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

George T. Opande¹
David M. Musyimi²
Kipruto L. Cherono³
Bonface K. Jiveri⁴
Daniel K. Buyela⁵

¹Research scientist, SPBS, Dept. of Botany, Maseno University, Kenya
²Research scientist, SPBS, Dept. of Botany, Maseno University, Kenya
³P.G. Student, SPBS, Dept. of Botany, Maseno University Kenya
⁴Technologist, SPBS, Dept. of Botany, Maseno University, Kenya
⁵Sn. Technologist, SPBS, Dept. of Botany, Maseno University, Kenya

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 antimicrobial 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 antisceptic 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).
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](image)

![Plate-2.4. Electronic weighing machine](image)

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$_2$SO$_4$ 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 1 ml of acetic aldehyde 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 1 cm 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% FeCl$_3$. 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$_2$SO$_4$ 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 \textit{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 \textit{P. syringae} impregnated Petri dishes.

<table>
<thead>
<tr>
<th>Source</th>
<th>D.F</th>
<th>Sum of squares</th>
<th>Mean of squares</th>
<th>F. Value</th>
<th>Pr&gt;value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>9</td>
<td>22.27</td>
<td>2.47</td>
<td>3.11</td>
<td>0.0166</td>
</tr>
<tr>
<td>Error</td>
<td>20</td>
<td>15.92</td>
<td>0.80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected total</td>
<td>29</td>
<td>38.19</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microorganism</td>
<td>1</td>
<td>5.90</td>
<td>5.90</td>
<td>7.41</td>
<td>0.0131</td>
</tr>
<tr>
<td>Treatments</td>
<td>4</td>
<td>13.16</td>
<td>3.29</td>
<td>4.13</td>
<td>0.0134</td>
</tr>
<tr>
<td>Microorganism by treatment</td>
<td>4</td>
<td>3.22</td>
<td>0.81</td>
<td>1.01</td>
<td>0.4246</td>
</tr>
</tbody>
</table>

Plate-3. 0% and 10% \textit{F. oxysporum}
Table 2. Antimicrobial effects of different concentrations of *S. nigrum* extract on the growth of *P. syringae* and *F. oxysporum*.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>6.00c</td>
</tr>
<tr>
<td>2.5%</td>
<td>6.77bc</td>
</tr>
<tr>
<td>5%</td>
<td>7.32ab</td>
</tr>
<tr>
<td>7.5%</td>
<td>7.12ab</td>
</tr>
<tr>
<td>10%</td>
<td>8.02a</td>
</tr>
<tr>
<td>L.S.D</td>
<td>1.07</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>microorganism</th>
<th>mean</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. syringae</em></td>
<td>7.49a</td>
</tr>
<tr>
<td><em>F. oxysporum</em></td>
<td>6.60b</td>
</tr>
</tbody>
</table>

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 Flavonoids, 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*.

<table>
<thead>
<tr>
<th>Phytochemical</th>
<th>Leaf Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Phenols</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
</tbody>
</table>

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 RECOMMENDATIONS

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 antimicrobial 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 flavonoids, 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.
REFERENCES


Views and opinions expressed in this article are the views and opinions of the author(s), Journal of Asian Scientific Research shall not be responsible or answerable for any loss, damage or liability etc. caused in relation to/ arising out of the use of the content.