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ANTIBACTERIAL ACTIVITY OF CRUDE EXTRACTS OF *Solanum incanum* AGAINST *Escherichia coli* and *Staphylococcus aureus*

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ABSTRACT

Human pathogenic microorganisms have developed resistance in response to indiscriminative use of commercial drugs. Plants produce many secondary metabolites with microbiocidal activity hence their use in traditional medicine. Herbalists in Kenya use medicinal plants including *Solanum incanum* in treating microbial infections. Though *S. incanum* has been used to treat different diseases in humans and animals, there is little information on antimicrobial activities of its extracts against *Escherichia coli* and *Staphylococcus aureu*s. In this study, phytochemical analysis and antibacterial activity of *solanum incanum* leaves, roots and seeds extracts were determined. Ethanolic and aqueous extracts of leaf, root and seed of concentrations 25, 50, 75 and 100, and amoxicillin 25 mg/ml (control) with three replications were used for antibacterial analysis by the agar-well diffusion method. The results were subjected to analysis of variance at $P < 0.05$. Phytochemical screening revealed the presence of alkaloids, flavonoids, glycosides, saponins, steroids and tannins. *Solanum incanum* exhibited significant antibacterial effect against the two test bacteria. Ethanol extracts were more active than extracts against the bacteria. Ethanol extracts at 100% inhibited growth of *Staphylococcus aureus* more than the *Escherichia coli*. The zones of inhibition for *Staphylococcus aureus* were 35.0±0.6 mm, 30.94±0.3 mm and 30.14±0.64mm for seed, root and leaves respectively.On the other hand, the zones of inhibition for *Escherichia coli*at 100% ethanol were 27.20±0.06, 23.14±0.12 and 21.0±0.4 seed, root and leaves respectively.The results validate the use of these plants in ethnomedicine and potential of this plant in treating infections caused by the two bacteria.

Keywords: Bacteria, growth inhibition, extracts, phytochemicals, solanum

INTRODUCTION

Plants generally produce secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs (**Bobbarala *et al.*, 2009**). According to World Health Organization (2001) medicinal plants are sources of a variety of drugs. Between 65% and 80 % of the people in developing countries rely primarily on medicinal plants for their basic health care (**Mwaura *et al*., 2020; Wood-Sheldon *et al*., 1997**). Phytomedicines continue to occupy an important position in the treatment of diseases worldwide (**Tyagi and Prasad, 2015**). Plants offer the local population with immediate and accessible therapeutic products (**Mwaura *et al.*, 2020; Bruck *et al*., 2004**). Medicinal plants contain chemicals such as saponins, tannins, essential oils, flavonoids, alkaloids and other chemical compounds (**Emitaro *et al*., 2020; Morrison, 2009**). Antimicrobials of plant origin have few side effects and therapeutic potential to heal many diseases (**Iwu *et al*., 1999**). Medicinal plants are plants whose plant parts extracts, infusions, decoctions, powders are used in the treatment of different diseases (**Nostro *et al*., 2000**). Biological and pharmacological activities of phytochemical compounds take into account different parameters and factors such as species, ecological factors and environmental conditions (**Musyimi *et al.*, 2008**). Most of the plants used in folk medicine are under studied in relation to their phytochemical composition yet they are pillars of traditional medicines (**Emitaro *et al*., 2020**).

Plants are used to treat infections and ailments in different countries and many potent and powerful drugs have been produced from these medicinal plants (**Gangadevi, 2008**). Biologically active compounds from plants have played a vital role to combat diseases. Therefore, such plants should be investigated to better understand their properties, safety and efficacy (**Nascimento *et al*., 2000**). There is a growing need to screen medicinal plants for bioactive compounds as a basis for further pharmacological studies.Although a number of studies have been undertaken on antimicrobial effects of plant extracts, many plants used in different traditional medicinal systems are yet to be studied. Because of the rise of multi-drug resistant strains of bacteria and an alarming increase in the incidence of new and re-emerging infectious diseases, there is need to discover new antimicrobial compounds (**Semere, 2006; Aliero and Afolayan, 2006**).

The potential for developing antimicrobials from higher plants is rewarding because of the development of new drugs which are needed today. Research is necessary to discover the active compounds within higher plants (**Abebe *et al.*, 2014**; **Jayalakshmi, 2011)**. Plants serve the health needs of approximately 80% of people globally (**WHO, 2001**). The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body (**Musyimi *et al*., 2008**). Screening of plants for activities against microbes may provide new and locally available drugs which can improve the health of our people in developing countries.

Africa has over 5000 plants which are known for use in medicinal purposes, but only a small percentage have been described or studied scientifically (**Taylor *et al.*, 2001**). Herbal medicine in Kenya has received very little attention in modern research and development and less effort has been paid to upgrade the traditional health practices. By the year 2011, Kenya had forty thousand traditional healers which included herbalists, bone setters, faith healers and birth attendants prescribing medicinal plants (**Mwaura *et al*., 2020**).

Solanum species are important sources of a large number of phytochemical compounds with substantial curative applications against human pathogenic diseases (**Abbas *et al.*, 2014; Bari *et al.*, 2010**). *Solanum incanum* (L) is one of the important traditional medicinal plants in Kenya (**Waithaka *et al.*, 2019**). It grows wild and most of its medicinal uses are based on its analgesic properties. The fruit contains numerous small soft seeds which contain insignificant amounts of narcotic alkaloids (**Aliyu, 2006; Auta *et al.*, 2011; Owini *et al.*, 2015**). Though information on the use of *S. incanum* as a traditional medicine to treat different diseases in humans and animals is available (**Alamri and Moustafa, 2012)**, there is little information on the effects of the extracts of different parts of this plant on specific pathogenic bacteria. In addition, the development of resistance to antibiotics by many pathogens has posed an alarming threat on the control of diseases. Therefore, there is need to search for new and safer antimicrobial compounds (**Bonjar, 2004; Bobbarala *et al*., 2009**). Even though information on the use of *S. incanum* to treat different diseases in humans and other animals is available, little is known about the antimicrobial activities of the various parts of *Solanum incanum* against *Escherichia coli* and *Staphylococcus aureus.* There is little information on the active metabolites responsible for the antimicrobial activity of this plant in Kenya. The main objective of this study was to investigate antimicrobial activity and phytochemical screening of crude leaf, root and fruit extract of *Solanum incanum.* It *was hypothesized that different plant parts of Solanum incanum* have significant antimicrobial and phytochemical properties.

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MATERIALS AND METHODS

Collection of plant materials

The leaves, root and seeds of *solanum incanum* used in this study were collected from area surrounding Maseno University, Siriba campus. The materials were cleaned off adhered soil or dust in the field by shaking and were placed inside polythene paper bags and taken to the Maseno University laboratory where the tissue specimens were washed with distilled water and allowed to dry under shade for three weeks. The experiments were conducted in the Botany laboratory of the department of Botany, Maseno University.

Preparation of crude extracts

One hundred grams (100g) of leaves, roots and seeds were separately cut into smaller sizes and pounded using electric grinder into fine powder and finally kept in air tight containers. The powdered contents were macerated with 80%v/v of ethanol and water with occasional shaking and filtered to extract bioactive compounds. Upon observing the homogeneity of the contents, the ethanol and water-based maceration and filtration procedures were repeated three times to increase maceration and filtration efficiency. The filtrates were then stored in stored in refrigerator until were needed for analysis.

Phytochemical analysis

Phytochemical screening for all the secondary metabolites was carried out according to **Opande *et al*. (2017)**. The presence of constituents was then tested using standard procedures for alkaloids, tannins, saponins, flavonoids, terpenoids, and steroids.

Test for steroids

10ml of chloroform extract of the test plant leaves was evaporated then again dissolved in 0.5ml Chloroform. Then 0.5ml acetic anhydride and 2ml concentrated sulphuric acid was added. Development of a blue or green color or a mixture of the two shades confirmedthe presence of steroid compounds.

Test for terpenoids

Five mL of extract was mixed with 2mLof chloroform and concentrated sulphuric acid (3mL) was carefully added to form a layer. A reddish brown colouration formed in the interface, which indicated the presence of terpenoids.

Test for Tannins

A 1cm3 of freshly prepared 10% KOH was added to a 1cm3 of the plant extract. A dirty white precipitate indicated the presence of tannins. Then 5ml of the extract was boiled for about 5minutes and 5% of Iron III chloride (FeCl3) added. A greenish precipitate formed indicating the presence of tannins.

Test for flavonoids

A small piece of magnesium ribbon was added to the extract, followed by a drop wise of concentrated hydrochloric acid. Development of colours varying from orange to red, crimson to magenta indicated the presence of flavones.

Test for alkaloids

10ml of plant extract was stirred with 5ml HCl on a steam bath, the obtained solution was filtered and 1ml of it treated with two drops of Mayer's reagent. The two solutions were mixed and made up to 100ml with distilled water. Development of turbidity of the extract confirmed the presence of alkaloids.

Test for saponins

About 10ml of the extract was added to 5ml distilled water and was vigorously shaken for 2mins. The formation of froth indicated the presence of saponins.

Test for glycosides

10ml of extract was put into two separate beakers. To one of the beakers 5ml dilute sulphuric acid was added and 5ml of water to the other. The two beakers were heat for 3-5mins then filtered into separate test tubes. The filtrate was made alkaline with 5% Noah and heat with Fehling's solution for 3min. Presence of reddish precipitate in the acid filtrate and absence of such precipitate in the aqueous filtrate indicated presence of glycosides.

Preparation of Nutrient Media

Bacterial colonies were inoculated into liquid nutrient broths and incubated in 200 rpm shaking incubator at 37°C over night before the date of inoculation. Then, each broth culture was adjusted to match to McFarland 0.50 turbidity standard to get approximately 1×108 CFU/mL. Mueller–Hinton (M-H) media was prepared according to the procedures given by its manufacturer as growth media for agar-well diffusion assay.

Antibacterial tests

The antimicrobial activities of the ethanol and aqueous crude extracts of the root, seeds and leaves of *S. incanum* was evaluated using the agar-well diffusion method according to **Hailu *et al*. (2005)** and **Nascimento *et al*. (2000)**. The aqueous and ethanolextractsfrom the different plant parts (root, leaf and seeds) were assayed for antimicrobial activities against *Staphylococcus aureus* and *Escherichia coli*. Three types of extracts at concentrations of 25,50,75, 100 mg/ml and amoxicillin 25 mg/ml was placed in 6 mm diameter wells in the Mueller-Hinton agar (MHA) plates. Each well was filled with 50 µl of specific concentration of extract. Plates were incubated at 370C for 24 hours. Amoxicillin was used as a control. The plates were replicated three times. The zones of inhibition formed following incubation were measured after 24 h of growth at 370C. Results were expressed as the mean zones of inhibition. 25 mg/ml of Amoxicillin was used as standard control in the experiment.

Data analysis

Data from the study was subjected to analysis of variance ANOVA using SASStatistical Package to analyze data by comparing the zone of inhibition of aqueous and ethanolic extracts of *Solanum incanum*. Means were separated ($p>0.05$) to determine if the treatments had any significant effect on the two test pathogens.

RESULTS

Phytochemical analysis

Qualitative phytochemical screening (table 1) confirmed that root extracts contained alkaloids, saponin, glycosides, flavonoids and tannins and lacked terpenoids and steroids. The leaf extract contained alkaloid, saponins, glycosides, steroids and tannins and lacked flavonoids. The seed extract contained all the seven chemical compounds determined.

Table 1 The phytochemical compounds of *Solanumincanum*

Phytochemicals	Root extract	Leaves extract	Seeds extract
Alkaloids	+	+	+
Saponins	+	+	+
Glycosides	+	+	+
Terpenoids	-	+	+
Steroids	-	+	+
Flavonoids	+	-	+
Tannins	+	+	+

KEY: (+) presence (-) absence

Table 2 Antibacterial activities of crude seed extracts against against *E. coli* and *S. aureus*

Test pathogen	Conc. of extracts(mg/ml)	Mean zone of inhibition		
		Water	Ethanol	Amoxicillin 25 mg/mL (control)
E. coli	100	17.74±0.06	27.20±0.06	21.46±0.30
	75	13.31±0.21	20.44±0.3	16.10±0.2
	50	8.87±0.15	13.6±0.20	13.73±0.15
	25	3.73±0.2	13.57±0.25	13.57±0.25
S. aureus	100	15.2±0.5	35.0±0.6	33.7±0.3
	75	11.4±04	26.25±0.5	25.25±0.23
	50	7.60±0.26	17.50±0.30	16.83±0.15
	25	4.07±0.12	9.77±0.06	11.57±0251

Antibacterial activities of the crude extracts

The antibacterial activities of the different extracts of *S. incanum* against the two pathogenic bacteria showed significant zones of inhibitions (Tables 2,3 and 4).

Table 3 Antibacterial activity of crude root extracts against *E. coli* and *S. aureus*

Test pathogen	Conc. of extracts(mg/ml)	Mean zone of inhibition		
		Water	Ethanol	Amoxicillin 25 mg/mL (control)
E. coli	100	14.14±0.5	23.14±0.12	21.46±0.3
	75	10.61±0.38	17.36±0.09	16.10±0.23
	50	7.07±0.25	13.57±0.06	13.73±0.15
	25	2.90±0.10	12.33±0.15	13.57±0.25
S. aureus	100	11.66±0.3	30.94±0.3	33.66±0.3
	75	8.75±0.23	23.21±0.23	25.25±023
	50	5.83±0.15	15.47±0.15	16.83±015
	25	2.03±0.06	8.03±0.06	11.57±0.25

Table 4 Antibacterial activities of crude extracts of the leaves of *Solanumincanum*against *E. coli* and *S. aureus*

Test pathogen	Conc. of extracts(mg/ml)	Mean zone of inhibition		
		Water	Ethanol	Amoxicillin 25 mg/mL (control)
E. coli	100	9.06±0.3	21.0±0.4	21.46±0.3
	75	6.80±0.23	15.75±0.3	16.10±0.23
	50	4.53±0.15	13.50±0.20	13.73±0.15
	25	2.53±0.06	11.73±0.12	13.57±0.25
S. aureus	100	10.34±0.64	30.14±0.64	33.66±0.3
	75	7.76±0.48	22.61±0.5	25.25±0.23
	50	5.17±0.32	15.07±0.31	16.83±0.15
	25	1.43±0.12	7.57±0.15	11.57±0.25

DISCUSSION

The present study showed the capacities of the different solvents and plant parts of *S. incanum*, especially the leaf, root and seed to inhibit microbial activity, which conformed to those reported by previous researchers (**Sahleand and Okbatinsae, 2017; Ghosal and Mandal, 2012**). The antibacterial effects of all extracts varied depending on test organism, solvent used in extraction and plant parts. This may be due to the solubility of secondary metabolites in different organic solvents. It has been suggested that aqueous and ethanolic extracts from plants used in allopathic medicine are potential sources antimicrobial agents due to the presence of these secondary metabolites (**Taylor *et al.*, 2001**). Similar studies have indicated that *Solanum incanum* possesses numerous biologically active compounds (**Ghosal and Mandal, 2012**). Phytochemical determination confirmed presence of alkaloids, steroids, saponins, glycosides, flavonoids and tannins. These results are more or less similar to the previous findings by other workers, who explored the antibacterial potential of plant extracts against wide ranges of microorganisms, particularly from various members of family Solanaceae (**AL-Janabi and AL-Rubeey, 2010; Koduru *et al.*, 2006**). Presence of alkaloids in significant amount such as saponins and flavonoids inhibit tumor growth (**Kemei and Ndukui, 2017)**). According to **Sahleand and Okbatinsae (2017)** phytochemical active compounds are responsible for antimicrobial activity against stomach pain and diarrhea pathogens. Ethanoic seed extracts exhibited the greatest diameter of zones of inhibition on tested bacteria compared to other parts, in agreement with the previous results by **Beaman-Mbaya and Muhammed (1976)**. Findings from this study are in agreement with findings of **Alamri and Moustafa (2012)**. Similar studies support these findings (**Yigezu *et al.*, 2014**). Ethanol extracts concentrations exhibited significantly higher zones of inhibition compared to amoxicillin.The activities of the ethanol and aqueous extracts of the different parts of the plant against the test pathogens also varied. The findings reveal that the plant parts extracted by ethanol provided more consistent antibacterial activities compared with those extracted using water. This indicates that the active ingredients of the plant are more readily dissolved and extracted in ethanol compared with water. The results also showed that different plant parts possess different levels of antibacterial activities. The greater activities recorded by seeds and root extracts over the leaves extracts in this study suggest that more of the bioactive ingredients may have been lodged in these parts. Active ingredients such as phenols confer broad spectrum activities in plants (**Alamri and Moustafa, 2012**). Among the tested bacteria for susceptibility to various crude extracts, *S. aureus* was found to be the most susceptible. The results of the present study indicate that the parts of the plant have promising antibacterial activities against *S. aureus* and *E. coli*. These results are supported by previous studies (**Yigezu *et al.*, 2014**). This study has demonstrated that *S. incanum* roots and seeds may serve as good sources of bioactive compounds compared to leaves.

CONCLUSION

The present investigation revealed that *Solanum incanum* exhibited significant antimicrobial effects against *E. coli* and *S. aureus*. In this study *Staphylococcus aureus* was found to be more susceptible to *S. incanum* extracts than *E. coli*. The two solvents employed for the extraction process i.e., water and ethanol showed different power in their extraction efficiency which could be due to their difference in polarity. Seed ethanoic extract was found to be the most suitable extract for the antimicrobial activity among the three parts. The phytochemical and antibacterial study of the extracts of leaf, root and seeds of the plant yielded results that strengthen the findings of previous studies. The positive findings from this study provide a scientific basis for the traditional use of *Solanum incanum*. Further studies are required to isolate the active principles from the crude extract for proper drug development.

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