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Evaluation of Different Botanical Plant Extracts and Other Material against Enset Bacterial Wilt (Xanthomonas campestris PV Musacearum) Disease in Oromia Regional State, Ethiopia

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ABSTRACT

Enset bacterial wilt (EBW) disease caused by Xanthomonas campestris pv. musacearum is a major constraint of Enset production in Toke-Kutaye District, Oromia Regional State. Farmer indigenous practices to controlling Enset bacterial wilt in the area is by using extracts of botanical plants which is recently becoming an important in development of new control measures. However, detailed study for identifying the potential botanicals against the disease was found necessary. Therefore, this study was conducted with the objective to evaluate the crud extracts botanicals, goat urine, and salt against the pathogen. In vitro, antibacterial activities of leaf and root extracts of the botanicals plant were examined against EBW isolates. The cruds extracts of botanicals such as such 'Solle' (Olinia rochetiana), 'Hadafiti' (Clematis simensis), 'Tembosuse' (Inula conferiflora), 'Etecha' (Dodonaea angustifolia), 'Kebericho' (Echinops kebericho), 'goat urine' and 'salt' individually and in combinations were tested using disc diffusion method. The results of vitro study showed that the inhibition zone (21.02mm) for the standard check (Penicillin) was significantly higher than all treatments under study. However, maximum growth inhibition zone 14.05mm was observed in the extract combination of Etecha + Kabericho followed by single extract of Etecha and combination of Solle + Hidafite + Tembosuse with inhibition zone of 12.08 and 11.78mm respectively. Results indicate the potential of these plants for further work on isolation and characterization of the active principle responsible for antibacterial activity and its exploitation as therapeutic agent.

Keywords: *Enset bacterial wilt, Xanthomonas campestris pv. Musacearum, Botanical extract, Disk diffusion, Antibacterial activity*

1. INTRODUCTION

Root and tuber crops play a major role as food source in southern, southwestern, western, and central part of Ethiopia (Spring et al., 1996). Enset (Ensete ventricosum (Welw) Cheesman) is one of the indigenous root crops widely cultivated for its food and fiber values in the above-mentioned parts of the country (Taye, 1996). It is estimated that a quarter or more than 15 million of Ethiopia's population depends on Enset as staple and co-staple food source, for fiber, animal forage, construction materials and medicines (Brandt et al., 1997) and the area of Enset production in Ethiopia is estimated to be over 180,000 hectares (CSA, 1994).

Enset is considered as a security food crop as it can withstand long periods of drought, heavy rains, and flooding, which normally devastates other crops (Degu and Workayehu, 1990). The sustainability of Enset agriculture is, however, threatened by a number of factors including population pressure, which is associated with more intense cultivation, degradation of the soil and environment (Quimio and Tessera, 1996). Diseases are collectively the most severe biological problem facing Enset. Several types of diseases are known to affect Enset plants such as fungal, nematode, viral and bacterial diseases were reported to cause damage at different degrees of intensity (Quimio, 1992). Among various diseases, Enset bacterial wilt disease is the major bottleneck for Enset production and it is very destructive as it kills Enset plants at all growth stages (Dereje, 1985).

Bacterial wilt of Enset (EBW), caused by Xanthomonas campestris pv. musacearum was first reported from Ethiopia by Yirgou and Bradbury (1968) and this disease currently found in all Enset growing regions and it is the most serious in terms of its effects on production. Since the diseases relentlessly speared throughout the major Enset growing region of the country causing significant yield losses and still remains to be the major problem particularly on Enset cultivation in the country (Korobko, 1997 and Kidist, 2003). Furthermore, studies that were carried out on other possible control strategies evaluation of traditional medicinal plant extracts against the Enset bacterial wilt disease is also very critical (Kidist, 2003).

Traditionally medicinal plants have produced a variety of beneficial compounds properties. The active compound that contain in medicinal plant can either inhibit the growth of pathogens or kill them and have no toxicity to the host cells are considered for developing new antimicrobial drugs (Nascimento et al., 2000). In addition, uses of medicinal plant extracts are important means to management disease, increase production, and minimize chemical use and environmental hazard. Therefore, farmers in different areas have their traditional practices to control different plant disease through plant extract and other material but these indigenous practices have not given focus by researchers, research centers for long time up until now.

Recently, evaluation of traditional medicinal plant extracts against Xanthomenas campastris pv. musacearum species is becoming an imperative at the

present. Thus a focus should also be given to indigenous practices of the farmer to look for their effectiveness. Especially the indigenous knowledge on medicinal plants such as Olinia rochetiana (A.Juss) locally called 'Solle', Clematis simensis (Fresen) locally called 'Hadafiti', Inula conferiflora (A.Rich) locally called 'Tembosuse', Dodonaea angustifolia (L.f) locally called 'Etecha', Echinops kebericho (Mesfin) locally called 'Kebericho', 'goat urine' and 'salt' is important. Some innovative farmers at Toke-Kutaye district used the extraction of those medicinal plants and apply on bacterial wilt infected Enset plant through cutting pseudostem. In addition, they use those herbs extract for disinfection contaminated tools. Therefore, such practices need to be proven through scientific method and it is better control strategies against the disease. The aim of current study was to evaluate the crud extracts of different botanicals, goat urine and salt against the Xanthomenas campastris pv. musacearum isolates.

2. MATERIALS AND METHODS

2.1 Enset Bacterial Wilt Sample collection

Enset fields in Toke-Kutaye district were inspected for plants with bacterial wilt symptoms. The samples were taken from infected Enset plant leaf petioles and pseudostem, which show early stage of the disease symptom to avoid some saprophytic microorganisms that grow in tissues killed by the primary pathogen (Quimio, 1992). The petioles and pseudostems containing bacterial ooze were taken from symptomatic Enset plants using surface sterilizing knife with 76% ethanol (Aritua et al., 2007). Finally, the collected samples were put in polythene paper bags, sealed and kept in an ice box and transfer in to the laboratory. The laboratory tests were carried out at the Bacteriology laboratory of Ambo Plant Protection Research Center (PPRC). The center is located at a distance of 123 km west of Addis Ababa.

2.1.1 Isolation and Identification of Xanthomonas campestris pv musacerum

In the laboratory, the collected samples were surface-sterilized by dipping in 76% ethanol for 2 minutes, rinsed three times in sterilized distilled water and blot-dried by spreading on the three layer of filter paper. Isolation of Xanthomonas campestris pv musacerum was done on to Yeast extract Dextrose Calcium Carbonate (YDC) agar plates. The YDC medium containing 10 g yeast extract, 20 g D-gulcose, 20 g $CaCo_3$ and 15 g Agar. The medium was dissolved in one litter distilled water of pH 7.0 and was autoclaved at 121^0C for 15 minutes, 15 lb/pressure (Goszczynska et al., 2000). The medium was poured onto 90 cm diameter Petri dishes and allowed to cool overnight. The following day, the suspected Enset plant parts (leaf petioles and pseudostems) were surface-sterilized by washing with cotton wool dipped in 70% ethanol and cross sections of each made by use of a sterilized blade. Bacteria were picked from the ooze with a sterilized tooth pick and put in 2 ml sterilized distilled water in a beaker. This suspension was streaked on toYDC agar plates with the help of loopful. After that, the plates were labelled and kept in incubator for 3 days at 28^0C (Aritua et al., 2007). The single bacterial colonies of yellow, light yellow, deep yellow and creamy were purified repeatedly on YDC agar medium and pure bacterial colonies were transferred to YDC slants which were prepared in the test tube incubated at 28^0C for 48-72 hours and preserved at 4^0 C till use. Identification of all the strains was confirmed by standard biochemical and cultural characterization methods.

2.2 Collection and identification of botanical plant

The botanicals plants were collected from Toke-Kutaye district Oromia regional state. They were authenticated by Professor Ensermu Kelbessa Head of National herbarium centre Addis Ababa University. Table 1 Show botanical plants and parts used

Table 1: The identified botanical plants and parts used

S/ N	Species	Family	Vernacular	Part use
1	Olinia rochetiana A.Juss	Oliniaceae	Solle	Young Leaves
2	Clematis simensis Fresen	Ranuculaceae	Hadafiti	Young Leaves
3	Inula conferiflora A.Rich	Asteraceae	Tembosuse	Young Leaves
4	Dodonaea angustifolia L.f	Sapindaceae	Etecha	Young Leaves
5	Echinops kebericho Mesfin	Asteraceae	Kebericho	Fresh Root

2.2.1 Extraction of the botanical plant material and preparation of 'Goat urine' and 'salt'

The botanical plants were collected from west show zone, Toke-Kutaye district in large amount for extraction purpose. The extraction of the tested plant was done in PPRC laboratory. From each collected sample 30g of young leaves of 'Solle', 'Hedafita', 'Tembosusa', 'Etecha' and roots of 'Kebericho' were taken separately and disinfected in 76% ethanol. After that 50 ml of sterilized distil water was added and crushed using mortar and pistil and mixed to form a suspension. The suspension was filtered with a cheese cloth. In addition, 50 ml of goat urine was measures by volume measurement and put separately. Besides, one spoonful of salt was also added in to 50 ml of sterilized distilled water and mixed to form dilution with the help of vortex and filter with whatman number one filter paper. The laboratory equipments used for extraction were sterilized in autoclaved at 121^0C for 15 minutes. Finally, the prepared crude extract, goat urine and salt were prepared separately and preserved at 5ºC until further use (Antibiotic and filter-paper disks test).

2.3 Antibacterial Activity Assay

The antibacterial activity of the various extracts of botanical plant, goat urine and salt was determined by the standard disc diffusion method (Mercan et al., 2006).

Muller Hinton Agar Medium (MHA) was prepared; the pH is maintained at 7.4 and then sterilized by autoclaving at 121ºC and 15 lbs pressure for 15 minutes. 20 ml of the sterilized media was poured into sterilized Petri dish and the medium was allowed to cool in a sterile condition for 24 hours and plates were then inoculated with $1x10^8$ cfu cultures of test bacteria. A sterile cotton swab is used for spreading the test microorganism from the nutrient broth evenly on the MHA plates. After swabbing the MHA plates and left for few minutes to allow complete absorption of the inoculums. 6 mm sterilize filter paper disc were prepared and soaked in to 1000 mpp (micropipette) concentration of the extracts however, each impregnated filter disc were absorbed 100 mpp/disc concentration of crude extract. The discs injected with extracts were placed on the inoculated MHA agar by pressing slightly and place at the center of the swapped plates and incubated at 37°C for 24 hours. At the end of the period, inhibition zones formed on the medium were measure in mm. A 6 mm sterilized paper disc were impregnated in to sterilized distal water they serves as control and the standard check (penicillin) disc (6 mg/disc) were used as positive reference to determine the sensitivity of bacterial tested. The botanical extract, goat urine and salt were examined individually and combination at known concentrations against the bacterial isolate or the crud extract of young leafs and root with goat urine and salt were mixed in equal proportions in combination of two, three, four, five, six, seven and individual of plant extracts were also test against the bacterial isolate (Table l).

2.4 Experimental Design

The antibacterial activity of botanical extracts, goat urine and salt was indicated by clear zones of inhibition and measured in mm. A total of 45 treatments were arranged in CRD with three replications. The Data was analyzed using (SAS Version 9.0) statistical software program, The treatment means were compared using Duncan's Multiple Range Test (DMRT) at P level = 0.05.

3 RESULTS AND DISSECTION

3.1 Antibacterial test of botanical plant extracts, goat urine and salt against Xanthomonas campestris pv. musacearum isolate

The antibacterial activities of botanical plant extracts, 'goat urine', 'salt', control group and standard antibiotic (Penicillin) were compared in vitro through disc diffusion method and the result was shown in Table 2. Among all treatment tests the standard check antibiotics (Penicillin) have shown strong antibacterial activity against Xanthomonas campestris pv musacearum isolate than other treatments with inhibition zone of 21.02 mm. In comparison, all single treatment and other treatment combination, Etecha + Kabericho were showed significant inhibitory effect followed to the standard check with 14.05 mm zone of inhibition. These could be due to the antibacterial effect that was accumulated more in these extract and which is highly aromatic. Some researchers also report that aromatic phenolic compounds have antimicrobial properties (Alma et al., 2003). On the other hand, the control disks inserted with sterilized distils water showed no inhibitory effect against Xanthomonas campestris pv. musacearum isolate.

In the same way, the single herb extract of Etecha, and combination of Solle + Hidafite + Tembosuse extracts significantly reduced the growth of the bacterial isolate next to standard check and Etecha + Kabericho with inhibition zone of 12.08 and 11.78 mm, respectively. This indicate that the botanical extracts from those plant part were also promising effect against bacterial isolate. Hence, further isolation and evaluation of the active ingredient from these botanical is important to know more about their inhibitory effect.

Furthermore, the combination of Solle + Hidafite + Tembosuse + Etecha, Solle + Kebericho, Hidafite + Kebericho, Hidafite + Etecha, Tembosuse + Kebericho, Hidafite + Tembosuse + Etecha + Kebericho + Urine and Kabericho + Salt have showed moderate inhibitery activeties against bacterial isolate as comparison to standard antibiotic (Penicillin) and with inhibition zone of 11.11, 10.50, 10.39, 10.23, 10.19, 10.07 and 10.16 mm, respectively. Similar study has been reported by Kidist, (2003). The result indicated that the crude extracts of the leaf, bract, stem and root of Pychnostacis abyssinica at 0.01g/ml concentration were evaluated for their inhibitory effect on Xanthomonas campestris pv. musacearum isolates following the agar disc diffusion method. The extracts from leaf and bract through hot and cold extraction with methanol showed some inhibitory effect against isolates 10-11mm inhibition zone by the leaf hot extract, and 12-13mm by bract hot extract.

The result of the present investigation revealed that, the antibacterial activity of many treatment combinations showed a weak antibacterial activity against the bacterial growth as comparison with standard antibiotic (Penicillin) with inhibition zone range between 7.10 – 9.87mm (Table 5).

whereas, the single plant extract such as Tembosuse, Hidafite, Kabericho, Solle, Goat urine, Salt and combination of Hidafite + Salt, Tembosuse + Urine, Solle + Salt, Solle + Urine, Solle + Tembosuse, Tembosuse + Salt, Tembosuse + Etecha, Urine + Salt, Hidafite + Tembosuse were not significantly different among treatments and they didn't show any antibacterial activity against the isolate at equal concentration of the combinations. This implies that, their combination of the treatment may have neutralized the active ingredients so much. While, in single extract test the chemical compounds in the extracts might not have synergetic effect against the isolate. Hence, no visible effects observed on the Xanthomonas campestris pv. musacearum isolate.

Based on this study the antibacterial activity of the single and combination of botanical extract, goat urine and salt varied significantly at (P = 0.05 level). These

variations could be due to the active ingredient or phytochemical differences between species extract and other material. For example the combination effect of the medicinal plant extract as indicate in the result in (Table 2) the treatment combination of Etecha + Kabericho have inhibitory effects against the isolate but when investigation single effect of each treatments the crude extract Etecha show 12.08 mm and Kabericho showed zero zone of inhibition. Therefore, when combining those extracts the potential of the combined crud extract increasing against bacterial isolate on some medicinal plant extract or when combination of the crud extract its result antibacterial effect can be increase against the test organism. This implies that when combination two different plant extract that contained differences active ingredient there is chemical reaction formed. This chemical reaction can be increasing the efficacy of those extracts aginset test isolate.

In generally, the results of the present study showed that a combination of Etecha + Kabericho, indiviual plant extract Etecha, and a combination of Solle + Hidafite + Tembosuse are important for control of Xanthomonas campestris pv. musacearum isolate in vivo condition. However, further characterization of bioactive chemicals responsible for their antibacterial activity and further study on the in vivo application is crucial.

The potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of a phytomedicine to act against microbes. Plant-based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials (Iwu et al., 1999). Continued further exploration of plant- derived antimicrobials is needed today. Further research is necessary to determine the identity of the antibacterial compounds from within these plants and also to determine their full spectrum of efficacy. However, the present study of in vitro antimicrobial evaluation of botanical plant extracts, 'goat urine', 'salt', forms a primary platform for further phytochemical and pharmacological studies.

Table 2: Inhibitory effect of botanical extract, goat urine and salt against Xanthomonas campestris pv. musacearum

No	Treatment	Mean zone of inhibition (in mm)
1	Penicillin	21.027^{a}
2	Etecha+Kabericho	14.050^{b}
3	Etecha	12.080^{bc}
4	Solle+ Hidafite+ Tembosuse	11.783^{bc}
5	Solle+ Hidafite+ Tembosuse+ Etecha	11.210^{dfc}
6	Solle+ Kebericho	10.500^{defc}
7	Hidafite+ Kebericho	10.397^{defc}
8	Hidafite+ Etecha	10.247^{hdefc}
9	Kabericho+Salt	10.193^{hdefc}
10	Hidafite+ Tembosuse+ Etecha+ Kebericho+Urine	10.167^{hdefc}
11	Tembosuse+ Kebericho	10.070^{hdefc}
12	Kebericho+Urine	9.870^{hdefcg}
13	Hidafite+ Tembosuse+ Etecha	9.860^{hdefcg}
14	Etecha+Kabericho+Urine+Salt	9.823^{hdefcg}
15	Etecha+Urine	9.467^{hdefcg}
16	Hidafite+ Tembosuse +Etecha+ Kebericho+ Urine+Salt	9.410^{hdefcg}
17	Etecha+ Kebericho+ Urine	9.153^{hefcg}
18	Solle+ Hidafite+ Tembosuse +Etecha+ Kebericho+ Urine+Salt	8.690^{hefg}
19	Hidafite+ Tembosuse +Etecha+ Kebericho	8.567^{hefg}
20	Tembosuse +Etecha+ Kebericho	8.340^{hefg}
21	Tembosuse +Etecha+ Kebericho+ Urine	8.340^{hefg}
22	Etecha+Salt	8.307^{heg}
23	Solle+Hidafite+ Tembosuse +Etecha+ Kebericho+ Urine	7.820^{heg}
24	Solle+Hidafite	7.800^{heg}
25	Solle+ Etecha	7.730^{heg}

CV=2.39 SE = ± 1.4033
Means with the same letter are not significantly different at p = 0.05

Continued from pervious page

No	Treatment	Mean zone of inhibition (in mm)
26	Solle+Hidafite+ Tembosuse +Etecha+ Kebericho	7.583^{hg}
27	Tembosuse +Etecha+ Kebericho+ Urine+Salt	7.447^{hg}
29	Hidafite+Urine	7.100^{h}
30	Hidafite+salt	2.670^{i}
31	Tembosuse+Urine	2.333^{i}
32	Tembosuse	0.000^{i}
33	Tembosuse +Etecha	0.000^{i}
34	Solle	0.000^{i}
35	Hidafite+ Tembosuse	0.000^{i}
36	Solle+Urine	0.000^{i}
36	Solle+Salt	0.000^{i}
37	Kebericho	0.000^{i}
38	Tembosuse+Salt	0.000^{i}
39	Kebericho+ Urine+Salt	0.000^{i}
40	Solle+Tembosuse	0.000^{i}
41	Urine	0.000^{i}
42	Urine+salt	0.000^{i}
43	Salt	0.000^{i}
44	Hidafite	0.000^{i}
45	Control	0.000^{i}

CV=22.39 SE = ± 1.4033

Means with the same letter are not significantly different at p = 0.05

In conclusion, the results of the present study are similar to a certain degree with the traditional use of the plants and the study was successful in identifying the potential of each botanical plant extracts, 'goat urine' and 'salt' in single and a combination against the isolate, Based on the in vitro test, the results showed the following plant extracts have better antibacterial effect against Xanthomonas campestris pv. musacearum isolate, next to standard check (penicillin). These are a combination of botanical plant extract Etecha + Kabericho as well as, the single botanical extract of Etecha and combination of Solle + Hidafite + Tembosuse. Therefore, based on such indicative results, isolation and evaluation of the active compound that responsible for antibacterial activity of Xanthomonas campestris pv. musacearum isolate could contribute in controlling the disease. In addition, other medicinal plants that were found effective against other Xanthomonas campestris pathovars should be evaluated against Enset bacterial wilt. In general, those midicenal plant extracts was utilized as a potential source of bactericidal compound against the tested bacteria isolate that cause serious damage on Enset crop in Ethiopia, but in vivo studies are required to support this.

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