

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

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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. *Streptococcus pyogenes* and *Staphylococcus aureus* showed the highest inhibition of (28 mm and 25 mm respectively) while *Streptococcus agalactiae* showed the least sensitivity of (6 mm). The plant extract showed the strongest MIC and MBC of 4.7 mg/ml for *S. pyogenes*. 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 gluco-alkaloid first isolated from *Solanum nigrum*, and it has also been isolated from other species such as; *S. gigantium*, *S. incanum*, *S. tuberosum* and *S. aculeastrum* [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; glycosidal 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. pneumoniae* (clinical isolate) *S. pyogenes* (ATCC 20592) *S. agalactiae* (ATCC 20593) *E. faecalis* (ATCC 25922) *P. aeruginosae* (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 shaken thoroughly using a magnetic stirrer [13]. 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 and the filtrate placed in rotary evaporator (Laborota 4000, SN 090816862, Germany) with the water bath set at 50 °C to evaporate the methanol. After evaporation a dark gelatinous material was left behind and this was used for antibacterial testing of the organisms it was done according to procedure described by [14].

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]. Mueller Hinton agar was used for culturing the bacteria. 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 microbial

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 Ciprofloxacin. The plates were then incubated at 37 °C for 24 hours [16].

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

Plant specimen	<i>S. aureus</i>		<i>S. pyogenes</i>		<i>S. agalactiae</i>		<i>E. faecalis</i>		<i>P. aeruginosae</i>		<i>K. pneumoniae</i>	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
<i>S. incanum</i>	4.7	18.8	4.7	4.7	37.5	75.0	18.8	37.5	18.8	18.8	18.8	18.8
Positive control	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7
Negative control	There was growth in all the tubes											

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

2.9 Testing for Solanine (Steroidal Alkaloid)

Wagner's Method

The presence of Solanine was determined by dissolving 200 mg of the plant extract in 10 ml methanol then allowed to stand for 4 hours followed by filtration 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).

3. Results

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).

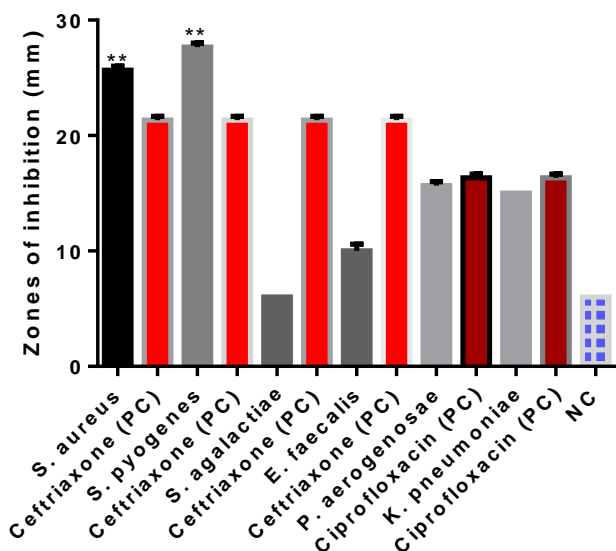


Figure 1: Antibacterial activity of *Solanum incanum*. PC: positive control; NC: negative control

The *solanum incanum* extract showed the strongest MIC and MBC of 4.7 mg/ml for *S. pyogenes*. The extract was also active against *P. aeruginosa* and *K. pneumoniae* with an MIC and MBC of 18.8 mg/ml respectively. However, the extract had the least activity against *S. agalactiae* with an MIC of 37.5 mg/ml and an MBC of 75.0 mg/ml.

4. Discussion

The antibacterial activity of *Solanum incanum* extract against the six bacterial species showed that the plant contains pharmacologically active components. The presence of these bioactive compounds in crude extracts is known to confer antibacterial activity against disease-causing microorganisms. The plant exhibited antibacterial activity against four test organisms. The results were comparable to those of the standard drugs (Ceftriaxone and Ciprofloxacin). Among the six bacterial strains tested for antibacterial activity, *S. pyogenes* and *S. aureus* were the most susceptible with inhibition zone of 28 and 25 mm respectively and their activity was better than that of standard drugs at a $P < 0.05$, while *S. agalactiae* was least susceptible organism to the plant extract with inhibition zone of 6 mm. The extract of *Solanum incanum* was found to be bacteriostatic and bacteriocidal against *S. pyogenes*, *S. aureus*, *K. pneumoniae* and *P. aeruginosa*. The strongest antibacterial activity was shown against *S. pyogenes* (MIC and MBC of 4.69 mg/ml) while the least antibacterial activity was shown in *S. agalactiae* (MIC of 37.50 mg/ml and MBC of 75.00 mg/ml).

Generally, activity against Gram positive bacteria was higher than Gram negative strains in most cases. This is in concurs with previous studies that plant extracts are more active against Gram positive bacteria than Gram negative bacteria. The higher sensitivity of Gram-positive bacteria could be attributed to their cell wall architecture which has outer peptidoglycan layer which is not an effective permeability barrier as compared to the outer phospholipid membranes of

Gram-negative bacteria [13,19,20]. Difference in sensibility was also evidence among tested strains in both crude and fraction extracts. This could be due to genetic differences between different strains. This proves the necessity of antibiogram prior to prescription as a precautionary measure in mitigating drug resistance development [21]

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.

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