-692.

Okoye, E. L., Uba, B. O., Uhobo, P. C., Oli, A. N and Ikegbunam, M. N. (2014). Evaluation of the antibacterial activity of methanol and chloroform extracts of *Alchornea cordifolia* leaves. *Journal of Scientific Research and Reports* **3**(1): 255-262.

Ortez, J. H. (2005). Disk diffusion testing. In: **Manual of Antimicrobial Susceptibility Testing**, pp. 39-52, (Coyle, M. B., ed). American Society for Microbiology, USA.

Rai, M. V., Pai, V. R., Kedilaya, H. P and 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.

Rajeshwar, Y., Gupta, M and Mazumder, U. K. (2005). *In vitro* lipid peroxidation and antimicrobial activity of *Mucuna pruriens* seeds. *Iranian Journal of Pharmacology Therapeutics* **4:** 32-35.

Rhouma, A., Ben Daoud, H., Ghanmi, S., Ben Salah, H., Romdhane, M and Demak M. (2009). Antimicrobial activities of leaf extracts of *Pistacia* and *Schinus* species against some plant pathogenic fungi and bacteria. *Journal of Plant Pathology* **91**(2): 339-345.

Rodriguez, H., Curiel, J. A., Landete, J. M., De Las Rivas, B., De Felipe, F. L., Gomez-Cordoves, C., Mancheno, J. M and Munoz R.(2009). Food phenolics and lactic acid bacteria. *International Journal of Food Microbiology* **132:** 79–90.

Schaad, N.W and Stall, R. E. (1988). *Xanthomonas.* In: *Laboratory guide for Identification of Plant Pathogenic bacteria* (2nd.ed), pp. 81-84, (Schaad, N. W., ed). APS Press St. Paul, Minnesota.

Sen, A and Batra, A. (2012). Evaluation of antimicrobial activity of different solvent extracts of medicinal plant: *Melia azedarach* L. *International Journal of Current Pharmaceutical Research* **4**(2): 67-73.

Smith JJ, DR Jones, E Karamura, G Blomme and FL Turyagyenda. 2008. An analysis of the risk from Xanthomonas campestris pv. musacearum to banana cultivation in Eastern, Central and Southern Africa. Bioversity International, Montpellier, France.

Stefanovic, O., Radojevic, I., Vasic, S and Comic, L. (2012). Antibacterial activity of naturally occurring compounds from selected plants. In: **Antimicrobial Agents**, pp. 1-24(Bobbarala, V. ed). InTech, Janeza Trdine 9, 51000 Rijeka, Croatia.

Sultana, B., Anwar, F and Ashraf, M. (2009). Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. *Molecules* **14:** 2167-2180.

Sutton, S. (2011). Measurement of microbial cells by optical density. *Journal of Validation Technology* **17**(1): 46-49.

Taguri, T., Tanaka, T and Kouno, I. (2006). Antibacterial spectrum of plant polyphenols and extracts depending upon hydroxyphenyl structure. *Biological and Pharmaceutical Bulletin* **29:** 2226-2235.

Temesgen Addis (2005). Biology of enset root mealybug *Cataenococcus ensete* and its geographical distribution in southern Ethiopia. M.Sc. Thesis. Alemaya University, Ethiopia.

Temesgen Addis, Turyagyenda, L. F., Tameru Alemu, Karamura E and Blomme G. (2010). Garden tool transmission of *Xanthomonas campestris* pv. *Musacearum* on Banana (*Musa* spp.) and enset in Ethiopia. *Acta Horticultureae* **879:** 367-372.

Thippeswamy, S., Praveen, P., Mohana, D. C and Manjunath, K. (2011). Antimicrobial evaluation and phytochemical analysis of a known medicinal plant *Samanea saman* (jacq.) merr. against some human and plant pathogenic bacteria and fungi. *International Journal of Pharma and Biosciences* **2**(2): 443-452.

Tian, F., Li, B., Ji, B., Zhang, G and Luo, Y. (2009). Identification and structure – activity relationship of gallotannins separated from Galla chinensis. *LWT–Food Science and Technology* **42:** 1289-1295.

Tiwari, P., Kumar, B., Kaur, M., Kaur, G and Kaur, H. (2011). Phytochemical screening and extraction. A review. *Internationale Pharmaceutica Sciencia* **1**(1)**:** 98-106.

Tripathi, L., Tripathi, J. N., Tushemereirwe, W. K and Bandyopadhyay, R. (2007). Development of a semi selective medium for isolation of *Xanthomonas campestris pv. musacearum* from banana plants. *European Journal of Plant Pathology* **117:**177-186.

Tripathi, L., Mwangi, M., Abele, S., Aritua, V., Tushemereirwe, W. K and Bandyopadhyay, R. (2009). Xanthomonas wilt: A threat to banana production in East and Central Africa. *Plant Disease* **93** (5): 440-451.

Visht, S and Chaturvedi S. (2012). Isolation of natural products. *Current Pharma Research* **2**(3):584-599.

Wadood, A., Ghufran, M., Jamal, S. B., Naeem, M., Khan, A., Ghaffar, R and Asnad G. (2013). Phytochemical analysis of medicinal plants occurring in local area of Mardan. *Biochemistry Analytical Biochem*istry **2:**144. doi: 10.4172/2161-1009.1000144.

Zieslin, N and Ben-Zaken, R. (1993). Peroxidase activity and presence of phenolic substances in peduncles of rose flower. *Plant Physiology and Biochemistry* **31:** 333-339.

Zongo, C., Savadogo, A., Somda, K. M., Koudou, J and Traore, A. S. (2011). *In vitro* evaluation of the antimicrobial and antioxidant properties of extracts from whole plant of *Alternanthera pungens* H.B. and K. and leaves of *Combretum sericeum* G. Don. *International Journal of Phytomedicine* **3:** 182-191.