J. Chem Soc. Nigeria, Vol. 44, No. 3, pp 407 -410 [2019]

An Acyclic Triterpene from *Alternathera sessilis* plant commonly used in Nigeria

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Received 06 February 2019; accepted 11 April 2019, published online 12 May 2019

Abstract

Alternathera sessilis whole plant sample (leaves,stems and roots) was extracted with methanol .The methanol extract of *Alternathera sessilis* was subjected to column chromatography technique. The methanol extract afforded a compound. The structure of the compound was elucidated using various spectroscopic techniques - Infra red spectroscopy (IR), carbon nuclear magnetic resonanance (^{13}C NMR), proton nuclear magnetic resonance (^{1}H NMR), heteronuclear multiple bond correlation (HMBC). The compound was identified as squalene, an acyclic triterpene .This is the first time the isolation of squalene is reported from *Alternathera sessilis*. The antioxidant activity of the compound was also investigated. It gave an IC_{50} value of 0.78 mg/mL.

Keywords: *Alternathera sessilis*, squalene, ^{13}C NMR, ^{1}H NMR

Introduction

Alternathera sessilis is a common weed that belongs to the family Amaranthaceae. It is used in the treatment of snake bites and as a vegetable by nursing mothers to increase breastmilk flow and as febrifuge (1),(2).Sterols, fatty acid and lupeol (3). Hydrocarbon and esters (4) were previously isolated from *Alternathera sessilis* found in India and middle east regions. There is no scientific justification of the pharmacological or medicinal uses of this plant. This work reports for the first time the isolation of squalene from *Alternathera sessilis* which could be responsible for the pharmacological or medicinal uses of this plant found in Nigeria.

Material and methods

General methods

The two-dimensional proton NMR spectra were recorded in deuterated solvents on a Bruker AVANCE NMR spectrometer. The spectra were recorded in deuteriochloroform ($CDCI_3$), and the chemical shifts were recorded in ppm (parts per million) relative to the solvents. The deuteriochloroform was referenced at δ 7.26 in the ^{1}H NMR spectrum and the central line at δ 77.23 in the ^{13}C NMR spectrum. Infrared spectra were recorded using a Perkin-Elmer (2000 FTIR) spectrometer. The liquid samples were sandwiched between NaCI plates at the. Laboratory grade solvents were used (Sigma – Aldrich, Germany). Silica gel 60 (Sigma Aldrich) and Sephadex^{TM} LH – 20 (GE Health Care Bio-sciences AB, Sweden) were used for column chromatography. Thin layer chromatography was performed on DC – Fertigfolien ALUGRAM® SIL G/UV_{254}, layer – 0.20mm silica gel 60 with fluorescent indicator UV_{254}, Germany. Detection of spots was carried out with by UV absorption and P-anisaldehyde in concentrated H_2S0_4 using sigma gun spray.

Plant material

The whole plant material of *Alternathera sessilis* was collected from Ijagun area in Ijebu-Ode, Ogun State, Nigeria. They were authenticated at FRIN (Forestry Research Institute of Nigeria) with FHI NO 109674. The whole plant (leaves, stem and roots) of *Alternathera sessilis* was dried and pulverized before extraction.

Extraction and isolation

The dried plant sample of *Alternathera sessilis* was ground and 2 kg of sample obtained. 1.5 kg of the plant sample was carefully poured into an aspirator bottle and soaked with hexane for 4 days. The same process was repeated with ethyl acetate and methanol. After 4 days, the mixtures were decanted and the extracts were recovered by distillation process. The column was packed by introducing glass wool into a clean column clamped vertically. The slurry of the silica gel in hexane was poured down the column through a funnel. Acid washed sand was poured at the top of the

column to prevent disturbance of the surface during loading. Methanol extract (15 g) was loaded on to the column. Different fractions were eluted using different solvent mixtures from 100 % hexane to 100 % ethyl acetate and from 100 % ethyl acetate to 50 % methanol in ethyl acetate. Eluent (50 ml) was collected in each case as fraction and these individual fractions distilled and collected. The separation was monitored using thin layer chromatography. TLC analysis was carried out on 0.2 mm silica gel, aluminium-backed plates (Merck Art. 5554). Then, the plates were analyzed using a UV-lamp at 254 mm and by fluorescence under UV-lamp at 365 mm and also developed using anisaldehyde spray reagent.

Each fraction was spotted using thin layer chromatography (TLC) to determine fractions of similar components which were then combined based on their R_f values .Elution was done with hexane, followed with gradient of 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% ethyl acetate in hexane.

Determination of DPPH Radical Scavenging Capacity

The effect of the extract on DPPH radical was estimated adopting the method of (5). A solution of 0.135 mL DPPH in methanol was prepared and 1.0 mL of this solution was mixed with 1.0 mL of extract in methanol containing 0.02-0.1 mg of extract. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 run. Ascorbic acid and was used as standards. IC_{50} value was calculated using Graph pad software. The ability to scavenge DPPH radical was calculated by the following Eq:

DPPH radical scavenging activity (%) = $[(Abs_{control} - Abs_{sample}) /(Abs_{control}]x100$

Where,

$Abs_{control}$ = Absorbance of DPPH radical + methanol

Abs_{sample} = Absorbance of DPPH radical + sample extract/standard

Results and Discussion

The Compound was isolated as yellow oil from the whole plant of *Alternathera sessilis*. The FTIR spectrum of the compound showed stretches absorption bands at 3415 cm^{-1} that was attributed to a hydroxyl group, 2954 and 2852 cm^{-1} attributed to CH stretches .The ^1H NMR spectrum of the compound showed six olefinic proton resonances at δH 5.11 which were assigned to H-3/22, H-7/18 and H-11/14 . Fifteen carbon resonances representing thirty carbons were observed in the ^{13}C NMR spectrum. Eight methyls, ten methylenes, six methines and six trisubstituted carbons were also observed in the distortionless enhancement by polarisation transfer spectrum DEPT. The presence of three methine carbon resonating at δ124.4, δ124.5 and δ124.6 and ten methylene proton (m, δ2.05, H-4, H-5, H-8, H-9, H-123,H-13, H-16, H17, H-20 and H-21), a singlet at δ1.68 (6H, s, H-1 and 24Me) together with a proton resonance at δ1.60s (9H, bs) in the (1H NMR spectrum) correspond respectively, to an in-chain, allylic methyl group and three out chain allylic groups of a polyprenoid system . The out of chain methyl groups resonating at δ17.9, δ16.5 and δ16.3 indicated the geometry of the six trisubstituted double bonds.The signals at δ25.9, confirmed its in-chain position in ^{13}C NMR spectrum . The ^1H and ^{13} C NMR spectral features of this compound were compared with the reported literature (6) as shown in Table 1 and were found to be similar to squalene, an acyclic triterpenoid, which is a very common compound in many natural sources, fish liver oils and many vegetables oil. It has been isolated previously from *Croton muscicapa* and Amaranths grain (7).The compound squalene showed the highest significant antioxidant activity at 1.0 mg/mL at 58.0%. It gave a significant IC_{50} value of 0.78 mg/mL . This indicates that the compound –squalene isolated for the first time from *Alternathera sessilis* is a good antioxidant that may be responsible for the ethno-medicinal uses of this plant. The reported antimicrobial activity of the methanol extract of *Alternathera sessilis* reported in previous work on this plant could be attributed to the presence of compound -squalene , known for its antimicrobial activity (8). This is the first time this compound squalene is

Compound :- ^{1}H NMR(500 MHz) and ^{13}C NMR(125 MHz) data,see Table 1

Table 1: Correlations table ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data[a] for compound 1: Squalene and literature[b] in $CDCL_3$

No	^{13}C NMR[a] (125 MHz) $CDCL_3$	^{13}C NMR[b] (125 MHz) $CDCL_3$	^{1} H NMR[a] (500 MHz) $CDCL_3$	^{1} H NMR[b] (500 MHz) $CDCL_3$
1/24	25.9 CH3	25.7CH3	1.68s	1.67s
2/23	131.2 C	131.2 C	-	-
3/22	124.4 CH	124.2CH	5.11m	5.11m
4/21	26.6 CH_2	26.7CH_2	2.10 m	2.10 m
5/20	39.7 CH_2	39.7CH_2	1.98 m	1.98 m
6/19	135.0 C	134.9C	-	-
7/18	124.5 CH	124.3CH	5.42 m	5.42 m
8/17	26.6 CH_2	26.6CH_2	2.07 m	2.07 m
9/16	40.0 CH_2	40.0CH_2	1.98 m	1.97 m
10/15	135.1 C	135.1C	-	-
11/14	124.6 CH	124.2 CH	5.11 m	5.11 m
12/13	28.2 CH_2	28.2CH_2	2.01 dd J = 3.3, 6.8	2.01 dd J = 3.3, 6.8
25/30	17.9 CH_3	17.6CH_3	1.60s	1.60s
26/29	16.5 CH_3	16.0CH_3	1.67s	1.66s
27/28	16.3 CH_3	16.0 CH_3	1.60s	1.60s

[a] Assignment aided by HMQC and HMBC experiments

[b] Literature

isolated from *Alternathera sessilis*. Squalene acts as a protective agent and has been shown to decrease chemotherapy induced side effects. Moreover, sqaulene exhibits chemopreventive activity. Although it is a weak inhibitor of tumor cell proliferation, it contributes either directly or indirectly to the treatment of cancer due to its potentiation effect (9).These reported pharmacological activities of squalene which isolation is reported for the first time in this work give a added value to the medicinal uses/usefulness of *Alternathera sessilis*. It also provides scientific justification for the medicinal uses of this plant.

CONCENTRATION mg/mL	ABSORBANCE (nm)			
	Sample	Ascorbic Acid	Scavenging Activity %	
			Sample	Ascorbic acid
1.0	0.769	0.151	56.5	91.5
0.5	0.770	0.182	56.4	90.0
0.25	0.780	0.172	55.9	90.3
0.125	0.793	0.179	55.1	89.8
0.0625	0.793	0.179	55.1	89.8

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