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Antibacterial Activity Of *Solanum incanum* Roots And Fruits Methanol Extracts Against Gastrointestinal Bacteria Causing Food Poisoning

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Abstract: *Food poisoning is a bacterial disease causing many deaths, reduced productivity and financial instability in the world. Antibiotics and proper hygiene are some of the control strategies used. Antibiotic resistance, toxicity and lack of hygiene awareness are the limiting factors to these control mechanisms. This has led to many deaths caused by food poisoning. The fruits and roots of Solanum incanum are extensively used in treatment of food poisoning. The present work was carried out for screening and comparing the antimicrobial potential of root and fruit extracts of Solanum incanum in treating food poisoning. The extracts were made using methanol and their ability to inhibit the growth of gram positive (Staphylococcus aureus) and gram negative bacteria (Escherichia coli, Salmonella typhimurium) was determined. The extent of antibacterial activity of both the root and fruit extracts was determined using disc diffusion technique. Mean value and Standard Deviation were calculated for the test bacterial isolates. Data were analyzed by one way ANOVA and P values were considered significant at* $P < 0.05$. *Among the three bacterial strains tested for antibacterial activity, S.aureus was most susceptible to both extracts with inhibition zones ranging from 14.0±1.0 to 3.0±2.0mm for the fruit and 16.67±0.33 to 2.0±1.0mm for the root extract. S.typhimurium was least susceptible to the fruit extract with zone of inhibition of 8.67±2.33 to 6.0±3.0mm. E.coli was least susceptible to the root extract with zones of inhibition from 15.0±0.67 to 2.67±1.33mm. The range of MIC was found to be 12.5 mg/ml-25mg/ml for both extracts. The root extract had a higher antibacterial activity than the fruit extract Result of the present study reveals that root and fruit extracts of Solanum incanum were showing antimicrobial potential against tested microorganism and can be used as future antimicrobial drug.*

Key Words: *Antibacterial activity, Solanum incanum, gastrointestinal bacteria, food poisoning*

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I. Introduction

Food poisoning is an illness caused by eating contaminated food. The contamination can be due to presence of microbes such as bacteria, virus or fungus, and chemicals occurring naturally or introduced in food. The people at a high risk of food poisoning are children younger than 1 year old, pregnant women, older people (≥70 years of age) with certain underlying conditions and people with compromised immune systems through chronic or acute ill health, some conditions and under treatments (Hayes, 1992). Some of the bacteria that cause food poisoning are *Escherichia coli, Staphylococcus aureus, Salmonella typhimurium* and *Clostridium perfringens* . In addition, intake of these pathogens is commonly from unclean water, inadequately cooked food and stale food, most of which are tightly associated with poverty.

Various symptoms are associated with food poisoning and more often the arising pathologies are similar among the microbes involved. These symptoms range from nausea, diarrhea, vomiting, stomach pains, cramps, sweating, fever, chills, headache and lethargy (Balch *et al.,* 2011) . Vomiting and diarrhea can lead to dehydration and possibly death if not addressed promptly. The diseases and consequences of food-borne pathogenic micro-organisms are of major global importance and concern, with a conservatively estimated 80 million cases of "food poisoning" occurring annually in the Western world (Varnam *et al.,* 1996) . In South Africa, 334 cases of food poisoning were reported in 1993 (Miliotis *et al.,* 2003). In 2009 there were 1,255 reported deaths due to food poisoning in Africa (Ilu, 2009). These deaths may be under estimated because of lack of health care services in some regions. These deaths reduce productivity in the affected areas and leave the families with financial instability if the affected person was the bread-winner of the family. In national and regional economy, the presence of contaminants in food products leads to export bans leading to loss of huge financial loss. FAO reported that in 1997, a ban imposed on Ugandan fish exports to the European Union markets due to their frequent cause of poisoning, resulted in losses amounting to US$36.9 million (Kadariya*et al.,* 2013).

The two approaches for controlling food poisoning are preventive and chemotherapy. Preventive methods involve proper cooking of food, personal hygiene such as hand washing and appropriate storage of left-overs (Scwartz*et al.,* 2001). For chemotherapy, antibiotics like cephalexin, cefaclor, cefridroxil, nafcillin, ciprofloxacin and ampicillin are used for treatment. The prevalence of antimicrobial resistance among food-borne pathogens is reported to have increased (Yucel*et al.,* 2005; Nyenje*et al.,* 2012a), probably as a result of selection pressure created by the use of antimicrobials in animal and plant health. In addition, improper use of antibiotics principally under dosing and administration of wrong drugs due to misdiagnosis, are contributing factors. This therefore calls for identification of novel antibiotic compounds for development of anti-microbial drugs. And one potential avenue is utilization of ethno-botanical knowledge.

The use of plant extract for medicinal treatment has been a long practice, and has been a source of some commercially available drugs such as penicillins. Notably, it offers a source of novel anti-microbial compounds that are alternatives to drug resistant antibiotics (Alam *et al.,* 2009). Many research efforts have been directed towards the provision of empirical proofs to back up the use of plants species in trade and medicinal practices in recent years (Ojo *et al.,* 2005). This is demonstrated by significant interest in plants used traditionally or by indigenous healers to support treatment of various diseases; scientific validations is necessary to allow full and commercial exploitation of bioactive agents from these medicinal ethno-botanicals. However, there still exist a large number of plants with tremendous medicinal potentials that have not been investigated despite herbal medicine dating as far as 3000 BC (Ayensu, 1978; WWF, 1993) and technological advancements. Presently, nearly 30% or more of the modern pharmacological drugs are derived directly or indirectly from plants and their extracts (Jabeen *et al.,* 2007; Banso, 2009; Ahamunthunisa and Hopper, 2010). Indeed, more needs to be done in this front, and ethno-knowledge presents a simple route.

Solanum incanum, locally known as bitter apple, is a medicinal plant used by Bantus namely Kamba and Kikuyus, in East Africa, specifically Kenya. The fruit sap and roots being mostly used to treat food poisoning and other bacterial infections namely wound among these communities. The plant is distributed throughout continental Africa (Lester *et al.,* 1990). Its fruits are yellow, green or white and are edible (Lester *et al.,* 1990). It furthermore occurs in the Middle East and India. With this kind of wide distribution, use is diverse and includes local medication for stomach ache, colic, pneumonia, rheumatism, tooth-ache, food poisoning and skin infection among others.

In the current study, I investigated the antimicrobial activity of *S. incanum* fruits and roots on microbes responsible for food poisoning namely *Staphylococcus aureus, Escherichia coli, Salmonella typhimurium* using disc diffusion technique. Root and fruit extracts of *Solanum incanum* have antimicrobial potential against these microbes, with root extract showing higher activity as compared to fruit fraction. This is important towards validating this plant as a medicinal plant that can be utilized by local communities who cannot afford formal medical health care due to various factors including cost.

II. Methods And Material

The fresh and disease free *Solanum incnum* roots and fruits were collected from Kabati, Kitui county-Kenya.The fruits were obtained by hand plucking while the roots by digging and carried in a polythene paper bag to the laboratory. The fruits were cut to remove the fruit sap, the roots were cut into smaller pieces using a sterile blade, air dried for 7 days at room temperature.

The dried fruit sap and roots were blended in the Chemistry Department Egerton University Njoro. 30 g of the blended powder (root and fruits) were soaked in 300 ml of 80% methanol (New Delhi, Okhla, India) each, for 5 days in a round bottomed flask at room temperature intermittent shaking to allow the active phytochemicals to dislodge in the solvent. Extracts were filtered through the filter paper. The filtrate was evaporated using a rotary evaporator from the Chemistry Department Egerton University Njoro set at 40- 50 ^{0}c to get the methanol extracts. The dried extracts were weighed and diluted with dimethyl sulphoxide (DMSO) (Sigma-Al-Drich, Munich, Germany) to concentrations of 200mg/ml, 100mg/ml, 50mg/ml and 25mg/ml. A negative control of DMSO and a positive control of Ciprofloxacin (Merck KGaA, Darmstadt, Germany)wereused.

Susceptibility tests was performed using three strains of microorganisms including Gram positive *Staphylococcus aureus* and Gram negative bacteria *Escherichia coli* and *Salmonella typhimurium.*These microorganisms were a gift from Ms. Mwajuma of Department of Biochemistry and Molecular Biology, Egerton University Njoro.

Disk Diffusion assay was used to determine the growth inhibition of bacteria by plant extracts as described by Mohammad and Dabai (2008). Muller Hinton agar (Hi media laboratories pvt.ltd, Mumbai, India) was prepared following the manufacturers' instructions for purposes of culturing bacteria and poured into plates then allowed to dry. The concentration of overnight test cultures was adjusted using normal saline to 0.5 $\times 10^{6}$ CFU/mL McFarland standards. The bacterial suspensions were seeded on Muller Hilton Agar plates using a sterilized cotton swab. Whatmann no.1 paper discs of 6.0 mm diameter each were punched and dipped in 200mg/ml, 100mg/ml, 50mg/ml and 25mg/ml concentrations of the extracts then seeded in each plate. This was done in triplicates and the inoculated plates were allowed to congeal for 30 min to allow pre diffusion time and then incubated at 37^{0}C for 24 hrs. The plates were examined for evidence of zones of inhibition which appear as a clear area around the discs (Cheesbrough, 2001). The diameter of such zone of inhibition was measured using a transparent meter ruler and the value will be recorded and expressed to the nearest millimeter.
The means of the diameter of inhibition zones for the root and fruit extract were compared using one- way ANOVA with $p<0.05$ to establish their spectrum of anti-bacterial activity.

III. Results And Discussion

3.1.1. Recovered Crude Extract

3g of the root extract and 5g of the fruit extract were obtained after evaporation. They were dissolved in 15ml and 25ml of DMSO respectively to make 200mg/ml concentrations of each. The extracts were crystalline but soluble in DMSO. The root extract was yellow in color while the fruit extract was brown in color as shown in **fig 3.1**.

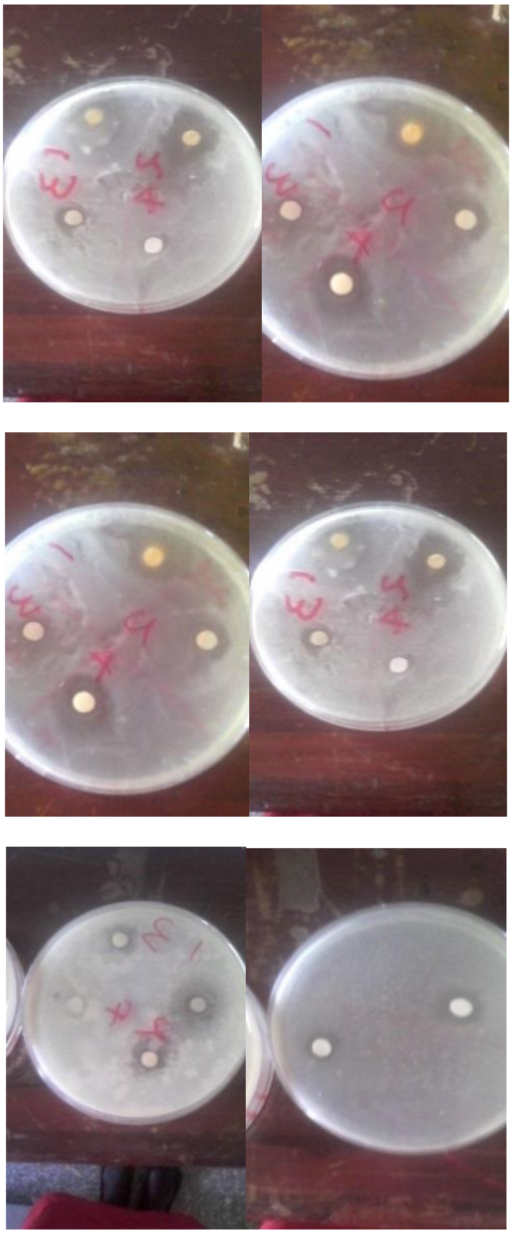
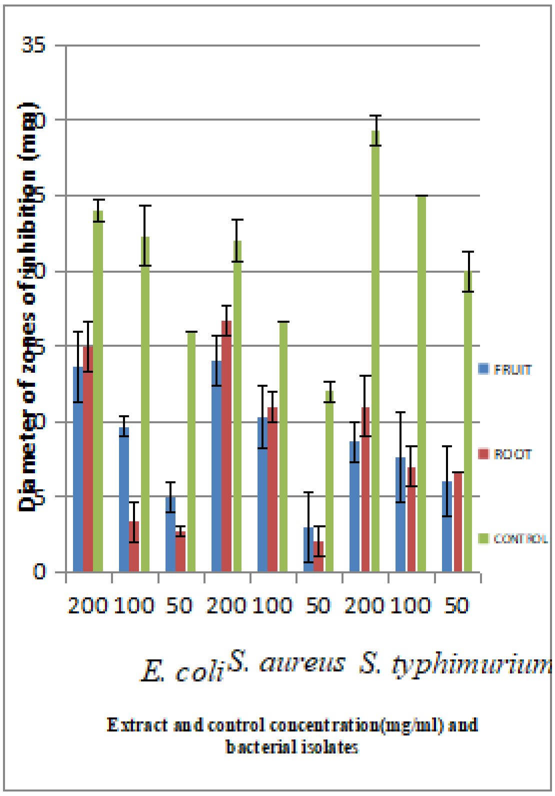


Figure 3.2 Antimicrobial activity of fruit and root extracts of *Solanum incanum* against gastrointestinal *Staphylococcus aureus, Escherichia coli and Salmonella typhimurium.* Different concentrations of methanol extracts of *Solanum incanum* roots and fruits were tested using disc diffusion system against three pathogens. Zones of inhibition of different concentrations of fruit extract **(a)** and root extract **(b)** on *S. aureus*, Zones of inhibition of different concentrations of fruit extract **(c)** and root extract **(d)** on *E. coli*, Zones of inhibition of different concentrations of fruit extract **(e)** and root extract **(f)** on *S. typhimurium.* Zones of inhibition of different Zones of inhibition of different concentrations of ciprofloxacin **(g)** and DMSO **(h)** on *E. coli*. The plates were divided into four quadrants each with a different concentration of the extracts or positive control. The concentrations were represented by numerals; **1**-200mg/ml, **2**- 100mg/ml **3**- 50mg/ml **4**-25mg/ml.

The *S. incanum* roots and fruits extracts showed antibacterial activity against *E. coli, S. aureus* and *S. typhimurium* determined using disc diffusion technique. The positive control (Ciprofloxacin) showed larger zones of inhibition compared to the roots and fruit extracts at all the concentrations. The values of the diameter of zones of inhibition are represented graphically as shown in **fig 3.3.**



IV. Conclusion

Although a conclusion may review the main points of the paper, do not replicate the abstract as the conclusion. A conclusion might elaborate on the importance of the work or suggest applications and extensions. Authors are strongly encouraged not to call out multiple figures or tables in the conclusion—these should be referenced in the body of the paper.

Fig 3.3. A graph of zones of inhibition (mm) of *Solanum incanum* root and fruit extracts and Ciprofloxacin against gastrointestinal *Staphylococcus aureus, Escherichia coli and Salmonella typhimurium*. The methanol extracts were tested against the three bacterial isolates and the zones of inhibition determined using disc diffusion technique.

3.1.3 Statistical analysis

The root and fruit extracts extend of antibacterial activity was analyzed using one way ANOVA. The data was grouped into two groups- the fruit and the root's zones of inhibition. The following hypotheses were used;

Null hypothesis

The antibacterial activity of the root extract = the antibacterial activity of fruit extract

Alternate hypothesis

The antibacterial activity of root extract is higher than that if fruit extract

The analysis was summarized in **Table 3.1** with significance level of at point 0.05.

Table 3.1- statistical analysis of the zones of inhibition of root and fruit extracts on different bacterial isolates.

	Sum of squares	**Degrees of freedom (d.f)**	**Mean square (s^2)**	**F**
SCORES **Between groups** **Within groups** **Total**	**137.256** **530.35** **667.606**	**1** **22** **23**	**137.256** **24.107**	5.69

V. Discussion

The antibacterial analysis revealed affectivity of *S. incanum* activity against *E. coli, S. aureus* and *S. typhimurium*. The diameter of the zone denotes the relative susceptibility of the test microorganism on the extracts. 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 observed antibacterial activity is attributed to the presence of bioactive compounds in the extracts of plant tested. Several constituents of *S. incanum* have been associated with antibacterial activity, mainly the steroidal alkaloids, glycosides, phenols, saponins, flavonoids and proteins (Britto *et al.,* 2001). The activity was directly proportional to the concentration of the extracts. This is due to the increase in the phytochemicals concentration in the extracts (Farnsworth, 1982). This then shows the potential ability of the bitter apple plant in treating food poisoning.

Among the three bacterial strains tested for antibacterial activity, *S. aureus* was most susceptible to both extracts with inhibition zones ranging from 14.0±1.0 to 3.0±2.0mm for the fruit and 16.67±0.33 to 2.0±1.0mm for the root extract. *S. typhimurium* was least susceptible to the fruit extract with zone of inhibition of 8.67±2.33 to 6.0±3.0mm. *E.coli* was least susceptible to the root extract with zones of inhibition from 15.0±0.67 to 2.67±1.33mm. The high susceptibility of *S. aureus* (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 (Trombetta*et al.,* 2005) like *E.coli* and *S. typhimurium.* Gram negative bacteria also contain the membrane efflux pumps which could have pumped out some of the bioactive antibacterial compounds of the plant extracts (Rownd*et al.,* 1971). The ability of these extracts to be sensitive to both Gram positive and Gram negative bacteria is a clear indication of their broad spectrum antimicrobial activity.

Methanol was able to extract the bioactive agents from the plant roots and fruits. Methanol has the ability to extract both polar and nonpolar bioactive compounds in *Solanum incanum* compared to ethanol and water (Anokwuru*et al.,* 2011). The present observation suggests that the methanol as an organic solvent extraction was suitable to verify the antimicrobial properties of medicinal plants and they are supported by many investigators (Krishna *et al*., 1997; Singh and Singh, 2000; Narayan *et al*., 2008). It also indicates that cheaper and simple methods of extraction like the use of methanol are effective in extracting the bioactive components of *S. incanum* hence producing cheaper antibacterial drugs to treat food poisoning.

From the study the minimum inhibitory concentration of both the root and the fruit extracts was between 12.5mg/ml to 25mg/ml. This indicates that even a small concentration of the plant extracts can be effective in killing food poisoning pathogens hence the bacterial isolates were not resistant to even the smaller concentrations of the extracts. This justifies the use of *S. incanum* in comparting bacterial resistance.

The standard antibiotic Ciprofloxacin showed activity in all the three bacterial isolates. Though its antibacterial activity is higher than that of bitter apple roots and fruit extracts, ciprofloxacin is expensive and only available in hospitals and chemists. Most of the uneducated people are not even aware of its availability. Bitter apple is a cheaper and easily available cure for food poisoning than Ciprofloxacin.

From the statistical comparative analysis, the root extract had a significantly higher antibacterial activity than the fruit extract. This is probably because the root is highly concentrated with phenols which are effective against *S. aureus* and flavonoids which are effective against *E. coli* as compared to the fruit as reported by (Tomoka*et al.*, 2002). Though the fruits have a significant activity against food poisoning pathogens, the roots are more effective in treating food poisoning than the fruits.

This study there justifies the affectivity of the methanol extraction method and the use of bitter apple roots in treating food poisoning.

Other medicinal uses

Bitter apple roots also have an anti- schistosomal effect on cercariae (Muchika*et al.,* 2011) and antifungal effect on *Trichophyton tonsurans, Cryptococcus neoformans* and *Candida albicans* (Mbaya and Muhammed, 1976). This gives evidence that the plant has the ability to cure most of the stomach problems apart from food poisoning.

Limitations

Bitter apple fruits are irritating hence posing a limitation to their use.

VI. Conclusions And Recommendation

6.1. Conclusion

The results of the study revealed that the *S. incanum* roots and fruits extracts have antibacterial activity against gastrointestinal *Staphylococcus aureus, Escherichia coli and Salmonella typhimurium* with root extract showing a higher activity. This is evidence that methanol extraction was effective.

The present finding can be of commercial interest to both pharmaceutical companies and research institutes in the production of new and cheap antimicrobial drugs using the plant. The study also provides support for the use of medicinal plants in the management of bacterial diseases like food poisoning.

6.2. Recommendation

I hereby recommend for;

- Use of methanol in extraction of the bioactive agents for drug production.
- Further research on the amount of bioactive agents especially the phenols and flavonoids which are highly present in the roots and their concentration in different locations of the plant.
- Research for the active compound for drug discovery.
- Trying the extracts with other bacteria causing food poisoning and other deadly bacteria like *Streptococcus pneumonia*, *Mycobacterium tuberculosis* and

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