ANTIMICROBIAL ACTIVITY OF *STRYCHNOS HENNINGSII* (GILG) LOGANIACEAE LEAF AND ROOT AQUEOUS EXTRACTS

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Abstract
Strychnos henningsii (Gilg) Loganiaceae plant extract has been used for treatment of various ailments such as rheumatism, gastrointestinal complications and syphilis. It is also used to prepare milk soups and fatty-meat. *S. henningsii* leaf and root water extracts have bioactive chemicals. This study was carried to validate antimicrobial activity of *S. henningsii* extracts in the treatment of bacterial ailments. Antimicrobial activity of aqueous extracts was tested using isolates of bacteria (*Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi).* Commercial discs (30µg Tetracycline, 10µg Gentamycin, 30µg Kanamycin, 30µg Chloramphenicol, 30µg Augumentin, 25µg Cotrimoxazole, 10µg Ampicillin and 30µg *Cenfuroxime) were used as positive controls. The antibacterial activity of aqueous extracts in* concentrations of (100, 50, 25 and 12.5) µg/ml was determined by agar disc diffusion assay. The most susceptible bacteria in extracts concentration of 100µg/ml were *S. typhi* (11.00 ± 1.00 and 10.67 ± 1.20) mm followed by *S. aureas* (10.00 ± 1.00 and 9.00 ± 1.00) mm. The most resistant bacteria were *P. aeruginosa* and *S. typhi* in leaf and root extracts respectively at concentration of 50µg/ml. *S. aureas* was active in all extracts concentrations. The inhibition zones for antibiotics (12.00 ± 0.1 to 21.00 ± 0.3)mm were larger than those of the plant extracts (6.67 ± 0.30 to 11.00 ± 1.00)mm except for Tetracycline (7.00 ± 0.0)mm. *S. aureas* was resistant to Kanamycin and Chloramphenicol, *S. typhi* was resistant to Tetracycline and Chloramphenicol, *P. aeruginosa* was resistant to Chloramphenicol. Gentamycin, Tetracycline, Kanamycin and Chloramphenicol were active against *E. coli*. Gentamycin was active in all the bacterial strain tested. The results show considerable antimicrobial activity against the bacteria species tested. The present study justifies the use of aqueous extracts by herbalists in the treatment of diseases caused by bacterial infections.

Key words: *Strychnos henningsii,* commercial disc, antibacterial activity, aqueous extracts.

1.0 Introduction

For centuries, therapeutic properties of various medicinal plants have been used for the treatment of human and livestock health disorders worldwide (Yadav *et al.*,

2014). The demand for herbal drugs worldwide is large and steadily growing (Kuria *et al*., 2012). In developing countries, 80% of the population use traditional medicine mainly due to challenges in accessing the conventional medicine attributed to their low income or poverty (Survase and Raut, 2011); (Maina *et al.*, 2013); (Tirop *et al*., 2018).

Infectious diseases such as tuberculosis, bacterial pneumonia, influenza and diarrhea are the world's main cause of human mortality (Ngoci *et al*., 2012). Doughari and Okafor, (2007) reported drug resistance against bacteria due indiscriminate use of commercial antimicrobial drugs in the treatment of infectious diseases. Today limited antimicrobial spectrum has been reported, cases of side effects and emergence and reemergence of infections (Huie, 2002). Plant-based antimicrobials represent a vast untapped source of medicines and hence further exploration of plant-based antimicrobials.

Strychnos henningsii (Gilg) is a member of the *Plantae* kingdom*;* Loganiaceae family; *Strychnos* genus*; henningsii* species (Orwa *et al.*, 2009). The local names are: Muteta (Kikuyu/Kamba), Maset (Kipsigis), Entuyesi (Maasai), Kapkamkam (Pokot), Muchimbi (Meru), Turukukwa (Tugen), Yapolis (Turkana), Nchipilikwa (Samburu) and Hadesa (Somali). Henning's Strychnos and Red bitter berry (English), Koffieharderpper (Afrikaanas) (Gachathi, 2007) are the common names (Maundu and Tengnas, 2005). *S. henningsii* (Gilg) Loganiaceae, is an endangered species which used to be found in many countries in the World and is considered a multipurpose tree whose main function is treatment of gastrointestinal complications (Kipkemoi *et al*., 2013). The stems, roots, leaves, bark and fruits of the plant are prepared in a number of ways depending on the condition being treated, though the most often used plant part is the bark (Schmelzer and Gurib-Fakim, 2008). In East Africa, bark, root, and stem extracts are used to prepare meat and milk soups among various communities. The Kikuyu, Maasai, and Kamba communities use it for treatment of painful joints, fitness and general body pains (Chapman *et al*., 1997). Further, the soup from the plant is used as an aphrodisiac and for treatment of nausea, colic and syphilis (Kuria *et al.*, 2012). The fruits of *S. henningsii* are used by Mbeere people of Kenya to flavor traditional beer (Chapman *et al*., 1997). Other medicinal uses of the plant include treatment of rheumatism and snake bites (Kipkemoi *et al*., 2013). The bark is used by herbalists as purgative (Schmelzer and Gurib-Fakim, 2008), while some alkaloids have muscle relaxing effects (Kuria *et al*., 2012). In livestock, the ground bark has been used as antiseptic and for treatment of cattle's wound (Kipkemoi *et al*., 2013).

Some of the phytochemicals found on stem and bark extracts include alkaloids, saponins, tannins, flavanoids, phenols, terpenes, steriods and tannins (Marles and Farnsworth, 1995). *In vitro* and *in vivo* studies of the plant extracts possess anti-inflammatory, antioxidant, antispasmodic and analgelsic activities (Tits *et al*., 1991);

(Oyedemi *et al.*, 2010). The mono and bis-indole alkaloids isolated from the plant have high antiplasmodial activities (Frederich *et al.*, 2008). According to the study by Tirop *et al.*, 2018, acute and sub-acute toxicity showed the leaves and root aqueous extracts to be relatively safe up to dose of 2500 and750 mg/kg respectively *S. henningsii* (Gilg) Loganiaceae leaves aqueous extract from sample collected from Eastern Province Kenya has been reported to have antimicrobial activity against *Staphylococcus aureas, Escherichia coli* and *Bacillus subtilis* (Kareru *et al.*, 2008) The composition of herbal medicines varies depending on geographic region and the growing conditions. Environmental factors such as: altitude, soil, rainfall pattern, length of daylight, seasonal variation in temperature, among others, can affect the concentration of specific phytochemicals in herbal plants (Zhang *et al.,* 2012). Moreover, other factors such as: part of plant used, processing method, seeding time, genetic make-up, planting density, use or non-use of pesticides and fertilizers, may have a similar effect (Barnes and Anderson, 2007).

In spite of its use, the scientific literature has limited information of the antimicrobial activity of *S. henningsii* leaf and root aqueous extracts. This study therefore sought to validate antimicrobial activity of *S. henningsii* leaf and root aqueous extracts.

2.0 Materials and Methods

2.1 Plant collection and extraction

Samples of *S. henningsii* leaves and root were collected from Kabiruini forest (on latitude 0°23'0"S and longitude 36°57'0"E) in Nyeri County, Central Kenya. Authenticity of the plant was confirmed by a taxonomist at the Department of Botany, Jomo Kenyatta University of Agriculture and Technology (JKUAT) Kenya and was given voucher specimen number (*S. henningsii* 001/2014) and deposited at the herbarium. Root barks were peeled off and root cut into small pieces. The leaves and root were shade-dried at ambient temperature. It was then ground using a plant mill into powder form. 200g of leaves and 500g root powders were obtained. The powders were then stored in sterilized sealed containers at room temperature (25°C). The samples were extracted using the previously described methods of (Kofi-Tsekpo *et al.*, 1985) and (Harborne, 1998). Briefly, 100g each of the powdered *S. henningsii* leaves and root material were boiled in 1L of distilled water at 98°C for 2 hrs. The solution was then allowed to cool. The extracts were then decanted into a 1L clean dry conical flask and filtered through a number 1 Whatman® filter paper **(**Whatman International Ltd Maidstone, England) under vacuum pump into a 500ml clean dry conical flask. Decantation and filtration processes were repeated until the sample became clear. The filtrate was centrifuged at 3000rpm for 5 minutes and the supernate obtained were 400ml of leaves and 500ml of root extracts. The supernate was quickly frozen at -40°C and dried for 48h using a freeze dryer (Alpha 1-4 LD Plus (Christ, Germany) to give a yield of 13.25g and 12.6g of dry root and leaves extracts respectively. The resulting extracts were reconstituted in distilled water to give

concentrations of (100, 50, 25 and 12.5) µg/ml. The samples were kept in fridge at 4°C before use in subsequent bioassay.

2.2 Bacteria Isolates

Preparation of Agar Medium

The recommended medium for disc diffusion testing is Mueller-Hinton agar (MHA) (NCCLS, 2000). MHA (Oxoid, UK) was prepared from a commercially available dehydrated base, according to the manufacturer's instructions. The prepared medium was autoclaved at 121°C, 15 Ibs pressure for 15 mins and immediately placed in a 50°C water bath. On cooling, the medium was poured into round plastic flat-bottomed petri dishes on a level surface to give a uniform depth of about 4mm and allowed to cool to room temperature.

2.3 Isolation of the Bacteria

The clinical samples of *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Salmonella typhi* were obtained from Department of Medical Microbiology, JKUAT. Using a wire loop, the specimens were removed from the tubes and inoculated onto plates containing MHA (Oxoid, UK), which were then incubated at a temperature of 25°C for 72 hrs.

2.4 Gram Staining Microscopy

The gram staining procedure of the isolates was carried out according to the Hucker method (Collins and Lyne's, 2004). The isolates were maintained at -80^{o} C in Trypticase broth until use.

2.5 Antimicrobial Assay

Agar Disc Diffusion Method

Sterilized whatmann No.1 (6mm) filter papers disks were saturated with filtered sterilized plant extracts (leaf and root) of desired concentration ranging from 12.5 to 100µg/ml (Ergene *et al*., 2006; Hudzicki, 2009). The impregnated sterilized filter papers with plant extracts (leaf and root) was placed on the surface of suitable solid agar medium Mueller Hinton (Mueller and Hinton, 1941), Then the media was pre-inoculated with the test isolates of the bacteria species (*Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi)*. The standard inoculum size was 1 x 10^{8} CFUs/ml of bacteria for inoculating diffusion plates (Baris *et al*., 2006). The process of drying impregnated paper disk was done overnight under a laminar flow cabinet (Basri and Fan, 2005). The plates were incubated for 24 hours at 37°C (Baris *et al*., 2006). All tests were performed in triplicate. After incubation a prominent point was noted, where there was 80% growth reduction. The zone diameter was measured to the nearest whole millimeter using a transparent ruler and recorded against the corresponding plant extracts (Das *et al*., 2010). The same procedure was repeated using commercial disc (30µg Tetracycline, 10µg Gentamycin, 30µg Kanamycin, 30µg Chloromphenical, 30µg Augumentin, 25µg

Cotrimoxazole, 10µg Ampicillin and 30µg Cenfuroxime) as positive controls (Kareru *et al*., 2008).

2.6 Data Analysis

The data obtained on pharmacological efficacy were analyzed using t-test. The data was then expressed as means ± standard deviation (S.D) of three replicates. Statistical analysis was conducted using IBM® SPSS Statistics Version 21 (International Business Machine Corporation, USA). The values obtained were considered significant at $P \leq 0.05$.

3.0 Results and Discussion

In African traditional medicine, *S. henningsii* leaves and root aqueous extracts have been used to treat a variety of conditions including syphilis, snake bites, gastrointestinal complications (purgative), rheumatism, snake bites, malaria, diabetes mellitus and dysmenorrhoea (Kuria *et al.*, 2012) . It is also used to prepare milk soups and fatty-meat (Chapman *et al*., 1997). The dosage used by Kenyans herbalists is 750mg/kg/day (Kuria *et al*., 2012).

3.1 Antimicrobial Activity

The inhibition zones of the plant extracts increased on increasing the concentration of the extracts except for leaves extracts using *E. coli* (Figure 1) showing a concentration dependent activity. This is an indicator that better activity can be achieved by increasing the concentration of extract or bioactive compounds. The most susceptible bacteria in extracts concentration of 100µg/ml were gram negative bacteria *S. typhi* (11.00 ± 1.00 and 10.67 ± 1.20) followed by gram positive bacteria *S. aureas* (10.00 ± 1.00 and 9.00 ± 1.00) (Figure 1). This is not in agreement with the findings of Mohamed *et al*., (2010) which report that extracts are more active against gram positive bacteria than gram negative bacteria. This is attributable to gram negative bacteria having lipoproteins and lipopolysaccharides that prevent large hydrophilic molecules from reaching an otherwise susceptible cellular target and also it has an outer membrane that serves as an impermeable barrier for many small molecules (Kaur and Arora, 2009). This could be explained by the fact that the molecules in the plants extracts were able to penetrate the impermeable barrier.

The most resistant bacteria were *P. aeruginosa* and *S. typhi* in leaves extracts and root extracts respectively at concentration of 50µg/ml. *S. aureas* was active in all the extracts concentrations (100, 50, 25 and 12.5)µg/ml (Figure 1). In the current study, the antimicrobial activity result confirms the considerable antimicrobial activity against the bacteria at the tested concentrations (100, 50, 25 and 12.5µg/ml) of *S. henningsii* extracts. This finding was similar to the previous study by (Yadav *et al*., 2014) which showed that alcoholic extracts of *Strychnos potatorum* had antibacterial activity against *Salmonella typhimurium, Escherichia coli* and *Staphylococcus aureas* at the tested concentration of (100, 200)µg/ml. The findings

of this study suggests that the compound responsible for antimicrobial activity was present in each extract at different concentrations as similarly observed by Rojas *et al*., (2006).

The four antibiotics (Gentamycin, Tetracycline, Kanamycin and Chloramphenicol) were active against *E. coli*. Gentamycin was active in all bacterial tested. Gentamycin is a broad spectrum antibiotic whose mode of action is by irreversibly binding the 30S sub unit of the bacterial ribosome, interrupting protein synthesis (Walsh, 2003). The zones of inhibition of the antibiotics (12.00 ± 0.10 to 21.00 ± 0.30) were greater than that of the plant extracts except for Tetracycline (7.00 ± 0.00) which was in the same range (between 6.67 ± 0.30 to 11.00 ± 1.00) as that of plants extracts. This finding suggests that the active components of the extracts comprised only a fraction in the extracts used.

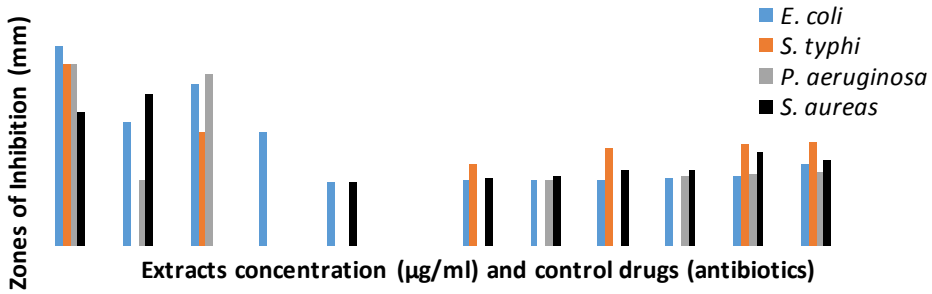

Figure 1: Antimicrobial activity of plant extracts

Note: 0.00 = No inhibition zone around the disc of either leaves or root aqueous extracts or controls.

Antibiotics: Briefly, 30µg Augumentin, 25µg Cotrimoxazole, 10µg Ampicillin and 30µg Cefuroxime were resistant to all of the bacteria strain tested.

A comparison of activities of different concentrations *S. henningsii* extracts revealed that;

Table 1: A comparison of Antimicrobial activities of different concentrations S. henningsii extracts using T-test

Different Aqueous Extracts Concentrations	**T- Test of Significant**
Leaves extracts at 100µg/ml	Not significant between the bacteria except between *S. typhi* and *P. aeruginosa* (*P*=0.035). Significant between the antibiotics and bacteria except; Tetracycline against *P. aeruginosa* (*P*=.0184), Kanamycin against *S. typhi* (*P*=0.184).

Leaves extracts at 50µg/ml	Not significant between other bacteria except between *S. aureas* and *S. typhi* (*P*=0.02). Significant between all antibiotics and bacteria except; Kanamycin against *S. typhi* (*P*=0.199).
Leaves extracts at 25µg/ml	Not significant between the bacteria. Significant between the antibiotics and bacteria except; Kanamycin against *S. typhi* (*P*=0.063).
Leaves extracts at 12.5µg/ml	Not significant between the bacteria Significant between Gentamycin (*P*=0.007) and Tetracycline (*P*=0.0001).against *E. coli.*
Root extracts at 100µg/ml	Not significant between the bacteria except between *S. typhi* and *P. aeruginosa* (*P*=0.034), *E. coli* and *S. typhi* (*P*=0.02). Significant between all antibiotics and bacteria except; Kanamycin against *S. typhi* (*P*=0.225).
Root extracts at 50µg/ml	Not significant between the bacteria. Significant between all antibiotics and bacteria except; Tetracycline against *S. typhi* (*P*=1.00).
Root extracts at 25µg/ml	Not significant between the bacteria. Significant between Gentamycin (*P=.010*) and Tetracycline (*P*=0.001) against *S. aureas*

S. aureas was resistant to Kanamycin and Chloramphenicol, *S. typhi* was resistant to Tetracycline and Chloramphenicol, *P. aeruginosa* was resistant to Chloramphenicol (Figure 1). Chloramphenicol is a basteriostatic by inhibiting protein synthesis. It prevents protein chain elongation by inhibiting peptidyl transferase activity of bacterial ribosome. It specifically binds to A2451 and A2452 residues in the 23S rRNA of the 50S ribosomal subunit, preventing peptide formation (Allison *et al.*, 1962). Resistance to Chloramphenicol is mostly due to inactivation of the antibiotic by Chloramphenicol acetyltransferase (CAT) enzymes that acetylate the antibiotic (Rossolini *et al.*, 2017).

4.0 Conclusion

The results show considerable antimicrobial activity against the bacteria species tested. The aqueous plant extracts had broad spectrum activity in that it inhibited growth of both gram positive and gram negative bacteria. The present study justifies the use of *S. henningsii* aqueous extracts by herbalists in treatment of infections caused by the tested bacteria.

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