Study on the Alkaloid, Allicin, Glycoside and Saponin Composition of the Leaves of *Sansevieria liberica* Gérôme and Labroy by Gas Chromatography.

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ABSTRACT

The alkaloid, allicin, glycoside, and saponin levels of the leaves of *Sansevieria liberica* were determined by gas chromatography. The leaves are rich in alkaloids (110.780 mg/kg wet weight and 317.420 mg/kg dry weight), with low allicin (1.332 mg/kg wet weight and 3.815 mg/kg dry weight) and saponin (0.675 mg/kg wet weight and 1.933mg/kg dry weight), and very low glycoside (0.026 mg/kg wet weight and 0.075 mg/kg dry weight) contents. Seventeen alkaloids were detected, consisting mainly of epoxy-3,7-dimethoxycrinane-11-one (about 22.34%), with moderate levels of buphanidrine (8.34%), ambelline (8.22%), augustamine (8.11%), crinamidine (6.52%), 6-hydroxyundulatine (5.67%), crinane-3α-ol (5.47%). Of the three allicins detected, diallylthiosulphinate (52.76%) was the most abundant, while the most abundant of the three saponins detected was avenacins B-1 (56.22%). These results show that the leaves are rich in alkaloids, lending credence to their medicinal uses.

(Keywords: alkaloids, allicins, glycosides, saponins, *Sansevieria liberica*)

INTRODUCTION

The genus Sansevieria (family Agavaceae, Ruscaceae or Dracaenaceae) whose common names include mother-in-laws tongue, devils tongue, and snake plant [USDA, 2008], is made up of about sixty (60) species [Evans, 2005]. It has special significance in horticulture, for it is grown as an experimental fiber crop, as an ornamental for interior use and, to a lesser extent, landscaping outside [Henley, 1982]. A number of species of bowstring hemp are grown as ornamental plants [Evans, 2005]. Among the ones commonly found in Nigeria is *Sansevieria liberica*. It has concave, short petioled leaves that are in part transversely banded with light and dark green, also linearly striated with whitish to light green and dark green striations [Reed, 1978]. The leaves contain over 2% fiber. This plant has long rhizomes with long fibrous roots and a rapid rate of growth. In Nigeria, the leaves and roots of *Sansevieria liberica* are used in traditional medicine for the treatment of asthma, abdominal pains, colic, diarrhea, eczema, gonorrhea, hemorrhoids, hypertension, monorrhagia, piles, sexual weakness, snake bites and wounds of the foot [Gill, 1992; Osabohien and Egboh, 2008; Adeyemi *et al*., 2009]. The sedative and anticonvulsivant activities of the roots have been studied by Adeyemi *et al*. [2007].

Preliminary phytochemical screening of extracts from the roots of *Sansevieria liberica* revealed the presence of alkaloids [Bero *et al*., 2009], oils, reducing sugars, alkaloids, saponins, anthraquinones, and tannins [Adeyemi *et al*., 2007, 2009]. However, these studies are mostly qualitative, and on the roots, with nothing in the literature on the contents of the leaves. So, in the present study, the alkaloid, allicin, and saponin contents of the leaves of *Sansevieria liberica* were evaluated.

MATERIAL AND METHODS

Collection of Plant Samples

Samples of fresh *Sansevieria liberica* were procured from a horticulturist by Air Force Gate, Aba Road, Port Harcourt, Nigeria and from within Alikor Estate, Choba, Rivers State, Nigeria. After due identification at the University of Port Harcourt Herbarium, Port Harcourt,

Nigeria, their leaves were collected, rid of dirt and stored for subsequent use. All reagents used were GC-grade purity.

Standard Solutions, Calibration, Identification and Quantification

Standard solutions were prepared in methanol for alkaloid and allicin, ethanol for saponin. The linearity of the dependence of response on concentration was verified by regression analysis. Separated peaks were identified by direct comparison of their retention times with those of standards. Quantification was performed by establishing calibration curves for each compound determined, using the standards. The result of the calibration of the GC machine is shown in Table 1.

Table 1: Calibration Data of the GC System.

Compound	Correlation constant	Relative resolution (%)	Equation
a. Alkaloids			
➢ 9-octadecenamide	0.99827	-11.764	Area=13.604448*Amt+0
➢ Dihydro-oxo-demethoxyhaemanthamine	0.99970	-4.868	Area=7.08082178*Amt+0
➢ Augustamine	0.99992	-2.530	Area=5.56479993*Amt+0
➢ Oxoassoamine	0.99969	-4.987	Area=6.4172004*Amt+0
➢ Crinane-3α-ol	0.99956	-5.909	Area=5.76675781*Amt+0
➢ Buphanidrine	0.99955	-6.018	Area=4.9754859*Amt+0
➢ Powelline	0.99968	-5.075	Area=63.21632*Amt+0
➢ Undulatine	0.99912	-8.416	Area=3.0988*Amt+0
➢ Ambelline	0.99987	-3.226	Area=0.496*Amt+0
➢ 6-Hydroxybuphanidrine	0.99949	-6.373	Area=0.34648*Amt+0
➢ 6-Hydroxypowelline	0.99987	-3.226	Area=0.992*Amt+0
➢ Crinamidine	0.99974	-4.603	Area=0.26416*Amt+0
➢ 6-Hydroxyundulatine	0.99982	-3.846	Area=0.832*Amt+0
➢ 1β,2β-Epoxyambelline	0.99983	-3.670	Area=8.72*Amt+0
➢ Epoxy-3,7-dimethoxycrinane-11-one	0.99733	-14.634	Area=0.984*Amt+0
➢ 6-Hydroxycrinamidine	0.99997	-1.544	Area=20.72*Amt+0
➢ Mitraphylin	0.99971	-4.828	Area=7.21937244*Amt+0
b. Allicins			
➢ Diallylthiosulphinate	0.99761	-8.333	Area=7000*Amt-14000
➢ Methylallylthiosulphinate	0.99936	-4.211	Area=23600*Amt-24333.333
➢ Allyl methylthiosulphinate	0.99965	-3.106	Area=170000*Amt-130000
c. Glycosides			
➢ Ouabain	0.99957	-5.858	Area=76.381*Amt+0
➢ Digitoxin	0.99986	-3.329	Area=74.381*Amt+0
➢ Digoxin	0.99983	-3.723	Area=64.4006*Amt+0
➢ Salicin	0.99987	-3.177	Area=74.409*Amt+0
➢ Amygdalin	0.99980	-3.972	Area=74.2626*Amt+0
➢ Arbutin	0.99990	-2.801	Area=84.409*Amt+0
d. Saponins			
➢ Avenacin A-1	0.99963	-3.185	Area=4.55E13*Amt-3.5667E13
➢ Avenacin B-1	0.99930	-4.418	Area=620*Amt-670
➢ Avenacin A-2	0.99999	-0.5571	Area=480*Amt-66.666667
➢ Avenacin B-2	0.99981	-2.247	Area=600*Amt-333.33333

Amt = amount.

Determination of Alkaloid Composition

The method reported by Tram *et al*. [2002] was adopted. 30 g of the ground sample was added to 250 mL of boiling deionized water and allowed to soak for 30 minutes, before filtration. The filtrate was acidified to pH 4 with acetic acid, before extracting with 30 mL of petroleum spirit and chloroform. The acidic aqueous phase was made alkaline (pH 9), with 25% aqueous ammonia, and then extracted three times with 30 mL of chloroform. The chloroform extract was concentrated to 1.0 mL, before chromatographic analysis. Chromatographic analyses was carried out on an HP 6890 (Hewlett Packard, Wilmington, DE, USA), GC apparatus, fitted with a flame ionization detector (FID) (range scanned: 220–500 nm), and powered with HP Chemstation Rev A 09.01 (1206) software, to quantify and identify compounds. The column was a ZP-5 Column (30 m × 0.32 mm × 0.25 µm film thickness). Injections were accomplished with a 20 µL fixed loop. Prior to GC analysis, all solutions were filtered through 0.45 µm membranes filter and then degassed in an ultrasonic bath for 30 min.

Determination of Allicin Composition

5.0 g of the pulverized sample was added to 50 mL of 98% ethanol, and allowed to stand for 48 hours before filtering with a Whatman No. 1 filter paper. The extract was concentrated, and the resultant residue was dissolved in methanol for chromatographic analysis.

GC analysis was performed with a Hewlett–Packard (HP 6890) Series system, with a flame ionization detector (FID), and powered by HP Chemstation Rev A 09.01 (1206) software. The column was ZP-5 (30 m × 0.32 mm × 0.25 µm film thickness). UV detection was performed at 240 nm.

Determination of Glycosides

1.0 g of the pulverized sample was extracted by pouring 10 mL of ethanol/water (7:3) mixture on it, and allowing to stand for 2 hours. The mixture was filtered with Whatman No. 1 filter paper, and the extract was purified by washing with lead acetate. The purified extract was further purified by adding sodium hydrogen phosphate, before concentrating to 1 mL, for chromatographic analysis. GC analysis was carried out on an HP 6890, GC apparatus, fitted with a flame ionization detector (FID) (range scanned: 220–500 nm) and powered with HP Chemstation Rev A 09.01(1206) software. The capillary column was a ZP-5 Column (30 m × 0.32 mm × 0.25 µm film thickness), detected at 205nm. The injection volume was 20 µL.

Determination of the Saponin Composition

The method of Hanafy and Lobna [2007] was adopted. The pulverized sampled was defatted with petroleum ether at 40^0C for 3 hours. After filtering the petroleum ether, the sample was extracted with methanol for 3 hours, with mild heating. The methanol extract was concentrated and re-extracted with methanol/acetone (1:5) mixture. The precipitate obtained was dried *in vacuo*, until it turned to a whitish amorphous powder, on complete drying. It was eluted on a silica gel (230-400 mesh) column, with chloroform/methanol/water (7:3:1).

The first fraction collected was air dried at room temperature and the residue obtained was treated as pure saponins. The residue was dissolved in methanol for chromatographic analysis. Chromatographic analysis was carried out on an HP 6890 (Hewlett Packard, Wilmington, DE, USA), GC apparatus, fitted with a flame ionization detector (FID) (range scanned: 220–500 nm), and powered with HP Chemstation Rev A 09.01 (1206) software. The capillary column was a ZP-5 Column (30 m × 0.32mm × 0.25µm film thickness), detected at 254 nm. The injection volume was 20 µL. The mobile was filtered and degassed prior to use.

RESULTS AND DISCUSSION

Table 2 shows the alkaloids composition of the leaves of *Sansevieria liberica*. Their alkaloid content is high, consisting mainly of epoxy-3,7-dimethoxycrinane-11-one (about 22.34%)[a], with moderate levels of buphanidrine (8.34%), ambelline (8.22%), augustamine (8.11%), crinamidine (6.52%), 6-hydroxyundulatine (5.67%), crinane-3α-ol (5.47%) and an unidentified component (13.03%). 9-

octadecenamide (3.97%), dihydro-oxo-demethoxyhaemanthamine (3.30%), oxoassoamine (3.10%), powelline (0.35%), undulatine (0.72%), 6-hydroxybuphanidrine (1.97%), 6-hydroxypowelline (3.28%), 1β,2β-epoxyambelline (3.67%), 6-hydroxycrinamidine (0.68%) and mitraphylin (1.29%) were present in low levels.

The total alkaloid content of *S. liberica* leaves was lower than that reported for *Acalypha wilkesiana* [Ikewuchi *et al*., 2011]. Their 6-hydroxyundulatine, 6-hydroxybuphanidrine and dihydro-oxo-demethoxyhaemanthamine contents were higher while the other components were either lower or comparable in quantities to those of *A. wilkesiana* leaves. Epoxyambelline both alone, and in 1:1 combination with ambelline, produces moderate to pronounced activation of mouse spleen lymphocytes [Ghosal *et al*., 1984].

The allicins composition of the leaves of *Sansevieria liberica* is given in Table 3. The leaves have moderate levels of allicin content. It consisted mainly of diallylthiosulphinate (52.76%), with moderately lower levels of allyl methylthiosulphinate (20.07%) and methylallylthiosulphinate (27.17%). The allicins contents/profiles of the leaves of *S. liberica* is comparable to that of *A. wilkesiana* [Ikewuchi *et al*., 2011]. Diallylthiosulphinate (mainly called allicin) is reported to have antimicrobial, insecticidal, hypotensive, hypolipidemic, anti-thrombotic, antioxidant, anti-ulcer, and anti-inflammatory activities [Eilat *et al*., 1995; Elkayam *et al*., 2003].

The glycoside composition of *Sansevieria liberica* is given in Table 4. The leaves have very low glycosides content. The main glycoside was amygdalin (39.63%); others detected include ouabain (4.51%), digitoxin (17.07%), digoxin (13.58%), salicin (6.04%), arbutin (19.18%).

Table 2: The Alkaloid Composition of the Leaves of *Sansevieria liberica.*

Compounds	R. time (min)	Composition (mg/kg) /Wet weight	/Dry weight
Total alkaloid	-	110.78	317.42
➢ 9-Octadecenamide	10.741	4.39	12.59
➢ Dihydro-oxo-demethoxyhaemanthamine	12.175	3.65	10.46
➢ Augustamine	13.625	8.98	25.73
➢ Oxoassoamine	15.086	3.44	9.85
➢ Crinane-3α-ol	16.473	6.06	17.36
➢ Buphanidrine	17.357	9.24	26.46
➢ Powelline	18.532	0.39	1.12
➢ Undulatine	19.338	0.80	2.30
➢ Ambelline	20.349	9.11	26.09
➢ 6-Hydroxybuphanidrine	21.008	2.18	6.24
➢ 6-Hydroxypowelline	22.191	3.64	10.42
➢ Crinamidine	23.044	7.22	20.68
➢ 6-Hydroxyundulatine	23.735	6.28	17.99
➢ 1β,2β-Epoxyambelline	24.802	4.06	11.64
➢ Epoxy-3,7-dimethoxycrinane-11-one	25.825	24.74	70.90
➢ 6-Hydroxycrinamidine	26.810	0.75	2.15
➢ Mitraphylin	27.757	1.43	4.09
➢ Unidentified component	2.619	14.43	41.36

R. time = retention time.

Table 3: Allicins Composition of the Leaves of *Sansevieria liberica.*

Compounds	R. time (min)	Composition (mg/kg) /Wet weight	/Dry weight
Total allicins	-	1.332	3.815
➢ Diallylthiosulphinate	16.523	0.703	2.013
➢ Methyl allylthiosulphinate	16.947	0.362	1.037
➢ Allyl methylthiosulphinate	18.098	0.267	0.766

R. time = retention time.

Table 4: Glycosides Composition of the Leaves of *Sansevieria liberica.*

Compounds	R. time (min)	Composition ($\times 10^{-3}$ mg/kg) /Wet weight	/Dry weight
Total glycosides	-	26.23	75.17
➢ Cardiac glycosides	-	9.22	26.43
• Ouabain	20.535	1.18	3.39
• Digitoxin	21.439	4.48	12.83
• Digoxin	23.270	3.56	10.21
➢ Salicin	18.790	1.59	4.54
➢ Amygdalin	19.475	10.40	29.79
➢ Arbutin	17.491	5.03	14.42

R. time = retention time.

Table 5: Saponins Composition of the Leaves of *Sansevieria liberica.*

Compounds	R. time (min)	Composition (mg/kg) /Wet weight	/Dry weight
Total saponins	-	0.675	1.933
➢ Avenacin A-1	7.692	0.000	0.000
➢ Avenacin B-1	9.864	0.379	1.087
➢ Avenacin A-2	11.126	0.074	0.212
➢ Avenacin B-2	11.488	0.221	0.635

R. time = retention time.

The leaves of *S. liberica* had higher total glycosides, ouabain, digitoxin, amygdalin and arbutin contents than *A. wilkesiana* [Ikewuchi *et al*., 2011]. Ouabain is a cardiotonic steroid [Dmitrieva and Doris, 2002].

Low saponin levels were recorded in the leaves (Table 5). This consisted mainly of avenacins B-1 (56.22%) and B-2 (32.82%), with a moderately lower level of avenacin A-2 (10.96%). Avenacin A-1 was not detected. The *S. liberica* leaves had higher total saponins, avenacin A-2, avenacin B-1 and avenacin B-2 contents than the leaves of *A. wikesiana* [Ikewuchi *et al*., 2011]. Saponins are reported to have broad range of pharmacological properties [Soetan, 2008]. Avenacins have antimicrobial properties [Armah *et al*., 1999; Mert Türk *et al*., 2005].

In conclusion, this study showed that *Sansevieria liberica* leaves are rich in alkaloids. This finding supports their use as medicinal plants.

Footnote:

a. Percentages are based on the weight of the compounds per its total extract, whether alkaloid, allicin, glycoside or saponin.

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