Phytochemical screening and antimicrobial activity of methanolic leaf extract of *Securidaca longipedunculata* Fresen (Polygalaceae)

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Abstract

The present occurrence of drug resistance by most microorganisms has posed an enormous problem in the health care and triggered the need for continuous research for better and safe therapeutic agents. *Securidaca longipedunculata* is locally used in treating skin infections. Thus this study was designed to investigate the phytochemical constituents and antimicrobial activities of methanol leaf extract of *Securidaca longipedunculata* against *Streptococcus pyogenes, Staphylococcus aureus* and *Candida albicans* which are known to cause skin infections. Antimicrobial activity against the organisms was determined using agar well diffusion method. Four concentrations of the leaf extract were prepared and used in the study (800 mg/ml, 400 mg/ml, 200 mg/ml, and 100 mg/ml). Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) of the extract were also analysed. Upon extraction, the phytochemical constituents of the leaf were found to be carbohydrate, saponins, flavonoids, alkaloids, steroids, cardiac glycosides, tanins and anthraquinones. There was no activity observed at 200 and 100 mg/ml concentrations of the extract on all the test organisms. For the Minimum Inhibitory Concentration (MIC) test against all the tested bacterial isolates, the MIC was found to be 400 mg/ml. In the MBC test, the test showed that *S. pyogenes* and *S. aureus* were not killed by the leaf extract but rather they were inhibited from growing this is the same with *C. albicans*

in the MFC. This study the use of the leaf of *S. longipedunculata in* traditional medicine for topical application in skin infections.

Key words: *Securidaca longipedunculata*, *Staphylococcus aureus, Streptococcus pyogenes, Candida albicans*, MIC, MBC, methanol leaf extract

Introduction

Medicinal plants over the years have shown great antimicrobial activities against various disease-causing organisms (Pei *et al*., 2021) and have attracted the attention of pharmaceutical and scientific communities. Plants and their extracts are known to be used for therapeutic purpose since the time immemorial economical, effective, and easily available (Saquib *et al*., 2019). Among the antimicrobial drugs approved from the year 1981-2019, 48% were developed from natural products or natural derivatives of compounds mimicking natural products (Newman and Cragg, 2020). Many plants have been used because of their antimicrobial properties, which are due to compounds synthesized in the secondary metabolism of the plant (Tiwari and Rana, 2015).

Securidaca longipedunculata Fresen belongs to the family Polygalaceae, it is a small tree with alternate leaves which varies in size, shape and crowded towards the tips of the stem (Van-Wyk *et al*., 2009). It is locally known as Uwar magunguna or Sanya (Hausa), Ipeta (Yoruba), Alali (Arabic) and Umfufu (Swahili), while its common names include Violet tree, Fibre tree and Rhodesian violet. Leaves of *S. longipedunculata* is locally used in the treatment of epilepsy, headaches, stomach ache, infertility, snakebite, toothache, cancer, skin infections, dislocated jaw, contraceptive purposes and to expel the placenta (Mustapha, 2013), while its stem bark is traditionally used to treat epilepsy, stomach ache, venereal diseases, skin diseases, dysentery, malaria, typhoid, inflammation, chest complaints, abortion, constipation, viral infections, snake bites and infertility problems (Kadiri *et al*., 2013).

The present occurrence of drug resistance by most microorganisms has posed an enormous problem in the health care and triggered the need for continuous research for better and safe therapeutic agents (Esposito *et al*., 2016). Evidence-based studies to verify the efficacy of medicinal

plants have provided insights into the synthesis of plant-based compounds with therapeutics application (Dhama *et al*., 2014). *Securidaca longipedunculata* is locally used in treating skin infections. Thus this study was designed to investigate the phytochemical constituents and antimicrobial activities of methanol leaf extract of *Securidaca longipedunculata* against *Streptococcus pyogenes, Staphylococcus aureus* and *Candida albicans* which are known to cause skin infections.

Materials and Methods

Collection and identification of leaf

Fresh leaves of *Securidaca longipedunculata* were collected from the main botanical garden of Ahmadu Bello University, Zaria, Kaduna State-Nigeria in July, 2021. The plant was identified by Mr. Namadi Sunusi of the herbarium section in the Department of Botany, Faculty of Life sciences, Ahmadu Bello University Zaria, with voucher number ABU900243. The plant leaves were adequately washed under tap water, rinsed with distilled water, dried under shade, and pulverized with a pestle and mortar and kept in a transparent sterile polythene bag at room temperature for use.

Phytochemical screening

Phytochemical screening of the extracts was carried out to determine the presence of tannins, saponins, cardiac glycosides, steroidal rings, alkaloids, flavonoids and anthraquinones in the leaf extract, as previously described by Trease and Evans (2009).

Preparation of extract

The extract was prepared as described by Okogun, (2002). One hundred (100) grams of the pulverized leaf was weighed and extracted with 500 ml of 70 % methanol by maceration method. The leaves were soaked in methanol for 72 hours. Thereafter, the extract was filtered, and the solvent was evaporated over a hot water bath at 40 °C. The obtained extract was stored in a clean container at room temperature.

Preparation of extract concentrations were carried out according to the method described by Srinivasan *et al*., (2009). Stock solution of the plant extract was prepared by adding 0.8 g of each crude plant extract in 1 ml sterile water. This was used to prepare 800 mg/ml, 400 mg/ml, 200 mg/ml, and 100 mg/ml concentrations using the two-fold serial dilution method (Srinivasan *et al*., 2009).

Test organisms

The test organisms *S. aureus* and *C. albicans* cultures were obtained from the Department of Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, while *S. pyogenes* culture was obtained from the Department of Veterinary Microbiology, Faculty of Veterinary Medicine both in Ahmadu Bello University, Zaria.

Standardization of inoculums

One ml of overnight cultures of *S. aureus, S. pyogenes* and *Candida albicans* were diluted with 9 ml of normal saline to obtain a 1:10 dilution, 1 ml from the 1 in 10 dilution was added to 9 ml of normal saline to obtain 1 in 100 dilution, 1ml from the 1 in 100 concentrations was then diluted with 9mls of normal saline to obtain 1 in 1000 dilution. 1 in 1000 dilution was used as the inoculum in determination of antimicrobial activity.

Determination of antibacterial activity using cup plate method

One ml of the standardized culture of the test organism was measured and used to flood the surface of the solidified medium. A sterile cork borer (10 mm) was used to make 5 holes unto which plant extract and the control antibacterial agent (ciprofloxacin 10 µg/ml) were introduced. With the aid of a sterile pipette, 0.1 ml of the 4 graded concentrations of the extract (800 mg/ml, 400 mg/ml, 200 mg/ml and 100 mg/ml) were used to fill the hole, the fifth hole was filled with 0.1 ml of ciprofloxacin 10 µg/ml as control. A pre diffusion time of 30 minutes was allowed after which the plates were incubated at 37 °C for 24 hours. The diameter of the zones of the extract and control that inhibited microbial growth was measured to the nearest millimeter using a ruler.

This procedure was carried out in duplicate for both bacterial organisms and average zone of inhibition for each concentration was recorded against the two organisms.

Determination of antifungal activity

About 20 ml of sterilized molten Sabouraud dextrose agar (SDA) was cooled to about 45°C and poured into sterile dried petri dish and allowed to solidify. One (1) ml of the standardized culture of organisms was measured and used to flood the surface of the solidified medium, excess of the inoculum was discarded into a container of disinfectant and a sterile cork borer was used to bore 5

wells in the agar. Two (2) drops of molten SDA was used to seal the base of the well so that the agent will not seep under the agar. The plant extract (0.1 ml) at four different concentrations (800 mg/ml, 400 mg/ml, 200 mg/ml and 100 mg/ml) and the control antifungal agent (Terbinafine 50 µg/ml) were added to the bored wells with the aid of a sterile micro pipette. Pre diffusion time of 30 minutes was allowed then plates were incubated at 37 °C for 24 hours. The diameter of the zones of the extract and control that inhibited microbial growth were measured to the nearest millimeter.

This procedure was carried out in duplicate for the fungal organism and average zone of inhibition for each concentration was recorded against the organism.

Determination of minimum inhibitory concentration

The Minimum Inhibitory Concentration (MIC) of the extract was determined using the broth dilution technique. Nutrient broth and Sabouraud dextrose broth were used as media for the bacterial and fungal culture respectively. Ten test tubes, each containing 5 ml of the broth medium, were used for each of the test organisms, with the first test tube containing double strength of the broth medium and also containing highest concentration of the extract. From the first test tube containing 10 ml of broth and antimicrobial agent, 5 ml was withdrawn into a second test tube, then 5 ml was withdrawn from the second test tube into a third this was done for all test tubes till the tenth test tube. Finally, 5 ml from the last test tube was withdrawn out and discarded in a solution of disinfectant so that each test tube contained 5 ml (mixture of broth and antimicrobial agent).

Each test tube was inoculated with 0.1 ml of the standardized culture then incubated at 37 °C for 24 hours for the nutrient broth and Sabouraud dextrose broth and a test tube containing only the broth was used as negative control for each organism. After the incubation period, a loop full from each tube was further sub cultured onto nutrient agar to confirm whether the microbial growth was inhibited

Determination of minimum bactericidal and fungicidal concentration (MBC and MFC)

MBCs and MFCs of the extracts were determined by sub-culturing a loopful from the test tubes that showed no visible

turbidity in the MIC assay on nutrient and Sabouraud dextrose agar plates which were then incubated at 37 °C for 24 hours. The concentration that showed no visible growth after incubation was taken as the MBC and MFC (Hugo and Rusell, 2011).

Results

Percentage yield of crude plant extract obtained from 70 % methanol by maceration method

S1= weight of powdered sample (in grams)

S2 = weight of extract obtained (in grams)

S1= weight of powdered sample =100 g

S2 = weight of extract obtained =20 g

Percentage yield = $\frac{Weight\ of\ extract\ obtained}{Weight\ of\ powdered\ sample} \times 100$

$= \frac{20}{100} \times 100 =$

20%.....eqn. 1

Table 1: Phytochemical analysis of methanolic leaf extract of *Securidaca longipedunculata*

S/N	Test	Observation	Inference
1	Molisch Test	A reddish colour at Interfacial Ring Is Formed	Carbohydrate present
2.	Frothing Test	A honeycomb froth that persists for 10-15 Mins	Saponins present
3	Ferric Chloride Test	Yellow solution is Formed	Flavonoids present
4	Wagners Test:	Whitish precipitate is formed	Alkaloids present
5	Liebermann-Burchard's Test	Greenish blue colour is formed	Steroids present.

6	Kella-Killiani's Test	Interphase for purple-brown ring was carefully observed Pale green Cclour in the upper acetic acid layer was seen	Cardiac glycosides present
7	Ferric Chloride Test	A greenish black Ppecipitate was observed	Tannins present
8	Borntrager Test	Bright pink colour in the aqueous upper layer is observed.	Anthraquinones present

Table 2: Zones of inhibition of methanolic leaf extract of *Securidaca longipedunculata* against *Streptococcus pyogenes and Staphylococcus aureus*

S/No.	Concentration (mg/ml)	Zones of inhibition (mm)	
		Streptococcus pyogenes	*Staphylococcus aureus*
1.	800	14	16
2.	400	10	10
3.	200	-	-
4.	100	-	-
5.	CIP 10 (mcg/ml)	20	28

Key: - = No activity, CIP= Ciprofloxacin

Table 3: Zones of inhibition of methanolic leaf extract of *Securidaca longipedunculata* against *Candida albicans*

S/No.	Concentration (mg/ml)	Zones of inhibition (mm)
1.	800	24

2.	400	20
3.	200	-
4.	100	-
5.	Terb (50 mcg/ml)	34

Key: - = No activity, Terb= Terbinafine

Table 4: Minimum inhibitory concentration of methanolic leaf extract of *Securidaca longipedunculata against S. aureus, S. pyogenes* and C. *albicans*

Concentration (mg/ml).	*S. aureus*	*S. pyogenes*	*C. albicans*
400	-	-	-

Key: - = No growth

Table 5. Minimum bactericidal and fungicidal concentrations of methanolic leaf extract of *Securidaca longipedunculata* against *S. aureus, S. pyogenes* and *C. albicans* respectively

Concentration (mg/ml)	*S. aureus*	*S. pyogenes*	*C. albicans*
400	+	+	-

Key: + = Growth, - = No growth

Discussion

Methanol leaf extract of *S. longipedunculata* exhibited antimicrobial activities against *S. pyogenes, S. aureus*, and *C. albicans* at 400 and 800 mg/ml with 400 mg/ml as the MIC and MFC. This is similar to the work done by Ndamitso *et al*., (2013) where they recorded no activity in the leaf extract at concentrations less than 200 mg/ml.

The presence of various secondary metabolites such as tannins, saponins, alkaloids, flavonoids in *S. longipedunculata* are responsible for the bioactivity of the leaf extract (Donald *et al.,* 2011). Flavonoids have been reported to be effective antibacterial substances *in vitro* against many microorganisms by the inhibition of membrane-bound enzymes (Cowan, 1999).

Varying factors could attribute to this difference in activity and medicinal plant efficacy against the test microbes and they include the part of the plant used, time of collection of the plant part, solvent used in extraction, the age of plant when harvested and the amount of the active constituent, which can vary in quality and quantity from season to season (Omoya and Akharaiyi, 2012). Although, the leaf extract showed the presence of essential phytochemicals, it had less antimicrobial activity compared to other parts of the plant such as stem bark as reported by Abubakar *et al*., (2022) and roots as reported by Musa *et al*., (2013). This could be attributed to the phytochemicals being present perhaps in lower concentrations than in the root and stem back of the plant

The extraction solvent, methanol, is not selective. It has the ability to extract many components from the very polar to non-polar compounds. As a result, it may have extracted non-bioactive components which may have diluted the concentration of the active antimicrobial component in the extract. This can explain the relatively high MIC and MFC. A more selective solvent may result in lower MIC and MFC respectively. Further purification of the extract followed by fractionation will serve to concentrate the active constituent which may be present in minute quantities. This also explains why the herbal preparations of this leaves are prescribed in large quantities by the practitioners.

MBC and MFC of the methanol leaf extract of *S. longipedunculata* showed that the extract had bacteriostatic and fungicidal properties (Table 5). This

supports the ethnomedicinal use of the plant

Conclusion

Antimicrobial susceptibility testing of the methanol leaves extract of *S. longipedunculata* showed bacteriostatic and fungicidal properties against the test organisms which support the ethnomedicinal use of the plant. Phytochemical analysis of the extract showed that the methanol leaf extract contained bioactive compounds including flavonoids that are known to possess antimicrobial properties

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