

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

William Omuketi Emitaro\*<sup>1</sup>, David Mutisya Musyimi<sup>2</sup>, Darius Andika Otiato<sup>3</sup>,  
Buyela Daniel Khasabuli<sup>2</sup>

<sup>1</sup>Department of Biological Sciences, School of Biological and Physical Sciences,  
Jaramogi Oginga Odinga, University of Science and Technology, Bondo, Kenya

<sup>2</sup>Department of Botany, School of Physical and Biological Sciences, Maseno University,  
Maseno, Kenya

<sup>3</sup>Department of Plant, Animal and Food Sciences, Jaramogi Oginga Odinga,  
University of Science and Technology, Bondo, Kenya

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

Publication date: September 30, 2018

### 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. pvvesicatoria*. *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.

\*Corresponding Author: William Omuketi Emitaro ✉ [omuketiw@gmail.com](mailto:omuketiw@gmail.com)

---

## 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 al.*, 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,  $\beta$ -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 scalpel 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°C 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 *Bidens pilosa* 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°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°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)
<i>B. Pilosa</i>	3.3 <sup>a</sup>	4.2 <sup>b</sup>
<i>E. Hirta</i>	3.6 <sup>a</sup>	5.9 <sup>a</sup>
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	<i>B. pilosa</i> (Zone of inhibition in mm)		<i>E. hirta</i> (Zone of inhibition in mm)	
	Water extract	Ethanol extract	Water extract	Ethanol extract
Leaf	3.04 <sup>b</sup>	3.79 <sup>b</sup>	3.29 <sup>b</sup>	6.21 <sup>a</sup>
Root	3.54 <sup>a</sup>	4.58 <sup>a</sup>	3.92 <sup>a</sup>	5.50 <sup>a</sup>
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			
	<i>B. pilosa</i> extract		<i>E. hirta</i> extract	
	Leaf	Root	Leaf	Root
0%	0.00 <sup>d</sup>	0.00 <sup>c</sup>	0.00 <sup>d</sup>	0.00 <sup>d</sup>
5%	1.67 <sup>c</sup>	3.67 <sup>b</sup>	2.33 <sup>cd</sup>	3.67 <sup>c</sup>
10%	3.00 <sup>bc</sup>	4.00 <sup>b</sup>	3.00 <sup>bc</sup>	4.33 <sup>bc</sup>
15%	3.33 <sup>bc</sup>	4.33 <sup>b</sup>	3.33 <sup>bc</sup>	5.33 <sup>b</sup>
25%	4.00 <sup>ab</sup>	4.67 <sup>ab</sup>	3.33 <sup>bc</sup>	5.33 <sup>b</sup>
50%	4.33 <sup>ab</sup>	4.67 <sup>ab</sup>	5.00 <sup>ab</sup>	5.67 <sup>ab</sup>
100%	5.33 <sup>a</sup>	5.67 <sup>a</sup>	6.67 <sup>a</sup>	7.00 <sup>a</sup>
Ridomil	4.33 <sup>ab</sup>	4.33 <sup>ab</sup>	4.33 <sup>ab</sup>	4.33 <sup>bc</sup>
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	<i>B. pilosa</i>		<i>E. hirta</i>	
	Leaf	Root	Leaf	Root
0.0mg/ml	0.00 <sup>d</sup>	0.00 <sup>d</sup>	0.00 <sup>d</sup>	0.00 <sup>f</sup>
12.5mg/ml	2.67 <sup>c</sup>	3.33 <sup>c</sup>	4.67 <sup>c</sup>	5.67 <sup>d</sup>
25mg/ml	4.00 <sup>bc</sup>	4.67 <sup>bc</sup>	5.00 <sup>c</sup>	5.67 <sup>d</sup>
50mg/ml	5.33 <sup>ab</sup>	5.67 <sup>b</sup>	5.33 <sup>c</sup>	6.67 <sup>cd</sup>
100mg/ml	6.67 <sup>a</sup>	7.33 <sup>a</sup>	7.00 <sup>b</sup>	7.33 <sup>bc</sup>
150mg/ml	6.67 <sup>a</sup>	7.67 <sup>a</sup>	8.00 <sup>ab</sup>	8.33 <sup>ab</sup>
200mg/ml	7.33 <sup>a</sup>	8.00 <sup>a</sup>	8.33 <sup>a</sup>	9.00 <sup>a</sup>
Ridomil	4.33 <sup>bc</sup>	4.33 <sup>bc</sup>	4.33 <sup>c</sup>	4.33 <sup>e</sup>
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.

## References

- Abubaka EM.** 2009. Antibacterial activity of crude extracts of *Euphorbia hirta* against some bacteria associated with enteric infections. *Journal of Medicinal Plants Research* **3(7)**, 498-505.
- AL-Saleh MA.** 2011. "Pathogenic variability among five bacterial isolates of *Xanthomonas campestris* pv. *vesicatoria*, causing spot disease on tomato and their response to salicylic acid." *Journal of the Saudi Society of Agricultural Sciences* **10(1)**, 47-51.
- Ashilenje DS, Omonyin ME, Okalebo JR.** 2012. Potassium nutrition: towards sustainable and profitable production of vegetable african nightshades (*Solanum* L. Section *Solanum*) in Western Kenya. *Journal of Chemical and Pharmaceutical Research* **4(4)**, 2306-11.
- Assaha DVM, Ueda A, Saneoka H.** 2013. Comparison of growth and mineral accumulation of two solanaceous species, *Solanum scabrum* Mill. (Huckleberry) and *S. melongena* L. (Eggplant), under salinity stress. *Soil Science and Plant Nutrition* **59(6)**, 912-920.
- Bauer AW, Kirby WM, Sherris JC, Turck M.** 1966. Antibiotic Susceptibility Testing By a Standardized Single Disk Method. *American Journal of Clinical Pathology* **45(4)**, 493-496.
- Bhalodia NR, Shukla VJ.** 2011. Antibacterial and antifungal activities from leaf extracts of *Cassia fistula* l. An Ethnomedicinal Plant. *Journal of Advanced Pharmaceutical Technology and Research* **2(2)**, 104-109.
- Didwania N, Deepti S, Trivedi PC.** 2013. Antibacterial activity of a few medicinal plants against *Xanthomonas Campestris* Pv. *Campestris*. *International Journal of Research in Pharmaceutical Scinces* **4**, 177-82.
- Edmonds JM, Chweya JA.** 1997. Promoting the conservation and use of under-utilized and neglected crops: Black nightshades (*Solanum nigrum* L.) and related species. *International Plant Genetic Resources Institute, Rome, Italy* 1-90.
- Gopinath SM, Rakesh CK, Patil GMA, Dayananda KS.** 2012. Preliminary phytochemical evaluation of leaf extracts of *Euphorbia Hirta*, *Syzygium cumini* of Siddarabetta, Tumkur district, Karnataka. *International Journal of Pharmacy and Bio Sciences* **3**, 431-435.
- Gosh SK.** 2015. Application of synthetic chemicals in agriculture and their toxic effect on the environment. *Bulletin of Environment, Pharmacology and Life Sciences* **4(9)**, 01- 06.
- Idowu MK, Oyedele DJ, Adekunle OK, Akinremi OO, Eilers B.** 2014. Effects of planting methods and seed density on vegetable yield and nutrient composition of *Solanum macrocarpon* and *Solanum scabrum* in southwest Nigeria. *Food and Nutrition Sciences* **5**, 1185-1195.
- Jadhav MD, Kedar PD.** 2013. Antibacterial activity of medicinal plant's against *Xanthomonas Citri*. *International Journal of Advanced Biotechnology and Research* **4(3)**, 315-18.
- Mahmud S, Parvin A, Mamun ANM. Sen K.** 2013. Evaluation of antibacterial sensitivity of *Euphorbia hirta* Linn. leaf extract. *Journal of Herbal Science* **2(1)**, 2278-2257.
- Mamun MSA, Ahmed M.** 2011. "Prospect of indigenous plant extracts in tea pest management indigenous plant extracts in tea." *International Journal of Agricultural Research Inovation and Technology* **1**, 16-23.

- 
- Mbega ER, Mortensen CN, Mabagala RB, Wulff EG.** 2012. The effect of plant extracts as seed treatments to control bacterial leaf spot of tomato in Tanzania. *Journal of General Plant Pathology* **78**, 277–286.
- Musyimi DM, Chemistokj, Buyela DK.** 2012. Growth and physiological response of African nightshades (*Solanum scabrum* Mill.) to sodium chloride salinity stress, global advanced research. *Journal of Food Science and Technology* **1(4)**, 066-073.
- Muthomi J, Musyimi DM.** 2008. Growth response of African night shades (*Solanum scabrum* Mill.) seedlings to water deficit. *Journal of agricultural and biological science* **4(5)**, 24-36.
- Nashwa SMA, Abo-Elyousr KAM.** 2012. Evaluation of various plant extracts against the early blight disease of tomato plants under greenhouse and field conditions. *Plant Protection Science* **48(2)**, 74–79.
- Ogbulie JN, Ogueke CC, Okoli IC, Anyanwu BN.** 2007. Antibacterial activities and toxicological potentials of crude ethanolic extracts of *Euphorbia hirta*. *African Journal of Biotechnology* **6(13)**, 1544-1548.
- Okoli RI, Turay AA, Mensah JK, Aigbe AO.** 2009. Phytochemical and antimicrobial properties of four herbs from Edo state, Nigeria. *Report and opinion* **1(5)**, 67–73.
- Opara EU, Obani FT.** 2010. Performance of some plant extracts in the control of bacterial spot disease of solanum. *Agricultural journal* **5(2)**, 45-49.
- Shenge KC, Mabagala RB, Mortensen CN.** 2010. Current status of bacterial –speck and -spot diseases of tomato in three tomato-growing regions of tanzania. *Agricultural Extention and Rural Development* **2(5)**, 84–88.
- Sung SH, Kyoung HK, Byong TJ, Sun HC, Jae HP, Dong HK, Hyuk JK, Sang HM.** 2012. Antibacterial and antioxidant activities of tannins extracted from agricultural by-products. *Medicinal Plants Research* **6(15)**, 3072–79.
- Ukwubile CA, Agu MO, Adamu SZ, Abdulrahman A, Kagaru DC, Matthew OJ.** 2014. Pharmacognostic, toxicity, antibacterial and antihemorrhagic evaluations of *Bidens pilosa* L. (Asteraceae). *Experimental Pharmacology* **4(3)**, 162-167.
- Yamdeu GJH, Julienne N, Charles DD, Daniel F, Sandrine PT, Romain FF, Henry AZP.** 2013. Antifungal potential and phytochemical analysis of extracts from seven cameroonian plants against late blight pathogen *Phytophthora Infestans*. *Current Microbiol Application Science* **2(5)**, 140–54.