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LC-MS analysis and free radical scavenging activity of extracts from *Anogeissus leiocarpus* (DC.) Guill. & Perr. and *Sansiviera liberica* Gerome & Labroy

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Abstract

Anogeissus leiocarpus and *Sansiviera liberica* are plants used in traditional medicine as antiviral agents. A preliminary study of the bioactive constituents was carried out using Liquid Chromatography – Mass Spectrometry (LC-MS) technique. Free radical scavenging activity of the crude extracts were determined using 2,2-diphenyl picrylhydrazyl radical method. Results obtained showed that *Anogeissus leiocarpus* and *Sansiviera liberica* were rich in alkaloids and their glycosides. Methanol extract of stem of *Anogeissus leiocarpus* (MSAL) at 1.0 mg/mL was more active than methanol extract of the twig of *Sansevieria liberica* (MTSL) with %inhibition of 79.5% and 78.1% respectively in the free radical scavenging assay. These activities were significant when compared with butylated hydroxylanisole and ascorbic acid. This study has given a clue to the traditional application of these two plants as antiviral agents in ethnomedicine.

Keywords: *Anogeissus leiocarpus*; *Sansiviera liberica*; Free Radicals; Alkaloids; Glycosides; LC-MS

1 Introduction

Anogeissus leiocarpus (DC.) Guill. & Perr. of the family Combretaceae is a deciduous shade tree 15–30 m high with light green foliage. It is known in English as "chew-stick". It is used for firewood, roofing and is good for tool-handles [1]. The powdered bark is applied to wounds, ulcers and toothache. A decoction of the bark from the twigs is used to clean sores and syphilitic chancres. Root-bark is used as an emulsifying agent, stimulant and aphrodisiac. In Northern Nigeria, some parts of the tree are boiled in water together with potash and the liquid is taken as a cure for stomach pains and for schistosomiasis. In veterinary medicine, the bark, or the fruit or seeds, is used as a taenicide for horses and donkeys. A decoction of leaves is applied to the skin in Ivory Coast to alter the pigmentation, and is used as an eye-wash for certain complaints. It was also reported that the plant possess antiviral activity [1, 2]. Preliminary phytochemical screening of the *Anogeissus leiocarpus* leaves and stem bark showed that, the plant was rich in tannins, flavonoids, terpenes and saponins. Polyphenolic compounds such as 3,3,4-tri-Omethylflavellagic acid, 3,3,4-tri-O-methylflavellagic acid-4--Dglucoside, gentisic, protocatechuic, gallic acids, chebulagic acid, chebulinic acid and ellagic acid were isolated. Flavogallonic acid, bislactone, castalagin and ellagic acid were isolated from the bark. Eight flavonoids, namely, 4H-1-Benzopyran-4-one, 7-[(6-deoxy- α-Lmannopyranosyl)oxy]-5-hydroxy-2-(4- hydroxy-3-methoxyphenyl), cathecin, quercetin, isoquercetin , rutin, vitexin, kaempferol , and procyanidin B2 were isolated from the leaves of the plant. Five triterpenes and triterpene glycosides were isolated, namely sericoside, its related aglycone sericic acid, rachelosperoside; its related aglyconerachelosperogenin, and arjungeni [3].

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Sansevieria liberica Gérôme & Labroy of the family Dracaenaceae or Liliaceae is native to tropical western Africa (i.e. from Morocco to Chad) and also extends into some parts of central and eastern Africa (i.e. the Central African Republic, Ethiopia, Kenya and Tanzania). It has common name African bow-string hemp or leopard lily. It is succulent and cultivated as an ornamental garden plant. In Nigeria, its leaf fibres, were used for making ropes, bowstrings and fishing-lines. In traditional medicine, juice pressed from the leaves or a decoction of the leaves is drunk for the treatment of gonorrhoea, earache and toothache. Leaf sap is applied to ulcers, sores and topically in case of earache and toothache. Fermented rhizomes are eaten to cure malaria. A root decoction is used as a remedy for convulsions. In Ghana, the roots are used as an abortifacient and administered during labour. As a fetish plant, it is grown on graves, at shrines and in homes [1; 4]. The proximate and phytochemical composition of *Sansevieria liberica* Gérôme and Labroy leaves was investigated and it revealed the presence of alkaloids, carotenoids, flavonoids (catechins and flavones), phytates, saponins and tannins [5]. [6 and 7] reported the sedative, anticonvulsant and antidiarrhoeal activities of the aqueous root extract of *S.liberica*. Protective effect of aqueous extract of the rhizomes of *S. liberica* on carbon tetrachloride induced hepatotoxicity in rats was also reported [5].The alkaloid, allicin, glycoside, and saponin levels of the leaves of *Sansevieria liberica* were determined by gas chromatography. Seventeen alkaloids were detected, consisting mainly of epoxy-3,7-dimethoxycrinane-11-one with moderate levels of buphanidrine, ambelline, augustamine, crinamidine, 6-hydroxyundulatine, crinane-3α-ol. Of the three allicins detected, diallylthiosulphinate was the most abundant, while the most abundant of the three saponins detected was avenacins B-1. These results show that the leaves are rich in alkaloids, lending credence to their medicinal uses [8].

Free radicals are reactive oxygen due to the presence of unpaired electrons and lead to chain reactions. Excess free radicals have been reported to play key role in the aging process in humans, in cardiovascular diseases, brain dysfunction, and cancer. Antioxidants have the ability to completely neutralize the free radicals thereby stopping the chain reactions [9; 10]. Hence, this study is aimed at determining the chemical composition of the two plant using Liquid Chromatography-Mass Sectrometry (LC-MS) and investigating the free radical scavenging activity.

2 Material and methods

2.1 Plant Material, Chemical and Reagents

Samples of *Anogeissus leiocarpus* (1 kg) and *Sansiviera liberica* (1 kg) were obtained from a farm in Ido Local Government Area of Oyo State, Ibadan, Nigeria in July 2016 and identified by a Taxonomist at the Botany Department, University of Ibadan, Nigeria. The plants were air-dried for four weeks, and kept in desiccator till when needed for analysis.

The following BDH chemicals and reagents were used: n-hexane, ethyl acetate, methanol, chloroform, dichloromethane, hydrochloric acid, conc. tetraoxosulphate (VI) acid, conc. hydrochloric acid, ammonia solution. General purpose chemicals obtained were distilled prior to use. Dimethylsulphoxide (M&B, England), and silica gel 30 - 260 microns (Merck, Germany) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) and butylatedhydroxylanisole (BHA) were obtained from Sigma Chemical Co (St Louis, MO).

2.2 Extraction of Crude extract

The powdered plant was extracted with methanol by cold extraction. The mixture was filtered to remove the marc. The combined filterate was evaporated to dryness in a rotatory evaporator at 37^0C and stored in a dessicator.

2.3 General Experimental Procedures and Analysis

LC-MS is a hyphenated technique which combines the separating power of High Performance Liquid Chromatography (HPLC), with the detection power of mass spectrometry. The crude methanol extracts of *Anogeissus leiocarpus*, methanol extract stem (MSAL) and *Sansevieria liberica* methanol extract twig (MTSL) were subjected to HPLC-HR MS analysis: The liquid chromatographic system was an Agilent 1200 Series (California, USA), which consisted of a binary pump, autosampler, vacuum degasser, thermostated column compartment, and a diode array detector which was coupled to a Finnigan LTQ Orbitrap mass spectrometer equipped with a HESI-II source. Separation was achieved on a Gemini-NX C18 column (150 mm x 2 mm i.d., particle size 3 µm) that had been maintained at 35^0C. The binary solvent system consisted of 50 mM aqueous AF (pH 3.5 adjusted with FA, mobile phase A) and 0.1% FA in MeCN (mobile phase B), with a linear gradient. The linear mobile phase gradient started at 5% B (0-1min), increased to 100% B (1-50 min), maintained at 100% B (5-14 min), ramped back down to 5% B. The flow rate was 0.35 mL/min, and the injection volume was 10 µL. High resolution MS using the Orbitrap mass spectrometer was carried out to detect the analytes. Nitrogen was employed as sheath gas (50 arbitrary unites) and auxiliary gas (5 arbitrary units). Ion spray, capillary and tube lens voltages were set to 4kV, 200 V and 75V, respectively. The ion source vaporizer and capillary temperatures were set to

350 and 275^{0}C, respectively. Mass spectra were acquired over the *m/z* 100-800 range in positive electrospray ionization (+ESI) and in Fourier transform mass spectrometry /FTMS) mode. The full-scan data were also gained at a high resolution power of 60,000 full width at half maximum /FWHM; at *m/z* 400) and at a scan rate of 1 s per spectrum. The following gradient was used; Time (min): H_2O+ 0.1 % FA (%): ACN+0.1 % FA (%): 0:95:5; 1:95:5; 49: 20:80; 51:0:100; 56:0:100; 56.5: 95:5;60: 95:5. Flow rate: 0.35 mL/min column: Luna C18 (2), 3µm, 50x3 mm. The data recorded were processed with Finnigan Xcalibur 2.0.7 software.

2.4 Free radical Scavenging activity using 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH)

The free radical scavenging activity was measured by using the 2,2-diphenyl-1-picryl-hydrazil (DPPH) radical method. Solution of DPPH (0.3 mM) was prepared in methanol. Five microlitres of each sample of different concentration (0.0625 – 1.0 mg/mL) was mixed with 95 µl of DPPH solution in methanol. The mixture was dispersed in 96 well plate and incubated at 37^{0}C for 30 min. The absorbance at 517 nm was measured by microtitre plate reader (Spectramax plus 384 Molecular Device, USA) and percent radical scavenging activity was determined by comparison with the methanol treated control. Butylated hydroxyl anisole (BHA) and Ascorbic acid were used as standard [9; 11].

2.5 Statistical Analysis

Absorbance measurements are expressed as mean absorbance ± SD of triplicate analysis. Inhibitory activity (%) was calculated from (1 − as/Ac) ×100 or Ac-As/Acx100 where, As is the absorbance in the presence of test substance or sample and Ac is the absorbance of control.

3 Results and discussion

3.1 LC-MS Analysis

LC-MS gives the secondary metabolites present in the extracts of the two plants. This also gives indication of the compounds that can be responsible for the antiviral activity. Figure 1 showed the chromatogram with relative abundance of compounds obtained from *A. leiocarpus* and *S. liberica* methanol extracts. Six and twelve compounds were obtained respectively. The mass list of compounds and there fragmentation patterns are given in Tables 1 and 2. The compounds are dominated with alkaloid and their glycosides. Secondary plants metabolites in plant extracts have been reported for numerous pharmacological activities. Alkaloid and their glycosides have been reported to show anti-inflammatory, anticancer, analgesics, local anesthetic and pain relief, neuropharmacologic, antimicrobial, antifungal activities [10].

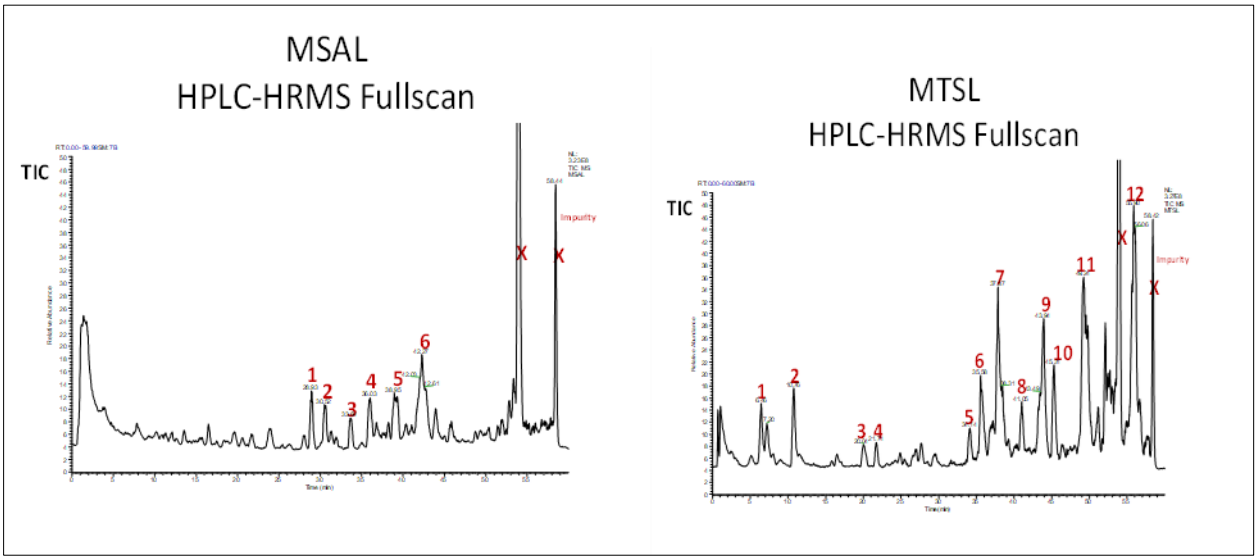

Figure 1 Chromatogram showing peaks obtained from *Anogeissus leiocarpus* and *Sansevieria liberica* methanol extracts

Table 1 List of Compounds detected by LC-MS^2 and LC-MS^3 of *Anogeissus leiocarpus* Stem methanol extract (MSAL)

No	sum formula	molecular weight M + H$^+$	Fragments	Comment
1	$C_{36}H_{58}O_{12}Na$	705.3820	543.3293, $C_{30}H_{48}O_7Na$	M-glucose
			499.3394, $C_{29}H_{48}O_5Na$	
2	$C_{36}H_{58}O_{11}Na$	689.3872	-	compound 1-O
3	$C_{30}H_{58}O_{11}Na$	689.3872	527.3346, $C_{30}H_{48}O_6Na$	Isomer compound 2 M-glucose
			483.3446, $C_{29}H_{48}O_4Na$	fragments as compound 1 further loss at H_2O
4	$C_{17}H_{13}O_9$	361.05530	346.0317, $C_{16}H_{10}O_9$	M-CH_3
	$C_{16}H_{36}O_2N$	274.2739	329.0289, $C_{16}H_9O_8$	329-H_2O
			256.2637, $C_{16}H_{34}ON$	M-H_2O
			102.0914, $C_5H_{10}ON$	
5	$C_{16}H_{29}O_3N_6$	353.2297	335.2191, $C_{16}H_{27}O_2N$	M-H_2O
			317.2086, $C_{16}H_{25}ON_6$	M-2x H_2O
			235.1308, $C_{10}H_{15}ON_6$	M-deoxysugar
6	$C_{18}H_{38}O_3N$	316.2845	298.2739, $C_{18}H_{36}O_2N$	M-H_2O
			280.2633, $C_{18}H_{34}ON$	M-2x H_2O
			262.2527, $C_{18}H_{32}N$	M-3xH_2O

Table 2 List of Compound detected by LC-MS^2 and LC-MS^3 of *Sansivieria liberica* Twig methanol extract (MTSL)

No	sum formula	molecular weight M + H$^+$	Fragments	Comment
1	$C_{17}H_{22}O_4N$	304.1542	156.1019, $C_8H_{12}O_2N$	
			138.0912, $C_8H_{12}ON$ 110.0965, $C_7H_{12}N$	
2	$C_{17}H_{24}O_3N$	290.1750	124.1121, $C_8H_{14}N$	similar to compound 1 -H_20 +4H 124-CH_3-NH_2
			93.0700, C_7H_9	
3	$C_{13}H_{13}O_2N_2$	229.0971	212.0706, $C_{13}H_{10}O_2N$	M-MH_3
			184.0757, $C_{12}H_{10}ON$	212-CO
			168.0808, C12$H_{10}N$	
4	$C_{18}H_{18}O_4N$	312.1228	177.0546, $C_{10}H_9O_3$	177-CH_3-OH
			145.0285, $C_9H_5O_2$	
5	$C_{19}H_{25}O_2$	285.1847	267.1742, $C_{19}H_{23}O$	M-H_2O
			121.0649, C_8H_9O	
6	$C_{51}H_{47}O_2N_5$	761.3717	599.319, $C_{44}H_{41}ON$	Mixture
7	$C_{31}H_45O_8N_6$	629.3294	467.2767, $C_{25}H_{35}O3N6$	M-glucose

	$C_{22}H_{34}O_5N$	392.2431	233.1535, $C_{15}H_{21}O_2$	M-sugar-NH_3
8	$C_{27}H_{28}O_4N_2Na$	467.1941	407.1729, $C_{25}H_{24}N_2Na$	M-2xCO_2-4H
	($C_{27}H_{28}O_4N_2$)			
9	$C_{32}H_{40}O_4N_7Na$	609.3033	300.2895, $C_{18}H_{38}O_2N$	M-H_2O
	$C_{18}H_{40}O_3N$	318.3001	282.2790, $C_{18}H_{36}ON$	M-2x H_2O
			264.2685, $C_{18}H_{34}N$	M-3xH_2O
10	$C_{29}H_{55}ON_9$	545.4524	527.4420, $C_{29}H_{53}N_9$	M-H_2O
			509.4313, $C_{31}H_{53}N_6$	527-NH_2
11	$C_{36}H_{53}O_{10}N_6$	729.3818	583.3245, $C_{30}H_{43}O_6N_3$	M-C_5 sugar
	$C_{30}H_{43}O_6N_6$	583.2339	451.2817, $C_{25}H_{35}O_2N_6$	M-C_5 sugar
12	$C_{37}H_{63}O_3N_6$	639.4959	359.2555, $C_{19}H_{31}ON_6$	

Oxidative stress due to excess free radicals is the main causative agents of many diseases. *A. leiocarpus* and *S. liberica* extracts were investigated for free radical scavenging activity. Table 3 shows the percentage inhibition of the crude methanol extract of stem of *A. leiocarpus* (MSAL), and methanol extract of the twig *of S. liberica* (MTSL). MSAL at 1.0 mg/mL was more active than MTSL with %inhibition of 79.5% in the free radical scavenging assay when activity was compared with butylated hydroxylanisole and ascorbic acid. These plants can be classified as natural antioxidants and can be used as remedy in curing diseases related to free radical scavenging activity. The results of this analysis suggest that the plants could have good antiviral activity being the plant's application in traditional medicine. Further research work to check the antiviral activity is ongoing. This work also support previous researches on these plants having effective protective activity on carbon tetrachloride induced hepatotoxicity in rats and the study on the alkaloid, allicin, glycoside and saponin composition of the Leaves of *Sansevieria liberica* Gérôme and Labroy by Gas Chromatography [12; 13].

Table 3 % Inhibition of samples and standards on 2,2-Diphenyl-1-picryl hydrazyl radical (DPPH)*

Concentration (mg/mL)	MSAL	MTSL	BHA	Ascorbic Acid
0.0625	64.8	50.3	91.6	60.3
0.125	67.9	54.8	92.7	65.0
0.25	70.6	59.9	93.0	70.7
0.5	72.8	64.6	94.11	78.7
1.0	79.5	78.1	95.9	90.1

* MSAL- *Anogeissus leiocarpus*, methanol extract stem MTSL- *Sansevieria liberica methanol* extract twig BHA-Butylatedhydoxyanisole, Absorbance of DPPH at 517 nm 0.822 ± 0.001

4 Conclusion

Bioactive constituents from *Anogeissus leiocarpus* and *Sansevieria liberica* obtained from Liquid Chromatography - Mass Spectrometry (LC-MS) afforded six and twelve constituents respectively and were dominated with alkaloids and their glycosides. Free radical scavenging activity of the crude extracts showed that they possessed significant activity when activities were compared with butylated hydroxylanisole and ascorbic acid used as standards in the assay. Therefore, it can be concluded that *Anogeissus leiocarpus* and *Sansevieria liberica* contain bioactive compounds responsible for the observed activity. Further work on bioactivity of the two plants would justify the antiviral activity.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors confirm that there are no known conflicts of interest.

Credit authorship contribution statement

- G.K. Oloyede.: Conceptualization, Methodology, Investigation, Data analysis, Writing - original draft, Supervision.
- D.O. Oluwayelu: Resources, Conceptualization, Investigation (Antiviral activity).
- P.A. Onocha: Conceptualization, chemical Analysis, Investigation, Data analysis,
- M. Oyelola: Laboratory Investigation, Practicals
- M. Spiteller: LC-MS- Equipment Analysis, Elucidation of Spectra

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