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Antimicrobial activities of hexacosane isolated from *Sanseveria liberica* (Gerome and Labroy) plant

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ABSTRACT

The antimicrobial activity of hexacosane isolated from *Sansevieria liberica* was determined using standard method. The compound was isolated by directing the fractionation of ethyl acetate extract of the air dried root with microbial sensitivity test. The results of antimicrobial test showed moderately high activities against all the test microbes with the compound having zone inhibition of 29, 27, 26 and 25 cm against *Klebsiela pnemoniae, Salmonela typhi, Mithecithinne staphaureus* and *Proteus vulgaris* respectively. The structure of the compound was identified from ^{13}C-NMR, ^{1}HNMR, IR and GC-MS spectral data. The isolation, structural elucidation, NMR spectral assignment and bioactivities are reported.

Keywords: *Sansevieria liberica*, antimicrobial activity, structural elucidation.

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INTRODUCTION

Sansevieria is a genus of about seventy species of flowering plants. It belongs to the family Asparagaceae, sub family Nolinoideae. *Sansevieria liberica* is a pretty plant with bright green leaves growing 45 to 100 cm tall, with a smooth texture and light grey tip. The leaves are typically arranged in a rosette (Chahinian, 2005). It is commonly called “Moda” in Hausa, “Ebubage” in Igbo, “Ijo-ikoko” in Yoruba and Bow string hemp or Mother-in-laws tongue in English. The medicinal uses of *S. liberica* depend on the region. Different regions have different uses for the species. In the Northern part of Nigeria the roots are used to treat menorrhagia, menstrual pains (Sambo and Ali, 2008). It is also used to normalise unusually menstrual period while in the Western and Eastern part of Nigeria it is used to treat diarrhea, abdominal pains, menorrhagia, gonorrhea, eczema, pile, snake bite, impotence, asthma, high blood pressure (Odugbemi, 2008). The sap of the species *Sansevieria ehrenbergii* has antiseptic qualities, and the leaves are used for bandages in traditional first aid. Leaves of *Sansevieria liberica* possess anti-inflammatory effects which may be due to its bioactive constituents such as alkaloids, saponins, flavonoids, terpenoids, steroids, glycosides, reducing sugars, tannins, resins, carbohydrates, proteins, acidic compounds, fats and oils (Chinasa et al., 2011). The aim this research is to determine the antimicrobial potentialities of *Sansevieria liberica* and isolate the active compound responsible for the activity.

MATERIALS AND METHODS

Plant materials

The plant was obtained from botanical garden of Ahmadu Bello University, Kaduna State, Nigeria, verified and authenticated by Musa Muhammed of Ahmadu Bello University herbarium and assigned voucher number of 900215.

Plant extraction

The freshly collected stem was separately cut into chips, and air-dried in the laboratory, grinded into powder, weighed and stored in polythene bags until needed. Percolation method of extraction was used for the extraction of the crude extracts from the stem and root. A portion (150 g) of the pulverized roots was introduced into 500 cm^3 conical flask. Methanol (400 cm^3) was added until the samples were well immersed and left on the bench for 48 h. Each solution was filtered. Portions (250 cm^3) of the filtrate was transferred into separatory funnel and 250 cm^3 of n-hexane was added, shaken thoroughly and left over night for the two layers to separate. The n-

hexane layer was drained into a conical flask and evaporated to dryness at 40°C using rotary evaporator. Chloroform, ethyl acetate and n-butanol fractions were obtained from the methanol extracts using the same procedure. Each of the extracts obtained was weighed in a sample bottle and kept in a refrigerator until needed (Garba and Okeniyi, 2012).

Column chromatography of ethyl acetate extract

Ethyl acetate fraction that showed higher activity in most of the tested microbes was subjected to column chromatography. A portion (2.0 g) of the fraction was mixed with 1.0 g of celite column (silica gel 60 g, internal diameter 2.5 cm). The column was first eluded with 200 cm^3 petroleum ether:chloroform (4:1). This was followed by 200 cm^3 petroleum ether:ethylacetate (9:1) and finally 200 cm^3 ethylacetate:methanol (5:1) and each were collected in fraction 50 cm^3. Each portion collected were evaporated using rotary evaporator (Cannell, 1998). These fractions were subjected to TLC and similar fractions were pooled together and labelled A to H. The pooled fractions were tested for bioactivity against those selected microbes.

Antimicrobial activity

The isolates of microbes were obtained from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital Zaria from which the antimicrobial activities of the extracts of *S. liberica* were determined against the collected isolates of *Salmonella typhi, Methicithinness staphaureus Candida albicans*, *Streptococcus pyogenes, Staphylococcus aureus*, *Escherichia coli, Pseudomonas flaurescense*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Candida thrusei*.

Procedure

The antimicrobial activities of the extract were determined using agar well diffusion methods as described by Navorro et al. (1996) and Okeke et al. (2001).

Preparative thin layer chromatography (PTLC)

Fraction G which was found to be more potent was further subjected to PTLC which was carried out using silica gel pre-coated glass plates.The plates were cut in sizes of 20 × 20 cm with layer and thickness of 0.25 mm. A thin line of about 1.5 cm from the bottom of the plate was drawn with a pencil. The sample to be separated was dissolved in minimum amount of solvent to give an approximate concentration of 20 mg/ml. It was then applied uniformly along the thin line using capillary tube. The TLC plate was run using petroleum ether:ethyl acetate and allowed to dry after which it was developed. The developed plate was air dried in a fume cupboard and the position of the band of interest with R_f value of 0.75 cm was marked with pencil and scraped off the back of the plate on to a foil paper. The scraped sorbent size was transferred to a sintered glass funnel and washed repeatedly with chloroform and the solution obtained was evaporated to give white crystals of the compound (Gibbons and Gray, 1998).

RESULTS AND DISCUSSION

The results of sensitivity test showed the all the fractions possess different activities against all the test microbes with ethyl acetate fraction showing higher activities of 30, 27, 24 and 22 mm against *Salmonella typhi, Klebsiella pneumonia, Proteus vulgaris*, *Streptococcus pyogenes* and *E coli* respectively. For instance, ethyl acetate fraction, n-butanol fraction and chloroform fraction gave higher activities with zone of inhibition diameter of 25, 24 and 20 mm against *Staphylococcus aureus*, *Methicithinnes staphaureus, Candida albicans,* respectively at a concentration of 700 µg/ml. This showed that ethyl acetate, n-butanol and chloroform fraction could be used for the treatment of various diseases caused by the tested microbes. However, low activities were recorded from all the fractions at lower concentrations indicating that the activity of this plant is concentration dependent and generally low activity was observed in the n-hexane fraction of the plant with half of the microbes tested showing no activity. Also no activities were observed from chloroform, methanol, and n-butanol extracts against *Streptococcus pyogenes*, *E. coli*, *S. typhi*, *Pseudomonas fluorescens* and *K. pneumonia* at all the prepared concentrations (Table 1).

Ethyl acetate fraction recorded a broad spectrum of antimicrobial activities against the test microbes. It was therefore selected for column chromatographic separation leading to the isolation of one active compound.

The results of column chromatography was obtained using three different solvent mixtures, total of 8 fractions were obtained with fraction A having the highest R_f value of 0.65, weighing 0.1 g (Table 2). Fraction G had the highest weight of fraction (5.6 mg) with R_f value 0.60 which also showed potential of higher activity against the test microbes was further subjected to PTLC leading to the isolation of pure compound whose spectral identity showed that its hexacosane.

The result of preparative TLC gave single compound with R_f value 0.75, the compound melts at 56°C (Table 3). Although not all the test microbes were used for testing the activity of isolated compound due to small amount of the compound, four microbes were used at same concentration as shown in Table 4.

Infra-red analysis

The IR spectra of isolated compound gave absorption at 2918.40 which corresponds with the absorption for C-$H_{stretching}$ vibration of CH_2. Absorption at 2850.88 is the C-$H_{stretching}$ for CH_3. The absorption at 1467.88 is bending vibration for CH_2, 723.33 is a CH_2 bending vibration of straight chain with carbon atoms more than seven (Figure 1).

H-nmr analysis

The proton NMR shows a peak at 0.8 ppm which is for CH_3, the most intense peak at 1.2 to 1.6 ppm is for CH_2

Table 1. Results of antimicrobial sensitivity test of root extract of *Sansevieria liberica* [zone inhibition in diameter (mm)] and some drugs against the microbes.

Solvent fraction/ reference standard	Concentration (µg/cm^3)	MS	SA	SP	EC	ST	PV	PF	KP	CA	CT
Chloroform	7×10^2	21	19	0	20	20	0	0	24	22	19
	6×10^2	11	15	0	12	11	0	0	13	11	10
	5×10^2	7	10	0	7	6	0	0	10	7	6
	4×10^2	0	3	0	0	0	0	0	6	6	0
Methanol	7×10^2	19	20	0	20	20	0	0	20	18	22
	6×10^2	15	10	0	10	9	0	0	10	9	11
	5×10^2	10	7	0	7	8	0	0	8	6	8
	4×10^2	3	5	0	4	0	0	0	3	0	0
n-hexane	7×10^2	18	20	0	17	0	0	0	18	0	0
	6×10^2	10	15	0	9	0	0	0	10	0	0
	5×10^2	0	7	0	0	0	0	0	7	0	0
	4×10^2	6	0	0	6	0	0	0	0	0	0
n-butanol	7×10^2	24	25	22	0	0	18	0	0	23	23
	6×10^2	12	13	11	0	0	12	0	0	12	13
	5×10^2	7	10	7	0	0	7	0	0	10	10
	4×10^2	0	3	0	0	0	6	0	0	10	6
Ethylacetate	7×10^2	24	20	22	22	30	24	0	27	24	25
	6×10^2	13	15	16	11	15	13	0	14	12	13
	5×10^2	10	9	10	10	13	10	0	10	7	7
	4×10^2	3	0	3	0	4	6	0	4	0	0
Sparfloxancin		27	35	34	0	0	19	30	37	0	0
Ciproloxacin		0	30	30	40	32	0	0	41	0	0
Fluconazole		0	0	0	0	0	0	0	0	37	39

Key: MS: *Methicithinness staphaureus;* SA: *Staphylococcus aureus;* SP: *Streptococcus pyogenes* EC: *Escherichia coli;* ST: *Salmonella typhi*; PV: *Proteus vulgaris;* PF: *Pseudomonas flourescens*; KP: *Klebsiella pneumonia*; CA: *Candida albicans;* CT: *Candida thrush.*

Table 2. Column chromatography of ethyl acetate extract of root of *S. liberica.*

Solvent	Fraction collected	R_f value	Weight of fraction collected (mg)
Pet. Ether : chloroform 4:1	A	0.65	0.1
	B	0.35	0.3
	C	0.30	0.1
Pet ether : ethylacetate 9:1	D	0.5	0.3
	E	0.25	1.1
	F	0.45	0.1
Pet. Ether: Methanol 5:1	G	0.60	5.6
	H	0.43	0.5

proton, an integration shows there are approximately 49 protons responsible for those peaks. The last peak that appears more down field at 7.2 ppm is for deuterated chloroform which is the solvent peak (Figure 2).

Table 3. Preparative TLC of fraction G with pet. ether and ethyl acetate.

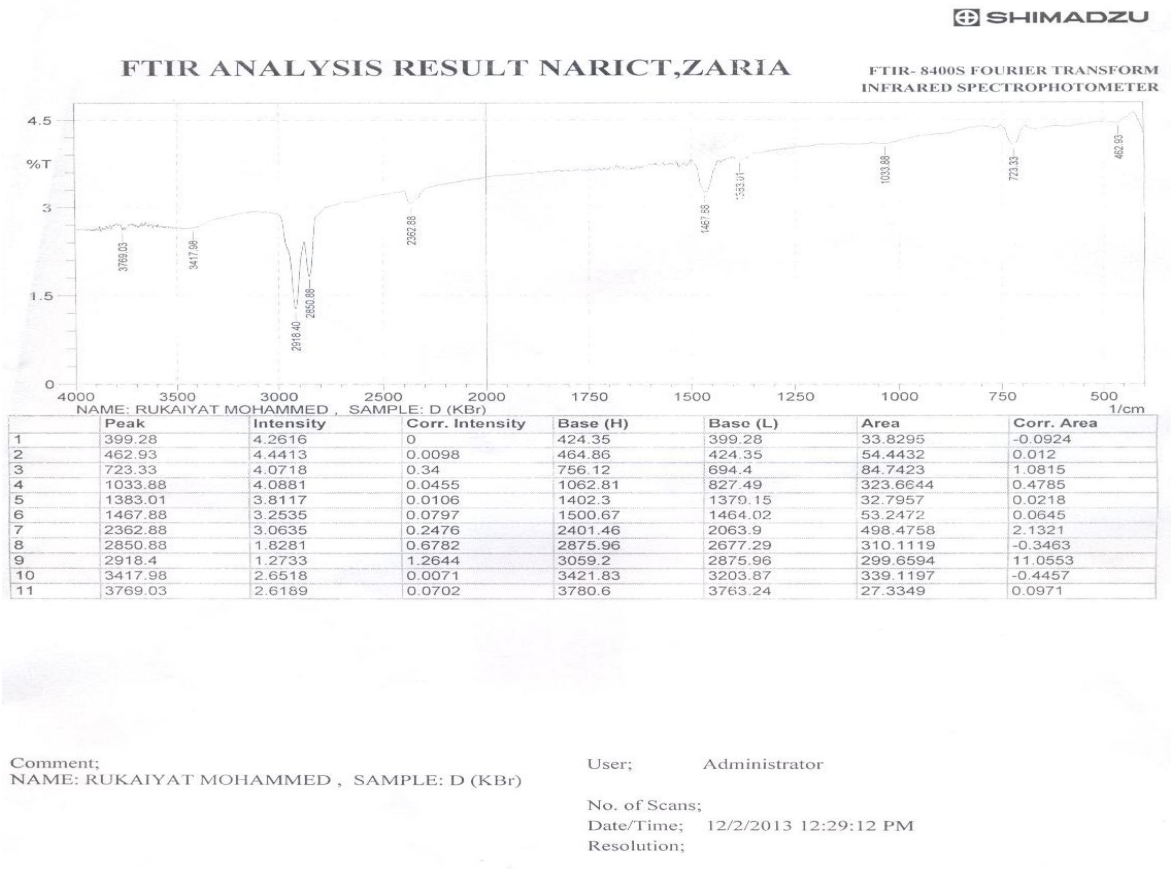
Fraction	Solvent	R_f Value	Weight of fraction (mg)	MP
1	Pet-ether: ethyl acetate	0.75	4.7	56°C

MP = melting point.

Table 4. Results of antimicrobial test of isolated compound.

Compound	Concentration (µg/cm^3)	MS	SA	CA	EC
Or SA	5×10^2	23	24	20	25
	4×10^2	16	18	10	17

Key: MS = *Methicithinness staphaureus*; SA = *Staphylococcus aureus*, CA = *Candida albicans;* EC = *Escheridia coli.*



	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	399.28	4.2616	0	424.35	399.28	33.8295	-0.0924
2	462.93	4.4413	0.0098	464.86	424.35	54.4432	0.012
3	723.33	4.0718	0.34	756.12	694.4	84.7423	1.0815
4	1033.88	4.0881	0.0455	1062.81	827.49	323.6644	0.4785
5	1383.01	3.8117	0.0106	1402.3	1379.15	32.7957	0.0218
6	1467.88	3.2535	0.0797	1500.67	1464.02	53.2472	0.0645
7	2362.88	3.0635	0.2476	2401.46	2063.9	498.4758	2.1321
8	2850.88	1.8281	0.6782	2875.96	2677.29	310.1119	-0.3463
9	2918.4	1.2733	1.2644	3059.2	2875.96	299.6594	11.0553
10	3417.98	2.6518	0.0071	3421.83	3203.87	339.1197	-0.4457
11	3769.03	2.6189	0.0702	3780.6	3763.24	27.3349	0.0971

Figure 1. IR spectrum of the isolted compounds.

^{13}C nmr analysis

Peak at 14ppm is CH_3 which appears more upfield, peak at 22 ppm is CH_2 which is directly attached to CH_3, whereas peaks at 30 and 32 ppm are peaks that represent all the CH_2 in the isolated compound (CH_2) = 20. And the peak at 76 is for carbon of deuterated chloroform used as solvent.

GC-MS analysis

From the GC-MS it was evident that the molecular ion peak which is also gives the molecular mass of the compound is 367. The compound fragments at 323 breaking off propyl radical with mass of 44. Taking all the above spectroscopic analyses into consideration the compound is proposed to be hexacosane and the

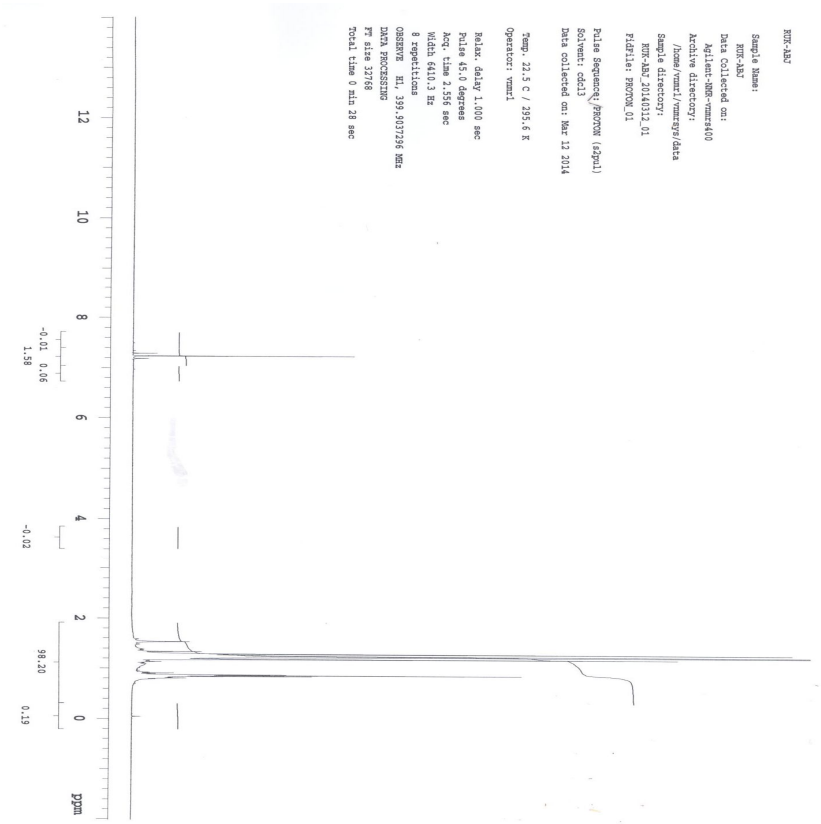


Figure 2. [1]Hnmr spectrum of the isolated compound.

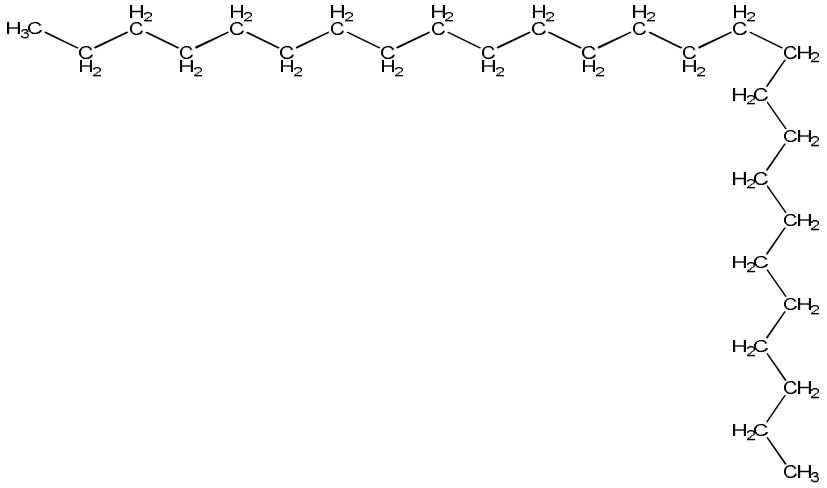


Figure 3. Hexacosane.

structure is shown in Figure 3.

CONCLUSION

The extracts of the root were found to have higher activities against the test microbes. Chromatographic separation and further preparative thin layer chromatography carried out on the ethyl acetate root fraction lead to the isolation of white amorphous compound with melting point of 56°C. Structural elucidation using [1]Hmnr, [13]Cmnr, IR and GC-MS showed

that the compound is hexacosane.

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