



## **Efficacy of *Securidaca longipedunculata* Fresen (Polygalaceae) against Two Standard Isolates of *Neisseria gonorrhoeae***

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. All the authors participated in the conception and design of the study. Author WEM carried out phytochemical extractions and sensitivity tests, and also contributed to statistical analyses, and wrote the first draft of the manuscript. Authors WEM and MO managed the protocols and administration of the study. Author ZNOA organized and formatted the article. All authors read and approved the final manuscript.*

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### **ABSTRACT**

**Aim:** A study was carried out to determine the *in vitro* antibacterial activity of ethanol extract of root and bark of *Securidaca longipedunculata* against two standard isolates of *Neisseria gonorrhoeae* NO.0296 and WHO- K.

**Materials and Methods:** Plant materials were collected and dried at room temperature, followed by ethanol extraction using a rotary pump. Antibacterial activity was done on the isolates using agar disc-diffusion method with Dimethyl Sulfoxide (DMSO) as a negative control and ceftriaxone as a positive control. Zone of inhibition was measured in millimeters. Minimum bactericidal concentration of the extract was determined using different concentrations of the extract then plated on *Neisseria gonorrhoeae* (GC) medium and a standard protocol was used to determine the presence of phytochemical compounds.

**Results and Discussion:** The results obtained showed that both the root and bark extracts of S.

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*longipedunculata* have antibacterial activity against the two bacterial strains with a zone of inhibition of  $\geq 10\text{mm}$  and a bactericidal activity at  $0.01\mu\text{g}^{-\text{ml}}$  of 10% crude extract. Comparison of susceptibility on tetracycline, penicillin and ciprofloxacin with the root and bark extract showed higher susceptibility to the extracts. The extracts showed presence of saponins, steroids, glycosides, flavonoids, terpenes, alkanoids, phenolics and tanins. The demonstrated antibacterial activity of *S. longipedunculata* against *N. gonorrhoeae* provides a scientific basis for the traditional use in treating venereal diseases in western Kenya.

**Conclusion:** This investigation and further studies will pave the way for use of this plant in antibacterial drug development for alleviating human suffering. We recommend further studies to identify the specific compound(s) responsible for the antibacterial activity.

**Keywords:** *Securidaca longipedunculata*; phytochemicals; *Neisseria gonorrhoeae*.

## 1. INTRODUCTION

*N. gonorrhoeae* is a sexually transmitted bacterium that causes infections in the cervix, the urethra, the rectum, pharynx of adults, and in newborn infants, it affects the eyes. It belongs to the genus *Neisseria* within the family Neisseriaceae [1]. It is a kidney bean shaped Gram-negative, non-spore forming, non-motile, encapsulated, and non acid-fast bacterium. It requires an aerobic environment with added  $\text{CO}_2$  and enriched media such as chocolate agar for growth. It is oxidase positive and produces  $\beta$ -lactamase. Small, smooth, and non-pigmented colonies are produced after 18-24 hours of incubation. WHO estimates that 340 million new cases of Gonorrhea, Chlamydia and other sexually transmitted infections occur every year, of which 85% are in developing countries [2]. Gonorrhea and Chlamydia account for 62 million and 92 million new infections respectively (2).

Antimicrobial resistance in gonorrhea is of increasing concern, and successful treatment of gonorrhea is becoming more difficult. Gonorrhea causes pelvic inflammatory disease in women, which can lead to severe reproductive health complications, e.g., ectopic pregnancy, chronic pelvic pain and even tubal infertility [3]. Though not common, gonococci infections can also result in localized septic arthritis, endocarditis, and meningitis and eye infections that can change course to blindness in infected infants. Gonococcal infections can increase the risk for sexual transmission of human immunodeficiency virus (HIV) [4,3].

Medicinal plants are the major sources of new medicines and may constitute an alternative to the usual drugs [5,6,7]. The violet tree, *Securidaca longipedunculata*, is the most popular of all the traditional medicinal plants and is used for almost every conceivable ailment [8,9]. It is

widely distributed in woodlands and arid savannas of tropical Africa, especially in the Northwest and in the Lamoon provinces of South Africa and Mozambique. The root and bark are taken orally either powdered or as the infusion for treating chest complains, inflammation, abortion, ritual suicide, tuberculosis, infertility, venereal diseases and for constipation [10,11]. A toothache can also be relieved by chewing the roots. Powdered roots are used to treat a headache by rubbing them on the forehead. Infusions of the root are used for washing topical ulcers [12]. Studies show that violet tree has antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The tree has been used traditionally to treat venereal diseases, thus the aim of this work is to evaluate the antibacterial potential of the root and bark extracts on *N. gonorrhoeae*, determine the phytochemicals in the extract and to test the different antibiotics used in comparison with the extracts.

## 2. MATERIALS AND METHODS

### 2.1 Bacterial and Plant Samples

Two standard isolates of *N. gonorrhoeae* from gonococcal surveillance laboratory were sub-cultured and pure cultures grown on modified Thayer-Martin agar media. Plant samples were collected from Bungoma County after being identified by the locals who use it. They were brought to the laboratory and dried at room temperature and ground into powder. Extraction was done using 90% Ethanol and solvent removed by using a rotary evaporator.

### 2.2 Preparation of the Plant Extracts

From each material 600 g of powder was weighed separately using a digital weighing machine and placed in a conical flask and 1500

ml of 90% ethanol added to it. The mixture was stirred gently, tightly covered and left for 24 hours. The mixture was filtered using Whatman filter paper No. 1. The filtrate was placed in a rotary evaporator at 40°C to remove the solvent [13].

### 2.3 Antibiotic susceptibility Testing

The bacterium was sub-cultured in modified Thayer-Martin agar then colonies picked and put in Muller Hinton broth. Turbidity was adjusted to fit the 0.5 McFarland standard and examined for susceptibility to penicillin (5 µg), ciprofloxacin (5 µg), tetracycline (30 µg), ceftriaxone (30 µg) and azithromycin (15 µg) on modified Thayer-Martin medium by Kirby Bauer disc diffusion method as per CLSI guidelines and protocol (2015). Data was presented as percentages.

### 2.4 Antibacterial Activity of the Extract

A 24 hour old culture of the bacterium was used as inoculums for the tests. The bacterial culture turbidity was adjusted to 0.5 McFarland. The Modified Thayer-Martin (MTN II) medium for bacterial growth was prepared and sterilized; it was then poured in sterile Petri dishes. Bacterial lawns were made aseptically on the agar plates using sterile swabs then three wells were made on each of the agar plates using sterile Durham's tubes. Water based 50 µl of 10% crude extract of root and bark were used. Ceftriaxone with water as a solvent was used as a positive control and DMSO as a negative control. 50 µl extract was placed in the wells and incubated at 37°C for 24 hours. After the incubation time, the plates were examined for the presence of inhibition as a property of antimicrobial activity. The following was used as the standard: no activity (<7 mm), 8–11 mm active, >12 mm very active.

Different concentrations of 10% crude extract thus  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$  and  $10^{-6}$  was prepared and also tested against the plant extract to confirm the minimum inhibitory concentration from the bark or roots of the aqueous extract of *S. longipedunculata*.

### 2.5 Determining the Bactericidal Activity of the Extract

The bactericidal activity of the extract was determined by preparing Modified Thayer-Martin agar with different concentrations of the 10% crude extract thus, 18ml of media and two ml of the extract starting with  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$

and  $10^{-6}$  and inoculated with *N. gonorrhoeae* and incubated for 24 hours before examining for the bactericidal activity by checking the plates for bacterial growth. The plate concentration at which the bacteria started growing was termed as minimum inhibitory concentration. Antibiotic susceptibility of the common antibiotics was done to determine which antibiotic is much more effective and to compare with the extract to confirm its strength. This was done by aseptically preparing GC media and putting discs of the different antibiotics that are used to treat gonorrhoea and cultured overnight. The zones of inhibition were then measured in millimeters and presented using a pie-chart showing percentages of the zones of inhibition. Data was analyzed by subjecting to Analysis of Variance (ANOVA) at p value = 0.05. The null hypothesis was that Ethanolic root and bark extracts of *S. longipedunculata* do not have activity against *N. gonorrhoeae*.

### 2.6 Phytochemical Analysis

The methods described by [14] were used to test for the presence of saponins, tannins, phenolics and alkaloids. Lieberman Burchard reaction as described by [15] was used to test for steroids and the Salkowski test was used to test for the presence of glycosides. Testing for saponins, each extract (0.5 g) was mixed with water in test tube. Foaming that persisted on warming was taken as an evidence for the presence of saponins. Testing for tannins and phenolics, each extract (0.5 g) was separately stirred with 10 ml of distilled water and then filtered. A few drops of 5% FeCl<sub>3</sub> reagent were added to the filtrate. Blue-black or blue-green coloration or precipitation was taken as an indication of the presence of phenolics and tannins. Testing for alkaloids, each extract (0.5 g) was stirred with 5mL of 1% hydrochloric acid (HCl) on a steam bath. The solution obtained was filtered and one 1mL of the filtrate was treated with a few drops of Mayer's reagent. The turbidity of the extract filtrate on addition of Mayer's reagent was taken as evidence of the presence of alkaloids in the extracts.

### 2.7 Testing for Steroids

To test for steroids, 0.5 g of each extract was separately added with 5 drops of acetic anhydride and then a drop of concentrated sulphuric acid H<sub>2</sub>SO<sub>4</sub>. The mixture was steamed for 1 hour and neutralized with sodium hydroxide (NaOH), followed by the addition of chloroform.

The appearance of a blue-green color indicated the presence of steroids. Testing for glycosides, 0.5 g of each extract was dissolved in 2 ml of chloroform. H<sub>2</sub>SO<sub>4</sub> was carefully added to form a lower layer. A reddish brown color at the interface indicated the presence of a steroidal ring, that is, a glycone portion of the cardiac glycoside.

### 3. RESULTS AND DISCUSSION

A number of phytochemical compounds were confirmed to be present in the root and bark extracts (Table 1). The root and bark extract of *S. longipedunculata* have antibacterial activity against *N. gonorrhoeae*, the causative agent for gonorrhoea. The bark showed a higher antibacterial activity compared to the roots. The bark and root extract of *S. longipedunculata* have a plethora of phytochemicals, which contribute to their antibacterial activities against *N. gonorrhoeae*. These phytochemical compounds have been documented as being biologically active and exhibiting their effects on physiological activity [16].

The extracts also showed bactericidal effects against the two standard isolates of *N. gonorrhoeae*, WHO standard K and the US standard isolate 0296, suggesting that a therapeutic concentration could be attained in a living host. The high antimicrobial activity of the ethanol extract observed is similar to the findings of [11], who attributed these activities to the high content of flavonoid. For the comparison with the common antibiotics used, the root and bark showed a higher antibacterial activity compared to penicillin, tetracycline and ciprofloxacin. This justifies its need to be used as a traditional medicine.

**Table 1. Phytochemical analysis of the ethanol extract from the stem bark and Root bark of the *S. longipedunculata***

Pytochemical components	Stem bark	Root bark
Saponins	+	+
Tannins	+	+
Phenolics	+	+
Glycosides	+	+
Flavanoids	+	+
Steroids	+	+
Alkaloids	+	+

Table 1 shows the phytochemical profile of *S. longipedunculata*. The ethanolic extracts from

stem bark and root bark of the plant contained alkaloids, flavonoids, glycoside, phenols, saponins, steroids and tannins.

#### 3.1 Antibacterial Activity of the Root and Bark Extract of *S. longipedunculata*

Both the root and bark extracts showed antibacterial activity to *N. gonorrhoeae* strains, thus WHO standard K and the US standard isolate 0296 from the urethral swab having shown some zone of inhibition with the bark extract having a higher activity against the microorganism compared to the root extract. Table 2 shows the antibacterial activity of 10% of the root and bark extract of *S. longipedunculata*.

One sample t-test was used to test the performance of the extracts against the null/negative control (DMSO) in order to statistically determine if the extracts were active against *N. gonorrhoea*. The results (Table 2) indicated that the mean zone of inhibition for extracts was significantly greater than the null value ( $t= 40.695$ ,  $P<.001$ ). As such it was concluded that the extracts were active against *N. gonorrhoea*.

Again, one sample t-test was used to test the performance of the extracts against the positive control (ceftriaxone). To facilitate this analysis, the mean zone of inhibition for ceftriaxone across all trials was calculated and used as the test value against the mean zone of inhibition for the extracts. The results (Table 3) indicated that the mean zone of inhibition for extracts was significantly lower than the positive control value ( $t= -59.787$ ,  $P<.001$ ). This means that the extracts were not as active against *N. gonorrhoea* as was ceftriaxone.

Further, one-way analysis of variance (ANOVA) was conducted to determine the difference in antibacterial activity between the root and bark extracts of *S. longipedunculata*. As indicated in Table 4, there was significant difference between the extracts with the bark being more active than the root ( $F= 102.081$ ,  $P<0.001$ ).

The bark showed a higher activity compared to the root extract, which was 19.1 mm and 13.8 mm respectively. This shows a promising herbal antimicrobial alternative in the face of increasing resistance to synthetic antibiotics. The bioassay showed that the bactericidal activity of the extract was found to be  $10^{-2}$  thus  $0.01 \mu\text{g ml}^{-1}$ . Antibacterial susceptibility to the common antibiotics showed that *N. gonorrhoeae* is highly

susceptible to ceftriaxone with the highest zone of inhibition of 42 mm with WHO Standard isolate K, while in US -0296 has a zone of inhibition of 39 mm. The bark showed a higher zone of inhibition on the two isolates compared to ciprofloxacin and tetracycline, while in penicillin the isolates showed that they are resistant with a 0mm zone of inhibition.

The 10% root and bark extract showed susceptibility to *N. gonorrhoeae* as shown in the Fig. 1 where the bark extract was found to have the same zone of inhibition with ciprofloxacin and root extract was found to be more susceptible than tetracycline. *N. gonorrhoeae* was not sensitive to Penicillin.

**Table 2. Performance of the extracts against negative control**

One-sample test						
Test value = 0						
t	df	Sig. (2-tailed)	Mean difference	95% confidence interval of the difference		
				Lower	Upper	
Diameter of zone of inhibition	40.695	71	.000	16.47778	15.6704	17.2851

**Table 3. Performance of the extracts against the positive control**

One-sample test						
Test value = 40.686						
t	df	Sig. (2-tailed)	Mean difference	95% confidence interval of the difference		
				Lower	Upper	
Diameter of zone of inhibition	-59.787	71	.000	-24.20822	-25.0156	-23.4009

**Table 4. ANOVA results with mean values of zone of inhibition for extracts**

Descriptives						
Diameter of zone of inhibition						
	N	Mean	Std. deviation	Std. error	95% confidence interval for mean	
					Lower bound	Upper bound
Back extract	36	19.1056	2.78095	.46349	18.1646	20.0465
Root extract	36	13.8500	1.41674	.23612	13.3706	14.3294
Total	72	16.4778	3.43574	.40491	15.6704	17.2851

ANOVA						
Diameter of zone of inhibition						
	Sum of squares	df	Mean square	F	sig	
Between Groups	497.176	1	497.176	102.081	.000	
Within Groups	340.929	70	4.870			
Total	838.104	71				

**Table 5. Comparison of zones of inhibition of the plant extracts and standard antibiotics in the market**

Antibiotic	WHO-K	US STC-0296	% CONC
Ciprofloxacin	12 mm	19 mm	100%
Tetracycline	19.1 mm	13.1 mm	100%
Penicillin	0 mm	0 mm	100%
Ceftriaxone	42 mm	39 mm	100%
BARK	20.1	19.9 mm	10%
ROOT	13.5 mm	14.8 mm	10%

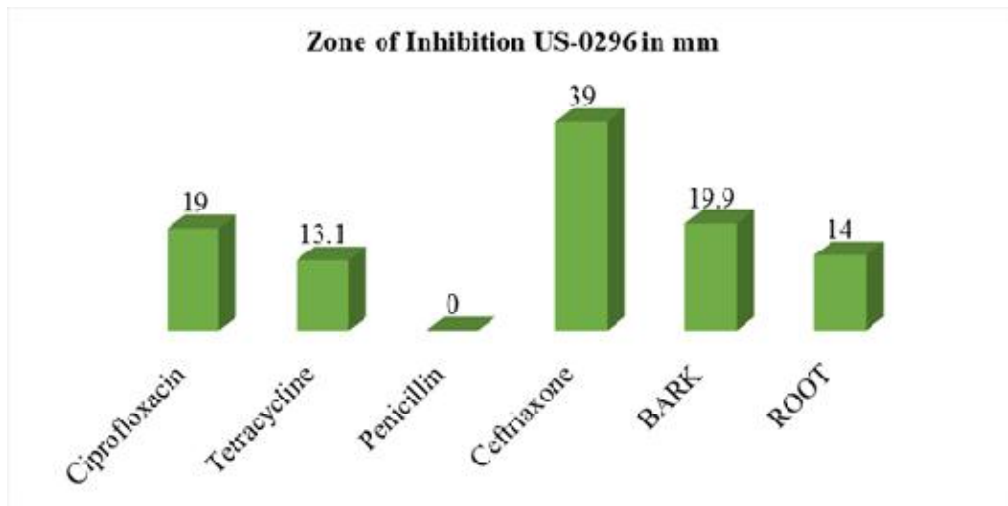


Fig. 1. Zones of inhibition on US STC-0296 expressed in percentages

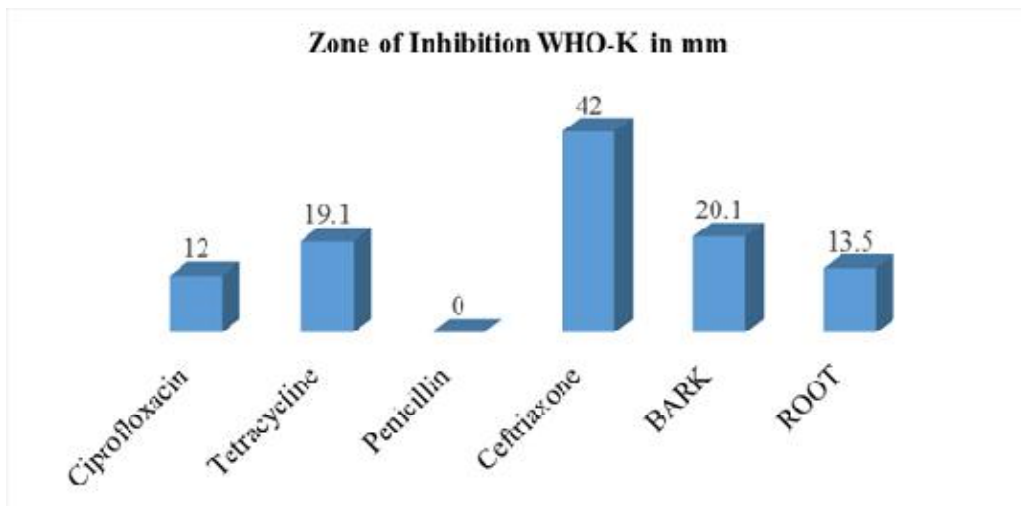


Fig. 2. Comparison of the zone of Inhibition of antibiotics to WHO-K

For WHO – K standard isolate was found to be more susceptible to ceftriaxone than the rest of the test antibiotic and the extracts as shown in Fig. 2 above. The activity of the plant extracts seemed to compare well with those of the antibiotics albeit in their crude form. Isolation and purification of the active compound is likely to improve pharmacological activity of the phytochemicals, and would be good candidates for drug development.

### 3.2 Bactericidal Activity of the *S. longipedunculata* Root and Bark Extract on *N. gonorrhoeae*

Different concentrations of the 10% extract was put in the modified Thayer-Martin media and

inoculated with WHO –K then incubated for 24hrs. The plates with a concentration of  $10^{-3}$  had bacterial growth, thus the minimum inhibitory concentration of root and bark extract after 24 hours was found to be  $10^{-2}$ , which is equivalent to  $0.01\mu\text{g ml}^{-1}$ .

### 4. CONCLUSION AND RECOMMENDATION

The ethanol root and bark extract of *S. longipedunculata* showed antimicrobial activity against *N. gonorrhoeae*.

Further studies would be required to isolate the specific compound(s) of the plant responsible for the bacteriocidal activity in order to standardize

the plant preparation for maximum therapeutic benefit. Based on these findings and the medicinal potential of this plant, further research should be done on this plant in order to determine its major active compound(s). Furthermore, this study concentrated on the bark and root, which would not be ecologically prudent to harvest for large scale utilization. Our next study will focus on the leaves, which can be harvested without endangering the plant life, and to identify the specific compounds responsible for the antibacterial activities.

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### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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