Antibacterial Activity of Methanolic Crude Extract of *Solanum incanum*: Kenyan Traditional Medicinal Plant

Owino J\(^1\), Omundi J\(^3\), Njeru S. Ngoc\(^4\)

\(^1\) Nakuru War Memorial Hospital, Department of Pathology, P. O. Box, 240, Nakuru - Kenya
\(^2\) Mount Kenya University Box 342-01000 Thika
\(^3\) Maseno University, P.O. Box 333-40105, Maseno - Kenya
\(^4\) Kisii University, Department of Medicine, P. O Box 408, Kisii – Kenya

Abstract: It has been shown that 80% of the world’s population use medicinal plants either in their crude unmodified form or partially in their modified semi-synthetic form for their medical care. This study aimed at extracting the active ingredients of *Solanum incanum*, determine the antibacterial activity by measuring the zones of inhibition, MIC and MBC. The active ingredients were extracted using methanol. The antibacterial activity of plant extract was assayed in vitro by agar disc diffusion method. *S. pyogenes* and *S. aureus* showed the highest inhibition of (28 mm and 25 mm respectively) while *S. agalactiae* showed the least sensitivity of (6 mm). The plant extract showed the strongest MIC and MBC of 4.7 mg/ml for *S. pygenes*. The extract was also active against *P. aeruginosa* and *K. pneumonia* with an MIC and MBC of 18.8 mg/ml respectively. However, the extract was least active against *S. agalactiae* with an MIC of 37.5 mg/ml and an MBC of 75.0 mg/ml.

Keywords: *Solanum incanum*, Antibacterial activity, Medicinal plant, glycosidal alkaloid, Plant extract

1. Introduction

Medicinal plants are used in both developing and developed countries as a source of drugs or as a source of herbal extracts for various therapeutic purposes [1]. Use of plant derived natural compounds as part of herbal preparations and as alternative sources of medicine continue to play major role in the general wellness of the people all over the world [2]. WHO estimates that 80% of the world population presently uses herbal medicine for some aspects of primary health care. This high co-dependence on herbal drugs has been facilitated by factors such as low cost of herbal drugs endearing them with the poor mass of under developed and developing world; the ‘green’ movement in the first world that campaigns on the intrinsic safety and desirability of natural products and the individualistic philosophy of western society that encourages self-medication, with many people preferring to treat themselves with herbal remedies [3,4]. In developing countries like Kenya, there is an increasing attempt to incorporate traditional medicine in health care systems [5]. WHO in 2003 resolution (WHA56.31) recommended the inclusion of traditional healers in management of health. This move was to help countries document traditional medicines and remedies and to ensure the safety and efficacies of these remedies is established [6].

It’s obvious that at least some plants contain compounds with pharmacological activity that can be harnessed as medicinal agents [7]. Isolation of and experimentation with a single constituent provides information that can be adapted to more holistic understanding of the herbs action [8]. *Solanum incanum* belongs to the genus *Solanum* that contains aglycone, which is a steroidal alkaloid (containing nitrogen atom). *Solanum incanum* contains solanine which is a steroidal alkaloid whose pharmacological activity is against many bacterial organisms [9]. Solanine is a bitter glucosoidal alkaloid first isolated from *Solanum nigram*, and it has also been isolated from other species such as; *S. gigantium, S. incanum, S. tuberosum* and *S. aculaestrum* [9]. This alkaloid is mainly concentrated in unripe fruits and in green potatoes and disappear in ripening process [7].

2. Methods and Materials

2.1 Study design

This study was conducted using an experimental study design [10].

2.2 Study Area

The plant species *Solanum incanum* and the plant’s unripe fruits were collected from Maseno Municipality Kenya. The study was carried out at Maseno University Biomedical laboratory and at Nakuru Medical Laboratory Department.

2.3 Plant collection and identification

The part of the plant collected was the unripe fruit of *Solanum incanum* which contains the active component; glucosoidal alkaloid called Solanine, [11]. The plant was taken for botanical identification at the department of Botany in Maseno University.
2.4 Test Microorganisms

The test microorganisms were obtained from the Biomedical laboratory of Maseno University and from isolate of culture samples done at the Nakuru War Memorial Laboratory. The Microorganisms included; *S. aureus* and *K. pnemoniae* (clinical isolate) *S. pyogenes* (ATCC 20592) *S. agalactiae* (ATCC 20593) *E. feacalis* (ATCC 25922) *P. aerugonosae* (ATCC 25852)

2.5 Culture media

Mueller Hinton agar, Nutrient agar and broth, MacConkey, and Blood agar were used according to Kumar et al [12].

2.6 Extraction process

Incisions were made on the unripe fruit using a sterile scalpel blade and the green viscous fluid was squeezed out of the fruit. The juice was put into a conical flask and methanol was added. The flask was then corked using a stopper and the mixture was then allowed to stand overnight at room temperature for extraction to take place. After the overnight stay, the mixture was then filtered using Whatman filter paper. 1 ml of the filtrate was mixed with 3 drops of Wagner’s reagent prepared by dissolving 2 g iodine and 6 g potassium in 1 litre [18]. The presence of brown or reddish brown precipitate indicated the presence of Solanine (Steroidal Alkaloid).

2.7 Determination of antimicrobial activity

The antibacterial activity of the plant extract was assayed in vitro by agar disc diffusion method Jebashree et al [15]. Normal saline solution was used to dilute a 24 hour culture of the bacterial cultures to attain a 0.5 MacFarland standard. Spread plate method was used to culture the microbrial suspension in the Petri dishes. Dry sterile discs (6 mm in diameter) were soaked in the plant extract (made by dissolving 300 mg of the extracts in 1000 µl of methanol), then air dried in a clean dust free covered Petri-dish and placed on the spread plates at reasonable distances. Discs impregnated with methanol were used as negative control and two standard conventional antibiotics were used as positive controls; Ceftriaxone and Ciprofloxazin. The plates were then incubated at 37 °C for 24 hours [16].

The MIC was determined using the tube dilution broth method. This was done when the plant extract showed strong antibacterial activity by the disc diffusion method. The tubes were filled with 1 ml of nutrient broth. The extract was prepared by taking 300 mg of the plant extract and mixing it with 1000 µl of DMSO (0.01%) for complete dissolution of the extract [17]. Then 1 ml of the plant extract suspension was dispensed into the first tube before serial dilutions were done by transferring 1 ml of the nutrient broth containing the extract from the first tube to the second tube, the procedure was repeated until the last tube (tenth tube). 10µl of the test isolate was then dispensed into each tube. One tube (without extract or drug) was used as a negative control, whereas two tubes with antibiotics Ceftriaxone (for Gram positive) and Ciprofloxazin (for Gram negative) were used as positive controls.

The tubes were then incubated at 37 °C for 24 hours. The MIC values were determined as the lowest concentrations of the extract capable of inhibiting bacterial growth. The MBC was determined by sub-culturing the tubes which did not show growth on nutrient broth. The lowest concentration of the plant extract that did not yield any colony on the solid media after sub culturing and incubating for 24 hours was taken as the MBC [2].

<table>
<thead>
<tr>
<th>Plant specimen</th>
<th>S. aureus</th>
<th>S. pyogenes</th>
<th>S. agalactiae</th>
<th>E. feacalis</th>
<th>P. aerugonosae</th>
<th>K. pnemoniae</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. incanum</td>
<td>4.7</td>
<td>18.8</td>
<td>4.7</td>
<td>4.7</td>
<td>37.5</td>
<td>75.0</td>
</tr>
<tr>
<td>Positive control</td>
<td>4.7</td>
<td>4.7</td>
<td>4.7</td>
<td>4.7</td>
<td>4.7</td>
<td>4.7</td>
</tr>
<tr>
<td>Negative control</td>
<td>There was growth in all the tubes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

MIC: Minimum inhibitory concentration; MBC: Minimum bactericidal activity.

2.8 Determination of Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC)

The MIC was determined using the tube dilution broth method. This was done when the plant extract showed strong antibacterial activity by the disc diffusion method. The tubes were filled with 1 ml of nutrient broth. The extract was prepared by taking 300 mg of the plant extract and mixing it with 1000 µl of DMSO (0.01%) for complete dissolution of the extract [17]. Then 1 ml of the plant extract suspension was dispensed into the first tube before serial dilutions were done by transferring 1 ml of the nutrient broth containing the extract from the first tube to the second tube, the procedure was repeated until the last tube (tenth tube). 10µl of the test isolate was then dispensed into each tube. One tube (without extract or drug) was used as a negative control, whereas two tubes with antibiotics Ceftriaxone (for Gram positive) and Ciprofloxazin (for Gram negative) were used as positive controls.

The MIC values were determined as the lowest concentrations of the extract capable of inhibiting bacterial growth. The MBC was determined by sub-culturing the tubes which did not show growth on nutrient broth. The lowest concentration of the plant extract that did not yield any colony on the solid media after sub culturing and incubating for 24 hours was taken as the MBC [2].

### Table 1: Minimum inhibitory concentrations and minimum bactericidal concentrations (mg/ml) of Solanum incanum against the six bacterial isolates

<table>
<thead>
<tr>
<th>Plant specimen</th>
<th>S. aureus</th>
<th>S. pyogenes</th>
<th>S. agalactiae</th>
<th>E. feacalis</th>
<th>P. aerugonosae</th>
<th>K. pnemoniae</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. incanum</td>
<td>4.7</td>
<td>18.8</td>
<td>4.7</td>
<td>4.7</td>
<td>37.5</td>
<td>75.0</td>
</tr>
<tr>
<td>Positive control</td>
<td>4.7</td>
<td>4.7</td>
<td>4.7</td>
<td>4.7</td>
<td>4.7</td>
<td>4.7</td>
</tr>
<tr>
<td>Negative control</td>
<td>There was growth in all the tubes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

MIC: Minimum inhibitory concentration; MBC: Minimum bactericidal activity.

2.9 Testing for Solanine (Steroidal Alkaloid)

Wagner’s Method

3.1 Antimicrobial Activity

The results were read after 24 hours of aerobic incubation. The zones of inhibition were measured as shown in the Fig 1. The plant extract showed varying degrees of antibacterial activity against the test organisms (Fig 1). Larger zones of inhibition were seen on the plate with *Streptococcus pyogenes* and *Staphylococcus aureus* (zones of inhibition of 28 mm and 25 mm respectively). The organism that showed the least zone of inhibition was *Streptococcus agalactiae* (7 mm).
Ceftriaxone (PC)

Zones of inhibition (mm)

10
30
0
10
20
30
40
50
60
70
80
90
100

S. aureus
Ceftriaxone (PC)
P. aeruginosa
Ciprofloxacin (PC)
S. pyogenes
Ceftriaxone (PC)
P. aerogenosae
Ciprofloxacin (PC)
K. pneumonia
Ciprofloxacin (PC)
NC

**

5. Conclusion

The results of the study revealed that the plant contains potential pharmacologically active substances with antibacterial properties. It also showed that there is a possibility of getting effective compounds from natural sources, which can be of value in the fight against bacterial infections. The study also provides support for the use of medicinal plants in the management of bacterial diseases.

6. Acknowledgement

We would like to express our gratitude to Dr. Orina, staff of Mount Kenya University at the School of Health Sciences Thika, Chief Technologist Biomedical Sciences of Maseno University for the support and Nakuru War Memorial Hospital bacteriology department for their support.

Reference


Author Profile

Owino, J is Laboratory manager and medical scientist at Nakuru War memorial Hospital and a Masters student at Mount Kenya University Thika Campus School of post graduate studies.

Njeru S. Ngoci is a Lecturer of Medical Biochemistry and Biomedical Sciences with Kisii University, School of Health Sciences-Kenya. Doctoral Student in Biochemistry (LGSA-FLI and the Friedrich Schiller University)

Omundi J is laboratory scientist and a Masters student at Maseno University-Kenya