Preliminary Phytochemical and Antimicrobial Examinations of Leaves of Securidaca Longipedunculata

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ABSTRACT

Secruidaca longipedunculata Fresn. (Polygalacaeae) "Ipeta," is commonly used by the Yoruba for treating various skin diseases as well as an anticonvulsant. Phytochemical examination of the plant indicates the presence of saponins and tannins. An attempt has been made to isolate the saponins in the leaves and relate them to their traditional use.

Securidaca Longipedunculata Fresn (Polygalacaeae) "Ipeta," is widely used as a medicinal plant. In East Africa, it is claimed that a decocoction of the root is used for cough, chest and body complaints and as a chewing stick for relief of toothache. In South West Africa, an infusion of the bark is used in treating siphilis and other veneral diseases.

The dry scrapings of the root are powdered and rubbed over the limb. A single treatment of this nature is said to be effective in relieving rheumatism and fibrositis, which interesting since the root is known to contain methyl salicylate (1).

In Ghana, the root is used in various ways as a charm in battle to dispel evil and to avert injury by weapons (2). In the Yoruba speaking part of this country it is used for treating various skin diseases as well as an anticonvulsant.

The presence of saponin in this plant which is a shrub of tropical Africa was first reported by Lenz in 1913 (3) and confirmed in 1958 by Prista and Alves (4) The only reported sapogenin isolated from the root of this plant is "Senegenin" by Davreux and Delaude in 1971 (5).

This paper presents a broad outline of the isolation of the various saponins in the leaves of this plant and the result of the antimicrobial testing against two microorganism. The characterisation of the various sapogenins and their antifungal as well as their anticonvulsant properties are still to be determined.

EXPERIMENTAL

Plant material:

The plant was collected on Ede-Iwo road. It was authenticated as **S. longipedunculata** by comparison with a sample in the museum and herbarium, Faculty of Pharmacy, University of Ife, as well as at the Forest Research Institute Herbarium, Ibadan.

Antimicrobial testing:

Four holes of 9mm in diameter were made in nutrient agar plates (10X10cm) which had been previously seeded with 0.1 ml. of test organism. Only two microorganisms **Staphylococcus aureus** and **Escherichia coli** were used to monitor the activity of each fraction obtained. 1ml. of each microorganism when suspended in water gives 50% transmission when examined at 650mm.

0.5ml. of each saponin fraction (100,ug/ml. of phosphate buffer pH 7.4) to be tested were then introduced aseptically into the bores. Blanks consisted of 0.5ml. of buffer only while 0.5ml. of phenol (10ug/ml. of phosphate buffer pH 7.4) and 0.5ml. of distilled water were used as controls. All agar plates (test, blank and controls) were examined for bacterial growth after 48hrs. of incubation at 37°C and the zone of inhibition measured from the centre of the bores. 10 replications were performed for each test organisms.

The diameters of zone of inhibition were expressed as follows.

(*i*) — (No activity)
(*ii*) + (When inhibition zone was up to 10mm)
(*iii*) ++ (When inhibition zone was between 10-15mm)

Results are shown in Table 1

TABLE 1

PRELIMINARY RESULTS OBTAINED WITH SAPONIN FRACTIONS FROM THE LEAVES OF S. LONGIPEDUNCULATA.

Fraction	*M.P °C*	*Chemical Type*	*Anti* Microbial*	*Glucose*	*Galactose*	*Xylose*	*Arab Inose*	*Fu- Cose*	*Rha- Mnose*
001	229—304	Steroidal	++	+	+	+	+	+	+
002	203—204	Triterpenoid	+	+	+	—	+	+	+
003	119—201	Steroidal	+	+	+	—	—	—	—
004	226—230	Triterpenoid	++	+	+	—	+	—	—
005	226—228	Triterpenoid	+	+	+	—	—	+	—
006	216—219	Triterpenoid	+	+	+	—	—	—	—

\+ = Positive — = Negative.
* + = Where inhibition zone was up to 10mm.
* ++ = Where inhibition zone was between 10 — 15mm.

Preliminary Phytochemical Screening:

Phytochemical examination of the leaves, root and stem of **S. longipedunculata** were carried out according to the method of Farnsworth 1966 (5) for alkaloids, saponins, tannins, anthraquinones and Phlobatannins. Only saponins and tannins were found in the leaves root and stem.

Extraction and fractionation.

1.5kg of air - dried powdered leaves of this plant was extracted with MeOH in a soxhlet for a week. The extract was concentrated to about 50ml. **in vacuo** and allowed to stand. Acetone was added to the extract and kept in the refrigerator for 3 hours. The acetone was removed and the precipitate washed thrice with acetone and dried in a vaccum dessicator to yield 400g of crude total saponin.

The total crude saponin was then extracted with boiling absolute EtOH on a water bath. The extract was filtered and allowed to cool to room temperature overnight. 150 mg of crystalline material was filtered off washed with cold absolute EtOH and dried in a vaccum dessicator to yield fraction 001, m.p. 229-304.

The mother liquor was reduced to about 100ml, heated so that the precipitate formed during the evaporation dissolved, left to cool for several hours after which 130mg of crystalline material was filtered off. (Fraction 002, m.p 203 - 204.). The mother liquor was further reduced and left in the refrigerator for 3hrs and 98 mg of Fraction 003 with m.p. 199-201 was obtained.

The material which did not dissolve during treatment with boiling EtOH was dissolved in boiling MeOH and the solution left overnight. 160mg of amorphous material was filtered off (Fraction 004.

The mother liquor was partly evaporated and EtOH was added in just the amount possible without causing a precipitate to form, when the mixture was heated to boiling point. The mixture was left to cool and 80 mg of amorphous material, m.p. 226-228 Fraction 005) was obtained.

The mother liquor was further reduced and the mixture was left in the refrigerator overnight when 110mg of Fraction 006, m.p 216—219 was obtained.

All the six saponin fractions obtained were subjected to antimicrobial testing.

Hydrolysis of saponins.

1ml of 3N H_2S04 was used for each mg. of saponin to effect hydrolysis by refluxing for 40 hours. The resulting hydrolysates were cooled and each was extracted with 3 portions of $CHCl_3$. (200ml $CHCl_3$/mg saponin hydrolysed). The 3 $CHCl_3$ phases from each saponin hydrolysate were combined and the solvent removed by evaporation **in vacuo** at 40°C. The resulting aglycones are still to be characterised and their biological activities determined if found pure.

The acid phase of the hydrolysates were neutralised with $BaCO_3$ and the precipitate of $BaSO_4$ removed by filteration. The filterate was deionised by passage through a column (1 × 20cm) of Amberlite IR - 120 resin - analytical grade, and then through a column (1 × 30cm) of Amberlite IR - 4B resin analytical grade.

The deionised solutions and washings were freeze dried and the powder subjected to paper chromatography. A series of 65hrs descending paper chromatography with n-butanol: ethanol : water (10:2:1) and sprayed with a mixture of 2% diphenylamine in acetone, then 2% aniline in acetone, and then at 110° for 5 minutes. The sugars detected in each saponin fraction by comparison with standard sugar references are listed in Table I.

Results and discussion:

Several types of compounds must be considered when testing for saponins. For example, steroidal and triterpenoid saponins and their respective sapogenins as well as saturated sterols, saturated terpenes, diterpenes and other steroidal plant constituents. Steroidal sapogenins are useful medicinally in that they could be converted to steroid hermones.

Saponins have several properties that can be used as a basis for simple detection tests. At every stage of extraction and fractionation two of these properties frothing and haemolysis, have been used to confirm that the fractions obtained are actually saponins. (Farnsworth 1966).

To determine the purity of the six saponin fractions obtained they were subjected to tlc analysis using various combination of the following solvent systems on silica gel G chromatoplates:-

(a) Acetic acid: butanol: H_20 (15: 60: 25)
(b) $CHCl_3$: MeOH: NN_3 (40: 40: 20)
(c) Butanene: ter-butanol: H_20 (50: 20: 30)
(d) Ethyl acetate: EtOH (2:3)

However, when the plates were sprayed with 300% $^{w}/_{v}$ solution of $SbCl_3$ in concentrated HCl and heated at 105° for 5 minutes, only Fractions 001 and 003 gave pink colouration while all the six fractions gave weak blue fluorescence under uv in daylight without spraying.

This lead us to believe that even though all the six saponin fractions are tlc pure, fractions 002, 004, 005, 006 are not steroidal saponins.

This was proved further by using the theory of Simes **et al** 1958 (7) which stated that although both triterpenoid and steroidal saponins are haemolytic, triterpenoid saponins do not froth as much as steroidal saponins but when you add an aqueous Na_2CO_3 to a boiled extract of triterpenoid saponin, it will result in the production of a stable and dense froth as with steroidal saponin.

This was the case with these four fractions. However, all the six fractions showed strong antimicrobial activities against **S** aureus and **E coli.** None of them showed antiviral activities and fractions 001 and 003 showed positive anticonvulsant actions.

Sugars detected after hydrolysis include glucose, galactose, arabinose, fucose, rhamnose and xylose. (Table 1)

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