JASEM ISSN 1119-8362

Full-text Available Online at
www.ajol.info and
www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage. *March 2014*
Vol. 18 (1) 93-97

Phytochemical and in-vitro antimicrobial screening of *Sanseviera liberica* Gérôme and Labroy (Agavaceae) root extract

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***KEYWORDS*:** *Sanseviera liberica,* Antimicrobial activity, Phytochemical screening, Extracts, Clinical isolates.

ABSTRACT: *Sanseviera liberica* is used in Nigerian folk medicine for the treatment of asthma, abdominal pain, diarrhea, wounds of the foot, gonorrhea, snake bites etc. Some of these diseases are caused by pathogenic bacteria. To validate the trado-medical applications of *S. liberica*, an investigation of the phytochemical and antimicrobial properties of the methanol and n – hexane extracts was conducted. Phytochemical screening revealed the presence of tannins, flavonoids, saponins, reducing sugars and carbohydrates in both extracts. Alkaloids were present only in the methanol extract. Glycosides, terpenoids and steroids were absent in both extracts. The proximate analysis of *S. liberica* showed total ash value of 6.0 %, acid insoluble ash of 1.10 %, alcohol extractive value of 6.80 %, water extractive value of 5.20 % and moisture content of 14.50 %.The broth microdilution technique was used to evaluate the antimicrobial activities of both the methanol and n – hexane extracts of *S. liberica*. Results showed that *Psuedomonas. aureginosa*, *Streptococci pyrognase* and *Staphylococcus aureus* were sensitive to the methanol extract while *Candida albicans*, *Klebsiella pneumonia*, *Salmonella typhii*, *Baccilus subtilis* and *Escherischia coli* were not sensitive. All the eight microorganisms involved in the antimicrobial assay were not sensitive to the n-hexane extract. © JASEM

http://dx.doi.org/10.4314/jasem.v18i1.13

Introduction: Antibiotic drug resistance to human pathogenic bacteria is growing due to the indiscriminate use of existing synthetic antimicrobial agents (Jigna *et al*., 2005) and the genetic ability of the bacteria to transmit and acquire resistance to pharmaceutical drugs (Sahgal *et al*., 2009). There is therefore a need to develop new drugs from natural sources for the treatment of infectious diseases.

Medicinal plant research has been and continues to be considered a fruitful approach in the search for new drugs (Svendsen and Schaffer, 1982). The World Health Organisation (WHO) considers medicinal plants as the best source to obtain a variety of drugs that can either inhibit the growth of pathogens or kill them and have little or no toxicity to host cells (Sahgal *et al*., 2009, Santos *et al*., 1995).

Sanseviera liberica (Family Agavaceae, Ruscaceae or Dracaenaceae) belongs to the genus Sanseviera which is made up of about sixty species (Evans, 2005). It is a woody perennial plant with short petioled leaves that are in part transversely banded with light and dark green and also linearly striated with whitish to light green and dark green striations. The plant has long rhizomes with long fibrous roots and a rapid rate of growth. It is widely distributed throughout the world and is commonly located in shady places near streams and rocky parts (Adeyemi *et al*., 2009).

In Nigeria, extracts from the leaves and roots of *Senseviarea liberica* are used in folk medicine for the treatment of asthma, abdominal pain, colic, diarrhoea, eczema, gonorrhea, hemorrhoids, hypertension, monorrhagia, piles, sexual weakness, snake bite and wounds of the foot (Gill, 1992; Adeyemi *et al*., 2009). Available knowledge on the use of medicinal plants in traditional medicine is enormous but if this is not rapidly researched and documented, there is a risk that the knowledge will be lost. Furthermore, from the scientific

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investigations undertaken based on ethnomedicinal applications of such plants, biologically active compounds have been isolated. Hence in an attempt to investigate the scientific basis for the use of *S. liberica* in folk medicine, we investigated the phytochemical and antimicrobial activity of the methanol extract of the roots.

MATERIALS AND METHODS

Plant collection and extraction: Sensaviera liberica roots were collected at Elele, Rivers State of Nigeria and identified at the Department of Pharmacognosy, Madonna University, Elele, where a voucher specimen was deposited. The collected roots were dried under shade for 7 seven days and milled into coarse powder. 250 g of the plant material was macerated in 2.5 litres of 90% methanol for 7 days with occasional shaking. The crude extract was filtered by suction and concentrated under reduced pressure in a rotary evaporator. A portion of the extract was stored in a refrigerator at 4^{o}C for antimicrobial screening while the remainder was used for qualitative phytochemical analysis.

Micro organisms: 8 clinical isolates of bacteria (*S. aureus*, *C. albicans*, *B. subtilis*, *P. aeruginosa*, *S. pyrogens*, *S. typhii, E. coli* and *K. pneumonia)* were obtained from the diagnostic laboratory of Madonna University Teaching Hospital (MUTH). The bacterial isolates were maintained on nutrient agar and subcultured every three days (Idris *et al*., 2009).

Phytochemical analysis: The test tube method for qualitative phytochemical screening of the extract was adopted using standard methods (Trease and Evans, 1989, Harborne, 1984). The sample was screened for: carbohydrates, reducing sugars, anthraquinone, cardiac glycoside, cyanogenic glycoside, tannins, saponin, alkaloids and flavonoids.

*Antimicrobial activity***:** The plate-hole assay was used to determine the growth inhibition of bacteria by the extract (Kudi *et al*., 1999; Ogundipe *et al*., 2000).The stock concentration of 500 mg/ml was prepared by dissolving 1 g of the methanol extract of *S. liberica* into 2 ml of distilled water. 25 ml of the prepared nutrient agar was poured into sterile petri dishes and allowed to solidify and dry. A sterile cork borer of 9 mm diameter was used to bore three equi-distant holes in the set nutrient agar per plate and inoculated with 0.5 ml suspension of the bacteria over night. Thereafter the wells were filled with the extract at varying concentrations of 500 mg/ml, 400 mg/ml and 300 mg/ml respectively. This was done in triplicates and the plates were incubated at 37^{o}C for 18 hours. The anti bacterial activities were observed and measured using transparent meter rule and recorded if the zone of inhibition was ≥ 10 mm (Vlietinck *et al*., 1995; Reuben *et al*., 2008; Kudi *et al*., 1999).

Minimum inhibitory concentration (MIC): The broth dilution technique was used (Usman *et al*., 2007; Reuben *et al*., 2008). The prepared stock plant extract of 500 mg/ml concentration was serially diluted to a various working concentrations ranging from 0.780 mg/ml to 200 mg/ml using nutrient broth and later inoculated with 0.2 ml suspension of the test organisms. After 18 hours of incubation at 37^{o}C, the test tubes were observed for turbidity. The least concentration where no turbidity was observed was noted as the minimum inhibitory concentration (MIC).

*Minimum Bactericidal concentration (MBC)***:** This was determined from the broth dilution resulting from the MIC tubes by sub culturing in antimicrobial free agar (Usman *et al*., 2007). In this technique, the contents of the test tubes resulting from MIC was streaked using a sterile wire loop on agar plate free of bacteria and incubated at 37^{o}C for 18 hours. The lowest concentration of the extract which showed no bacterial growth was noted and recorded as the MBC (Reuben *et al*., 2008).

RESULTS AND DISCUSSION

The results of the phytochemical screening of the crude methanol and N-hexane *S. liberica* extract are shown in table 1. Both extracts contain tannins, flavonoids, saponins, reducing sugars and carbohydrates. Alkaloids were present only in the methanol extract. Glycosides, terpenoids and steroids were absent in both extracts.

Table 1. Phytochemicals present in the methanol extract and n – hexane fractions of *S. liberica*

Phytochemicals	Methanol extract	N-hexane fraction
Alkaloids	+++	-
Tannins	+++	++
Flavonoids	+++	+++
Glycosides	-	-
Saponins	+++	+++
Terpenoids	-	-
Steroids	+++	-
Reducing sugar	+++	+++
Carbohydrates	+++	+++

+++ = present, - = absent

Table 2: Proximate analysis on *S. liberica*

Parameters	Values (%)
Ash value	6.00
Acid insoluble ash	1.10
Alcohol extractive value	6.80
Water extractive value	5.20
Moisture content	14.50

The presence of some of the phytochemicals is responsible for the antimicrobial activities of the extracts. For example, flavonoids are synthesized by plants in response to microbial attack (Idris *et al*., 2009), and their mechanism of action is by inhibition of bacterial cell wall synthesis (Cowan, 2002). Tannins have anti – irritant, antisecretolytic, antiphlogistic, antimicrobial and antiparasitic activities hence they are used to treat non specific diarrhea, inflammation of mouth and throat and slightly injured skin (Idris *et al*., 2009, Westendarp, 2006). Therefore the broad spectrum of antibacterial activities of the extracts of S. liberica can be explained by the presence of alkaloids, tannins, flavonoids and saponins.

Table 3: Minimum inhibitory concentrations for methanol extract of *S. liberica* using broth dilution method

Test organism	Concentrations (mg/ml)				
	0.125	0.25	0.50	1.00	2.00
Pseudomonas aureginosa	++	++	--	--	--
Candida albicans	++	++	++	++	++
Klebsiella pneumonia	++	++	++	++	++
Salmonella typhii	++	++	++	++	++
Baccilus subtilis	++	++	++	++	++
Streptococcus pyrognase	--	--	--	--	--
Escherichia coli	++	++	++	++	++
Staphylococcus aureus	++	++	++	--	--

++ = present, -- = absent

Table 4: Minimum inhibitory concentrations for n-hexane fraction of *S. liberica* using broth dilution method

Test organism	Concentrations (mg/ml)					
	0.125	0.25	0.50	1.00	2.00	
Pseudomonas aureginosa	++	++	++	++	++	++
Candida albicans	++	++	++	++	++	++
Klebsiella pneumonia	++	++	++	++	++	++
Salmonella typhii	++	++	++	++	++	++
Baccilus subtilis	++	++	++	++	++	++
Streptococcus pyrognase	++	++	++	++	++	++
Escherichia coli	++	++	++	++	++	++
Staphylococcus aureus	++	++	++	++	++	++

++ = present,

Table 5: Minimum bactericidal concentrations of methanol extract of *S. liberica* using broth dilution method

Test organism	Concentrations (mg/ml)				
	0.125	0.25	0.50	1.00	2.00
Pseudomonas aureginosa	++	++	++	++	++
Streptococcus pyrognase	++	++	++	++	++
Staphylococcus aureus	++	++	++	++	++

++ = present,

Table 6: Effect of ciprofloxacin on some of the organisms

Test organism	Concentrations (mg/ml)				
	0.125	0.25	0.50	1.00	2.00
Pseudomonas aureginosa	--	--	--	--	--
Streptococcus pyrognase	--	--	--	--	--
Staphylococcus aureus	--	--	--	--	--

- - = absent

Three out of the eight microorganisms tested were sensitive to the methanol extract while five were not sensitive to the methanol extract. The sensitive ones were: *P. auregenosa, S. pyrognase and S. aureus.* These organisms are causative agents to diseases that occur to man. For example *Pseudomonas auregenosa* causes urinary tract infections, respiratory system infections, dermatitis, soft tissue infections, bacteremia, bone and joint infections, gastrointestinal infections and a variety of systemic infections, particularly in patients with severe burns and in cancer and AIDS patients who are immunosuppressed; *Streptococci pyrognase* causes sore throat and Staphylococcus aureus causes urinary

tract infection (UTI) in young women (Todar, 2012; Okunrobo *et al*., 2012).The resistant organisms to the methanol extract were *C. albicans, K. pneumonia, S. typhii, B. subtilis* and *E. coli.*
All the microorganisms were resistant to the n-hexane fraction of the methanolic extract. This implies that the polar extract is more potent than the non polar fraction. This justifies the use of alcohol to prepare the extract by some traditional medicine practitioners. The methanol extract of *S. liberica* contains phytochemicals with both bactericidal and bacteriostatic effects.

The proximate analysis of *S. liberica* showed total ash value of 6.0 %, acid insoluble ash of 1.10 %, alcohol extractive value of 6.80 %, water extractive value of 5.20 % and moisture content of 14.50 % (Table 2). Total ash value of 6.0 % reveals that the plant has low inorganic components and a high organic content. The low acid insoluble ash of 1.0 % indicates that a large portion of the ash content is acid soluble and hence may be physiologically important as salts in the body when consumed. The alcohol extractive value of 6.80 % which is higher than the water extractive value of 5.20 % shows that alcohol is a better solvent for extraction. The moisture content of 14.50 % which is lower than 20 % shows that the extract can be stored for a long period with lower chances of microbial growth.

Conclusion: The presence of a wide range of phytochemicals in the extracts of *S. liberica* with broad spectrum of activity against pathogenic microorganisms indicate that the plant extract could serve as a lead for the development of antimicrobial agents for use in chemotherapy.

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