ChemSearch Journal 11(2): 90 – 98, December, 2020
Publication of Chemical Society of Nigeria, Kano Chapter

Received: 10/11/2020 Accepted: 05/12/2020
http://www.ajol.info/index.php/csj

Phytochemical and Antimicrobial Activity of *Securidaca longipedunculata* Root against Urinary Tract Infection Pathogens

[1*]Namadina, M. M., [2]Shawai, R. S., [3]Musa, F. M., [4]Sunusi, U., [6]Aminu, M. A., [1]Nuhu, Y. and [5]Umar, A. M.

[1]Department of Plant Biology, Bayero University, Kano, Nigeria.
[2]Department of Crop Science, Kano University of Science and Technology, Wudil
[3]Department of Microbiology, Kaduna State University
[4]Department of Biochemistry, Faculty of Basic Medical Science, Bayero University, Kano
[5]Department of Remedial and General Studies, Audu Bako Collage of Agriculture, Dambatta
[6]Department of Biological Sciences, Bayero University, Kano
***Correspondence Email:** mnmuhammad.bot@buk.edu.ng; hajiyaiyalle@gmail.com

ABSTRACT

Urinary tract infection caused by bacteria leads to inflammation and over growth of uropathogens and prevalence of infection for both genders, but women is more vulnerable especially at the sexually active ages. But unfortunately, the continuous emergence of antibiotic resistant bacterial strains brings most serious public health concerns. It is therefore, important to look for more alternative, effective, safer and safer treatments. The aim of the present study was to investigate antimicrobial activity of *Securidaca longipedunculata* root extracts against some human pathogenic bacteria and fungi using agar well diffusion method and agar dilution for minimum inhibitory concentration (MIC). Phytochemical and acute toxicity studies were carried out using the standard methods. Phytochemicals which include alkaloids, flavonoids, saponins, tannins, carbohydrates and triterpenes were detected in both aqueous and methanolic extracts. The antimicrobial results revealed that, the methanolic extract had promising antibacterial activity. For intense *Escherichia coli* was found to be the most susceptible bacteria in both methanol and aqueous extracts at 500mg/ml with inhibition zones of 20 mm and 16mm, *Staphylococcus aureus* was next most susceptible bacteria to methanol extract of the root with inhibition zone of 16 mm and MIC of 31.25 mg/ml respectively. The extracts does not showed activity against all the tested fungal isolates at lowest concentration 62.5 mg/ml. The LD_{50} of *Securidaca longipedunculata* was found to be greater than 5000 mg /kg and could be considered safe for consumption.

Keywords: Acute toxicity, Antibacterial activity, Inhibition zones, Phytochemical, *Securidaca longipedunculata*, Urinary tract infection

INTRODUCTION

Urinary Tract Infection (UTI) is classified as the most common and occurring nosocomial bacterial infection in human population around the world (Khan *et al.*, 2014). UTI is a condition caused by pathogenic invasion of the epithelium, which lines the urinary tract from the minor calyx to prostatic urethra. The proliferation of bacteria in the urothelium which causes inflammatory response characterized by a wide range of symptoms including, fever, lethargy, anorexia and vomiting (Onu *et al.*, 2013). Although, both genders are susceptible to this type of infection, women are more prone due to their reproductive anatomy and physiology. In fact, it had been shown that half of all women by 32 years age had experienced at least an infection history (Vasudevan, 2014). The commonest pathogens found in the urine are *E. coli, S. saprophyticus* (Kibret and Abera, 2014). Other bacterial species namely, *Klebsiella sp., Proteus, Staphylococcus aureus* and *Pseudomonas aeruginosa.* These bacterial species are more common in most of the cases and frequently cause to complicated cystitis and pyelonephritis (Balakrishnan and Hill, 2010). Considering that UTI infection is mostly associated with Gram negative uropathogenic *E. coli* (Stamm and Hooton, 1993). Consequently, antibiotics which are effective against Gram negative uropathogens are mostly prescribed. Actually however, Gram-positive bacteria are well documented as listed in the foregoing (Kline and Lewis, 2015).

Securidaca longipedunculata is a plant commonly known as violet tree in English. In Nigeria, the plant is called Uwar magunguna, Ezeogwu, Aaalali, Ipeta by the Hausa, Igbo, Fulani, and Yoruba people respectively. *Securidaca longipedunculata* is a medium size tree measuring 8 to 9 m height with visible violet or white flowers, pale smooth bark, common in North-Central Nigeria and is generally widespread in hot

temperate part of Africa. *S. longipedunculata* stem bark and roots are still found amongst the most traded medicinal plants in Africa (Tabuti *et al.*, 2012). The root extract is used for treating venereal diseases, skin cancer, skin infections, constipation, flu, coughs and fever. It is also used for sexual boost, contraceptive and abortion purposes. Other uses of the root extract are for the treatment of toothache, tuberculosis, rheumatism, pneumonia and as blood purifier. The powdered stem bark exhibited antimicrobial activity against a variety of organisms including *Neisseria gonorrhea, Candida albicans*, *Trichomonas vaginalis* and the agent for syphilis (Hedimbi and Chinsembu, 2012). In this work an attempt has been made to establish the activity of the root extract of *S. longipedunculata* against some common UTI pathogens.

MATERIALS AND METHODS

Collection of Clinical Specimen

Ethical clearance with the number MOH/Off/797/T.I/645 was obtained from the ethical committee of Kano State Hospital Management Board, Kano State Ministry of Health for all the samples collected. Thus, patients were asked to clean their external genitalia with disinfectant and their midstream urine was collected in a sterilized cap. Samples were kept in an ice bag and transported to microbiological laboratory (Karzan *et al.*, 2017).

Urine culturing

Urine samples were cultured on Nutrient agar, blood agar and MacConkey agar media and incubated over night at 37°C. Significant growth was evaluated as ≥ 105 colony-forming units CFU/mL of midstream urine (Karzan *et al.*, 2017).

Culture characteristics

Each of the color, size, elevation, margins and texture of colonies were screened. The morphological different colonies on MacConkey agar, nutrient agar and blood agar were sub-cultured into nutrient agar medium, in order to purify the isolated bacteria from each patient urine specimen (Karzan *et al.*, 2017).

Microscopic examination

Pure isolates were examined microscopically, on the basis of their cell wall composition and presence of capsule (Karzan *et al.*, 2017).

Microbiological analysis

According to Gram staining technique, isolates were cultured on numerous selective and differential media to find out their color, colony morphology and ability of fermentation (Karzan *et al.*, 2017).

Isolation of Bacteria Species

The specimens were cultured on sterile blood agar, chocolate agar and Mac-conkey agar plates at 37°C for 24 h in an incubator. Discrete colonies were picked based on their morphology and further sub-cultured on blood agar and chocolate agar to obtain pure strains. The isolated colonies were Gram stained and based on their Gram reactions were inoculated on different selective media — mannitol salt agar, cetrimide agar, eosin methylene blue agar. Different biochemical tests were conducted (catalase, coagulase, and oxidase tests). All the isolates that grew on selected agar media were then placed on nutrient agar and chocolate agar slants and maintained in a refrigerator at 4°C (Cheesbrough, 2006).

Identification and Characterization of Test Organism using Rapid Test Kits

Identification and characterization of the bacteria was carried out using Microgen Identification Kit (XYZ). The test was performed according to the manufacturer‘s specifications (API biomerieux). It was performed by adding saline suspension of the test organisms to each of the wells, and appropriate wells (1, 2, 3 and 9) were overlaid with sterile paraffin oil. After overnight incubation (18-24 hours) at 37°C, suitable reagents such as Nitrate A and B, Kovacs, Typtophan deaminase (TDA), Voges-proskauer (VPI and II) were added to wells 8, 10 and 12 for additional tests and colour changes of the different tests recorded. The results were converted into four to eight digits codes depending on the organisms being tested and interpreted using the Microgen Identification Software Package (MID-60) (Sylvester, 2016).

Collection and Identification of Plant Materials

The roots of *Securidaca longipedunculata* were collected from local farm in March, 2017 at Babura Local Government Area, Jigawa State, Nigeria. The plant was identified and authenticated in the herbarium of the Plant Biology Department of Bayero University, Kano, Kano State, Nigeria and a voucher specimen number BUKHAN13 was deposited.

Preparation of Plant extracts

The roots of the plant was cleaned, air dried and ground to coarse powder using grinding machine. The powder was stored in air tight containers for further use. Fifty grams (50g) each of the powdered root was soaked into 500ml each of methanol and distilled water. The mixtures were allowed to stand for 3 days at room temperature (28 ±2^{o}C) with hourly agitations. Each extract was sieved through a muslin cloth, filtered through a Whatman (No.1) filter paper, poured unto a clean evaporating dish and placed on a water bath at 50^{o}C until all the solvent evaporated.

Qualitative Phytochemical screening of Aqueous and Methanolic extract of *Securidaca longipedunculata* Root

The plant extracts were subjected to phytochemical screening in order to identify the phytochemical constituents of the plant using the methods described below.

Tests for carbohydrates

Molish's (General) Test for Carbohydrates:

To 1.0 ml of the filtrate, 1.0 ml of Molish's reagent was added in a test tube, followed by 1.0 ml of concentrated sulphuric acid down the test tube to form a lower layer. A reddish colour at the interfacial ring indicates the presence of carbohydrate (Evans, 2009).

Tests for Saponins

Frothing test:

About 10ml of distilled water was added to a portion of the extract and was shaken vigorously for 30 seconds. The tube was allowed to stand in a vertical position and was observed for 30 mins. A honeycomb froth that persists for 10-15 mins indicates presence of saponins (Evans, 2009).

Test for Flavonoids

Shinoda Test:

A portion of the extract was dissolved in 1-2 ml of 50% methanol in the presence of heated metallic magnesium chips and a few drops of concentrated hydrochloric acid were added. Appearance of red color indicates the presence of flavonoids (Evan, 2009).

Test for Alkaloids

Wagner's Test:

Few drops of Wagner's reagent were added into a portion of the extract, whitish precipitate indicates the presence of alkaloids (Evans, 2009).

Test for Steroids and Triterpenes

Liebermann-Burchard's test:

Equal volumes of acetic acid anhydride was added to the portion of the extract and mixed gently. Concentrated sulphuric acid (1 ml) was added down the side of the test tube to form a lower layer. A colour change observed immediately and later indicates the presence of steroid and triterpenes. Red, pink or purple colour indicates the presence of triterpenes while blue or blue green indicates steroids (Evans, 2009).

Test for Cardiac Glycosides

Kella-killiani's test:

A portion of the extract was dissolved in 1ml of glacial acetic acid containing traces of ferric chloride solution. This was then transferred into a dry test tube and 1ml of concentrated sulphuric acid was added down the side of the test tube to form a lower layer at the bottom. Interphase for purple-brown ring was carefully observed, this indicates the presence of deoxy sugars and pale green colour in the upper acetic acid layer indicates the presence of cardiac glycosides (Evans, 2009).

Test for Tannins

Ferric chloride test:

Exactly 3-5 drops of ferric chloride solution was added to the portion of the extract. A greenish black precipitate indicates the presence of condensed tannins while hydrolysable tannins give a blue or brownish blue precipitates (Evans, 2009).

Test for Anthraquinones

Borntrager's test:

Exactly 5ml of chloroform was added to the portion of the extract in a dry test tube and shaken for at least 5mins. This was filtered, and the filtrate shaken with equal volume of 10% ammonium solution, bright pink colour in the aqueous upper layer indicates the presence of free anthraquinones (Evans, 2009).

QUANTITATIVE DETERMINATION OF PHYTOCHEMICAL CONTENTS OF *SECURIDACA LONGIPEDUNCULATA* ROOT

Determination of Alkaloids

About 5g of the sample was weighed into a 250ml beaker and 200ml of 10% acetic acid in ethanol were added and covered and allowed to stand for 4hours. This was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation is completed. The whole solution was allowed to settle, and the precipitates were collected, washed with dilute ammonium hydroxide solution and then filtered. The residue was the alkaloids, which was then dried and weighed (Haborne, 1973).

Determination of Flavonoids

About 10g of the plant sample was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter upper No. 42 (125mm). The filtrate was later be transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight (Bohm and Kocipal – Abyazan, 1994).

Determination of Saponins

The method of Obadoni and Ochuko (2001) was used. Out of the ground samples 10g was weighed for each into a conical flask and 100ml of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4hours with continuous stirring at about 55oC. The mixture was filtered and the residue re-extracted with another 200ml, 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90^{o} C. The concentrate was transferred into a 250ml separatory funnel and 20 ml of diethyl ether

was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and 60ml of n – butanol was added. The combined *n*-butanol extracts were washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight. The saponins content was calculated as percentage.

Determination of Tannins

About 500 mg of each sample was weighed into a 50 ml plastic bottle and 50 ml of distilled water was added and shaken for 1hour on a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtrate was pipetted out into a test tube and mixed with 2ml of 0.1M $FeCl_3$ in 0.1M HCl and 0.008M potassium ferro-cyanide. The absorbance was measure at 120 mm within 10 mins (Van-Burden and Robinson, 1981).

Determination of Total Phenols

The fat free sample was boiled with 50 ml of ether for 15 minutes. About 5 ml of the extract was pipetted into a 50 ml flask, and then 10 ml of distilled water was added. About 2 ml of ammonium hydroxide solution and 5ml of concentrated amyl alcohol were added. The sample was made up to mark and allowed to react for about 30 minutes for colour development. This was measured at 505 nm.

ANTIMICROBIAL SUSCEPTIBILITY TEST

Preparation of Extract Concentration

This was carried out according to the method described by Srinivasan *et al.,* (2009). Stock solution of the plant extracts were prepared by adding 0.5g of each crude plant extract in 1ml dimethyl sulphuroxide (DMSO). From each of the stock solutions, 500mg/ml, 250mg/ml, 125mg/ml and 62.5mg/ml concentrations were prepared using Two-fold serial dilution method (Srinivasan *et al.,* 2009).

Standardization of bacterial Inoculum.

Using inoculum loop, over-night grown agar culture (bacteria and fungi) was transferred into a test tube containing normal saline until the turbidity of the suspension matched the turbidity of the 0.5 McFarland Standard as described by the National committee for clinical laboratory standard (NCCLS, 2008).

Susceptibility Test of Bacterial and Fungal isolates to Different Concentrations of the Extracts

The antimicrobial activity of *Securidaca longipedunculata* crude extract (Methanol and aqueous) against *Klebsiella sp., Staphylococcus aureus, Shigella sp., Escherichia coli, Candida glabrata, Candida tropicalis, Candida krusei, Candida kfyer* and *Candida albicans* were evaluated using agar well diffusion method of susceptibility test (Srinivasan *et. al.,* 2009*)*. Mueller-Hinton agar and Sabouraud Dextrose agar plates were inoculated with 0.1ml of standardized inoculum of each bacterium and fungus respectively (in triplicates) using 0.1ml pipette and spread uniformly with sterile swab sticks. Three wells of 6mm size were made with sterile cork borer (6 mm) into the inoculated agar plates. Using micropipette, 0.1ml volume of the various concentrations; 500mg/ml, 250mg/ml, 125mg/ml and 62.5mg/ml each of the crude extracts were dispensed into wells of inoculated plates. DMSO was used as negative control. Commercially available standard antibiotic, ciprofloxacin and fluconazole were used as positive control parallel with the extracts. The prepared plates were then left at room temperature (37 ^{o}C) for 10 minutes, allowing the diffusion of the extracts into the incubation at 37 ^{o}C for 24 hrs in an incubator. The diameter of inhibition zones (DIZ) were measured and expressed.

Determination of Minimum Inhibitory Concentration (MIC)

The method used was the tube dilution method (Adesokan *et al*., 2007). Thus, the plant extracts were serially diluted from 500 mg/ml solution to obtain varying concentration. The concentrations were; 250 mg/ml, 125 mg/ml, 62.5mg/ml, and 31.25 mg/ml. Doubling dilutions of the extract were incorporated in Muller Hinton broth (Oxoid, UK), and then inoculated with 0.1ml each of standardized suspension of the test organisms into the various test tube containing varying concentrations. Another set of test tubes containing only Mueller Hinton broth were used as negative control, and another test tube containing Mueller Hinton broth and test organisms were used as positive control. All the test tubes and controls were then incubated at 37 ^{o}C for 24hrs. After incubation period, the presence or absence of growth on each tube was observed. A loop full from each tube was further sub cultured onto nutrient agar to confirm whether the bacterial growth was inhibited.

Determination of Minimum Bactericidal Concentration (MBC)

The MBC was determined by collecting 1ml of broth culture from the tubes used for the MIC determination and sub culturing into fresh solid nutrient agar plates. The plates were incubated at 37 ^{0}C for 24 h. The least concentration that did not show any growth after incubation was regarded as the MBC (Adesokan *et al*., 2007).

ACUTE TOXICITY STUDIES OF METHANOL EXTRACT OF *S. LONGIPEDUNCULATA* ROOT

Lethal Dose (LD_{50}) Determination

The method of Lorke (1983) was employed. Thus, the phase I involved the oral administration of three different doses of 10, 100 and 1,000 mg/kg of the crude extract, to three different groups of three adult wister albino rats. In a fourth group, three adults male wister albino rats were administered with equivalent/volume of distilled water to serve as control. All the animals were orally administered with the extracts using a curved needle to which acatheter had been fixed. The animals were monitored closely every 30 minutes for the first 3 hours after administration of the crude extracts, and then hourly for the next 6hours for any adverse effects. Then the animals were left for 72 hours for further observation.

When no death occurred, the phase II was employed, only one animal was required in each group. Groups 1–4, animals were orally given 1,500, 2,200, 3250 and 5,000mg/kg dose levels of the crude extract. All the animals were left for observation as in stage one.

RESULTS AND DISCUSSION

Phytochemical screening of the aqueous and methanol extracts (Table 1) revealed the presence of alkaloids, flavonoids, saponins, cardiac glycosides, triterpenes, steroids and tannins. Phenols and anthraquinones were absent in the aqueous extract but present in the methanol extract. These secondary metabolites (alkaloids, saponins, tannins, anthraquinones and flavonoids) are known to have activity against several pathogens and therefore aid the antimicrobial activities of *Securidaca longipedunculata* and support their traditional use for the treatment of various illness (Sharma *et al.,* 2012; Usman and Osuji, 2007; Hassan *et al.,* 2004). In all the two extracts, tannins were present which are known to inhibit of cell protein synthesis as it forms irreversible complexes with prolinerich protein (Shimadu, 2006; Sharma *et al.,* 2012). Tannins containing herbs have been reported in treating intestinal disorders such as diarrhea and dysentery (Dharmananda, 2003; Sharma *et al.,* 2012), treatment of inflamed or ulcerated tissues (Parekh and Chanda, 2007; Sharma *et al.,* 2012). It has been observed that tannins have anticancer activity and can be used in cancer prevention (Sharma *et al.,* 2012), thus suggesting that *Securidaca longipedunculata* has potential as a source of important bioactive molecules for the treatment and prevention of cancer. The presence of tannins in *Securidaca longipedunculata* supports the traditional medicinal use of this plant in the treatment of different ailments. These observations therefore support the use of *Securidaca longipedunculata* in herbal cure remedies. Table 2 shows the results for the quantitative phytochemical content of the *Securidaca longipedunculata* root. The alkaloids (106.0 mg/g) was the highest phytochemical detected in the plant while the lowest was saponins and phenols (4.0 mg/g). These phytochemicals are known to exhibit medicinal activity as well as pharmacological activity. Alkaloids have a wide range of pharmacological activities including antimalarial (e.g., quinine), anticancer (e.g., homoharringtonine) (Kittakoop *et al.,* 2014), antibacterial (e.g., chelerythrine) (Cushnie *et al.,* 2014), and antihyperglycemic activities (e.g., piperine) (Qiu *et al.,* 2014). The antibacterial test results presented in Table 3 and 4 showed good activity against Gram-positive and negative bacteria with low antifungal activity. The methanol extracts exhibited considerable level of inhibition against the entire test organism compared to the standard drug. This is suggestive of the presence of some compounds or groups in the extract with similar mechanism of action to that of the standard drug used. In addition, microorganisms show variable sensitivity to chemical substances related to different resistance levels between strains (Sharma *et al.,* 2012). Mujumdar *et al.* (2001) also reported that the crude methanol extract from the root of *Securidaca longipedunculata* exhibited anti-diarrhea activity in mice through the inhibition of prostaglandin biosynthesis and the reduction of osmotic pressure. Aiyelaagbe *et al.* (2007) reported that the presence of some secondary metabolites in the root extract of *Securidaca longipedunculata* inhibited some microorganisms isolated from sexually transmitted infections. This may be attributed to the presence of soluble phenolic and polyphenolic compounds (Kowalski and Kedzia, 2007; Sharma *et al.,* 2012). However, it may be suggested that plant extracts exhibiting diameters of zones of inhibition larger than 10 mm are considered active (Usman and Osuji, 2007; Sharma *et al.,* 2012). Thus, it is believed that the extract is a better antimicrobial agent for various pathogenic fungus and bacteria. Among these, methanol extract was showing better antibacterial and antifungal properties compared to aqueous extract of *Securidaca longipedunculata*.

Table 1. Qualitative Phytochemical screening of the Aqueous and Methanol extracts of *Securidaca longipedunculata* Root bark

Phytochemicals	Extracts	
	Aqueous	Methanolic extract
Alkaloid	+	+
Flavonoid	+	+
Saponins	+	+
Cardiac glycoside	+	+
Tannins	+	+
Steroid	+	+
Triterpenes	+	+
Phenol	-	+
Anthraquinones	-	+
Carbohydrate	+	+

Key: + Present, - Absent

Table 2. Quantitative Phytochemical Contents of *Securidaca longipedunculata* Root

Metabolite	Quantity (mg/g)
Alkaloids	106.0±0.33
Flavonoids	142.0±0.22
Saponins	4.0±0.12
Tannins	194.0±0.33
Phenols	4.0 ±0.88

Table 3. Antimicrobial activity of Aqueous extract of *Securidaca longipedunculata* Root

Clinical isolates	**Concentration/Diameter zone of inhibition(mm)**						**MIC**	**MBC**
	500	250	125	62.5	CPR/FLC	DMSO		
S. aureus	14	12	10	8	41/*	6	31.25	62.5
E. coli	15	13	11	9	40/*	6	31.25	62.5
Klebsiella sp.	13	11	9	7	36/*	6	31.25	62.5
Shigella sp.	14	12	9	6	38/*	6	-	-
C. albicans	11	10	6	6	*/40	6	-	-
C. glabrata	10	8	6	6	*/38	6	-	-
C. krusei	12	9	6	6	*/37	6	-	-
C. tropicalis	10	8	6	6	*/38	6	-	-
C. kfyer	10	10	8	6	*/39	6	-	-

Key: - Not tested for MIC and MBC because there was no inhibition at lowest concentration
*= Not test CPR (Ciprofloxacin was only used against bacterial isolates), FLC (Fluconazole was used against fungal isolates).

Table 4. Antimicrobial activity of Methanol extract of *Securidaca longipedunculata* Root

Clinical isolates	**Concentration/Diameter zone of inhibition (mm)**						**MIC**	**MBC**
	500	250	125	62.5	CPR/FLC	DMSO		
S. aureus	16	13	10	8	41/*	6	31.25	62.5
E. coli	20	15	11	9	40/*	6	31.25	62.5
Klebsiella sp.	13	10	9	6	36/*	6	-	-
Shigella sp.	16	14	9	7	38/*	6	31.25	62.5
C. albicans	12	10	8	6	*/40	6	-	-
C. glabrata	12	10	8	6	*/38	6	-	-
C. krusei	14	12	10	8	*/37	6	31.25	62.5
C. tropicalis	12	10	8	6	*/38	6	-	-
C. kfyer	12	10	8	6	*/39	6	-	-

Key: - Not tested for MIC and MBC because there was no inhibition at lowest concentration
*= Not test CPR (Ciprofloxacin was only used against bacterial isolates), FLC (Fluconazole was used against fungal isolates).

From the results of the acute toxicity studies (Table 5), no death was recorded in the first phase. In the second phase, doses of 1500, 2250, 3250 and 5000mg/kg were used and no death was also recorded. The oral median lethal dose (LD_{50}) for the methanol root-extract of *Securidaca longipedunculata* was therefore estimated to be

greater than 5000mg/kg and no sign of behavioural changes were also observed.

These results agree with the study conducted by Junaid (2008) that the aqueous root extract showed a relatively moderate zone of inhibition on *E. coli* and *S. aureus* (15 mm and 14 mm). The result from Table 5 shows that, methanol extract at 500 mg/ml showed maximum antibacterial activity against *S. aureus, Klebsiella sp., Shigella sp.* and *E. coli.* This result agreed with that of Akpemi (2013) that methanol extract of *S. longipedunculata* contains potential antimicrobial agents capable of treating drug resistant strains of some pathogenic bacteria, which include methicillin resistant- *Staphylococcus aureus* (MRSA) and *P.aeruginosa*. In this study crude methanol extract showed higher inhibitory activity against *E. coli* and *S. aureus*. The root extract of *S. longipedunculata* strongly inhibits Gram-negative bacteria (*E. coli*) which is resistant bacteria for many antibiotics. The inhibition zone of ciprofloxacin against *E. coli* showed (40 mm) which is two times the inhibition zone of methanol extract followed by *S. aureus* (41 mm) which is 2.05 times that of plant extract. *Klebsiella* (36 mm) and *Shigella dysentariae* (38 mm) showed 3.77 and 2.4 times higher than that of plant extract. Therefore, the inhibition zone of methanol root extract against *E. coli* showed a little bit similarity with the control than aqueous extract. Junaid (2008) reported the MIC value of aqueous extract of the root as 200mg/ml. These results are not in agreement with this study as the MIC value of 31.25 mg/ml was recorded in *S. aureus*, *E. coli* and *Klebsiella*. There was no MIC value for all the fungal isolates in both methanol and aqueous extract of the root because there was no activity at lowest concentration 62.5mg/ml.

The oral median lethal dose value for the methanol root extract of *Securidaca longipedunculata* obtained in rats was found to be above 5000mg/kg. This suggests that the plant extract is non-toxic as no death was recorded. Acute toxicity studies are usually carried out to determine the dose that will cause death or serious toxic manifestations when administered singly or severally at few doses in order to establish doses that should be used in subsequent studies (Wanda *et al.,* 2002). The Organization for Economic Cooperation and Development (OECD), recommended the chemical labelling and classification of acute systemic toxicity based on oral median lethal dose values as: very toxic if ≤ 5 mg/kg, toxic if > 5 mg/kg but ≤ 50 mg/kg, harmful if > 50 mg/kg but ≤ 500 mg/kg, and non-toxic or not harmful if > 500 mg/kg or ≤ 2000 mg/kg (Walum, 1998). The LD_{50} was found to be greater than 5000 mg/kg when administered orally in rats (Table 5) and all the animals remain alive and did not manifest any significant visible signs of toxicity at these doses. These studies showed that the extracts *S. longipedunculata* root are practically non-toxic when administered using the oral route. This is based on the toxicity classification which states that substances with LD_{50} values of 5000 to 15,000 mg/kg body weight are practically non-toxic (Loomis and Hayes, 1996). The pharmacological activities of the drug may be contributed to the presence of secondary metabolites (Sharma *et al.,* 2012). Hence, the presence of some metabolites in *Securidaca longipedunculata* suggests its activities against microbes. It is concluded that root of *Securidaca longipedunculata* could be a potential source of active antimicrobial agents and a detailed assessment of its *in vivo* potencies and toxicological profile is ongoing.

Table 5. Acute toxicity studies of methanolic extract of *Securidaca longipedunculata* Root

Treatment	Group	Number of Animals	Dose (mg/kg)	Mortality recorded after 24hrs
Phase I	I	3	10	0/3
	II	3	100	0/3
	III	3	1000	0/3
Phase II	I	1	1500	0/1
	II	1	2250	0/1
	III	1	3250	0/1
	IV	1	5000	0/1

CONCLUSION

From the above results, it can be concluded that the traditional medicinal plant *S. longipedunculata* possess diverse components in its root with antimicrobial activity against the selected bacterial and fungal strains. The pattern of inhibition also showed that, *E. coli* was the most susceptible bacterial strain followed by *S. aureus and Shigella dysentariae* in both aqueous and methanol extract of root and *Klebsiella sp.* was the most resistant bacteria in all extracts. Among solvents used methanol shows the highest inhibition zone, this means methanol extract was more effective against the tested organisms. Acute toxicity is one of the major concerns of indigenous therapeutic preparations and it can be confirmed that the LD_{50} of the methanol extract was above 5000 mg/kg and did not cause mortality in all the tested rats.

REFERENCES

Adesokan, A. A., Akanji, M. A. and Yakubu, M. T. (2007). Antibacterial potentials of aqueous extract of Enantia chlorantha stem bark. *African Journal of Biotechnology*, 6(22).

Aiyelaagbe, O.O., Adeniyi, B.A., Fatunsin, O.F., Arimah, B.D. (2001). *In vitro* antimicrobial activity and photochemical analysis of *Jatropha curcas* roots. *Intern. J. Pharmacol.* 3(1):106-110.

Akpemi, A., Adebayo, O., Iloegbulam, G. and Sabo, E. (2013). Phytochemical screening and antimicrobial activity of solvent fractions of *Securidaca longipedunculata* (Fresen) root bark methanol extract. *Journal of Chemical and Pharmaceutical Research* 5(10):28-33.

Andreu, A. (2005) Pathogenesis of urinary tract infections. Enfermedades infecciosasy microbiologia clinica 23: 15-21.

Balakrishnan, Hill, V. (2010) Dealing with urinary tract infections. *The Pharm J.* 287: 687-690.

Dharmananda, S. (2003). Gallnuts and the uses of tannins in Chinese medicine. In: proceedings of Institute for Traditional Medicine, Portland, Oregon.

Evans, W. C. (2009). *Trease and Evans pharmacognosy*, 16th edition, W. B. Saunders Ltd., London, 10 – 11.

Guglietta, A. (2017) Re-current urinary tract infections in women: Risk factors, etiology, pathogenesis and prophylaxis. Future Micro 12: 239-246.

Harborne, Z. B. (2009). *Phytochemical methods: A guide to modern techniques of plant analysis*, 3rd edn. Chapman and Hall Press, London. Pp. 13, 53, 53 195, 283.

Hassan, M.M., Oyewale, A.O., Amupitan, J.O., Abduallahi, M.S., Okonkwo, E.M. (2004). Preliminary phytochemical and antibacterial investigation of crude extracts of the root bark of Detarium microcarpum, *J. Chem. Soc. Nigeria*. 29:26-29.

Hedimbi, M., Chinsembu, K.C. (2012). An ethnomedicinal study of plants used to manage HIV/AIDS-related disease conditions in the Ohangwena region, Namibia. *Int. J. Med. Plants Res.* 1: 004-011.

Junaid, S., Ofodile A., Olabode A., Echeonwu G. and Okwori A. (2008). Evaluation of *Securidacalongipenduculata* leaf and root extracts for antimicrobial activities. *African Journal of Microbiology Research.* 2 (12) 322-325.

Karzan, M. K., Faeza, B. O. and Shahida, N. Y. (2017). Isolation and Identification of Urinary Tract Infectious Bacteria and Exploring their Anti-drug Potential against Some Common Antibiotics. *J Microb. Biochem. Technol*. 9(6): 285-289.

Khan, I.U., Mirza, I.A., Ikram, A., Afzal, A., Ali, S. et al. (2014) Antimicrobial susceptibility pattern of bacteria isolated from patients with urinary tract infection. J Coll Physicians Surg Pak 24: 840-844.

Kibret M, Abera B (2014) Prevalence and antibiogram of bacterial isolates from urinary tract infections at dessie health research laboratory, Ethiopia. *Asian Pac J Trop Biomed* 4: 164-168.

Kline, K.A., Lewis, A.L. (2015) Gram-positive uropathogens, polymicrobial urinary tract infection, and the emerging microbiota of the urinary tract. Micro spec: 4.

Kowalski, R., Kedzia, B. (2007). Antibacterial activity of *Silphium perfoliatum* extracts. Pharm. Biol. 45:495-500.

Lorke, D. (1983). A New Approach to Practical Acute Toxicity Testing. *Archives of Toxicology*. Pp. 275-287.

Maroyi, A. (2013). Traditional use of medicinal plants in South-central Zimbabwe: Review and perspectives. *J. Ethnobiol. Ethnomed*. 9:31-210.

Mongalo, N.I., McGawb, L.J., Finnieb, J.F., Van Stadenb, J. (2015). *Securidaca longipedunculata* Fresen (Polygalaceae): A review of its ethnomedicinal uses, phytochemistry, pharmacological properties and toxicology. *J. ethnopharmacol.* 165:215-226.

Mujumdar, A.M., Misar, A.V., Salaskar, M.V., Upadhye, A.S. (2001). Antidiarrhoeal effect of an isolated fraction (JC) of *Jatropha curcas* roots in mice. *J. Nat. Remedies.* 1:89-93.

Mustapha, A.A (2013a). Ethno-medico-botanical uses of *Securidaca longipenduculata* (Fresen) (Family Polygalaceae) from Keffi local government, Nasarawa State, Nigeria. *J. Nat. Remed*. 13:133-137.

Mustapha, A.A. (2013b). Ethno-medicinal field study of anti-fertility medicinal plants used by the local people in Keffi local government, Nasarawa State, Nigeria. Int. J. Med. Plants Res. 2:215-218 Nature Reviews; vol 7: pp. 926-935.

National Committee for Clinical Laboratory Standards (2008). Performance Standards for Antimicrobial Susceptability Testing; Ninth Informational Supplement. NCCLS document M100-S9. National Committee for Clinical Laboratory Standards, Wayne, PA.

National Committee for Clinical Laboratory Standards NCCLS (2000). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: approved standards- fifth edition. NCCLS document M7-A5. NCCLS. 2000: Wayne, PA, USA

Obadoni, B. O and Ochuko, K. (2001). Phytochemical studies and comparative efficacy of the crude extract of some haemostatic plants in Edo and Delta States of Nigeria. *Global Journal of Pure and Applied Science* 8: 203-208.

Ogunmefun, O.T., Gbile, Z.O. (2012). An ethnobotanical study of anti-rheumatic plants in South Western States of Nigeria. *Asian J. Sci. Technol.* 4(11):63-66.

Onu, G.A., Korir, S.C., Cheruiyot, J.C., Ratemo, O.D., Mabeya, B.M. et al. (2013) Isolation, identification and characterization of urinary tract infections bacteria and the effect of different antibiotics. J Nat Sci Res 3: 150-159.

Parekh, J., Chanda, S. (2007). In vitro antibacterial activity of crude methanol extract of Woodfordia fruticosa Kurz flower (Lythacease). *Braz. J. Microbiol.* 38:2.

Sharma, A.K., Mayank, G., Ragini, T., Gopal, N., Akhoury, S., Kumar, S., Yamini, B. T., Dharmendra, K. (2012). Comparative *in vitro* Antimicrobial and Phytochemical Evaluation of Methanolic Extract of Root, Stem and Leaf of *Jatropha curcas* Linn. *Pharmacognosy Journal*. 4(30) 34-40

Shimada, T. (2006). Salivary proteins as a defense against dietary tannins. *J. Chem. Ecol*. 32 (6):1149-1163.

Srinivasan, L., Sasaki, Y., Calado, D.P., Zhang, B., Paik, J.H., DePinho, R.A., Kutok, J.L., Kearney, J.F., Otipoby, K.L. and Rajewsky, K. (2009). PI3 kinase signals BCR-dependent mature B cell survival. *Cell*, 139:573-586.

Stamm, W.E., Hooton, T.M. (1993) Management of urinary tract infections in adults. *N Engl J. Med.* 329: 1328-1334.

Sylvester, N.M. (2016). Phytochemical and Antivenom activity of Albizia chivelieri stem bark on *Naja nigricollis* Broadley Envenomated Mice. M.sc Research Dissertation (Unpublished) Department of Pharmacognosy and Drug Development Library. Ahmadu Bello University, Zaria. Pp: 47-48.

Tabuti, J.R.S., Kukunda, C.*B.*, Kaweesi, D., Kasilo, O.M.J. (2012). Herbal medicine use in the districts of Nakapiripirit, Pallisa, Kanungu and Mukono in Uganda. *J. Ethnobiol. Ethnomed.* 8(1):35-42

Terraf, M.C.L., Tomás, M.S.J., Rault, L., Le, Loir, Y., Even, S. et al. (2017) *In vitro* effect of vaginal lactobacilli on the growth and adhesion abilities of uropathogenic *Escherichia coli. Arch Microbiol.* 1-8.

Togun, V.A., Egbunike, G.N. (2006). Seasonal variations in the sperm production characteristics of Zebu (white Fulani) cattle genitalia in the humid tropical environment. *Middle-East J. Sci. Res.*1:87-95.

Usman, H., Osuji, J.C. (2007). Phytochemical and in vitro antimicrobial assay of the leaf extract of *Newbouldia leavis, Afr. J. Trad*. CAM.4:476-480.

Van-burden, T. P and Robinson, W. C. (1981). Formation of Complexes between protein and Tannic acid. *Journal of Agriculture Food Chemistry*. 1:77.

Vasudevan, R. (2014) Urinary tract infection: An overview of the infection and the associated risk factors. J Micro Exp 1: 8.

Walum, E. (1998). Acute oral toxicity. *Environmental Health Perspectives* 106: 497- 503.

Wanda, M.H., Colin, G.R., and Mathew, A.W. (2002). *Handbook of toxicologic pathology*,2nd edition,academic press.p714.