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Pharmacognostic evaluation and antisickling activity of the leaves of *Securinega virosa* Roxb. ex Willd. (Euphorbiaceae)

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***Securinega virosa* (Euphorbiaceae) together with condiments from natural sources serve as antisickling remedies in Nigeria. This study was aimed at establishing the pharmacognostic profile as well as the antisickling activity of the leaves of *S. virosa* Roxb. ex Willd (Euphorbiaceae). Evaluation of the fresh, powdered and anatomical sections of the leaves were carried out to determine the macromorphological, micromorphological and chemomicroscopic characters. Chemical tests were employed in phytochemical investigations. Evaluation of the antisickling activity involved the inhibition of sodium metabisulphite-induced sickling of the HbSS red blood cells obtained from confirmed sickle cell patients who were not in crises. Concentrations of the crude extract and its fractions were tested with normal saline and p-hydroxybenzoic acid serving as controls. Microscopical studies showed anomocytic stomata arrangement and glandular trichomes. Phytochemical evaluation revealed the presence of tannins, flavonoids, alkaloids, saponins and cardiac glycosides. Percentage sickling inhibitions of the aqueous methanol extract of *S. virosa* as well as all the fractions, except the petroleum ether fractions were significant all through the period of assay p < 0. 05 compared to normal saline. These results are suggestive of a potential role for *S. virosa* in the management of sickle cell disorders and a candidate for further investigations.**

Key words: *Securinega virosa*, euphorbiaceae, pharmacognostic standardization, sickle cell disorders.

INTRODUCTION

Despite all the progress in synthetic chemistry and bio-technology, plants are still an indispensable source of medicinal preparations, both for prevention and cure. Lifestyle and eating habit alterations among the people make it vital to refer to herbal medicines as an alternative or complementary therapeutic measure. Nearly 70% of the world's population (mainly in the developing countries) relies entirely on such traditional medical therapies as their primary form of health care. Various herbs are also part of the socio-cultural and socio-economic heritage. Even in the present times, rural populations turn to herbal medicine as the most preferred therapeutic source (Meena et al., 2012).

Sickle cell disease (SCD) is a potentially devastating

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condition that is caused by an autosomal recessive inherited hemoglobinopathy, which results in the hallmark clinical sequale of vasoocclusion. It is seen worldwide, but occurs most frequently in Africa and less commonly in those of Mediterranean, Latino, East Indian and Arab descent. It is estimated that 16% of the population in Africa has a sickle hemoglobinopathy which is the highest proportion worldwide. The Americas and the East Mediterranean region represent the next highest proportion of sickle cell hemoglobinopathy as delineated by the World Health Organisation (Angastiniotis and Modelle, 1998).

Despite a variety of antisickling agents acting at different levels of the sickling mechanism, there is still a paucity of antisickling medicines. This is because of the potential toxicities associated with most of these agents. Apart from the general mutagenic and carcinogenic tendencies of gene modifiers, hydroxyurea, a classical example that possesses antisickling activity, causes bone marrow suppression which greatly limits its use (Strouse et al., 2008). On the other hand, this is gradually paving way for the consideration of condiments from natural sources as antisickling remedies. The increasing interest in these condiments is not unconnected with the general innocuous nature of their sources, which most often are herbs and even at times food crops.

The genus *Securinega* (Family Euphorbiaceae) comprises more than 20 species, including *Securinega virosa* Roxb. ex Willd, which is found growing in moderately fertile, well drained soil in temperate and subtropical regions of the world. Common names include bushweed (English), Iranje (Yoruba), Njisinta (Ibo), Gussu (Hausa) and Kartfi-Kartfi (Shuwaarabs).

S. virosa has been investigated for its efficacy in other studies. Studies of the methanol extract of *S. virosa* leaves on streptozotocin-induced diabetic rats showed significant reductions in blood glucose levels (Tanko et al., 2008). The anti-diarrheal activity of the methanol extracts of the leaves, stem and root barks on castor oil-induced diarrheal model showed the leaves and root bark extracts possess pharmacological activity against diarrhea (Magaji et al., 2007). The n-butanol fraction of *S. virosa* root bark contains bioactive principles that possess anticonvulsant activities which may be beneficial against absence seizure, further lending credence to its ethnomedicinal use in the management of epilepsy (Magaji et al., 2013). The leaf extracts of *S. virosa* have high antioxidant activities, with the ethanol extract having the greatest antioxidant activity compared to the hexane and ethyl acetate extracts (Uzama et al., 2013). Two cytotoxic alkaloids, virosecurinine and viroallosecurinine have been isolated from the leaves of *S. virosa* (Kuo-Hsiung et al., 1991).

This study is the pharmacognostic and biological evaluation of *S. virosa* Roxb. ex Willd (Euphorbiaceae), one of the recipes which has been used with acclaimed success by traditional healers in Nigeria in managing Sickle Cell Anaemia.

MATERIALS AND METHODS

Preparation of plant extract

The leaves of *S. virosa* Roxb. ex Willd (Euphorbiaceae) were collected in Benin City, Edo State, Nigeria. The plants were authenticated by the curator at the Forest Research Institute of Nigeria (FRIN), Ibadan where voucher specimens were deposited with the Herbarium specimen number FHI109685. The fresh leaves were air-dried for 72 h and powdered using an electric mill.

Macroscopic and microscopic examination

The following macroscopic characters for the fresh leaves were noted: size and shape, colour, surfaces, venation, presence or absence of petiole, the apex, margin, base, lamina, texture, odour and taste (Wallis, 1985; Evans, 2006).

The outer epidermal membranous layer (in fragments) were cleared in chloral hydrate, mounted with glycerin and observed under a compound microscope. The presence/absence of the following was observed: epidermal cells, stomata (type and distribution) and epidermal hairs (types of trichomes and distribution). The transverse sections of the fresh leaves through the lamina and the midrib as well as a small quantity of the powdered leaves were also cleared, mounted and observed (African Pharmacopoeia, 1986).

Examination of the powder for starch grains, lignin, mucilage, calcium oxalate crystals, cutin and suberin were carried out using standard techniques (Evans, 2006).

Phytochemical investigation

Chemical tests were employed in the preliminary phytochemical screening for various secondary metabolites such as tannins (phenazone; iron complex; formaldehyde and modified iron complex tests were carried out on the aqueous extract to detect the presence of hydrolysable, condensed and pseudo tannins), cardiac glycosides (Keller - Killiani and Kedde tests were carried out on the methanolic extract to detect the presence of a deoxy sugar, whose natural occurrence is to date, known only in association with cardiac glycosides and to indicate the presence of a lactone ring on the cardenolides respectively), alkaloids (Mayer's, Dragendorff's, Wagner's and 1% picric acid reagents to detect the presence of alkaloidal salts and bases), saponins glycosides (frothing of the aqueous extract when shaken and haemolysis test on blood agar plates were carried out to indicate and confirm the presence of saponins), anthracene derivatives (Borntrager's test for combined and free anthraquinones, where aglycones were extracted using chloroform and shaken with dilute ammonia) and cyanogenetic glycosides (sodium picrate paper test were used to test for the presence of hydrocyanic acid in the sample. Conversion to sodium isopurpurate indicates the presence of cyanogenetic glycosides) (Evans, 2006; Brain and Turner, 1975; Ciulei, 1981; Harborne, 1992).

Extraction and fractionation

The powdered leaves of *S. virosa* (3.60 kg) were extracted with MeOH-H_2O (50:50). Evaporating the solvent yielded an extract (0.52 kg) which was subsequently re-suspended in water and successively partitioned into petroleum ether (3 X 2L), Chloroform (3 X 2L) and n-BuOH (3 X 2L).

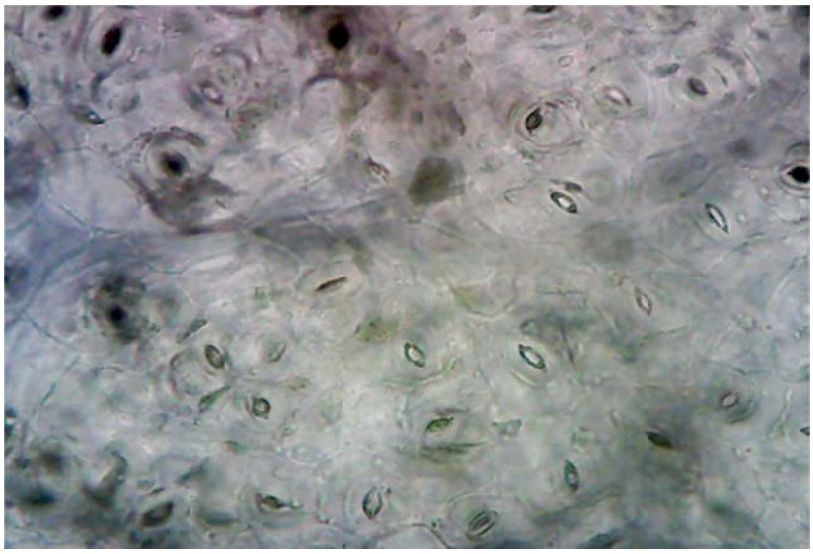

Figure 1. Diagnostic epidermal characters of *S. virosa* showing epidermal cells and stomata arrangement.

HbSS blood samples

HbSS Blood samples were collected by venipuncture from confirmed sickle cell patients not in crises on their clinic days at the Consultant Outpatient Department (COPD) of the University of Benin Teaching Hospital, Benin City, Nigeria. None of the patients used was recently transfused with HbAA blood.

Antisickling activity evaluation

The evaluation of the leaf extract and fractions of *S. virosa* for antisickling activities was carried out using a modified method of Moody and co-workers (Moody et al., 2003). Venipuncture blood samples from sickle cell anaemia patients not in crises were collected into EDTA bottles. Collected samples were centrifuged to remove the serum. The resulting packed erythrocytes were washed three times with sterile normal saline and centrifuged each time to remove the supernatant. A 0.5 ml sample of the washed erythrocytes were mixed each with 0.5 ml of the different concentrations of the aqueous methanol extract of *S. virosa* (100, 300 and 500 mg/ml) or fractions (500 mg/ml) in uncovered test tubes. A 5 mg/ml solution of ρ-hydroxybenzoic acid (PHBA) in normal saline was used as the positive control while normal saline served as negative control. Samples were taken from the different mixtures and the remaining portions of the mixtures incubated for 3 h, shaken twice during the period.

A 0.5 ml sample of 2% sodium metabisulphite was added to each mixture to deoxygenate the system, mixed thoroughly and sealed with liquid paraffin. Samples were taken in quadruplicates from the different mixtures at 0 min and at subsequent 30 min interval until seven readings were obtained.

Each sample was smeared on a microscopic slide, fixed with 95% methanol, dried and stained with giemsa stain. Each slide was examined under the oil immersion light microscope and counting of 100 red cells in each sample. The numbers of both sickled and unsickled red blood cells were counted and the percentage of unsickled cells determined.

Statistical analysis

Data are expressed as mean ± SEM. The differences between the means were analyzed using one way analysis of variance (ANOVA). Values of $P < 0.05$ were taken to imply statistical significance between compared data.

RESULTS

Macroscopic description

S. virosa leaves are simple, petiolated with variable shapes, obovate or orbicular. The margin was entire, apex acuminate, base cuneate and venation was reticulate. Average leaf size was 6. 3 cm ± 0. 8 (length) and 3. 5 cm ± 0. 3 (breadth). The taste and odour of the fresh leaf was characteristic.

Microscopic description

Micromorphological features revealed that anticlinal walls were thick and straight that is have straight epidermal cells. Stomata were present in both lower and upper epidermi. The stoma had two subsidiary cells with their long axis parallel to the pore and sometimes a 3rd subsidiary cell, indicating paracytic stomata arrangement (Figure 1). Glandular trichomes (Figure 2) were present on both surfaces.

A transverse section of the leaf across the mid-rib showed an upper and lower epidermi consisting of cells of similar sizes. It had an isobilateral structure that is both surfaces are identical. The mesophyll, consisting of upper and lower palisade layers and a median spongy mesophyll embedded a crystal sheath. The mid-rib bundle was surrounded by a zone of collenchyma cells on both surfaces. The phloem vessels embedded the

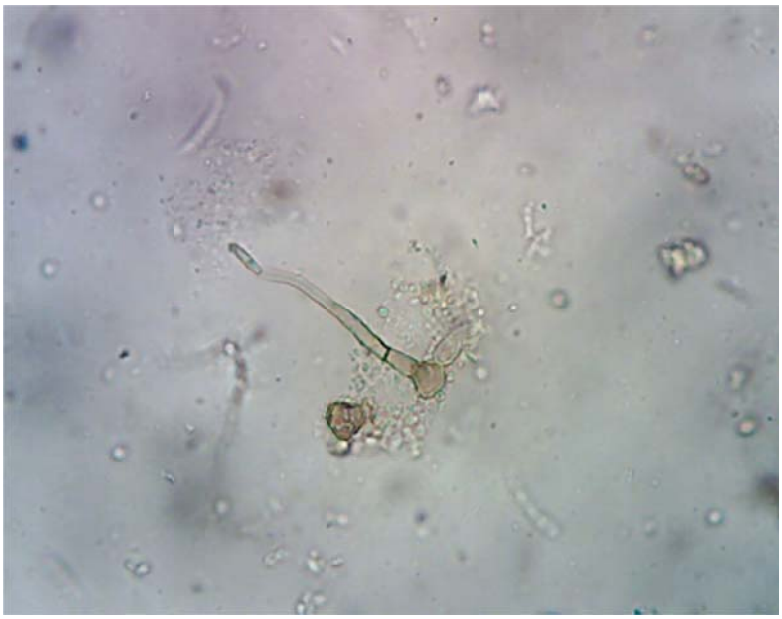

Figure 2. Glandular trichomes of *S. virosa.*

Table 1. Phytochemical constituents of *S. virosa* leaves.

Classes of secondary metabolites	Inferences
Alkaloids	+
Tannins	+
Flavonoids	+
Anthracene derivatives	-
Saponin glycosides	+
Cardiac glycosides	+
Cyanogenetic glycosides	-

- = Absent; + = present.

xylem vessels. Chemomicroscopic examination of the leaves revealed the presence of starch, calcium oxalate crystals, mucilage, tannins and cellulose.

Phytochemical screening

Phytochemical screening of the leaves of *S. virosa* for secondary plant metabolites revealed the presence of alkaloids, tannins, flavonoids, saponins and cardiac glycosides (Table 1).

Sickling inhibitory activities of crude extracts and fractions of *S. virosa*

Percentage sickling inhibition of the various doses of *S. virosa* extracts and fractions were significant all through the period of assay $p < 0.05$ compared to normal saline, except for petroleum ether fraction (Table 2); $n = 4$.

DISCUSSION

The types and distributions of Pharmacognostic characters in plants aid in their classification and identi-fication. Before any crude drug can be included in a Herbal Pharmacopoeia, pharmacognostic parameters and standards must be established (Abere et al., 2007). The results of these pharmacognostic investigations could, therefore serve as a basis for proper identification, collection and investigation of the plant. The macro- and micromorphological features of *S. virosa* described, distinguishes it from other members of the genera. These features described in this study are in tandom with those found in the Euphorbiaceae family (Inamda and Ganggadhara, 1978).

The Pharmacological activities of a given plant are associated with the type and nature of secondary plant metabolites present. The need for phytochemical screening has become imperative, since many plants accumulate biologically active chemicals in their tissues. Phytochemical evaluation of *S. virosa* revealed the presence of tannins, flavonoids, alkaloids, saponins and cardiac glycosides. These compounds detected in the plant are known to possess medicinal properties and health promoting effects (Danlami et al., 2013).

The *in-vitro* technique adopted in the antisickling efficacy bioassay was based on the simulation of the major *in-vivo* sickling-precipitating factor (that is, reduction of oxygen tension), using sodium metabisulphite as a physiologically acceptable reducing agent. The use of erythrocyte suspension instead of whole blood was particularly essential in ruling out the possibility of interactions of plasma component and products of their

Table 2. The sickling inhibitory activities of *Securinega virosa* crude extracts and fractions.

Time of incubation (min)	Percentage inhibition (%)							
	A	B	C	D	E	F	G	H
0	32.0±0.12	55.6±1.04	53.5±0.24	65.5±1.59	75.0±0.32	60.9±1.02	86.4±0.49	87.6±1.12
30	31.0±0.26	60.2±0.23	52.5±1.77	63.5±1.38	71.5±0.78	67.1±1.73	88.0±1.36	75.5±1.45
60	30.0±0.37	54.0±1.15	54.5±0.40	69.3±0.73	70.5±0.53	80.0±0.21	87.5±1.05	81.6±0.67
90	29.0±1.01	50.0±0.09	62.2±1.13	70.1±0.18	76.0±1.80	76.0±0.44	89.6±1.30	85.0±1.80
120	27.0±0.20	46.0±0.74	55.5±0.39	67.5±1.41	65.0±0.47	74.0±0.83	86.7±0.91	70.3±0.22
150	25.0±0.71	38.2±1.16	54.5±0.14	69.5±1.12	67.0±0.90	79.0±1.05	76.0±1.15	69.3±0.18
180	25.0±1.22	32.4±1.70	51.0±1.52	56.0±0.35	74.4±1.04	73.0±1.30	79.0±0.33	73.5±1.05

A= Blood + normalsaline + sodium metabisulphite; B = blood + PHBA + sodium metabisulphite; C = blood + crude extract of *S. virosa* leaf at 100 mg/ml + sodium metabisulphite; D = blood + crude extract of *S. virosa* leaf at 300 mg/ml + sodium metabisulphite; E = blood + crude extract of *S. virosa* leaf at 500 mg/ml + sodium metabisulphite; F = blood + chloroform fraction + sodium metabisulphite; G = blood + N-butanol fraction + sodium metabisulphite; H = blood + aqueous fraction + sodium metabisulphite. The results of the petroleum ether fraction were indeterminable.

several immunological reactions and certain metabolic co-factors in general with the red blood cells (Coker et al., 2007). Such interactions could significantly affect the shape and size of red blood cells and in the process inadvertently produce false negative or false positive results. The aqueous methanol extracts of *S. virosa* showed significantly inhibitory effect at the concentrations (100, 300 and 500 mg/ml) on sodium metabisulphite-induced sickling.

The fractions of the crude extract of *S. virosa* inhibited sodium metabisulphite induced sickling of HbSS red blood cells to varying degrees. The inhibitory activity of *S. virosa* could be due to the presence of bioactive compounds. Phenolic compounds which are present in *S. virosa* have been reported to possess antioxidant activity. Antioxidants have been reported to be major components of medicinal plants with known antisickling activity (Tatum and Chow, 1996). The antisickling activity could be linked to their ability either to inhibit *in-vitro* polymerization of haemoglobin or to some structural modification linked to the environment of haemoglobin by the extracts (Bianchi et al., 2007).

Conclusion

The pharmacognostic parameters of *S. virosa* which have been reported could be useful in its standardization. On the basis of the biological results, aqueous methanol extracts as well as the chloroform, n-butanol and aqueous fraction of *S. virosa* have been found to possess an antisickling activity, indicating that it has a role in the treatment of sickle cell disorders and a good candidate for further investigations.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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