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Antibacterial effect of *Solanum incanum* root extracts on bacteria pathogens isolated from portable water in Egerton University, Kenya

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ABSTRACT

Background

Contaminated water is a major source of enteric diseases. This study aimed at isolating pathogenic bacteria from portable drinking water in Egerton University. In addition, the study aimed at subjecting the isolates to sensitivity test of root extracts from *Solanum incanum* besides carrying out minimum inhibitory test of the root extracts.

Material and methods

The bacterial pathogens were isolated from water using membrane filtration. The roots were obtained from *Solanum incanum* plants in the field and dried at room temperature under shade. The root extracts were obtained using methanol, ethanol and water. Sensitivity test of the isolates to the extracts was carried out using disk diffusion technique. Minimum inhibitory technique was carried out using broth tube dilution technique.

Results

The bacterial pathogens such as *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella sp.* were isolated from the water samples. The crude extracts contained tannins, alkaloids, glycosides, flavonoids, resins, phenols and steroids. There was no significant difference between the zones of inhibition produced by the test bacterial pathogens when subjected to crude extracts obtained using methanol, ethanol and water ($F=28.57$ $P=0.07$). However, there was a significant difference between the MIC of methanol, ethanol and water extracts.

Conclusion

Portable water in and around Egerton University is contaminated with potential bacteria pathogens. However, extracts from *Solanum incanum* can be used as a remedy to the problem. There is need for determination of the structure of active ingredients in the extracts obtained from *Solanum incanum*.

Keywords

antibacterial, bacteria, pathogens, *Solanum incanum*

Introduction

Solanum incanum comprises of over 1000 species and has a cosmopolitan distribution except in boreal Alpine and aquatic habitats [1]. At least 100 species are found in Tropical Africa. The principal centres of diversity are located in central and South America with secondary centres in Africa and Australia. *Solanum* has been subdivided into 7 subgenera and numerous sections and series. *Solanum incanum* belongs to sub genus Leptostemonum section melongena. It is considered as a single polymorphic species. However, some authors distinguish 4 groups within a species while others consider each of these groups a different species [2]. *Solanum incanum* is said to be an ancestor of eggplant (*Solanum melongena L*) and the two species are therefore considered by some as a single species in the ethno botanical literature. There are several synonyms and common names used in description of *Solanum incanum* such as *Solanum bojeri* dunal, *Solanum campylacanthum* hochst, *Solanum delagoense* dunal, *Solanum lichtensteinii* willid and *Solanjum panduriforme* among others [3]. The plant is also known by common names from different communities that is bitter apple, poison apple, snake apple, thorn apple (Ndebele), mutongu (kikuyu), mtuguja mwitu (Kiswahili), Ocok (Luo) and umdulukwa munhomboro (Shona) [4].

Solanum incanum is a common weed found around houses, in overgrazed areas, along roadsides, along the forest edges and in bushed grasslands. It's found from sea level up to 2500m altitude [5]. It's considered as an indicator for low fertility of soils by some people. *Solanum incanum* is an herb or soft wooded shrub up to 1.8m in height with spines on the stem and calyces and with velvet hairs on the leaves. The flowers are pale blue or purple. The leaves are alternate, egg shaped in outline with broad end at base (crate) with slightly wavy margins especially on young leaves with a grey green upper surface and a green-white lower surface [6].

Solanum incanum is the scientific name while sodom apple is the common name. It belongs to the family solanaceae together with tomato groups [7]. It was naturalized and introduced in East Africa, though seems likely to be native to Kenya, Uganda and Tanzania [8]. *Solanum incanum* though found growing wild, it is very vital to indigenous communities as an indigenous herbal medicine and they use it in treatment of a number of diseases [9]. Most of its medicinal uses are based on its analgesic properties. Throughout Tropical Africa it heal or treats the following ailments; a sore throat, stomach ache, headache, painful menstruation, liver pain, pain caused by onchcerciasis and pneumonia [10].

The above ailments are treated using roots, fruits, stems and leaf decoctions which are gargled or drunk [11]. Roots are chewed and sap swallowed, leaf paste, root infusions and pounded fruits are applied externally or rubbed into scarification, leaf sap is rubbed on the gums or smoke of burning seeds is inhaled. Hiccups are suppressed by licking a mixture of ash of burned leaves and salt [12].

Solanum incanum is used in treatment of venereal diseases where roots decoctions are drunk, roasted pulverized roots are taken in water, leaf decoctions and fruit sap are drunk and the fruit sap is applied externally [13]. Different parts are widely used in the treatment of skin infection, whitlow, ringworm, burns, rushes wounds, warts carbuncles ulcers and benign tumours. In Senegal pounded macerations of the leaves is used as an eye bath to cure ophthalmia, in Malawi, fruit sap is rubbed into scarification around the eye to treat conjunctivitis [14]. In Uganda, Tanzania and South Africa, extracts of leaves or flowers are used as ear drops to cure inflammations. In Kenya, Uganda and Zimbabwe different parts are used to treat snake bites, the roots are drunk or chewed and sap is swallowed. Young chewed leaves or purple fresh roots are applied to the bite wound [15].

The conventional medicine used in treating diseases is failing today due to the increased drug resistance. As a result this study was designed to isolate bacterial pathogens from portable water in Egerton University and carry out their sensitivity to extracts obtained from *Solanum incanum*.

Material and methods

Study period and area

The study was conducted at Egerton University, main campus Njoro in Kenya in the year 2019. Egerton University is located in Njoro Sub County with coordinates as 0º 23' south, 35º 35' and altitude of 2000m above sea level. Temperatures range between 17-22ºC while the average annual rainfall is 1000mm [16].

Collection of water and isolation of bacterial pathogens

Portable water was collected from water drawing points using sterile bottles in Egerton University. The isolation of bacterial pathogens was carried out using membrane filtration technique [17]. The identification of the pathogens was carried out using colonial morphology and Analytical Profile Index (API) biochemical characterization bioassay [18].

Collection and extraction of *Solanum incanum* extracts

The *Solanum incanum* root collected and dried under shade for 14 days. The roots were ground into fine powder using a grinder and stored for further analysis. The fine powder (100g) was infused in 50ml of methanol for 10h. The extract was filtered using Whatman No.1 filter paper. About 5ml of the filtrate was measured and stored for phytochemical analysis. The solution was concentrated using a rotary vacuum evaporator at 600C to obtain a concentrated extract, which was stored for further analysis. This was repeated using ethanol and distilled water.

Phytochemical screening of the extracts
The presence or absence of the phytochemical constituents in all extracts was analyzed using standard procedures for tannins, alkaloids, glycosides, flavonoids, resins, phenols and steroids as described by Growther and Growther [19].

Test for tannins
About 0.5ml of plant extract was dissolved in 1ml of water. Two drops of ferric acid solution was added. A blue or green blue colour indicated a positive test.

Test for Alkaloids
About 2g of the dry powder was dissolved in 2ml of 2% HCL. Heating in a water bath for 10minutes was carried out. Five drops of Meyers reagent was added to the filtrate of the crude extract. Observation for appearance of turbidity was done.

Test for glycosides
To 2ml of the extract, 1ml of glacial acetic acid was added followed by few drops of ferric chloride and concentrated sulphuric acid. Observation for appearance of red brown colour was done.

Test for flavonoids
To 2ml of plant extract, few drops of concentrated HCL and Mg ribbon was added. Appearance of pink tomato red colour indicated a positive test.

Test for resins
To 1ml of extract 1ml of distilled water was added. Presence of turbidity indicated positive results.

Test for phenols
Test extract was heated with 4 drops of alcoholic ferric chloride solution. Formation of a blue black or green indicated a positive test.

Test for Steroids
About 1ml of the extract was dissolved in 5 ml chloroform. An equal volume of concentrated sulphuric acid was added from the side of the test tube. For positive results, the upper layer turns red and sulphuric acid layer turns yellow with a green fluorescence.

Antimicrobial screening
The antibacterial activity of methanol, ethanol and aqueous root extracts of *Solanum incanum* was determined by disc diffusion technique as described by Tel *et al.* [20]. Aseptically, 100µl from the working bacterial test pathogens were mixed with 20 ml of sterile Mueller Hinton agar and then poured into 15mm sterile culture plates. The media was left to solidify at room temperature. 6 mm discs previously prepared from Whatman No.1 filter paper and saturated separately with the extracts of methanol, ethanol and aqueous extracts were loaded on the seeded Petri plates. The plates were incubated at 37^{o}C for 24h. The zones of inhibition of were measured in millimetres (mm).

Minimum inhibitory concentration (MIC)
MIC of crude ethanol extract and their fractions were determined by serial dilution technique against *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli* and *klebsiella sp*.

Data management and statistical analysis
All statistical analyses were carried out using Statistical Package for Social Sciences Software (SPSS) version 17.0 software. Data was analyzed using ANOVA.

Ethical committee approval
This study was approved by the ethical approval committee, Egerton University.

Results

Characteristics of the isolated bacterial pathogens
All the isolated bacterial pathogens tested positive for catalase test (Table 1). *Staphylococcus aureus* positive for Gram stain, *P. aeruginosa* (Citrate and oxidase tests), *E. coli* (Indole), *Bacillus subtilis* (Gram stain) and *Klebsiella sp.* (Citrate). All the isolates tested negative for H_2S production, *S. aureus* for citrate, indole and oxidase, *P. aeruginosa* (Gram stain and indole), *E. coli* (Gram stain, citrate and oxidase), *B. subtilis* (citrate, indole and oxidase) and *Klebsiella sp.* (Gram stain, indole and oxidase.

Phytochemical Screening of *Solanum incunum* extracts
The results on phytochemical tests of the extracts are presented in table 2. All the tested phytochemical compounds were present in methanol, ethanol and water extracts.

Table 2: Phytochemical compounds of Extracts from *Solanum incunum*

S. No	Test	Present/absent
1	Tannins	+
2	Alkaloids	+
3	Glycosides	+
4	Flavanoids	+
5	Resins	+
6	Polyphenols	+
7	Steroids	+

Antimicrobial activity of the extracts from *Solanum incunum*
The zones of inhibition of the isolated bacterial pathogens are presented in table 3. In methanol extracts, the zones of inhibition varied from 20±0.1mm in *Pseudomonas aeruginosa* to 22±0.2mm in *Staphylococcus aureus.* In ethanol extracts, the zones of inhibition varied from 18±1mm in *Staphylococcus aureus* to 20±0.3mm Bacillus subtilis.

Table 1: Morphological and biochemical characteristics of the isolated bacterial pathogens

Colony characteristics	H_2S gas	Gram stain	Catalase	Citrate	Indole	Oxidase	Isolate identity
White, smooth, creamy and round	-	+	+	-	-	-	*S. aureus*
Green, glossy pigmented and thin	-	-	+	+	-	+	*P. aeruginosa*
White, moist with glistening growth	-	-	+	-	+	-	*E. coli*
White glossy membranous	-	+	+	-	-	-	*Bacillus subtilis*
Translucent-creamy, mucoid and round	-	-	+	+	-	-	*Klebsiella* sp.

However, in water extracts the zones of inhibition ranged from 16±0.3mm in *Pseudomonas aeruginosa* to 18±0.2mm in *Klebsiella* sp. There was no significant different between the zones of inhibition produced by the test pathogens when subjected to methanol, ethanol and water extracts (F=28.57 P=0.07).

Table 3: Zones of inhibition (mm) of the bacterial isolates by the extracts from *Solanum incunum*

Pathogen	Methanol	Ethanol	Water
Staphylococcus aureus	22±0.2	18±0.1	17±0.2
Pseudomonas aeruginosa	20±0.1	18±0.2	16±0.3
Escherichia coli	21±0.3	19±0.1	17±0.1
Bacillus subtilis	20±0.2	20±0.3	16±0.1
Klebsiella sp.	21±0.1	19±0.1	18±0.2

MICS (mg/ml) of the extracts from *Solanum incunum* when tested against the isolated bacterial pathogens

The minimum inhibitory concentration in the methanol extracts ranged from 200±0.1mg/ml in *B. subtilis* to 230±0.3mg/ml in *S. aureus* (Table 4). However, the range in ethanol extracts was 254±0.1mg/ml in *E. coli* to 270±0.2mg/ml in *P. aeruginosa*. In addition, the MIC in the water extracts varied from 280±0.2mg/ml in *S. aureus* to 300±0.2mg/ml in *Klebsiella sp.* There was a significant difference between the minimum inhibitory concentration of methanol, ethanol and water extracts (F=75.27 P=0.0007).

Table 4: Minimum inhibitory concentration (mg/ml) of the extracts from *Solanum incanum*

Pathogen	Methanol	Ethanol	water
Staphylococcus aureus	230±0.3	250±0.1	280±0.2
Pseudomonas aeruginosa	220±0.2	270±0.2	290±0.3
Escherichia coli	220±0.3	254±0.1	300±0.1
Bacillus subtilis	200±0.1	268±0.2	320±0.1
Klebsiella sp.	210±0.2	270±0.1	300±0.2

Discussion

Characteristics of the isolated bacterial pathogens

The bacterial pathogens isolated from portable water in the current study were typical of aquatic pathogens (Table 1). The results agree with a previous study carried out in Kenya [21]. Possible reason for the similarity might have originated from similar points of contaminants. Panneerselvam and Arumugam [22] added that, seepage from pit latrines contributes highly to contamination of portable water. Running of sewage systems together with pipes that transport water contributes immensely to contamination of portable water [23].

Phytochemical screening of *Solanum incunum* extracts

Vinayak *et al.* [24] obtained phytochemical results that differed with those of the current study (Table 2). Possible reasons to the difference could be the soil physicho-chemical characteristics of the regions where the plant were growing. In addition, Pronob and Islam [25] asserted that the environmental conditions of a certain area influences the phytochemical substances that *Solanum incanum* accumulates.

Antimicrobial activity of the extracts from *Solanum incunum*

The results of zones of inhibition produced by the extracts from *Solanum incanum* in the current study are presented in table 4. The results on growth inhibition of the test pathogens by methanol extracts of *Solanum incanum* differed with those of a previous study [26]. This may be have been caused by variations in the technique used in extracting the extracts. However, the results of extracts obtained using ethanol concurred with a previous study carried out in India [27]. This may have been caused similarity of the affinity ethanol had on the active ingredients [28]. The zones of inhibition produced by water extracts in the current study differed with a study carried out by Indhumathi and Mohandass [29] in Pakistan. The ability of the active ingredients in the extracts to dissolve in water may be a contributing factor.

MICS (mg/ml) of the extracts from *Solanum incunum* when tested against the isolated bacterial pathogens

The results of the current study on minimum inhibitory concentration of the bacterial isolates by the extracts from *Solanum incanum* (Table 4) disagreed with a previous study. The possible reason could have been the concentration of the active ingredients of the extracts [30]. Mojab *et al.* [31] further argued that the composition of the nutrients in which *Solanum incanum* is growing determines the type and concentration of the active ingredients the plant accumulates.

Conclusion

Portable water in and around Egerton University is contaminated with potential bacteria pathogens. However, extracts from *Solanum incanum* can be used as a remedy to the problem.

Abbreviations

Analysis of Variance (ANOVA), Analytical Profile Index (API), Minimum Inhibitory Concentration (MIC), Statistical Package for Social Sciences (SPSS).

Authors' contribution

PN conceived the study, collected data, participated in data analysis, manuscript writing and revision and final approval of the manuscript, BM participated in study panning, data acquisition, data analysis, manuscript revision and final approval of the manuscript, EM did the planning, data acquisition, data analysis and final approval of the manuscript, MF participated in data acquisition, data analysis and final approval of the manuscript. All the authors approved the final document.

Competing interests

None declared.

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Limitation and future scope of the study

Water around and in Egerton University need proper treatment. There is need for determination of the structure of active ingredients in the extracts obtained from *Solanum incanum*.

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