Journal of Asian Scientific Research
ISSN(e): 2223-1331
ISSN(p): 2226-5724
DOI: 10.18488/journal.2.2017.77.271.278
Vol. 7, No. 7, 271-278

URL: www.aessweb.com

ANTIMICROBIAL ACTIVITIES OF CRUDE LEAF EXTRACT OF *SOLANUM NIGRUM* ON *FUSARIUM OXYSPORUM* AND *PSEUDOMONAS SYRINGAE* CULTURES IN MASENO (KENYA)

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Article History
Received: 26 May 2017
Revised: 10 July 2017
Accepted: 18 July 2017
Published: 31 July 2017

Keywords
Phytochemicals
Antimicrobial
Leaf extract
Potentials
Medicinal
Black nightshade
Extraction
Vegetable
Ethanol extraction
Weed.

ABSTRACT

Solanum nigrum (black nightshade) is a common short lived perennial plant found in the Kenyan highlands and worldwide in distribution. It is a small highly branched plant that rarely adopt a habit of more than a foot in height. In more than 60 countries, it is considered a weed yet it is utilized as an important medicinal or food plant in barely over 30 countries. In Kenya it is consumed as a delicious vegetable and traditional medicine with therapeutic potentials in anti-ulcer, cancer among other diseases. Such medicinal potentials have created a need to better understand anti-microbial activities of this plant. Before this study, little was known about the antimicrobial potentials of its crude leaf extract against *F. oxysporum* and *P. syringae*, this study was therefore initiated to better understand these properties in varying concentrations. Plant leaves collected from Maseno University farm were shade dried and crushed to obtain a fine powder from where leaf extracts were obtained by cold method of extraction with ethanol as the solvent, at concentration ratio of 10%, 7.5%, 5%, and 2.5%. A control experiment was set with distilled water. Disc method was used for inoculation, replication of each leaf concentration was done and the data subjected to analysis of variance (ANOVA) and means separated and compared at Least Significant Difference (LSD) at $P<0.05$. *S. nigrum* extract has antimicrobial properties against the two test microorganisms. Flavanoids, terpenoids, tannin, saponin, phenol and steroid were detected in the leaf extract. The extracts showed that there is need to conduct studies in future studies leading to identification of active ingredients in this extract capable of controlling microbial growth and concentration of the extract does affect antimicrobial activities.

1. INTRODUCTION

Solanum nigrum the black nightshade is found in many countries throughout the world. It is accorded different names depending on the ethnic groups; **Kenya:** Isoyot (Kipsigis) Olmomoit (Maasai) managu (Kikuyu) namaska (Bukusu) Lisutsa (Luhya) osuga (Luo) Hindi (Makoi), and mnavu or tungujamito in Swahili; **Tanzania:** Soko, Kibondei (in Tanga Prov.); **Uganda:** Eshwiga (Kigezi Distr.), Ensugga, crevechiene in French and ballerina in Spanish.

S. nigrum has edible leaves that may vary in size and shape depending on conditions of its growth. It belongs to; family Solanaceae, genus *Solanum* and species *nigrum*. Solanaceae is a large family that consists of more than 90 genera and approximately 2000-3000 species.

S. nigrum has shown a wide spectrum of medicinal properties such as; anticancer, antioxidant, neuroprotective, antimicrobial, and anti-pyretic properties among others [1] and is one of the emerging food sources in Africa and other parts of the world [1]. *S. nigrum* leaves and berries are a potential source of inks; dyes etc. Plants are rich in proteins, fibers, vitamins and amino acids [2]. It is reported to possess various compounds such as; glycoalkaloids, glycoproteins, polysaccharides, polyphenolic together with compounds such as garlic acid, catechin, protocatechuic acid (PCA), caffeinic acid, epicatechin, rutin and naringenin that are responsible for diverse activities [3]; [4].

The widely reported toxicity of *S. nigrum* has been attributed to the alkaloid solanine, that has varying degrees of poisoning in humans, cattle, pigs, goats, ducks and chickens, with death resulting in some cases [5]; [6]. In India the plant is noted for its antiseptic and anti-dysenteric properties. In Kenya, boiled leaves of these Solanums are apparently recommended for pregnant women, while pounded leaves when soaked in water, fermented and used for the treatment of boils, ulcers and swollen glands is very effective. Unripe berries are used to treating worms while various parts of the plant are also believed to cure malaria, black fever, dysentery and urinary infection [7]. The fact that *S. nigrum* has medicinal potentials has created a need to better understand antimicrobial activities of this wonderful plant. It is therefore true to state that the health benefits *S. nigrum* to humans are many.

Before this study, little was known about antimicrobial potentials of the leaf extract against *F. oxysporum* and *P. syringae*, therefore this study was initiated not only to determine this unknown but also show if concentration variation has any effect on such activity.

2. MATERIALS AND METHODS

2.1. Field Collection of Leaves

Leaves of plants identified to be *S. nigrum* were collected from the Maseno University farm, examined and soil shaken off in the botany Laboratory. Maximum care was taken to avoid destruction (Plate-2.1).

Plate-2.1. *S. nigrum* leaves before shade drying

Plate-2.2. Extraction of *S. nigrum* leaf extract

2.2. Preparation of Leaf Extracts

The leaves of *S. nigrum* used for extraction were shade dried, after which the dried leaves were powdered and stored in a sterile bottle at room temperature [8]. Powdered dried leaves were weighed using an electronic weighing machine (plate 2.4), and ethanol used in the extraction. 100g of powdered dry leaves was extracted with 200 ml of ethanol and then concentrated using a rotary vapor pump (plate 2.3).

Plate-2.3. Rotary evaporator pump

Plate-2.4. Electronic weighing machine

2.3. Sources and Maintenance of Microorganisms

The *F. oxysporium* used in this study was obtained from existing cultures in the botany laboratory at Maseno University and its identity and systematic position confirmed by the morphological characteristics of the mycelium. While the *P. syringae* used in this exercise were obtained from infected tomatoes obtained from the Maseno University farm that showed disease symptoms. The host pathogen relationship for both microorganisms was confirmed in conformity to Koch's postulation. Though molecular identification for *P. syringae* using 16S ribosomal RNA would have provided an accurate identification when reviewed in Bergey's Manual of Systematic Bacteriology (BMSB), the identity of this prokaryote was established using morphologically using Bergey's Manual of Determinative Bacteriology (BMDB).

2.4. Culture Medium

Nutrient Agar (NA) was the culture medium for the *P. syringae* and Potato Dextrose Agar (PDA) was the culture medium for the fungus. These media after being prepared according to the manufacturer's instructions were autoclaved and dispensed at 20 ml per plate in 12 cm × 12 cm Petri dishes. Set plates were incubated overnight to ensure sterility before use.

2.5. Antimicrobial Bioassays

The extract was diluted in distilled water to make a stock solution after which different concentration of the plant extract were prepared by diluting the stock extracts in the following ratios (1:10(10%), 1:15(7.5%), 1:20(5%), 1:30(2.5%) and 0%. Each labeled medium plate was uniformly inoculated with a test organism in a form that lawn

growth can be observed. Susceptibility testing was carried out by measuring the inhibitory zone diameters on the PDA and NA medium using conventional paper by disc method. Inhibitory zone distances measured in millimeters, were rounded off to the nearest whole number (mm). The measurements were done by rulers and the treatments replicated three times. To measure the minimum inhibitory concentration (MIC) values, different concentrations of the stock, were assayed against the microbes. The minimum inhibitory concentration was defined as the lowest concentration able to inhibit any visible microbe growth [8].

2.6. Inhibition Zone Measurements

Circular discs of diameter measuring 6mm each were cut from a laboratory grade filter paper using a paper punch and each dipped in a known concentration of the extracts for about 2 minutes. The discs were then gently transferred to the center of the inoculated agar mediums. The diameter of the inhibition zone was measured in millimeters and compared with the control disc.

2.7. Screening for Phytochemicals in the Leaf Extracts

2.7.1. Flavanoids

A few drops of dilute NaOH was added to 1 ml of the leaf extract, and an intense yellow color produced in the plant extract on addition of a few drops of dilute acid The extract turns colorless as an indication of the presence of flavanoids.

2.7.2. Terpenoids

5 ml of each plant extract was mixed with 2 ml of chloroform and 3 ml of concentrated H_2SO_4 added to form a layer. A red brown precipitate coloration at the interphase formed indicated the presence of terpenoids.

2.7.3. Tannin

3 ml of the plant extract diluted with chloroform and 1ml of acetic aldehyede added, if addition of sulphuric acid by the side of the test tube produced a green color indicating the presence of tannins.

2.7.4. Saponin

The extract was diluted with 20 ml distilled water and then agitated in a graduated cylinder for 15 minutes. The formation of 1cm layer of foam indicated the presence of saponins.

2.7.5. Phenol

To 1 ml of extract, 2 ml of distilled water was added followed by few drops of 10% FeCl3. The appearance of a blue or green color indicated the presence of phenols.

2.7.6. Steroid

1 ml of extract dissolved in 10 ml chloroform and equal volume of concentrated H_2SO_4 was added by the sides of the test tube. No changes occurred indicating the absence of steroids.

3. RESULTS AND DISCUSSIONS

There was no significant difference between the means of treatments at 0% and 2.5% concentrations as indicated in table 2. There is also no significant difference for the means of the treatments at 2.5%, 5%. 5.0% and 7.5%, as clearly indicated in table 2. The f values of microorganisms and their treatments are significant ($0.01<0.05$) and ($0.01<0.05$) but the microorganism by treatment interaction is not significant ($0.42>0.05$) see table 1. The difference in means for *P. syringae* and *F. oxysporum* is significant Table 3.

3.1. Disc Diffusion Assay

The antimicrobial activities of *Solanum nigrum* leaf extract was examined against the microorganisms and their potency accessed by the presence or absence of an inhibition zone the results are shown in plates 3.1 and 3.2

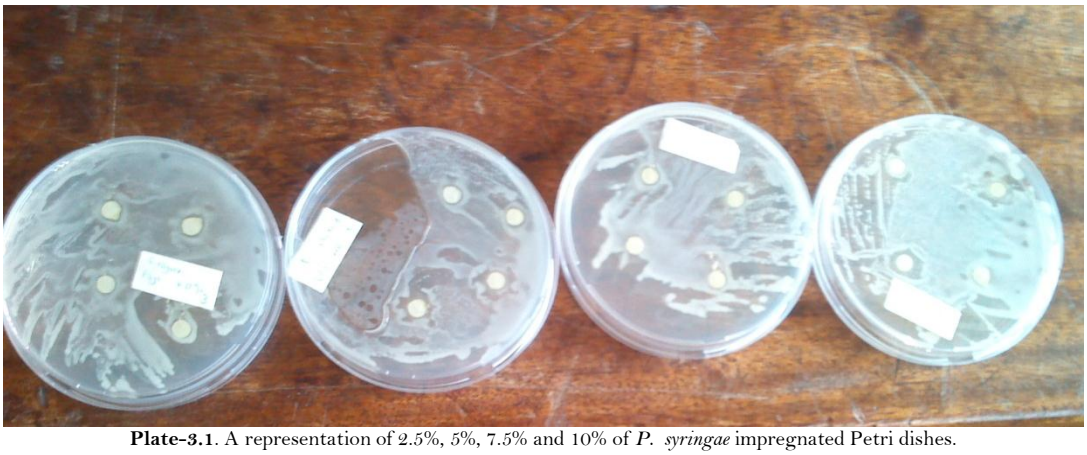

Plate-3.1. A representation of 2.5%, 5%, 7.5% and 10% of *P. syringae* impregnated Petri dishes.

Table-1. Microorganisms by treatment

Source	D.F	Sum of squares	Mean of squares	F. Value	Pr>value
Model	9	22.27	2.47	3.11	0.0166
Error	20	15.92	0.80		
Corrected total	29	38.19			
Microorganism	1	5.90	5.90	7.41	0.0131
Treatments	4	13.16	3.29	4.13	0.0134
Microorganism by treatment	4	3.22	0.81	1.01	0.4246

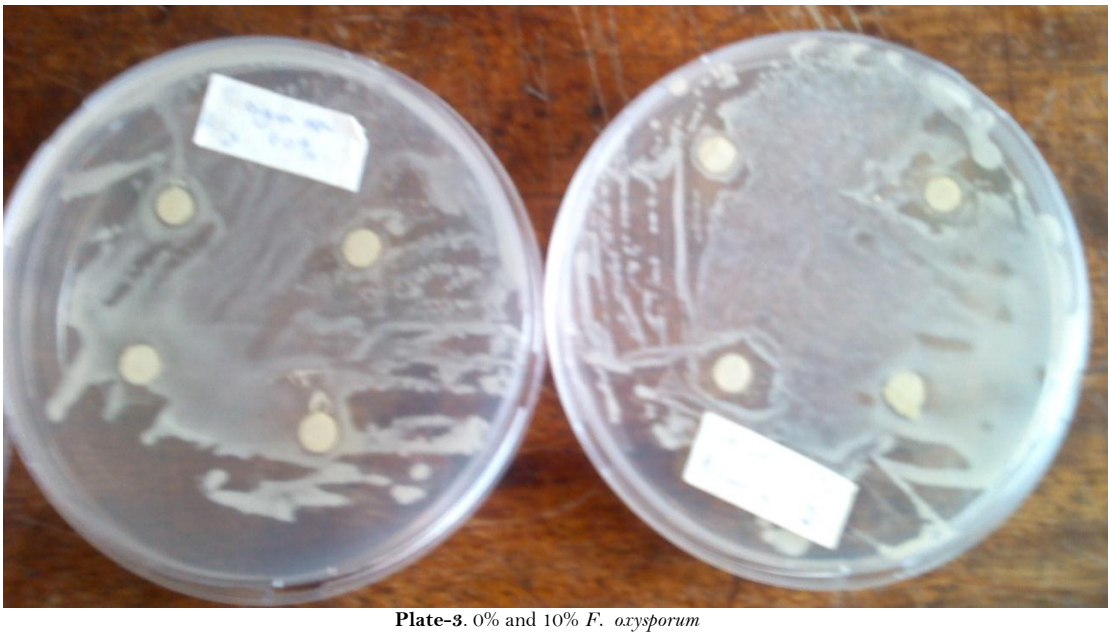

Plate-3. 0% and 10% *F. oxysporum*

Plate 4. 7.5% *P. syringae* inhibition zones

Table-2. Antimicrobial effects of different concentrations of *S. nigrum* extract on the growth of *P. syringae* and *F. oxysporum.*

Treatment	Mean
0%	6 00c
2.5%	6.77bc
5%	7.32ab
7.5%	7.12ab
10%	8.02a
L.S.D	1.07

Table-3. Difference on the means of treatments between *P. syringae* and *F. oxysporum*

microorganism	mean
P. syringae	7.49a
F. oxysporum	6.60b

3.2. Screening of Leaf Extract

The leaf extracts were tested for the presence of certain phytochemicals and the results obtained tabulated (table 4). The *S. nigrum* leaf extract screening confirms the presence of Flavanoids, Terpenoids, Tannin, Saponin, Phenol and Steroid. These are compounds that may be responsible for the antimicrobial effects of *S. nigrum.*

Table-4. Phytochemicals present or absent in leaf extracts of *S. nigrum*

Phytochemical	Leaf Extract
Alkaloids	+
Tannins	+
Steroids	_
Saponins	+
Cardiac glycosides	+
Phenols	+
Terpenoids	+
Flavonoids	+

Key: +____ phytochemicals present

-____ phytochemicals absent

Steroids were not detected from the leaf extract obtained during these studies, steroids when administered to animals exhibit marked physiological activity [9] ; [10]. Alkaloid content in *S. nigrum* could be responsible for their much acclaimed medicinal values though the exact mode of action is poorly understood. Saponins are a special class of glycosides which have soapy characteristics [11]. It has the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of forms in aqueous solution, haemolytic activity, cholesterol binding properties and bitterness [12]; [10]. These properties bestow high medicinal activities on extract from *S. nigrum*.

Tannins (commonly referred to as tannic acid) are polyphenols found present in this extract that are known antimicrobial agents; they are water soluble and found in several plant foods. They precipitate proteins. Tannins prevent the development and growth of microorganisms they precipitate protein and make nutritional protein unavailable to the microbes [12]. The growth of many fungi, yeasts, bacteria and viruses was inhibited by tannins [13].

Flavonoids is another phytochemical found in *S. nigrum* extract, they are potent water soluble antioxidants that prevent oxidant cell damage have strong anticancer activity [14].

It is clear from the screening conducted during this study, a concurrence of this study with other studies done in screened *S. nigrum* and *S. myriacanthus* for their phytochemical properties. These phytochemicals present in this plant may be responsible *S. nigrum* successfully used for the treatment of many ailments in herbal medicine. It is however, important to note that there is no certainty established in this study to confirm that the phytochemicals present in this plant are the ones directly responsible for the antimicrobial activity of *Solanum nigrum*, or their mode of action.

5. CONCLUSION AND RECOMENDATIONS

This study contributes to knowledge pertaining to antimicrobial activities of leaf extract of *S. nigrum* on *F. oxysporum* and *P. syringae* in laboratory cultures. The information obtained during this study clearly show that *S. nigrum* has antimicrobial properties, and we recommend its continued use in traditional treatment as an herbal drug and a delicious vegetable not only in Africa or Asia but the whole world. There is need to investigate this plant as a potential treatment medication because it has shown promising anti-microbial potentials in this studies. Its anti-microbial effects on other microorganisms affecting commercially important plants and crops needs to be investigated further since the results from this research only indicate that *S. nigrum* has antimicrobial effects against *P. syringae* and *F. oxysporum* at an effective concentration of 10% this creates a need to test efficacy of *S. nigrum* leaf extract in increased concentrations. Though *S. nigrum* contains many other phytochemicals, only flavanoids, terpenoids, tannin, saponin and phenols were isolated and confirmed to be present during these studies, while the presence of steroids was not confirmed in the leaf extract.

Since ethanol was the only solvent used in the extraction in this research, there is a need to conduct more research using other solvents that can easily evaporate like methanol to save on time spent evaporating it.

More research on the antimicrobial effects of the extracts of stem, fruits and roots of *S. nigrum* should be carried out on *P. syringae* and *F. oxysporum* to ascertain which extract is more potent in comparison to the leaf extract. Additionally, field studies should be carried out to see if the results from this research can be duplicated outside the laboratory

Funding: This study received no specific financial support.
Competing Interests: The authors declare that they have no competing interests.
Contributors/Acknowledgement: All authors contributed equally to the conception and design of the study.

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