Evaluation of Antifungal and Phytochemical Properties of Violet Tree (*Securidaca Longepedunculata* Fres)

By Junaidu, S., Shehu K., Alero, A. A., Bawa, J. A. & Suleiman, I.

*Federal University Dutsin-Ma, Nigeria*

**Abstract**- This study was undertaken to investigate the antifungal activities of aqueous and ethanol extracts of *Securideca longepedunculata* leaves and root bark against two *Aspergillus* species (*Aspergillus niger* and *Aspergillus flavus*). Agar incorporation method was used for antifungal testing. The results of phytochemical screening demonstrated the presence of flavonoid, saponin, alkaloids, cardiac glycoside and saponins glycosides. Highest growth inhibition (1.16+1.15mm) at 300 mg/ml, (1.67+2.88mm) and higher increase (3.16+0.57mm) at 100 mg/ml were observed. The results showed significant effect (p<0.05) of antifungal activities. In the same study, the results however, applied that the phytochemical constituents of *S. longepedunculata* leaves and root bark extracts can be used as potential antimicrobial agents in the management of microbial diseases caused by pathogenic *Aspergillus* species which can become an alternative to chemical antibiotics.

**Keywords**: antifungal, violet tree, phytochemical, aqueous, ethanol extracts, aspergillus.

**GJSFR-C Classification**: FOR Code: 780105
Evaluation of Antifungal and Phytochemical Properties of Violet Tree
(Securidaca Longepedunculata Fres)

Junaidu, S.ª, Shehu K.ª, Aliero, A. A.ª, Bawa, J. A.º & Suleiman, I.º

Abstract - This study was undertaken to investigate the antifungal activities of aqueous and ethanol extracts of Securidaca longepedunculata leaves and root bark against two Aspergillus species (Aspergillus niger and Aspergillus flavus). Agar incorporation method was used for antifungal testing. The results of phytochemical screening demonstrated the presence of flavonoid, saponin, alkaloids, cardiac glycoside and saponins glycosides. Highest growth inhibition (1.16±1.15mm) at 300 mg/ml, (1.67±2.88mm) and higher increase (3.16±0.57mm) at 100 mg/ml were observed. The results showed significant effect (p<0.05) of antifungal activities. In the same study, the results however, applied that the phytochemical constituents of S. longepedunculata leaves and root bark extracts can be used as potential antimicrobial agents in the management of microbial diseases caused by pathogenic Aspergillus species which can become an alternative to chemical antibiotics.

Keywords: antifungal, violet tree, phytochemical, aqueous, ethanol extracts, aspergillus,

I. INTRODUCTION

Violet tree (Securidaca longepedunculata) commonly called Krinkhout in Africa. It is a slender tree with beautiful flowers, belonging to the family polygalaceae. The tree is highly regarded for its medicinal purpose, especially by the vhaVenda people of the Sudan (Elkalifa, 2006). A cooperative approach by ethnobotanists, ethnopharmacologists, physicians and phytochemists is thereby essential to spur the progress of medicinal plants research (Ndou, 2006). Medicinal plants have traditionally occupied an important position in the socio-cultural, spiritual and medicinal arena of rural and tribal lives in Sudan. The Sudan has witnessed the fusion of many cultures, Pharonic, Islamic and Christianity along with the local indigenous cultures. With this unique history and vast variety of climate and flora, traditional medicine together with use of medicinal plants became an important part of the cultural heritage of the Sudan (Elkalifa et al., 1999). The abundance of information on traditional medicinal uses of plants in Africa is in danger of disappearing since the knowledge of how to use medicinal plants is mostly passed down orally and even to date is poorly documented (Gurib-Fakim, 2006), although written information has been produced for some specific regions. Moreover, the most serious threat to local medicinal plant knowledge, however, appears to be cultural change, particularly the influence of modernization and the western world view (Voeks and Leony, 2004) which has contributed to under mining traditional values among the young (Giday et al., 2003).

Plants are very good sources of medicinal compounds that have continued to play a dominant role in the maintenance of human health since Ancient times (Moriita et al., 2011). Plant extracts or their active constituents are used as folk medicine in traditional therapies of about 80% of the world’s population and Over 50% of all modern clinical drugs are of natural product origin (Kumar and Chandrashekar, 2011). The effect of plant extracts on microorganisms have been studied by a very large number of researchers in different parts of the world (Kumar et al., 2006; Mathabe et al., 2006) and the use of a variety of plant extracts and phytochemicals, both with known antimicrobial properties can be of great significance in therapeutic treatments. Many plants have been used because of their antimicrobial properties, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances, such as, the phenolic compounds which are part of the essential oils, as well as in tannin (Nascimento et al., 2000).

This study was design to investigate and determine the phytochemical properties and antifungal activities of violet tree Securidaca longepedunculata on Aspergillus species.

II. MATERIALS AND METHOD

a) Description of Study Area

Katsina State, covering an area 23,936 sq. km., is located between latitudes 11°08’N and 13°22’N and longitudes 6°52’E and 9°20’E. The state is bounded by Niger Republic to the north, by Jigawa and Kano States to the east, by Kaduna State to the South and by...
Zamfara State to the West. A cool dry (harmattan) season from December to February; a hot dry season from March to May; a warm wet season from June to September; a less marked season after rains during the months of October to November, characterized by decreasing rainfall and a gradual lowering of temperature.

b) Collection, Identification and Processing of Plant Material

Fresh roots and leaves of *Securida longipedunculata* were collected during the month of May, 2013 at 5:30pm-6:05pm from Kudewa, Kurfi Local Government Area, Katsina State, Nigeria. The plant was preserved, identified and authenticated at the Herbarium Section, in the Botany Unit of the Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria. Samples deposited at the Herbarium have a Voucher No. D-01SL-7.

The plant materials were properly washed under tap water, rinsed with distilled water, dried under shade and pulverized with a pestle and mortar and kept in a transparent sterile polyethylene bag at room temperature for use.

i. Preparation of Extract

Two hundred grams (200g), each of dried plant material was extracted by soaking in 1000 ml of ethanol and water (solvent) in 1000 ml of conical flask, and covered with aluminum foil and allowed for 24 hours.

The extracts were filtered and the solvents removed by warming in oven at 40°C for 3 days. The evaporated extract was stored for 48-hours in sterile universal bottles at room temperature, this methods is adopted by Okogun (2000) and Shariff (2001).

c) Qualitative Phytochemical Tests

The plant extracts were screen for the presence of secondary metabolites using standard method (Odebiyi and Sofowora, 1978; Trease and Evans, 1989).

i. Source and Maintenance of Microbial Test Strains

Stocked isolate of fungal strains *Aspergillus* species (*A. niger*, *A. flavus*) was obtained from Mycology Laboratory of Botany Unit Usmanu Danfodiyo University Sokoto, Nigeria. The isolate was maintained on Potato Dextrose Agar.

ii. Sterilization of Glassware

The glassware were adequately washed with liquid soap and sufficiently rinsed with tap water and distilled water respectively, air dried and sterilized in hot air oven at 160°C for 1 hour, while the conical flask was autoclaved.

d) Preparation of Media

i. Preparation of Sarboraud Dextrose Agar (SDA)

The sarboraud dextrose agar (SDA) was prepared according to manufacturer’s instructions, SDA (65g) was dissolved in 1000 ml distilled water and 0.5g streptomycin solution was added to inhibit bacterial growth. The conical flask was plugged with cotton and capped with aluminum foil, sterilizing using lender autoclave at 121°C for 15 minutes, cooled to 45°C before been poured into sterilized plates and kept at 30°C (Cheesebrough, 1985).

ii. Preparation of Potato Dextrose Agar (PDA)

Potato dextrose agar (PDA) was prepared according to manufacturer’s instructions, 39g PDA was dissolved in 1000 ml of distilled water, the suspension was mixed until completely homogenized and 0.5g of streptomycin was added to inhibit the growth of bacteria. The conical flask containing the media were plugged with cotton wool and capped with aluminum foil, sterilized using lender autoclave at 121°C for 15 minutes, cooled for 45°C and pouring in to sterile plates. The plates were kept at 30°C (Cheesebrough, 1985).

e) Antifungal Testing

Antifungal testing of the aqueous and ethanolic extracts of leaves and root bark were determined by Agar incorporation method as described by Brantner (1994) for antifungal testing.

i. Activity of *S. longipedunculata*

Food poison technique was used to determine the antifungal effects of different concentrations of the extracts. Into 100 ml conical flask, 15 ml of media were added. The flasks were plugged with cotton wool, capped with aluminum foil and allowed to stand for 24 hours. The four flasks of 100mg, 200mg, and 300mg of the extract were added. The fourth flask contained only the media.

Five (5ml) each of varying concentration of the leaves and root bark extracts were incorporated in to each flask containing 15 ml of the media, this was then poured in to pre-sterilized petri-dishes and kept at room temperature of 27°C to 30°C, the growing cultures was punch with sterile inoculating needle and then deposited in the centre of the petri dishes containing varying concentration. The control plate was sterilized and containing 20 ml of the media were place at the centre of treated plates.

The results was measured in millimeters (mm) by measuring the fungal growth from two lines vertical and horizontal, the mean were recorded (Singh and Tripathi, 1999). For each treatment 3 replicate were maintained. Mean of three 3 replicates served as the result of each of the varying concentration.

Results obtained were subjected to statistical analysis using one-way analysis of variance ANOVA, with SPSS 16.0 Version. p<0.05 considered as significant followed by Duncan’s Multiple Range Test to detect significant differences among the means as well as the interactions between the variable.
### III. Results

**a) Antifungal activities of the different solvent extracts of S. longepedunculata**

The growth inhibitions of *Aspergillus* species due to the application of *S. longepedunculata* are presented which revealed that leaves and root bark extracts with different solvents inhibited the growth of all the fungal species Table 1. It was also indicated in the same Table 1, that the phytochemical properties of the extracts appeared more unless where they are not present in the composition.

#### Table 1: Phytochemical Composition of *S. longepedunculata* Leaves and Root bark Extracts

<table>
<thead>
<tr>
<th>Phytochemical</th>
<th>Leaves</th>
<th>Root</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoid</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Saponin</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponin Glycosides</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Balsams</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Volatile oil</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Keys: -Not present, + Present

Antifungal activities of *S. longepedunculata* was exhibited in root bark extracts on *A. niger* appeared high (at 300 mg/ml 8.83+2.46) and 13.00+00 root bark extract. In *A. niger*, the highest growth inhibition was found in the ethanol leaf extract at 300mg/ml of 3.33 + 0.57mm, as seen in Table 2.

#### Table 2: Antifungal activities of *S. longepedunculata* Leaves and Root bark Extracts on *A. niger*

<table>
<thead>
<tr>
<th>Extract</th>
<th>Conc. (mg/ml)</th>
<th>Leaf extracts M ±SD(mm)</th>
<th>Root bark extracts M ±SD(mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous</td>
<td>0</td>
<td>21.3³ + 2.46</td>
<td>21.3³ + 2.46</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>10.00abcd+1.00</td>
<td>14.00abcd+3.50</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>9.17abcd+3.25</td>
<td>13.00abcd+0.50</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>8.83abc+2.46</td>
<td>13.00abc+0.50</td>
</tr>
<tr>
<td>Ethanol</td>
<td>0</td>
<td>21.3³a + 2.47</td>
<td>21.3³abc + 2.46</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>9.00abc + 3.12</td>
<td>4.33abc + 1.52</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>6.00ab + 2.59</td>
<td>4.33ab + 1.75</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>4.83ab+1.15</td>
<td>3.33ab+0.57</td>
</tr>
</tbody>
</table>

ab⁵c⁶ Means in a column with different superscripts are significantly different (p<0.05) Values are means ± standard error of three replications

The antifungal activity of *S. longepedunculata* in both the root bark and leaf extracts increase as a result of increase in the concentrations in mg/ml, Table 1. It could be deduced that, he aqueous and ethanolic extracts of the root bark and leaf significantly different (P<0.05) in increase of the antifungal activities in *A. niger* and *A. flavus* respectively.

In *A. flavus* highest growth inhibition was observed in the aqueous root extract at (300mg/ml of 6.33+1.44) and root bark (3.16+0.57) as seen in Table 3.
Table 3: Antifungal activities of S. longepedunculata Leaves and Root bark Extracts on A. flavus

<table>
<thead>
<tr>
<th>Extract</th>
<th>Conc. (mg/ml)</th>
<th>Leaf extract M ±SD(mm)</th>
<th>Root extract M ±SD(mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous</td>
<td>0</td>
<td>24.66±2.02</td>
<td>24.67±2.02</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>13.83\textsuperscript{ab}±2.51</td>
<td>8.50\textsuperscript{ab}±3.50</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>8.17\textsuperscript{c}±4.80</td>
<td>7.00\textsuperscript{bc}±4.92</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>4.67\textsuperscript{a}±2.75</td>
<td>3.00\textsuperscript{abc}±0.50</td>
</tr>
<tr>
<td>Ethanol</td>
<td>0</td>
<td>6.67\textsuperscript{ab}±4.31</td>
<td>6.16\textsuperscript{ab}±2.56</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>6.67\textsuperscript{ab}±4.31</td>
<td>6.16\textsuperscript{ab}±2.56</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>6.33\textsuperscript{ab}±5.39</td>
<td>4.50\textsuperscript{bc}±0.00</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>6.33\textsuperscript{ab}±1.44</td>
<td>3.16\textsuperscript{ab}±0.57</td>
</tr>
</tbody>
</table>

\textsuperscript{a,b,c} Means in a column with different superscripts are significantly different (p<0.05)

Values are means ± standard error of three replications.

IV. DISCUSSION

The present study revealed the phytochemical and antifungal screening of Securidaca longepedunculata samples, which co-opt the rich sources of bioactive compounds in potential use of diseases management. It was reported in this study, that the presence of various secondary metabolites like tannins, saponins, alkaloids, flavonoids and others in qualitative analysis extracted from S. longepedunculata might be responsible for great medicinal importance. These findings are in conformity with those reported by (Donald et al., 2011; Auwal et al., 2012) on phytochemical composition and acute toxicity of root bark extracts of S. longepedunculata. The present study demonstrated that the crude ethanol extracts possess remarkable activity. Antifungal activities of the ethanol extracts appeared to be more effective then aqueous extracts, since ethanol could extracts a wide variety of active component as compared to aqueous. Flavonoid together with the other secondary metabolites identified in the presence study have been severally reported to show curative activity against diverse pathogens, used traditionally analgesic antimicrobial, anti tumor headache, venereal diseases, constipation and coughs. This report is in line with findings of Abubakar et al. (2011) who investigated the growth inhibition and broad spectrum activity (14 to 27 mm) of Vernonia spp., from the crude ethanol extracts and chloroform fractions against some clinical bacterial strains and found the activity of chloroform fraction to be higher on Coryn bacterium ulcerans and Klebsiella pneumoniae (27 mm), while the chloroform fractions of V. oocphala and V. ambigua were more active on Proteus mirabilis (27 mm) and Salmonella typhi (22 mm), respectively. They added that the minimum inhibitory concentration (MIC) values ranged from 1.25-2.5 mg/mL for all the organisms tested.

However, the phytochemical screening and antifungal activities of the concentrations at 100, 200 and 300mg/ml of the extracts used in this research revealed the presence of active compounds like tannins, saponins, alkaloids, flavonoid, steroids/terpenes, tannins and glycosides. The antifungal activity exhibited amongst these substances suggests that S. longepedunculata has medicinal potential; this is due to the fact that each of the compounds identified has one or more therapeutic usage. Absent of anthraquinone worth nothing medically as earlier observed by (Ajiboye et al., 2010).

Results of the antifungal activities of ethanol and aqueous extracts of S. longepedunculata root bark and leaves ware tested against the organisms A. niger and A. flavus at three different concentrations, the extracts indicate significant effects (P<0.05) inhibitory activities of aqueous and ethanol extracts. This might be due to the fact that the extracts can exhibit remarkable activity. Antifungal activities of the ethanol extracts appeared to be more effective then aqueous extracts, since ethanol could extracts a wide variety of active component as compared to aqueous. Flavonoid together with the other secondary metabolites identified in the presence study have been severally reported to show curative activity against diverse pathogens, used traditionally analgesic antimicrobial, anti tumor headache, venereal diseases, constipation and coughs. This report is in line with findings of Abubakar et al. (2011) who investigated the growth inhibition and broad spectrum activity (14 to 27 mm) of Vernonia spp., from the crude ethanol extracts and chloroform fractions against some clinical bacterial strains and found the activity of chloroform fraction to be higher on Coryn bacterium ulcerans and Klebsiella pneumoniae (27 mm), while the chloroform fractions of V. oocphala and V. ambigua were more active on Proteus mirabilis (27 mm) and Salmonella typhi (22 mm), respectively. They added that the minimum inhibitory concentration (MIC) values ranged from 1.25-2.5 mg/mL for all the organisms tested.

The results of this study confirm the potential use of Violet tree, Securidaca longepedunculata as antifungal agents against infections caused by Aspergillus niger and Aspergillus flavus. The presence of these importance substances suggests that S. longepedunculata may possess myriads of therapeutic tendencies and ability to manage numerous malaises caused by Aspergillus species. The overall result concludes that the extracts used in this research are of potent antifungal activity. Thus, should be explored further for pharmaceutical uses as this is important in combating the recent observed emergence of drug resistance organisms.

V. CONCLUSION

The results of this study confirm the potential use of Violet tree, Securidaca longepedunculata as antifungal agents against infections caused by Aspergillus niger and Aspergillus flavus. The presence of these importance substances suggests that S. longepedunculata may possess myriads of therapeutic tendencies and ability to manage numerous malaises caused by Aspergillus species. The overall result concludes that the extracts used in this research are of potent antifungal activity. Thus, should be explored further for pharmaceutical uses as this is important in combating the recent observed emergence of drug resistance organisms.

VI. ACKNOWLEDGEMENT

The Authors appreciated the effort of Mr. Auwal Umar (Biological Sciences, Botany Unit, Usmanu Danfodiyo University Sokoto Nigeria) in carrying out the field work of this research.
REFERENCES  Références  Referencias


27. Ojewole JAO, Ilesanmi ORS, Olayiwola G (2000) Pharmacology of African Medicinal plants: Neuromuscular and cardiovascular properties of...


