Antibacterial assays of an African medicinal plant: *Securidaca longepedunculata*.

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**Abbreviations:**
- DMSO: dimethyl sulfoxide; GI tract: Gastrointestinal tract; ZI: Zone of inhibition; MIC: Minimum inhibitory concentration;  
- MBC: Minimum bactericidal concentration; ATCC: American type culture collection; ELISA: Enzyme linked immunosorbent assay

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**Abstract**

*Securidaca longepedunculata* is a savanna tree found mainly in Africa and it is referred to as the mother of all medicines by the local Africans. There are only a few studies regarding antibacterial activities of this plant. The aim of the experiment is to determine the antibacterial activity of the root extract of the plant against *Staphylococcus aureus*, *Shigella sonnei*, and *Enterococcus faecalis* which are known pathogenic bacteria in human beings. An ethanol extract of the root of *S. longepedunculata* was prepared and after alcohol evaporation and dissolving in DMSO, antibacterial activity was tested by determination of MIC. Control studies were also done with DMSO. The study revealed significant antibacterial activity of the plant against the tested Gram positive bacteria namely *S. aureus* and *E. faecalis* and not against the Gram negative tested bacteria *S. sonnei*. The findings of this study will help the suffering humanity to combat antibacterial drug resistance resulting lots of treatment failures which leads to increased morbidity and mortality in recent times. As this is only an *in vitro* study, further *in vivo* experiments are necessary before its actual application in human beings.

**Citation:**

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1. Introduction

*Securidaca longepedunculata* is a semi deciduous tree of size of about 6-12m, it is commonly known as the violet tree with a characteristic pale gray bark. It is a native of the Limpopo province lying in the northern region of the South Africa. This plant is referred as the ‘king of all medicines’ by the people of Africa. The taxonomic classification of the tree has been described in the Table 1. They have leaves of varying sizes and colour which are borne in clusters on dwarf branchlets, mostly spined-tipped. They have fine hairs when young but are shed off when they mature. Flowers are small, pink/purple in colour, sweetly scented, hermaphrodite (consist of both male and female sex organs) and are of 10mm in size. The flowers consist of five sepals, out of which the two lateral ones are winged petals and the other three petals being hooked together. The eight stamens are joined together forming tube like structure.. The flowers grow on a long peduncle hence accounts
for the name of the species: longepedunculata. The flowering season is during the early summer. The Fruits are round with a distinct membranous wing of about 40mm, purple green when young and gradually turns pale straw coloured between April and August. Fruits hang on the branches for many months and those which remain longest germinate the best. The plant is best cultivated in moist soil.

Table 1: Taxonomy of Securidaca longepedunculata

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phylum</td>
<td>Tracheophyta</td>
</tr>
<tr>
<td>Class</td>
<td>Magnoliopsida</td>
</tr>
<tr>
<td>Order</td>
<td>Polygales</td>
</tr>
<tr>
<td>Family</td>
<td>Polygalaceae</td>
</tr>
<tr>
<td>Genus</td>
<td>Securidaca</td>
</tr>
<tr>
<td>Species</td>
<td>longepedunculata</td>
</tr>
</tbody>
</table>

1.1 Distribution
(Baloyi and Tshisikhawe, 2009) Angola, Benin, Burundi, Cameroon, Republic of Congo, Ethiopia, Gambia, Ghana, Guinea, Kenya, Malawi, Mozambique, Namibia, Niger, Rwanda, Zambia, South Africa. It also grows in other countries like Cuba, Malaysia.

1.2 Active ingredients
The phytochemical screening detected the presence of important secondary metabolites like alkaloids in both ethylacetate and methanol fractions. The major constituents in the ethyl acetate extract of the root of the S. longepedunculata are the flavonoids:1,7 -dihydroxy xanthone and 1,5 dihydroxy-3,4,6,7,8-pentamethoxy xanthone, 5-O-prenyl-1-hydroxy-2,3,6,7,8-pentamethoxyxanthone, β-sitosterol,3-hydroxy-6-methoxyxallic acid, quercetin-3-O-β-galactopyranoside, saponine aegycones, presenegenin, elynoclavine, sinapic acid , securinine, methyl salicylate (Meli et al., 2007). Further the acetone extract of the root yields compounds like 1,3,6,8-tetrahydroxy-2,5-dimethoxynanthone, 1,6,8-trihydroxy-2,3,4,7-tetramethoxyxanthone (Meyer et al., 2009 ). The aqueous and methanol extract of the fresh root contains gallic acid, chlorogenic acid, caffic acid, epicatechic acid, rutin, p-coumaric acid, cinnamic acid, apigenin, quercetin glucosyl and quercetin dehydrate (Muanda et al., 2010 ). 2-methoxy-3,4- benzyldihydrobenzoic acid, benzyl 2-hydroxy-6-methoxybenzoate, 6-hydroxy-2-methoxy benzoic acid, 1,6,8-trihydroxy-2,3,4,5-tetramethoxyxanthone, 1,6-dihydroxy-2,3,4,5,8-pentamethoxyxanthone, 8-hydroxy-1,4,5,6-tetramethoxy-2,3-methylenedioxyxanthone, 4,6,8-trihydroxy-1,2,3,5-tetramethoxyxanthone, 4,8-dihydroxy-1,2,3,5,6-pentamethoxyxanthone, benzyl 3-hydroxy-2-methoxybenzoate and some other xanthones were also isolated from the chloroform extract of the root(Dibwe et al., 2013 ).

Triterpene saponins such as 3-O-[β-D-glucopyranosyl presene- genin 28-O-[β-D-apiofuranosyl-(1-3)-β-D-xylopyranosyl-(1-4)-[β-D- apiofuranosyl-(1-3)]-α-L-rhamnopyranosyl-(1-2)-[4-O-([E]-3,4,5trimethoxyxynamoy]]]-β-D-fucopyranono ester were obtained from the 70% methanol extract of the root(Mitaine-Offer et al., 2010 ). Recently benzyl 2-hydroxy-5-methoxy benzoate is also found to be present in this plant (Keshebo and Choudhury 2015).

Sinapoic acid, caffic acid, 4,5-dicaffeoyl-D-quinic acid, 3,4,5-tricaffeoyl-D-quinic acid and a large number of monosaccharides and polysaccharides have been extracted from the methanol fraction of the stem (De Tommasi et al., 1993 ).

The anthraquinones present accounts for improved digestion and used as laxative, reduce inflammation in arthritis patients, naturally occurring anthraquinones have anti cancerous activity. The saponins help in cholesterol reduction by binding with the bile salt thereby forming small micelles helping in easy absorption in the GI tract. The non-sugar part of the saponin has direct anti-oxidant activity. They react with the cholesterol rich membrane of the cancerous cells limiting their growth. The flavonoids can regulate carbohydrate and lipid metabolism alleviate hyperglycemia (Salib et al., 2013), have anti cancerous activity, reduces risk of cardiological complications and reduce oxidative stress by quenching the free radicals and hence used as a folk medicine in Egypt as anti-diabetetic(Mohammad and Elham, 2013), and check resistance to insulin(Jennings et al., 2014 ). Elymoclavine is an ergot alkaloid produced from Pennisetum typhoideum. It is a precursor in the biosynthesis of D-lysergic acid. The elymoclavine is derived from L-tryptophan (Ahimsa-Müller et al., 2007).

Reported by Daper et al., the tannins form complex with proteins through hydrogen bonding, covalent and other hydrophobic interactions and thereby preventing protein synthesis. This explains the antibacterial activity of the plant extract and further protein precipitation; vasoconstriction checks ulcer development (Dapar et al., 2007).

Cardiac glycosides present in the methanol extract, aqueous extract, and chloroform extract of the root except in the hexane fraction account for its anti-bacterial activity especially if the aglycone portion is steroidal (Klen and Martinkova, 2001). Presence of alkaloids and flavonoids in both the methanol and aqueous extracts of the root of the plant reveals the efficacy of the plant in the field of antimicrobial activity (Ndamitso et al., 2013).
1.3 Medicinal use of the plant

Roots and barks are usually used in powdered form for treating chest infections, inflammation, abortion, tuberculosis, infertility, venereal diseases, headache, conjunctivitis, malaria, urethral discharge, rheumatism, fibrosis, sleeping sickness, depression (Maroyi, 2013). In the Limpopo province the aborigines use the roots for treating mental disorders. The root extract is mostly mixed with salt and water and used as antidote against snake bites (Augustino et al., 2011). Due to the severe pesticide resistance observed in different parts of South Africa, several aromatic plants especially like S. longepedunculata is used. The active ingredient isolated for the insecticidal nature is methyl salicylate (Bossou et al., 2013). The 70% methanol extract of the leaves yield a potent MIC of 0.313mg/ml and MBC of 0.625mg/ml was reported against Escherichia coli and Salmonella typhi. The chloroform leaf extract on the other hand yielded a ZI of 18mm against Pseudomonas aeruginosa at a concentration of 7.5mg/disc (Ndamitso et al., 2013). Moreover, the hexane and ethyl acetate fractions had ZI values over a range from 14 to 19mm against Streptococcus pyogenes, Pseudomonas fluorescens, and Klebsiella pneumoniae (Musa et al., 2013). The aqueous extract of the leaf yielded a MIC (Minimum Inhibitory Concentration) of 6.25mg/ml and MBC (Minimum Bactericidal Concentration) of 62.5mg/ml against Salmonella typhi. The MIC of 0.313mg/ml and MBC of 0.625mg/ml was reported against Staphylococcus aureus and Pseudomonas aeruginosa. Furthermore, the acetone root extract exhibited a total activity of 19.200ml/g against these target organisms (Ndamitso et al., 2013). The total activity indicates the degree of dilution of the active ingredient per gram of the plant extract which is still bacteriostatic. The 70% methanol extract of the leaves yield a potent MIC of 0.45mg/ml and 0.23mg/ml against Serratia marcescens and Shigella flexneri (Karou et al., 2012). A striking value in the MIC in the range of 0.0312 to less than 0.0250 mg/ml was noted against Mycobacterium species like Mycobacterium tuberculosis, M. bovis, M. avium, M. smegmatis (Ferreira et al., 2012). Thus S. longepedunculata has strong potential effects in the treatment of several bacterial infections.

1.5 Toxicity

In a summary reported in 1944, a toxicity risk is imposed if taken in excess. Saponin obtained in the root extract can cause severe damage to bone marrow and haemolysis when comes in contact with blood. The root extract also contains 0.4% methyl salicylate (Smith et al., 1979). Severe poisoning can occur due to ingestion of 10-30ml of methyl salicylate. Extensive use of the extracts of the plant by the African native has led to cortical necrosis, acute interstitial nephritis, diarrhoea, dehydration, and collapse (Kamsu-Foguema and Fougwu, 2014). Toxicity study in animal model also demonstrated toxic activities in higher doses (Keshebo et al., 2014). In the new database (Plants for a future), revised in 2006, S. longepedunculata has been recognized as a plant with no hazards when used optimally.

2. Objective of research

This study is justified due to the fact that newer antibacterial agents are necessary in the recent upsurge of antibiotic resistant microbes throughout the globe.

3. Materials and Methods

The powdered and dried form of the root of the plant taken for the experiment was prepared by two Authors (MKC, DLK) in Ethiopia, Africa. Micro-organisms used were Staphylococcus aureus ATCC 25923, Shigella sonnei (WHO EQAS, National Food Institute, Denmark), Enterococcus faecalis ATCC 29212.

3.1 Extract preparation

100ml of 95% ethanol was added to a conical flask containing 5g of the plant material (Fig.1). The solution is then shaken vigorously to homogenize it evenly and kept for 72 hours in the dark with shaking at regular interval of 24 hours. After 72 hours the solution was filtered using Whatman filter paper number 1. The filtrate was then stored in the deep freeze (~ 4°C) for 24 hours. Then alcohol was evaporated in a steam bath at a temperature of about 100°C. The processed plant material was weighed and a stock solution of 42mg/ml was prepared with pure DMSO (dimethyl sulfoxide) and the solution was uniformly homogenized by using vortex.
3.2 Minimum inhibitory concentration assays
0.5 McFarland opacity suspensions of the bacterial strains were prepared. The MIC assay (Choudhury et al., 2016; Chatterjee et al., 2016; Chatterjee et al., 2015) was performed in microtitre plate, and after the serial dilution of the extracts, 10µl of culture was inoculated in each well. It was mixed properly by shaking and was incubated at 37 °C for 20 hours. The absorbance was measured at 0 hour and at 20th hour and the absorbances using an ELISA plate reader were taken at 620 nm.

4. Results
This study performed on the test organisms showed the MIC values 10mg/ml and 5mg/ml for S.aureus and E.faecalis respectively. S.sonnei was not inhibited (Figure 2-4).

**Figure 1:** Dried powdered form of the root of S. longepedunculata

**Figure 2:** Absorbances of the growths of S. aureus in different concentrations of S. longepedunculata (deviations from 0 hr baseline)

**MIC of the extract of Securidaca on S.aureus**

![Graph](image)

**Figure 3:** Absorbances of the growths of E. faecalis in different concentrations of S. longepedunculata (deviations from 0 hr baseline)

**MIC of the extract of Securidaca on E.faecalis**

![Graph](image)
5. Discussion

The MIC values indicate that the plant extract shows antibacterial potency against Gram positive \textit{S. aureus} and \textit{E. faecalis} while it shows no effect against Gram negative \textit{S. sonnei}. Thus the results show that the root extract of the plant \textit{S. longepedunculata} has bioactivity and pharmaceutical action. However, in a previous study MIC value of the acetone extract against \textit{S. aureus} was found less (Ngonda et al., 2012), than that in our study. There are also reports regarding remarkable action of this extract against both Gram positive and Gram negative bacteria (Musa et al., 2013), but in our study we did not get any action against \textit{S. sonnei}, which is a Gram negative bacteria. The presence of outer lipopolysaccharide membrane (LPS) in Gram negative bacteria serves as an impermeable barrier against most drugs. This is a possible reason due to which the MIC values indicate that the plant has no action against \textit{S. sonnei}. This is the first report of the action of this plant on \textit{S. sonnei} and \textit{E. faecalis}. According to some flavonoids and saponins may be responsible for antimicrobial activities (Ngonda et al., 2012). Synthesis of flavonoids is triggered by infection in nature thus these may be important agents for such antimicrobial activity (Bennett and Wallsgrove, 1994). The lipophilic nature of flavonoids may lead to damage of the cell membrane (Tsukiya et al., 1996).

Conclusion

Antibiotic resistance has led to the upsurge among the researchers to discover new chemotherapeutic agents to counteract the spreading of the resistant pathogenic strains and thereby research on herbal medicine has gained momentum over the years which were traditionally used by the local people for medication. Further research should be carried out to identify the key active ingredients of \textit{S. longepedunculata} which are responsible for these antibacterial activities so that it can be used as a novel antibacterial formulation. Extensive work on the \textit{in vivo} experiments and clinical trials on human also need to be carried out so that this plant can play a promising role in the field of antimicrobial therapy.

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Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Research Highlights

Antibacterial studies of \textit{Securidaca longepedunculata} of the Limpopo province of northern region of the South Africa which is also referred as the ‘king of all medicines’ by the people of Africa is explored in this study. This study performed on the Gram positive bacteria \textit{S. aureus} and \textit{E. faecalis}, and one Gram negative bacteria \textit{S. sonnei}. Alcoholic extract of the plant was prepared, followed by removal of alcohol and solution was prepared in DMSO. MIC values for \textit{S. aureus} and \textit{E. faecalis} were 10mg/ml and 5mg/ml respectively while \textit{S. sonnei} was not inhibited. The findings may help to use it as a new antimicrobial agent in future to fill up the gap of newer antimicrobial agents in recent times.
Limitations

*In vivo* study is essential before its application in human beings. Further experiments with chemical components of the plant may reveal interesting findings.

**Recommendations**

*In vivo* study should be done and a detailed study of the chemical components is also recommended.

**Funding and Policy aspects**

The Government should encourage a detailed study of this plant.

**References**


xanthones from *Securidaca longopedunculata* (Polygalaceae). Planta Medica, 73, 411.


