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Antibacterial effect of *Euphorbia hirta* and *Bidenspilosa* extracts against *Xanthomonas campestris* Pv *vesicatoria* isolated from diseased African nightshade (*Solanum scabrum*)

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Keywords: Antimicrobial activity, *Bidenspilosa*, Disc diffusion, *Euphorbia hirta*, Leaf extract, *Solanum scabrum*.

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Abstract

Solanum scabrum Mill is widely cultivated for consumption as a leafy vegetable, for medicinal purposes and as a source of income. Its productivity is faced with many challenges as farmers in Kenya have been recording low yield ranging between 1-3 tones/ha due to low soil fertility, pests and diseases. Bacterial leaf spot disease caused by *Xanthomona scampestris* pv *vesicatoria* accounts for 40-70% loss of yield of *Solanum* species including *Solanum scabrum*. The disease is controlled by application of synthetic chemicals which have adverse environmental effect. The aim of this study was to determine the antimicrobial effect of water and ethanol extracts from *B. pilosa* and *E. hirta* on *Xanthomona scampestris* pv *vesicatoria.* Leaves and roots of *Bidenspilosa* and *Euphorbiahirta* were obtained dried and used to make different treatments for water and ethanol extracts. Disc diffusion technique was used to determine antimicrobial activity of the extracts. Extracts from *B. pilosa* and *E. hirta* leaves and roots significantly inhibited the growth of *Xc*. pv*vesicatoria*. *Euphorbia hirta* extract was more effective than *B. pilosa* extracts in inhibiting bacterial growth. Root extracts had higher antimicrobial activity compared to leaf extracts. Higher concentrations for both water extract (100%) and ethanol extract (200mg/ml) significantly inhibited growth of *Xc*. pv *vesicatoria* than other lower concentration. The results obtained confirmed the use of *B. pilosa* and *E. hirta* in control of plant pathogens.

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Introduction

Solanum scabrum Mill. is one of the species of African nightshades that is extensively cultivated in West, East and Central Africa for consumption as leafy vegetable and for export. It is popularly used as a leafy vegetable accompanying starch staples because of its high calcium (Ca) content, rich in iron (Fe), methionine and vitamin A (Assaha *et al*., 2013; Musyimi *et a*l., 2012). 100g edible portion of *Solanum scabrum* is: water 87.8g, energy 163kj (39kcal), protein 3.2g, fat 1.0g, carbohydrate 6.4g, fiber 2.2g, Ca 200mg, P 54mg, Fe 0.3mg, β-carotene 3.7mg, ascorbic acid 24mg (Muthomi and Musyimi 2009). Leaf extracts are used to treat diarrhoea in children and certain eye infections and jaundice while raw fruits are chewed and swallowed to treat stomach ulcers or stomach-ache (Idowu *et al.,* 2014). Infusion of leaves and seeds can be rubbed onto the gum of children with crooked teeth to correct the condition (Muthomi and Musyimi 2009). Despite the numerous advantages attributed to *Solanum scabrum*, its productivity continues to be faced with many challenges.

According to Ashilenje *et al.* (2012), optimal yield of *S. scabrum* is 20-40tones/ha but farmers in Kenya have been recording low yield ranging between 1-3tones/ha due to low soil fertility, poor cultural practices, low quality seeds, pests and diseases (Ashilenje *et al.*, 2012; Edmonds and Chweya 1997). Bacterial leaf spot disease caused by *Xanthomona scampestris pv vesicatoria* accounts for 40-70% loss of yield in tomatoes, pepper and other *Solanum* species (Mbega *et al.*, 2012; Shenge *et al.,* 2010).The disease is controlled by copper based synthetic chemicals which have adverse effect to the environment as they persist for long in the soil (Ghosh, 2015). In regard to the deleterious effect of synthetic chemicals, there is a need to seek for alternative agents especially botanicals bacterial leaf spot disease.

Many plants have useful products for crop protection but they are often neglected in favour of synthetic products (Nashwa and Abo-elyousr, 2012). These products have useful properties as being cheap and easily available, cause little disturbance to the natural balance, and often harmless to humans and animals (Didwania *et al*., 2013). Extracts from *B. pilosa* and *E. hirta* have been reported to be active against plant pests, fungi and bacterial pathogens. *Euphorbia hirta* has been used to control citrus canker caused by *Xanthomona saxonopodis* pv*. citri* (Jadhav and Deobhankar, 2013) while *B. pilosa* has been used to control late blight in tomatoes and potatoes caused by *Phytophthora infestans* (Yamdeu *et al.*, 2013) but there is no information on efficacy and control of bacterial leaf spot caused by *Xanthomona scampestris* pv *vesicatoria* in *S. scabrum* using these plant extracts. This study therefore was to determine the effect of *B. bilosa* and *E. hirta* extract on *Xanthomona scampestris* pv *vesicatoria* and their use in controlling bacterial leaf sport disease in *S. scabrum*.

Materials and methods

Isolation of the pathogen from infected S. scabrum leaves

Six samples of infected leaves of *S. scabrum* showing water-soaked and necrotic areas were collected from farmer's field around Jaramogi Oginga Odinga University of Science and Technology and taken to microbiology laboratory for isolation and characterization according to Opara and Obani (2010) method. The leaf samples were washed separately in sterile water, surface-sterilized in 70% ethanol and rinsed in several changes of sterile water. From each leaf sample, small pieces of the infected portions were cut from the margin of the lesion using steril escalpel then teased apart with sterile dissecting needle in 1ml sterile water and left to stand for 30 minutes. The suspension was then streaked onto the surface of modified Tween B media and then incubated at 30^0C for 48 hours. The pathogens were then purified on Yeast dextrose calcium carbonate media.

Pathogenecity test

Seeds of *S. scabrum* were planted in pots containing a sterile mixture of top soil and farmyard manure and watered twice daily until when they were 4 weeks old. About 1ml of 10^{-4} suspension of the bacterial inoculum was forced into the underside of the leaf using hypodermic syringe without a needle. One milliliters of sterile water was also infiltrated in another seedling and left for 48hrs to test for the virulence of the bacterial organism according to Opara and Obani (2010). The inoculated plants were covered in plastic bags to maintain humidity at its maximum (Gracelin *et al*., 2012).

Pathogen identification

The pathogen isolates were morphologically and biochemically identified according to Opara and Obani (2010), Gracelin *et al*., (2012) and AL-Saleh (2011) methods.

Plant material collection and test concentration preparation

Fresh leaves and roots of *Bidenspilosa* and *Euphorbia hirta* were collected separately from and around Bondo campus, Jaramogi Oginga Odinga University of Science and Technology, taken to botany laboratory where they were identified by a taxonomist and later confirmed and authenticated at the Museums of Kenya herbarium, Nairobi. They were thoroughly washed with tap water, dried under shade for 14 days and then powdered using an electric motor. Fine powder collected was used for extraction in water and ethanol solvents (Okoli *et al.*, 2009; Abubakar 2009). Mamun and Ahmed (2011); Mahmud *et al.,* (2013) method of extraction was used. 10 grams of powdered leaf and root materials were separately kept in 500mL conical flask and 100mL of distilled water and ethanol added respectively. The mouth of the conical flasks was covered with aluminum foil, mixed thoroughly and left to stand overnight for complete elucidation of active materials. The extract was then filtered using muslin cloth followed by What man no 1 filter paper. For ethanol filtrate, the solvent was removed using rotary vacuum evaporator with the water bath temperature of 45^{o}C. The filtrate was used to make different concentrations of 5%, 10% 15%, 25%, 50% and 100% water extract and 12.5mg/ml, 25mg/ml, 50mg/ml, 100mg/ml, 150mg/ml, and 200mg/ml for ethanol extract.

Disc diffusion sensitivity test

The antibacterial activity assay was performed by agar disc diffusion method (Bauer *et al*., 1966). Colonies from pure culture were lawn spread on nutrient agar plates and discs impregnated with 10µl of each test concentration placed on the surface aseptically. Petri plates were arranged in a completely randomized design (CRD), incubated for 48 hours at 30^{0}C and zone of inhibition measured in millimeters using a transparent ruler.

Results and discussion

Extracts from *B. pilosa* and *E. hirta* exhibited antimicrobial activity against *Xanthomonas campestris* pv *vesicatoria*. Water extract of both *B. pilosa* and *E. hirta* had no significant difference while with ethanol extract *E. hirta* significantly inhibited the growth of *Xc*. pv *vesicatoria* than *B. pilosa* extract (Table 1).

Table 1. Effect of plant species on *Xc.* pv *vesicatoria*.

Plant species	Water extract Zone of inhibition (mm)	Ethanol extract Zone of inhibition (mm)
B. Pilosa	3.3^{a}	4.2^{b}
E. Hirta	3.6^{a}	5.9^{a}
P value	0.072	<.0001
LSD	0.344	0.429

Means with different superscript letters along the column are significantly different (P<0.05).

The antimicrobial activity of *B. pilosa* and *E. hirta* is attributed to the presence of phytochemical substances in these plants. The phytochemicals such as alkaloids, saponins, tannins, flavonoids and many others for *B. pilosa* (Ukwubile *et al*., 2014) and alkaloids, flavonoids, tannins, saponins,

terpenoids and Quinone for *E. hirta* (Gopinath *et al*., 2012) have antimicrobial activity which inhibited the growth of *Xanthomonas campestris* pv *vesicatoria*. Extracts from *B. pilosa* are effective in controlling plant pathogenic fungi *Phytophthora infestans* of potato and tomatoes Yamdeu *et al.* (2013) On the other hand, *E. hirta* has been found to be effective in controlling *Xanthomonas citri* the causative agent of citrus canker in citrus plants (Jadhav and Deobhankar, 2013)

Roots and leaves of *B. pilosa* and *E. hirta* water and ethanol extract were significantly active against *Xanthomonas campestris* pv *vesicatoria* but roots had higher antimicrobial activity than leaves (Table 2). The difference in performance was probably as a result of difference in distribution of phytochemicals in different parts of the plant. Roots have high concentration of tannin (Ukwubile *et al*., 2014) which exhibit greater antimicrobial active on both animal and plant pathogens. In plants, tannins are thought to function as chemical defenses against pathogens and herbivory (Sung *et al*., 2012).

Table 2. Effect of plant parts on *Xc.* pv *vesicatoria.*

Plant parts	*B. pilosa* (Zone of inhibition in mm)		*E. hirta* (Zone of inhibition in mm)	
	Water extract	Ethanol extract	Water extract	Ethanol extract
Leaf	3.04^{b}	3.79^{b}	3.29^{b}	6.21^{a}
Root	3.54^{a}	4.58^{a}	3.92^{a}	5.50^{a}
P value	0.04	0.014	0.009	0.115
LSD	0.474	0.623	0.442	0.904

Means with different superscript letters along the column are significantly different (P<0.05).

With different concentration of *B. pilosa* and *E. hirta* leaf and root water and ethanol extract, it was observed that the zone of inhibition increased with increase in the concentration of the extract (Table 3 and 4). The highest concentrations (100%) for water and (200mg/ml) ethanol extract produced the largest zones of inhibition compared to lower concentrations and synthetic chemical Ridomi®. This could be attributed to increased availability of active compounds which exerted antimicrobial activity more than lower concentration. The largest zones of inhibition in highest concentrations could also be due increased synergism of active compounds therefore exerting their antimicrobial activity over a wider area. The bacterium being a pathogen of a medicinal plant could have developed some resistance thus not being susceptible to low concentrations of the extracts. The results are in agreement with those of Ogbulie *et al*. (2007) and Bhalodia and Shukla (2011) that increased concentration of crude plant extract results in larger diameter of zone of inhibition against bacterial pathogens.

Table 3. Effect of different concentrations of *B. pilosa* and *E. hirta* water extract on *Xc.*pv *vesicatoria.*

Concentration	Zone of inhibition			
	*B. pilosa*extract		*E. hirta*extract	
	Leaf	Root	Leaf	Root
0%	0.00^{d}	0.00^{c}	0.00^{d}	0.00^{d}
5%	1.67^{c}	3.67^{b}	2.33^{cd}	3.67^{c}
10%	3.00^{bc}	4.00^{b}	3.00^{bc}	4.33^{bc}
15%	3.33bc	4.33^{b}	3.33^{bc}	5.33^{b}
25%	4.00^{ab}	4.67^{ab}	3.33^{bc}	5.33^{b}
50%	4.33^{ab}	4.67^{ab}	5.00^{ab}	5.67^{ab}
100%	5.33^{a}	5.67^{a}	6.67^{a}	7.00^{a}
Ridomil	4.33^{ab}	4.33^{ab}	4.33^{ab}	4.33^{bc}
P value	0.001	<.0001	0.001	<.0001
LSD	1.695	1.224	2.343	1.368

Means with different superscript letters along the column are significantly different (P<0.05).

Table 4. Effect of different concentrations of *B. pilosa* and *E. hirta* ethanol extract on *Xc.* pv *vesicatoria.*

Concentrations	*B. pilosa*		*E. hirta*	
	Leaf	Root	Leaf	Root
0.0mg/ml	0.00^{d}	0.00^{d}	0.00^{d}	0.00f
12.5mg/ml	2.67^{c}	3.33^{c}	4.67^{c}	5.67d
25mg/ml	4.00^{bc}	4.67^{bc}	5.00^{c}	5.67d
50mg/ml	5.33^{ab}	5.67^{b}	5.33^{c}	6.67cd
100mg/ml	6.67^{a}	7.33^{a}	7.00^{b}	7.33bc
150mg/ml	6.67^{a}	7.67^{a}	8.00^{ab}	8.33ab
200mg/ml	7.33^{a}	8.00^{a}	8.33^{a}	9.00a
Ridomil	4.33^{bc}	4.33^{bc}	4.33^{c}	4.33e
P value	<.0001	<.0001	<.0001	<.0001
LSD	2.029	1.619	1.274	1.06

Means with different superscript letters along the column are significantly different (P<0.05).

Conclusion

From this study, it is evident that medicinal plants can provide an alternative method of controlling plant pathogens with little disturbance to the ecological balance. Crude plant extracts at high concentrations is effective in inhibiting growth of *Xanthomona scampestris* pv *vesicatoria* the causative agent of bacterial leaf spot disease.

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