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

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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 Limpopo Province where it occurs (Ndou, 2006). A cooperative approach by ethnobotanists, ethnopharmacologists, physicians and phytochemists is thereby essential to spur the progress of medicinal plants research (Gilani and Rahman, 2005). Medicinal plants have traditionally occupied an important position in the socio-cultural, spiritual and medicinal arena of rural and tribal lives in Sudan (Nelson-Harrison *et al.,* 2002). Through its long history, 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 (Baker *et al*., 1995: 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 23,938 sq. km., is located between latitudes 11Â°08'N and 13Â°22'N and longitudes 6Â°52'E and 9Â°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

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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 *Securidaca longepedunculata* 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 polyethene 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^{0}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 was 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^{o}C for 1hour, while the conical flask was autoclaved

d) Preparation of Media

i. *Preparation of Sarboroud Dextrose Agar (SDA)*

The sarbouraud 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^{0}C for 15 minutes, cooled to 45^{0}C before been poured into sterilized plates and kept at 30^{0}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^{0}C for 15 minutes, cooled for 45^{0}C and pouring in to sterile plates. The plates were kept at 30^{0}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. longepedunculata*

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^{0}C to 30^{0}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 Tripatti, 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

Phytochemical	Leaves	Root
Flavonoid	+	+
Tannins	+	-
Saponin	+	+
Glycosides	+	-
Alkaloids	+	+
Cardiac glycosides	-	+
Steroids	+	+
Saponin Glycosides	+	+
Balsams	+	-
Anthraquinones	-	-
Volatile oil	+	+

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

Extract	Conc. (mg/ml)	Leaf extracts M ±SD(mm)	Root bark extracts M ±SD(mm)
Aqueous	0	21.3^{fg} + 2.46	21.33^{fg} + 2.46
	100	10.00^{abcd}+1.00	14.00^{d}+3.50
	200	9.17^{abcd}+3.25	13.00^{ef} + 0.50
	300	8.83^{abc}+ 2.46	13.00^{fg}+00
Ethanol	0	21.33^{e} + 2.47	21.33^{de} + 2.46
	100	9.00^{bc}+ 3.12	4.33^{b}+1.52
	200	6.00^{ab} + 2.59	4.33^{b} + 1.75
	300	4.83^{ab}+1.15	3.33^{ab} + 0.57

^{a,b,c} 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 3.00 +0.50mm), least growth inhibition (13.83+2.51mm) was observed in aqueous leaves extract at 100mg/ml, to the more higher concentrations (at 300mg/ml) of leaf (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*

Extract	Conc. (mg/ml)	Leaf extract M ±SD(mm)	Root extract M ±SD(mm)
Aqueous	0	24.66^{g} + 2.02	24.67^{f} + 2.02
	100	13.83^{cde}+2.51	8.50^{bc}+ 3.50
	200	8.17^{ab}+4.80	7.00^{ab}+ 4.92
	300	4.67^{a}+2.75	3.00^{a}+0.50
Ethanol	0	6.67^{ab} + 4.31	6.16^{b}+2.56
	100	6.67^{ab} + 4.31	6.16^{b}+2.56
	200	6.33^{ab} + 5.39	4.50^{b} + 0.00
	300	6.33^{ab} + 1.44	3.16^{ab} + 0.57

^{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 of bioactive compounds is an indication 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 *Corynbacterium ulcerans* and *Klebsiella pneumoniae* (27 mm), while the chloroform fractions of *V. oocephala* 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 against the organisms *A. niger* and *A. flavus* and the susceptibility of these organisms may be a pointer to their potentials as a component or drug against the organisms tested in this study.

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

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References Références Referencias

1. Ajiboye TO, Salau AK, Yakubu MT, Oladiji AT, Akanji MA, Okogun JI (2010) Aqueous Extract of *Securidaca longepedunculata Root induce Redox* Imbalance in Male Rats Liver and Kidney. *Journal of Human and Experimental Toxicology* 29 (8): 67-688.
2. Abubakar BA, Mikhail SA, Hamisu I, Adebayo OO (2011) Phytochemical Screening and Antibacterial Activities of *Vernonia ambigua*, *Vernonia blumeoides* and *Vernonia oocephala* (Asteraceae). *Acta Poloniae Pharmaceutica Drug Research*, 68 (1): 67-73.
3. Auwal SM, Atiku MK, Wudil AM, Sule MS (2012) Phytochemical composition and Acute Toxicity Evaluation of Aqueus Root Bark Extract of *Securidaca longepedunculata. Bayero Journal of Pure and Applied Sciences,* 5 (2): 67-72.
4. Baker J, Borris R, Carte B (1995) Natural product drug discovery and development. New Perceptive on International Collaboration, *Journal of National Production*, 58: 1325-1328.
5. Brantner A, Pfeiffer K, Brantner H (1994) Application of Diffusion methods required by Phamacopoeiasis for testing Antibacterial activity of Natural compounds. *Phamazie*, 49 (7): 512-516.
6. Cheesebrough M (1985) Medical Laboratory Manual for Tropical Countries, 17: 203-305.
7. Donald Z, Blackson LK, Thokozani-Gudeta WS, Zewge T, Dominic SB, Gondwez VS, Philip CS (2011) Propagation of the African medicinal and pesticidal plant, *Securidaca longepedunculata; African Journal of Biotechnology* 10 (32): 5988-5992.
8. Duncan RC, Knapp RG, Miller MC (1977) Test of hypothesis in population Means. In: Introductory Biostatistics for the Health Sciences. John Wiley and Sons Inc. NY: 71- 96.
9. Edeoga HO, Okwu DE, Mbaebie BO (2005) Phytochemical Constituents of Some Nigerian Medicinal Plants. *African Journal of Biotechnology*, 4(7): 685-688.
10. El Khalifa MY (1999) Home remedies in Khartoum State. Unpublished Research Data, APRI Reports.
11. Evans CW (1996) Trease and Evans Pharmacognosy, 14th edition. W.B. Saunders Company Ltd., London. pp 268-270.
12. Giday M, Asfaw Z, Elmqvist T, Woldu Z (2003) An Ethnobotanical Study of Medicinal Plants used by the Zay People in Ethiopia. *Journal of-Ethnopharmacology.*, 85: 43-52.
13. Gilani AH, Rahman AU (2005). Trends in ethnopharmacology. *Journal of Ethnopharmacology*, 100: 43-49.
14. Gurib-Fakim A (2006) Medicinal Plants: Traditions of Yesterday and Drugs of Tomorrow. *Mol. Aspi. Medicine*, 27: 1-93.
15. Harborne JB (1998). Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. Chapman and Hall Ltd., London. pp 279.
16. Hassan LG, Kamba AS (2010) Phytochemical Screening and Antimicrobial activities of *Euphorbia balsamifera* Leaves, Stem and Root against Some Pathogenic Microorganisms. *African Journal of Pharmaceutical Sciences and Pharmacy* 1:85-95.
17. Kamba AS, Hassan LG (2010) Anti-bacterial screening and brine shrimp (*Artemia salina*). Toxicity of *Securidaca longepedunculata* (Polygalaceae) Root bark, *African Journal of Pharmaceutical Science pharmacology*, 1(1): 85-95.
18. Kumar P, Chauhan S, Padh H, Rajani M (2006) Search for Antibacterial and Antifungal agents from Selected Indian Medicinal plants. *Journal of Ethnopharmacology*, 107: 182-188.
19. Kumar T, Chandrashekar K (2011) *Bauhinia purpurea* Linn. A Review of its Ethnobotany, Phytochemical and Pharmacological Profile. *Research Journal of Medicinal plants* 5(4) 420-431.
20. Mariita R, Ogal C, Oguge N, Okemo P (2011) Methanol Extract of Three medicinal plants from Samburu in Northern Kenya show significant Antimycobacterial, Antibacterial and Antifungal Properties. *Research Journal of Medicinal Plants*, 5(1) 54-64.
21. Mathabe M, Nikolova R, Laly N, Nyazema N (2006) Antibacterial activities of Medicinal Plants used for the treatment of diarrhoea in Limpopo Province, South *Africa*, *Journal of Ethnopharmacology*, 107: 286-293.
22. Nascimento GGF, Locatelli J, Freitas PC, Silva GL (2000) Antibacterial Activity of Plant Extracts and Phytochemical on Antibacterial-resistant Bacteria. *Brazilian Journal of Microbiology*, 31(4): 247-256.
23. Ndou PA (2006) Walter Silsilu: National Botanical Guarden. Retrived from www.plantzafrica.com/-plantqrs/securidlong.htm.
24. Nelson-Harrison ST, King SR, Limbach C, Jackson C, Galiwango A, Kato SK, Kanyerezi BR (2002) Ethnobotanical Research into the 21st century. In: Iwu MM, Wootton JC (Eds.), Ethnomed. Drug Discov. Elsevier, Amsterdam.
25. Okogun JI (2000) Methods of Medicinal Plant Extract Preparation. National Institute for Pharmaceutical Research and Development (NIPRD) Idu-Abuja, Nigeria. 20:145-48.
26. Ojewole JAO (2008) "Analgesic, Anti-inflammatory and Hypoglycaemic Effects of *Securidaca longepedunculata* (Fresen.) [Polygalaceae] Root Aqueous Extract." *Inflammopharmacology* 16(4): 174-181.
27. Ojowole JAO, Ilesanmi ORS, Olayiwola G (2000) Pharmacology of African Medicinal plants: Neuromuscular and cardiovascular properties of

Securidaca longepedunculata, *Nigerian Journal of Natural Product and Medicine*. 4 (Abstract)
28. Ojowole JAO, Olayiwola G, Ilesanmi ORS (2001) Pharmacological properties of *Securidaca longepedunculata*: Neuromuscular and cardiovascular properties of *Securidaca*. *Nigerian Journal of Natural Product and Medicine* 2001: 5 (Abstract)
29. Shariff ZU (2001) Modern Herbal therapy for Common ailments. Nature Pharmacy Series, 1 Ibadan, Nigeria/ United Kingdom: in Association with Safari Books (Export) Limited Spectrum Books Limited; pp. 79–84.
30. Trease GE, Evans WC (1989) Pharmacognosy. 13th Edition, Bailliere Tindal Ltd, London. pp 176-180.
31. Voeks RA, Leony A (2004) Forgetting the Forest: Assessing Medicinal plant erosion in Eastern Brazil. Economic Botany 58: 294-306.
32. WHO (1999) World Health Organization: Consultation Meeting on Traditional Medicine and Modern Medicine: Harmonizing the Two Approaches. Geneva, WHO, TM/ICP/TM/001/RB/98-RS/99/GE/32(CHN).