PRILIMINARY PHYTOCHEMICAL SCREENING AND ANTIFUNGAL SUSCEPTIBILITY OF *SECURINEGA VIROSA EXTRACT ON SOME UROPATHOGENS*

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ABSTRACT
*Securinega virosa*is commonly called "Bush weed" and is one of the great African medicinal plants described as a true "cure all". This study is aimed at evaluating the phytochemical screening and antifungal susceptibility of *Securinega virosa* extract on some uropathogen. Evidence of this study shows that *Securinega virosa* contains some phytochemicals such as alkaloids, saponin, glycosides, tannins, steroids, although, qyinones and flavonoids are absents. Fungal isolates were obtained from the microbiology laboratory, Federal University of Agriculture, Makurdi. Susceptibility test was carried out using Agar well diffusion method. The MIC of the potent extract was determined according to the macro broth dilution technique. At the concentration of 200mg/ml, *Candida albicans* had the zone of inhibition of 9.00±1.00 higher than the zone of inhibition of *Aspergilus niger*. The zone of inhibition of *Aspergilus niger* at 250mg/ml (12.92±0.57) was higher than that of *Candida albicans*(12.33±0.57). The potency of the aqueous leaf extract of *Securinega virosa* which reduced with decreased in concentration, 500mg/ml has the highest zone of inhibition across the isolates while 100mg/ml showed the lowest zone of inhibition. The study reveals that *S. virosa* show significant antifungal activities against Candida infection with a minimum inhibitory concentration of about 25%.

KEYWORDS: *Securinega virosais, Susceptibility, Candida albicans, Aspergilus niger, Phytochemical, Uropathogen.*

1. INTRODUCTION

Securinega virosa is one of the great African medicinal plants described as a true "cure all", of which all parts are used as remedies, particularly the root (Neuwinger, 2016). It belongs to the family, Euphorbiaceae of the order, Geraniales. *S. virosa* is a dense, low branching, with many branched shrub, sometimes a small spreading tree up to about 6 meters high, although, more commonly 2 to 3 meters, evergreen or deciduous. It is widely distributed throughout tropical Africa, also in India, Malaya, China and Australia (Dalziel, 2013).

In Nigeria, it is found in virtually all parts of the country. The common vernacular names of *S. virosa* include "*tsuwaawun karee*, *gussu*, *gwiiwar karee*" (Hausa), "*iranje*" (Yoruba), "*njisi nta*" (Ibo), "*shim shim*" (Kanuri), "*kartfi-kartfi*" (Shuwa Arabs) and "*camal*, *cambe*, *came*" (Fulani). In many parts of Africa including the north Eastern Nigeria, the root and leafy twig decoctions are used for the treatment of epilepsy. The plant is said to have a hallucinogenic effect and the decoction of the root with other plant is used in Northern Nigeria for the treatment of mental illness (Neuwinger, 2016).

The plant is also known as common bush weed and rich in Antioxidants which protect the human body against free radicals that may cause pathological conditions. This plant (*Securinega virosa*) produces definite phytochemicals with physiological actions in the human body. The medicinal values of the plants lie in the component of their phytochemicals. Phytochemicals are secondary metabolites produced by plants (Hill, 2010). Research interest has been directed to the use of natural phytochemical for the formulation of antioxidant-based drugs (Mondon *et al.*, 2012). The majority of the active antioxidant compounds are flavonoids, flavones, isoflavones, anthocyannins, cumarins, lignans, catachins, phenols and isocatachins. Flavonoids and flavones which are some of the constituent of the plant are widely distributed secondary metabolites with antioxidant and antiradical properties (Augustin *et al*, 2015). The leaves are used in the treatment of fever, body pain, stomachache, rheumatism, diarrhea, pneumonia, diabetes and epilepsy.

Among the most common infectious diseases, urinary tract infections (UTIs) are a commonly encountered diseases by clinicians in developing countries with an estimated annual global incidence of at least 250 million (Ronald *et al.,* 2001). UTIs refer to the presence of microbial pathogens within the urinary tract and it is usually classified by the infection site:-bladder (cystitis), kidney (pyelonephritis), or urine (bacteriuria) and also can be asymptomatic or symptomatic, UTIs that occur in a normal genitourinary tract with no prior instrumentation are considered as "uncomplicated," whereas "complicated" infections are diagnosed in genitourinary tracts that have structural or functional abnormalities, including instrumentation such as indwelling urethral catheters, and are frequently asymptomatic (Gonzalez and Schaeffer, 1999). It has been estimated that globally symptomatic UTIs result in as many as 7 million visits to outpatient clinics, 1 million visits to emergency departments, and 100,000 hospitalizations annually (Wilson and Gaido, 2004).

The aim of this study is to evaluate the preliminary phytochemical screening and antifungal susceptibility of *Securinega virosa* extracts on some uropathogens.

3.0 MATERIALS AND METHODS

3.1 Study Area

The plant sample (*Securinega virosa*) was collected from Federal University of Agriculture, Makurdi, Benue state. The plant sample was then taken to microbiology research laboratory and identified by a botanist before analysis.

3.2 Equipment/ Reagents

The equipment and reagents are as follows, Text tube rack, Conical Flask, digital weighing balance, Pipette, mortar, Heating mantle, concentrated sulphuric acid, glacial acetic acid, dilute hydrochloric acid, distilled water, Acetic anhydride, Ferric Chloride, distilled water and Methanol.

3.3 Preparation of plant sample

The samples (*Securinega virosa*) were washed under running water, dried for 3 days under room temperature and grinded into powdery form using mortar and pestle. The grinded sample was sieved and stored in an airtight bottle.

3.3.1 Extraction

180g of the plant sample was weighted and soaked in 500ml of methanol and water respectively. The soaked sample was allowed to stand for 2 days (48hr), it was filtered and concentrated in a water bath at 49.7^0c

3.3.2 Procedure for Phytochemical Screening

Major metabolites classes such as alkaloids, glycosides, flavonoids, Tannins saponins, steroids, Quinones, protein, phenol and terpenoids were screened according to the methods described by a guide to modern techniques of plant analysis, Medicinal Plants and Traditional Medicine in Africa and Pharmacognosy

3.3.3 Test for Phenol

2ml of the extract was pipette into a test tube, few drops of dilute ferric chloride solution was added. The formation of a red, blue, green, or purple coloration indicates the presence of phenols.

3.3.4 Test for Alkaloids

2ml of the extract was pipette into a test tube the filtrate was carefully tested with Mayer's reagent (potassium mercuric iodide). yellow coloured precipitate indicates the presence of alkaloids.

3.3.5 Test for Flavonoids
To 2ml of extract, few drops ammonia solution was added. Appearance of yellow or orange colour indicates the presence of flavonoids.
3.3.6 Test for Tannins
To 2 ml water extract of all plant parts, 2 ml of 10% ferric chloride solution was added in a test tube. Blue-black precipitate indicates the presence of tannins.
3.3.7 Test for Saponin
To 2ml methanolic extract of the plant, 2 ml distilled water was added in a test tube and vigorously shaken. Persistent froth volume produced, checked each 10 minutes for 30 minutes indicates the presence of saponin.
3.3.8 Test for Steroids
1g of the plant extract was dissolved in a few drops of acetic acid. It was gently warmed and cooled under the tap water and a drop of concentrated H_2SO_4 acid was acid was added along the side of the test tube. The appearance of green colour indicates the presence of steroid.
3.3.9 Test of Terpenoids
Few drops of chlorofoam were reacted with 2ml of the latterly and few drops of concentrated H_2SO_4 was added. A reddish-brown precipitate produced immediately indicate the presence of terpenoids

3.4 ANTI-MICROBIAL ACTIVITY OF THE PALNT EXTRACT

3.4.1 Preparation of test organisms
Cold stored agar slant cultures of Gram positive and Gram-negative organism and fungi were used in this study. The fungal isolates were obtained from the microbiology laboratory, University of Agriculture Makurdi.
Viability test of each organism was carried out by resuscitating the organisms in buffered peptone both and there after sub-cultured into nutrient agar medium and incubated at 37°c for 24hrs. The probable identity of the clinically sourced isolates was further confirmed by exposing the cultures to an array of biochemical tests which includes coagulase,indole methylised,citrate, action and oxidase tests as described by Collins *et al* 2004 and Sharma (2009). The result of the biochemical reaction elicited by the test isolate were compared with standard identification keys as described by Collins *et al*., 2004 on Sabourand dextrose agar to check the purity.

3.4.2 Preparation of Concentration of the Plant Extracts.
One gram (1g) each of the aqueous and methanolic extracts was added to 2ml of distilled water and methanolic respectively to give a concentration of 250mg/ml,100mg/ml and 58mg/ml were prepared by double broth dilution method as described by Udochukwu *et al.* (2015).

3.4.3 Anti-Microbial Susceptibility testing of the extract with the test organisms.

ANTI FUNGAL ASSAY
Susceptibility testing was carried out using agar well diffusion method. In this method, the inocula were prepared by inoculating the test organisms in dextrose broth and allowed to stand for 72hours. The cultures were diluted to 0.5 Mcfarltand turbidity standards. About 0.5ml each of the cultured fungi was pipette into Petri dish after which prepared Sabourand dextrose agar was pour plated and allowed to solidify.

After the cultured plates have gelled, wells were bored on the surface of the agar plates using 4mm core borer. About 0.2ml of the different concentration of each extract were transferred into the well using pasture pipette. The wells were sufficiently spaced to prevent the resulting zone of inhibition from overlapping. Fluconazole (25mg) was used as positive control. The plates were allowed to stand for 24 hours. The experiment was performed in triplicates and resulting zone of inhibition measured at the diameter of the well using a ruler.

Minimum Inhibitory Concentration (MIC)
The MIC of the potent extracts was determined according to the macro broth dilution technique described by Baron and Finegold (1990). Standardized suspension of the test organism was inoculated into a series of five sterile test tubes of nutrient broth containing two-fold dilution of the extracts and incubated at 37% for 24hours. The MIC was recorded as the least concentration that inhibited the growth of test organism.

3.5 Statistical Analysis
Data were analyzed for mean and standard deviation. Difference in parameter was tested for statistical difference at $p<0.05$ using T-test. All the analysis was done using statistical package service solution (SPSS) version 21.

RESULTS

4.1 Phytochemical Screening

Table 1 indicates the phytochemical constituents of each extract of *Securinega virosa* for the presence of secondary metabolites. Quinones and Flavonoid are absent in both methanolic and aqueous leaf extract of *Securinega virosa.*

Zone of inhibition of methanol leaf extract of *Securinega virosa* is presented on Table 2. Candida albicans have the highest zone of inhibition at the concentration of 500mg/ml which is significantly higher than the control. At the concentration of 200mg/ml, *Candida albicans* has the zone of inhibition of 9.00±1.00 higher than the zone of inhibition of *Aspergillus niger.* The zone of inhibition of *Aspergillus niger* at 250mg/ml (12.92±0.57) is higher than that of *Candida albicans* (12.33±0.57). There was however no significant difference in the mean zone of inhibition at different concentrations of methanolic leaf extracts of *Securinega virosa at P>0.05.*

Table 3 shows the potency of the aqueous leaf extract of *Securinega virosa* which reduced with decrease in concentration. 500mg/ml has the highest zone of inhibition across the isolates while 100mg/ml showed the lowest zone of inhibition. *Aspergillus niger* has the highest zone of inhibition across all concentrations except at 100mg/ml (9.33±1.55mm). There was significant different in the mean of inhibition at different concentrations of aqueous leaf extract of *Securinega virosa at* $P \leq 0.05$

Table 1: phytochemical properties of *Securinega virosa* leaf

Phytochemical	Methanol	Aqueous
Glycosides	+	+
Steroids	+	+
Saponins	+	+
Quinones	-	-
Phenol	+	+
Terpenoids	+	+
Protein	+	+
Alkaloids	+	+
Flavonoid	-	-
Tannin	+	+

Key:
(+) = present
(-) = Absent

Table 2: Zone of inhibition of methanol leaf extract of *Securinega virosa* on selected organisms

Test organism	zone of inhibition (mm)					
	Control (Fluconazole)	**500mg/ml**	**250mg/ml**	**200mg/ml**	**125mg/ml**	**100mg/ml**
C. alblicans	1.20±12.16	17.33±2.082	12.33±0.577	9.00±1.00	1.33±0.577	11.00±0.00
A.niger	1.15±11.03	16.00±2.000	12.92±1.564	12.33±1.528	10.33±0.577	9.00±1000
(p> 0.05) Df=5						p=0.182

Table 3: Zone of inhibition of aqueous leaf extract of *Securinega virosa* on selected organisms

Test organism	zone of inhibition (mm)					
	Control (Fluconale)	**500mg/ml**	**250mg/ml**	**200mg/ml**	**125mg/ml**	**100mg/ml**
C. alblica	1.33±7.60	13.33±2.309	11.33±0.577	10.33±0.577	10.33±0.577	11.00±0.00
A.niger	.88±11.87	15.67±1.528	14.33±1.528	12.33±1.528	10.33±1.528	9.33±1.55
(p≤ 0.05) Df=5					p=0.026	

5.1 DISCUSSION

Evidence of the study indicated that *Securinega virosa* plant contains phytochemicals such as alkaloid, saponin, tannin, glycoside although, quinones and flavanoids are absent. These phytochemicals are found in the leaf of the plant in various concentrations. This phytochemical gives the plant the therapeutic capacity which makes them a good medicinal source. The finding of the study also corroborates with the finding of Kiran Kumar *et al*. (2013) who also reported the presence of alkaloids, flavanoids, phenols, glycosides, tannins, saponins and lignins in the leaf extract of *Mirabilijalapa.*

The relative susceptibility of *Candida albicans* to antibiotics is dependents on the age of culture because the culture environment is rapidly changing and the cell population become more physiologically heterogenous and hence, more resistant with age. So, it is beneficial to test the effect of the plant extract on *Candida albicans* culture in its stationary phase of growth.

The study of biological and chemical properties showed that the Nigerian flora has a real therapeutic and nutritional potential and can be used to treat or prevent many diseases. These plants have a real interest in human health for their antibacterial. Antifungal, antiplasmodial and antioxidant activities.

The antifungal activity of the extracts of leaves of *S. virosa* was evaluated on two species of fungiwhich are: *C. albicans, Aspergillus niger*. These saprophytes and opportunistic yeast are present in the mucosa where they take advantage of an imbalance of existing flora or immune deficiency to multiply and cause infections. Resistance to azoles, including fluconazole, may explain clinical failures (Vanessa, 2001). Determining the diameter of the inhibition zones and determination of antifungal parameters (MIC) were used to assess their sensitivity. In view of the results, we note that the best extraction yields are obtained with the methanol extracts, as well as the best antifungal activity is obtained with ethanol extracts.

The methanol extracts were the most active; indicating that they concentrate most of the compounds. Methanol would be the best extraction solvent of active substances from this plant. It may also be explained that the activity of antibiotics in plant extracts against fungi or growth may be due to their mechanism of action, chemical structure or spectrum of activity.

As *S. virosa* induces diameters of inhibition zone greater than 10 mm and the MIC range from 100mg/ml. These results suggest that the plant has an antifungal activity. This corroborates with the results of Agassounon *et al.* (2001) which have shown that this plant has fungicidal properties.

The diameters of inhibition zones are 17 mm for methanolic extract the most active extract and 13 mm for aqueous extract at a concentration of 500 mg/ml. Kubmarawa *et al.* (2007) showed that the methanol extract of the leaves of *S. virosa* has significant inhibitory activity against *C. albicans* and *Aspergillus niger.* These results are supported by the work of Agassounon *et al.* (2001) which showed that the methanolic extract concentrate is better than aqueous extract of the leaf of plant. It should be noted that the antimicrobial activity of medicinal plants varies from one geographic region to another.

5.2 CONCLUSION

Study of *Securinega virosa* showed significant antifungi activities against multidrug resistant fungi that complicate Candida infection with an MIC of about 25%

Evidence from this study clearly shows that *Securinega virosa* contains phytochemicals that are essential for both the plant and makes the plant a good source of medicine for man. At 500mg/ml *Securinega virosa* extract has a minimum fungicidal concentration of 50%

5.3 RECOMMENDATION

From the result of this study, it is recommended that:

i. Further studies should be carried out on the quantitative and molecular analysis of organisms associated with urinary tract infection.
ii. In-vivo study of the plant extract should be carried out to determine its toxicity on human organ such as the bladder.
iii. Research should be carried out on the antiviral effect of *Securinegavirosa* extract on some selected organism particularly Hepatitis and rubella diseases.

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