



Original Research Article

Antimicrobial activity and phytochemical screening of *Solanum incanum* fruit extract against clinical samples of *Staphylococcus aureus* collecting from Nakuru Provincial General Hospital Laboratory, Kenya

Received 2 June, 2017

Revised 18 July, 2017

Accepted 21 July, 2017

Published 18 September, 2017

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The use of *Solanum incanum* for thousands of years to treat various diseases such as; sore throat, stomach ache, head-ache, painful menstruation, liver pain, pneumonia and rheumatism throughout tropical Africa has been great. This has led to great efforts towards validating the claimed medicinal value as demonstrated in this present study. In this current study we evaluated the percentage yield, phytochemical composition and use of methanolic and aqueous extract of *S. incanum* as remedy in management of *S. aureus* infections of patients attending Nakuru provincial General Hospital. In the current study qualitative phytochemical analysis by modified methods of Trease and Evans were used. The zones of inhibition and minimum inhibitory concentration activity against *S. aureus* and obtained by use of Agar well diffusion assay. The aqueous extract of *S. incanum* had a higher yield of 5.3% as compared to the methanolic extract that had a yield of 4.6%. From phytochemical analysis the basic alkaloids, were strongly present, followed by saponins and Steroid glycosides in parenthesis. While the terpenoids, flavonoids and cardiac glycosides were weakly present. The zones of inhibition of the methanolic and aqueous extract ranged from 6-12mm and 6-9.25mm respectively at a concentration of 0.01 to 100mg/ml with the positive control having a diameter of 12.35mm against the *S. aureus*. The MIC of *S. incanum* methanolic and aqueous fruit extract was found to be 0.1mg/ml and 1mg/ml respectively. The current study support the continuous use of *S. incanum* in management of wound infection in Kenyan communities caused by *S. aureus* infections.

Keywords: *Solanum incanum*, *Staphylococcus aureus*, Methanolic extract, Aqueous extract, phytochemical composition, Zones of inhibition, Minimum inhibitory concentration

INTRODUCTION

Due to the current emergence of resistant microbes to most antimicrobial agents, the search for alternative remedies is on call. This has led to proportional increase in demand for herbal products both locally and internationally as alternative source to orthodox/conventional remedies. The demand has been due to antimicrobial resistance microorganisms (AMR), poverty, increasing awareness to herbal products, high cost of modern medicine and limited access for trained doctors (Kumara et al., 2012). Thus, there is

need to encourage the use of medicinal plants as potential source of new drugs.

According to world health organization greater than 80% of the total world's population depends on the traditional medicine in order to satisfy their primary health care needs (Swamy and Sinniah, 2015). *Solanum* species are the most potent plants against pathogenic microorganisms. *Solanum incanum* L. is one of the important traditional medicinal plants which belong to

Solanaceae family. The plant is said to contain *solanine* which is a steroidal alkaloid whose pharmacological activity is against many bacterial organisms (Owini et al., 2015). *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. tuberosum* and *S. aculaestrum*. According to John Britto and Senthilkumar, 2001 and Pavitra et al., 2012, antimicrobial activity and phytochemical analysis of *Solanum incanum* was studied. However the antimicrobial activity and phytochemical screening of *Solanum incanum* methanolic and aqueous fruit extracts against *Staphylococcus aureus* species has not been studied.

Staphylococcus aureus is notorious for its ability to become resistant to antibiotics. Infections caused by antibiotic-resistant strains often occur in epidemic waves initiated by one or a few successful clones, methicillin resistant *staphylococcus aureus* (MRSA) is prominently featured during these epidemics hence there is need to find a promising herbal remedy such as *S. incanum* fruit extract against *S. aureus* resistant strains. In this current study, we evaluated the percentage yield, phytochemical composition and use of methanolic and aqueous extracts of *S. incanum* in management of *S. aureus* clinical samples of patients attending Nakuru provincial General Hospital.

MATERIALS AND METHODS

This study was carried out at the Department of Medical laboratory Sciences, Microbiology section and Botany department, GoK Laboratory, JKUAT in November, 2016-February, 2017.

Identification and Collection of *S. incanum* fruit

The collected *S. incanum* plant sample was taken to JKUAT Herbarium, Botany Department and was identified and authenticated by Mr. Kamau (Botanist) with voucher sample specimen number deposited. Ripe fruits of *S. incanum* were collected from JKUAT farm and then carried in a polythene bag to GoK Laboratory-JKUAT. On arrival to the laboratory, the fruits were washed with clean running tap water to free them from dust and debris. The fruits were wiped and dried using adsorbent tissue paper which were later sliced and chopped into small pieces using a sharp scalpel blade and shade dried in a greenhouse until it acquired a dry constant weight. The dried fruit was ground coarsely into fine powder form using locally made electric grinder with the weight of the powder determined and recorded. The pre-weighed ground fruit samples were labelled and stored in a closed cupboard awaiting bioassays.

Aqueous extraction

The aqueous extraction was carried out using method described by Asuzu and Njoku (1996). Fifty grams (50g) of

the fruit powder was extracted by macerating in hot distilled water for 24hrs. The mixture was thereafter, filtered using what man no:1 filter paper. The collected filtrate was concentrated in an oven at 60 °C into a slurry. The slurry was weighed and percentage yield determined and stored in a refrigerator at 4°C awaiting the bioassays.

Methanol extraction

Extraction was done using a method described by Harbone (1998). Fifty grams (50g) of fruit powder was extracted using 250ml of absolute methanol for 72hrs with twice daily through shaking. Thereafter, the mixture was filtered and the filtrate concentrated using a rotary evaporator (Rotavapor R-200, BOCHI) set at 50-60°C. The concentrate, was fully dried in an oven set at 35-40°C. The dried concentrate was weighed, percentage yield determined and scooped into a well labeled bijoux bottle and stored in a refrigerator at 4°C awaiting for bioassays.

Percentage yield determination of the extracts

This percentage yield was determined out for both the methanolic and aqueous extract as described by Ndukui et al. (2013b). Whereas;
Percentage yield = $W_2/W_1 \times 100$

Test micro-organism

The test micro-organism used was clinical isolates obtained from the Microbiology Laboratory Department of Nakuru Provincial General Hospital, Kenya. *S. aureus* was the only bacterial micro-organism under study. The isolated *S. aureus* was identified using the schemes of Cheesbrough (2006) and then sub-cultured into nutrient agar slants for further use.

Screening for various phytochemical groups

Identification of phytochemical in the fruit extracts was determined by using the following tests *Trease and Evans' pharmacognosy* (Evans, 2009). Chemical tests were carried out on the extracts of *S. incanum* using standard procedure, to identify the constituents described below;

Alkaloids

Alkaloids were tested for by mixing 50g of the powder with 250ml of 1% sulphuric acid. It was allowed to stand and then filtered. 10mls of the filtrate was shaken and added to Meyer's reagent. Formation of a whitish yellow precipitate indicated presence of alkaloids.

Flavonoids

A few drops of dilute HCL and a small piece of magnesium was added into a test tube containing 2ml extract and boiled for a few minutes. In the presence of flavonoids,

reddish pink or dirty brown color was produced.

Saponins

2ml of the crude extract was dissolved in 50ml of tap water and vigorously shaken. A honey comb formation persisting for few minutes indicated the presence of saponins.

Cardiac Glycosides

2ml of the crude extract was dissolved in 1ml of distilled water and aqueous sodium hydroxide solution added. Formation of a yellow color indicated the presence of glycosides.

Terpenoids

2ml of the crude extract was mixed in 2ml of chloroform; 3ml of concentrated sulphuric acid was added carefully to form a layer. Formation of a reddish brown color at the interface indicated the presence of terpenoids.

Steroids

2ml of the crude extract was placed into a test tube in which 0.5ml sulphuric acid, acetic anhydride and chloroform in similar amounts was added. A green coloration indicated the presence of steroids.

Antimicrobial activity

Antimicrobial assay of the extracts

The antimicrobial activity assay was done using the method described by Idu and Igeleke (2012). The plates were prepared by pouring nutrient agar media into sterile petri plates and allowed to set. The micro-organism was inoculated on the plates using a swab stick. A 4mm cork borer was used to bore holes on the medium, and the bottom of each hole was sealed with a drop of molten agar to avoid seepage of the extract. Four holes were made on each petri plate, adequately spaced out. About 0.2 μ l of the different concentrations (100, 10, 1, 0.1, and 0.01) mg/ml were introduced into the well. The petri plates were incubated at 37°C for 24hr, after which the zones were measured using a meter rule.

Determination of minimum inhibitory concentration (MIC)

The MIC of the crude extracts was determined by broth dilution method. Test tubes were labeled and 10ml of nutrient broth was introduced into each test tube, 0.5ml of bacteria suspension (1.0×10^6) was inoculated. This was followed by the adding 1ml of different concentrations (100, 10, 1, 0.1, and 0.01) mg/ml of the extracts to the sterile nutrient broth test tubes (Andrews, 2001).

The final volumes in all the test tubes were adjusted up to

10ml with Distilled water. The mixtures in all the test tubes were properly mixed before incubation at 37°C for 24hr. Observation for turbidity was carried out. The MIC was determined by the lowest concentration of the extract that prevented visible growth (Andrews, 2001).

Data analysis

Data was collected, summarized and stored in excel spreadsheets. Thereafter, exported to version 12.0 programme for inferential statistical analysis. ANOVA was used to analyze data by comparing the zone of inhibition of aqueous and methanolic extracts of *Solanum incanum* fruit. Data was presented as means and standard error of means in tables or graphs. $P \leq 0.05$ at 95% confidence interval was considered statistically significant.

Results

Percentage yield

The percentage yields of both aqueous and methanolic extracts were presented as W/W%. The aqueous extract of *S. incanum* had the highest of 5.3% as compared to the methanolic extract that had a yield of 4.6% as shown in Table 1.

Phytochemical screening of the fruit extracts

Various phytochemicals were screened for their presence i.e. alkaloids, flavonoids, saponins, cardiac glycosides, terpenoids and steroids. The Alkaloids were found to be strongly present, followed by saponins and Steroid glycosides. While the terpenoids, flavonoids and cardiac glycosides were weakly present respectively as in Table 2.

Antimicrobial assay

The 100mg/ml of the methanolic extract generated the highest zone of inhibition as compared to positive control. However, there was concentration dependent activity with the extract, as depicted in Table 3 below.

The aqueous extract showed a dose dependent activity, although it was less effective as compared to the positive control as shown in Table 4 below.

The zones of inhibition of methanolic and aqueous fruit extracts of *S. incanum* are represented in the graphs below (Figure 2).

DISCUSSION

Percentage yield

The need to determine the percentage yield of plant extracts is of great significance towards drugs discovery

Table 1. Percentage yield determined of the plant extracts

Aqueous extract	Methanolic extract
$\frac{1.6g * 100}{30g} = 5.3 \text{ w/w}\%$	$\frac{2.3g * 100}{50g} = 4.6 \text{ w/w}\%$

Key: W/W-weight over weight; % - percentage

Table 2. Phytochemical screening of the methanolic fruit extract of the *S. incanum*

Test	Inference	Color change observed
Alkaloids	+++	Whitish Yellow ppt
Flavonoids	+	Reddish-brown
Saponins	++	Brown
Cardiac glycosides	+	Yellow
Terpenoids	+	Reddish- brown
Steroids	++	Green

Key: +-Weakly Present; ++-Moderately present; +++-Strongly present

Table 3. Halos of inhibition of methanolic fruit extract (mm)

Concentration of methanolic extract (mg/ml)	Halos of inhibition (diameter) in mm \pm SEM.
100	12.00 \pm 0.5
10	10.50 \pm 0.5
1	8.75 \pm 0.25
0.1	6.75 \pm 0.25
0.01	6.0 \pm 0.0
Positive control	12.35 \pm 0.05
Normal control	6.0 \pm 0.0

Key: Positive control- Doxycycline; Normal control – dH₂O; mm- millimeters; SEM –Standard Error of the mean**Table 4.** Zones of inhibition of aqueous fruit extract (mm)

Concentration of aqueous extract(mg/ml)	Zones of inhibition (diameter) in mm \pm SEM.
100	9.25 \pm 0.25
10	6.75 \pm 0.25
1	6.75 \pm 0.25
0.1	6.1 \pm 0.25
0.01	6.0 \pm 0.0
Positive control	12.35 \pm 0.05
Normal control	6.0 \pm 0.0

Key: Positive control- Doxycycline; Normal control – dH₂O; mm- millimeters; SEM –Standard Error of the mean.

and development. This helps one to estimate the amount of plant materials required to generate a required amount of active compound. In this study the aqueous extract was found to have a better percentage yield as compared to the methanolic extract. This could be due to presence of glucides, starch and fibers present in aqueous extracts. According to Idris and Mohammed (2015), fresh aerial parts of *Artemisa afra* were extracted by both aqueous and methanol solvents. In this study the aqueous extracted powder provided a higher yield of 9.5% w/w while the crude methanolic extract had a yield of 7.8% w/w. This

agrees with the present study which shows that the *S. incanum* fruit extract extracted using distilled water had a higher percentage yield as compared to the methanol extract.

Phytochemicals present in *S. incanum* methanolic and aqueous extracts

S. incanum possesses numerous biologically active compounds which could serve as potential source of natural drugs in herbal medicine (Ghosal and Mandal,



Figure 1: Color change to indicate various Phytochemicals tested in the methanolic plant extracts

2012). It was reported that most of the plants of *Solaniceae* contain alkaloids, tannins, steroids, saponins, as well as reducing sugars (Tripathi, 2005). In this study the qualitative phytochemical tests confirmed the presence of the aforementioned phyto-active agents. The methanolic fruit extract was identified to have strong presence of alkaloids and moderate presence of saponins and steroids. However, the flavonoids, cardiac glycosides, terpenoids and steroids were weakly present as illustrated in Figure (1) and Table (1). John (2001), shows that *S. incanum* leaf extracts contain the following phytochemicals, alkaloids, flavonoids, terpenoids, saponins, tannins and cardiac glycosides, which corroborates with our current results. The presence of alkaloids in significant quantities is used as antimalarial, analgesics and stimulants. The presence of glycosides moieties like saponins, cardiac glycosides and flavonoids are known to inhibit tumor growth and serve also to protect against gastrointestinal infections are of pharmacological importance and give evidence to the use of the plant in ethno medicine. These compounds have potentially significant application against human pathogens including those that cause enteric infection Papadopoulou et al. (2005). This shows that different parts of the *S. incanum* plant has almost similar phytochemicals present which are attributed to its antimicrobial activity and hence there is need for more studies to be done on the roots and leaf extracts of *S. incanum* to evaluate their antimicrobial and antifungal properties.

In recent years, secondary plant metabolites (Phytochemicals), with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents (Saxena et al., 2013). Thus it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for treatment of the bacterial infections (Saxena et al., 2013). Phytochemicals have been extensively found at different levels in many medicinal plants which have exhibited antioxidant (Nam and Kang, 2004) as well

as antimicrobial activity (Chan et al., 2008).

Antimicrobial activity of *S. incanum* methanolic and aqueous extract

S. incanum fruit extracts produced auspicious results with the ability to inhibit the growth of *S. aureus* isolates. A clear zone of inhibition was noted around the agar well due to diffusion of drug and to the growing bacteria. The diameter of the zones of the zones of inhibition denotes the relative susceptibility of the test micro-organism to a particular antimicrobial agent. The term susceptible implies that an infection caused by strain tested may be expected to respond favorably to the indicated antimicrobial agent for that type of infection and pathogen. The results of the antimicrobial activity of methanolic and aqueous extracts of *S. incanum* fruit was presented in Table (3) and (4), Figure (2) and (3). These zones of inhibition produced by the different concentration of methanolic extract against *S. aureus* ranged from 6.0mm to 12.5mm and for the aqueous extract which had much smaller zones of inhibition ranged from 6.0mm to 9.0mm. According to Indhumathi and Mohandass (2014), *S. incanum* ethanolic fruit extracts was found to have antimicrobial activity against other human pathogens and apart from *S. aureus*. The zones of inhibition of the tested micro-organisms were as follows; *S. aureus* (26mm), *Bacillus subtilis* (12mm), *Pseudomonas aeruginosa* (10mm), *Salmonella paratyphi* (25mm) and *Vibrio cholera* (17mm). The zones of inhibition due to the crude ethanolic extract ranged from 10-26mm, at a concentration of 100ug/disc. The crude ethanolic extract showed the highest antibacterial activity (26mm) against *S. aureus* and also showed good antibacterial activity (10-25mm) against all pathogenic bacteria. This almost agrees with the present study in which the *S. incanum* methanolic fruit extract has a zone of inhibition of 13mm and 9mm in aqueous extract at a concentration of 100mg/ml. The

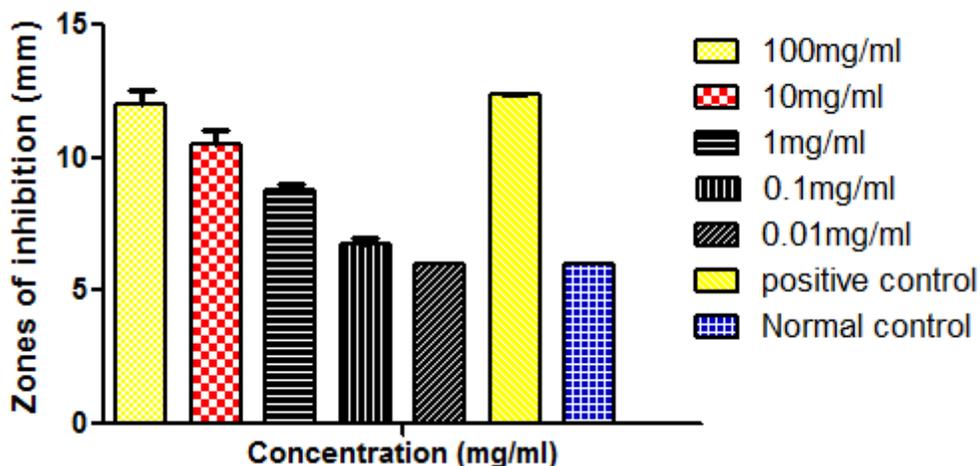


Figure 2: Zones of inhibition of methanolic fruits extract of *S. incanum*

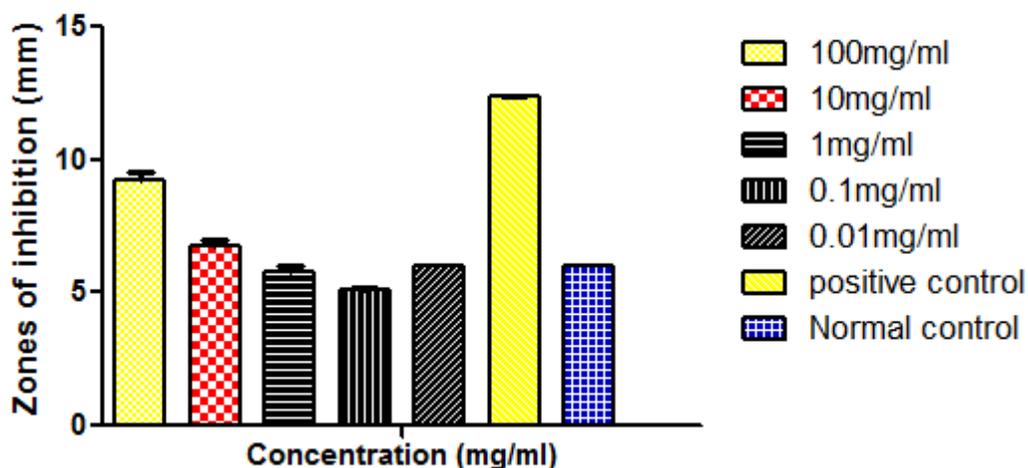


Figure 3: Zones of inhibition of aqueous fruits extract of *S. incanum* Minimum inhibitory concentration

profounding, activity of the methanolic extract as compared to aqueous extract can be attributed to the ability of the methanol solvent to extract both lipophilic and lipophobic phytoactive agents. Therefore, these lipophilic phytoactive agents were able to penetrate the peptidoglycan layer of the gram positive *S. aureus* micro-organisms which is not an effective barrier for both extracts (Parekh and Chanda 2007; Trombetta et al., 2005).

The presence of antimicrobial substances in the higher plants is well established since it has provided a source of inspiration for novel drug compounds as plants derived

medicines has made significant contribution towards human health (Ghosal and Mandal, 2012). Successive extraction and isolation of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. The traditional healers uses primarily aqueous (water solvent) but in this study it is evident that the methanolic extract of *S. incanum* fruit provided more consistent antibacterial activity. The antimicrobial activity of *S. incanum* fruit may be attributed to the various phytochemical constituents present in the crude extract. The purified components may have even

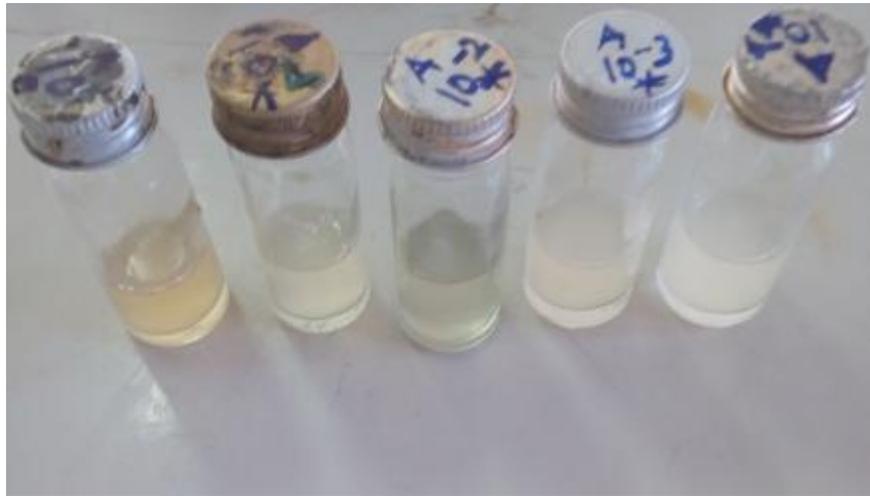


Figure 4: Minimum inhibitory concentration of the aqueous fruit extract

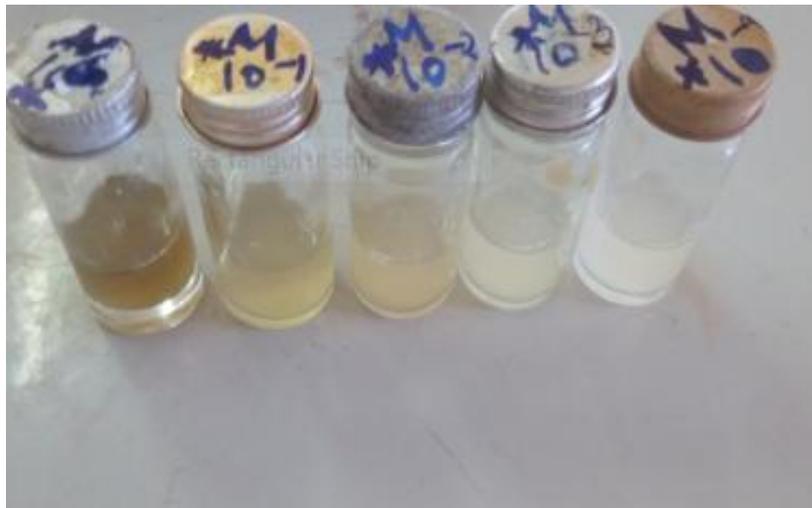


Figure 4: Minimum inhibitory concentration of methanolic fruit extract

more potency with respect to inhibition of microbes (Manske, 1960).

Minimum Inhibitory Concentration (MIC) of *S. incanum* methanolic and aqueous extract

Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial drug that will inhibit the visible growth of a micro-organism after overnight incubation. In this study MIC of *S. incanum* fruit methanolic and aqueous extracts were determined via serial dilution technique. The results of Minimum inhibitory concentration (MIC) of *S. incanum* fruit methanolic and aqueous extracts against *S. aureus* were 0.1mg/ml in methanolic extract and 1mg/ml in aqueous extract as shown in Table (4), Figure (4) and (5). According to Indhumathi and Mohandass (2014), they ascertained that the Minimum Inhibitory

Concentrations (MIC) of *S. incanum* ethanolic fruit extract against *S. aureus*, *Pseudomonas aeruginosa*, *Salmonella paratyphi* and *Vibrio cholera* were found to be 500ug/ml. But Minimum Inhibitory Concentrations (MIC) of *S. incanum* against *Bacillus subtilis* was found to 250ug/ml which falls within our ranges of study. Minimum Inhibition Concentration is very essential while evaluating the antimicrobial activity of plant extracts as a guide towards predicting the efficacy of a promising product. If pharmacokinetic and Pharmacodynamics (PKPD) principles are met by careful selection of a specific promising antimicrobial extracts while given at an appropriate dosage, this will relate to clinical cure, eradication of carrier status of a specific micro-organism, as well as prevention of selection of resistance. Interpretation of the MIC gives an understanding of the mode of activity of a given plant extract (Chan et al., 2008). Therefore, the

present study offers scientific bases for continuous use of *S. incanum* in management of *S. aureus* wound infections.

Conclusion

In the present study the methanolic and aqueous plant extracts of *S. incanum* revealed the strong presence of basic alkaloids whose presence is associated with the antimicrobial activities of the plant against *S. aureus*. It was also evident that plant methanolic extracts showed greater activity as compared to aqueous extracts. This supports the continuous use of *S. incanum* in management of wound infection in Kenyan communities, caused by *S. aureus*. Consequently, there is dire need to scientifically validate the claimed medical value of plants commonly used in local communities.

Ethical approval

The permission and approval was obtained from Jomo Kenyatta University of Agriculture and Technology Ethics Review Committee and the Medical Laboratory Sciences student review committee.

Acknowledgement

We do appreciate the technical support of Mr. Muthanga (GoK Lab) and Ms. Millicent Ogutu (MLS-Microbiology section) for their technical support and guidance during the course of this work. We also thank the Medical Laboratory Sciences (MLS) and Botany department staffs for the bench space to do this work and providing the necessary material for this work.

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