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Isolation and NMR Characterisation of a Pentacyclic Triterpenoid from chloroform fraction of *Ageratum conyzoides* Linn stem bark

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ABSTRACT

This study was carried out to identify the bioactive constituents of the stem barks of *Ageratum conyzoides Linn* a medicinally important plant of the Asteraceae family. This plant was selected on the basis of its widespread use in traditional herbal medicine A pentacyclic triterpenoid lupeol was isolated from the chloroform soluble fraction of the ethanol extract of the stem barks of *Ageratum conyzoides* a plant know to have potent pharmacological properties, antiangiogenic, antioxidative and anti-inflammatory in nature using a combination of silica gel column chromatography and preparative TLC. The structure of this compound was elucidated using NMR spectroscopic analysis (1D& 2D) FTIR and MS, by comparison with reported data. This is the first report of isolation of this compound from *Ageratum conyzoides*.

Keywords: Medicinal plant, Ageratum conyzoides, chloroform fraction, NMR, anti-inflammatory.

INTRODUCTION

Plant-based remedies enjoy a reputable position today, particularly in the developing countries, where basic health care facilities are inadequate. Herbal medications which are more effective, safe and cheap are gaining ground in both urban and rural communities [1]. Information from ethnic groups or traditional medicine practitioners has played an important role in the development of novel bioactive medicinal products from herb as therapeutic agents [2].

Medicinal plants are in high demand in the developed as well as developing countries for basic healthcare need, because of their wide biological and medicinal properties, higher safety margins and lesser costs. Over the years, the WHO suggested that countries should get involved in the use of herbal medicine with the aim of exploiting the secondary metabolites that provide effective and safe solutions for diseases of both non-microbial and microbial origins [3]. Medicinal plants have been revealed to have valid utility and over 80% people living in rural communities rely on its efficacy for their primary health care. The efficacy of herbal medicines against ill health is possible due to presence of so many bioactive constituents such as nutrients, phytochemicals, etc, which have pharmacological activities in the body of living organisms [4].

Ageratum conyzoides Linn is among the annual herb with a long history of traditional medicinal applications in both sub-tropical and tropical zones of the world. *Ageratum* is coiled from the Greek words 'a geras' meaning non-aging which refers to long life-time of plant. It is generally refered to as billy goat weeds. *Asteracease family comprises over 35 species with Ageratum conyzoides as one of the genera in the family* [5]. Among the species is the *Ageratum conyzoides* L., is generally refered to as "billy goat weed", "mentrasto" and "catinga-de-bode" [6].

It can survive in both tropical and subtropical areas where it thrives as ornamental during summer [7]. The species is now common in Europe, Africa, North America, Central America, the Caribbean, South America, and Oceania. *A.conyzoides* is generally used in folk medicine in many nations of the world as a analgesic, febrifuge, anti-inflammatory, purgative [5]. The plant when mature is used for its antispasmodic haemostatic, anti-inflammatory, antiasthmatic, properties for bacterial infections and the treatment of wounds [8-9]. *Ageratum conyzoides* is used for treatment of infective hepatitis, constipation, intestinal worms, filariasis and cuts. The whole plant possesses antiallergic, antinematocidal, anticoagulant, smooth muscle relaxant, haemostatic, analgesic, antifungal, antibacterial and hypothermic activities [9,10]. Phytochemical examination of *A. conyzoides* indicated presence of alkaloids, resins, saponins, tannins, glycosides and flavonoids [11].

The plant leaves have been reported to exhibit anti-inflammatory properties, with no apparent hepatotoxicity [12]. An extract of the dried plant or the fresh juice of the plant is used in the treatment of allergic rhinitis and sinusitis and also in treating post-partum uterine haemorrhage. *Ageratum conyzoides* is the only plant Igede people in Nigeria use in the treatment of HIV/AIDS [13].

Ageratum is coiled from the Greek "*a geras*," meaning non-aging, referring to the longevity of the flowers or the whole plant [14].

English: Chicken weed, Billygoat weed, Goat weed; Hindi: Jangli pudina, uchunti; also in Spain (El Salvador): Mejorana, sunsumpate (Columbia): Yerba hemostatica Portuguese: Mentrasto, Tropic ageratum;African Vernacular Names West Africa (Igbo): Nri-ewu (Yoruba): Imieshu or Imí esúyarnigbei. It is traditionally called “ufu opioko” and “otogo” by the Igedes in Benue state, Nigeria [15-16, 14]. In many part of the world, *Ageratum conyzoides* are reported to have a wide range of pharmacological uses, although applications vary from one region to another [17]. Traditionally in many parts of the world, this herb has been used to treat so many ailments especially in parts of the world like South America, Africa and Asia and as folk medicine. In India, among the rural and urban communities this specie is used as a anti-dysentery, bactericide and anti-lithic [18]. In Brazil, also its aqueous extract has been widely used in the treatment of fevers, colic, cold, diarrhoea, rheumatism and spasms [19, 20, 21]. Additionally, in Cameroon and Congo, its traditional use is to treat fever, rheumatism, headache and colic [22-23]. In parts of Central Africa, it is used in treatment of cold and pneumonia, however the common use is to cure wounds and burns [24].

MATERIALS AND METHODS

Sample Collection

The leaves and stems of *Ageratum conyzoides* Linn were harvested from the field of Abia State University,Uturu on the 14th January, 2017. Authentication of plant materials was done by Dr. Emmanuel, O. of Department of Plant Science and Biotechnology, Abia State University Uturu, Nigeria. Voucher specimen [1879] was deposited at the herbarium of the botanical Department of biological sciences school of the same University.

Preparation of Stem Bark Extract

The plant stem barks were washed with running tap water for 5 min to remove the dust and debris and rinsed with sterile distilled water. The fresh plant stem barks sample was air dried on the laboratory bench for ten days at temperature below 30 ^{0}C to avoid decomposition of thermo labile compounds. The sample was milled using and electric blender to coarse powder and powdered sample was kept in a clean closed container pending extraction. 500 g of pulverized stem barks material was mixed with 750 ml of solvent (95 % methanol) and kept in rotary shaker at 100 rpm overnight and filtered with What man No.1 filter paper The extract was concentrated under reduced pressure using Digital Heidolph Rotary evaporator (4000 series) and the supernatant plant extract was decanted after complete removal of the solvent. The extract was suspended in water and was successively partitioned using petroleum ether, chloroform ethyl acetate and methanol to obtain petroleum ether, chloroform ethyl acetate and methanol soluble fraction. the chloroform soluble fraction was used for this study

Isolation and purification of the compounds

The column used was 280 mm in height and 35 mm in diameter. The column was washed and rinsed with solvents of different polarities and finally with the solvent to to used. After drying, small cotton wool was used to cover the base of the column on which the packing will rest on, so that the silica gel does not wash out of the bottom of the column. Then 300 g of the silica gel (200 meshes) was mixed with petroleum ether in a beaker. The gel was poured into the column and tapped to remove any trace of air bubbles. 5.2 g chloroform fraction was subjected to silica gel column chromatography. The column was eluted with petroleum ether of 100%, then petroleum ether -chloroform mixture (95:5, 90:10, 85:15, 80:20, 75:25, 70:30, 65:35,60:40, 55: 45, 40:60, 35:65, 30:70, 25:75, 20:80, 15:85, 10:90, 5:95) successively chloroform-ethyl acetate solvent mixture; (95:5, 90:10, 85:15, 80:20, 75:25, 70:30, 65:35,60:40, 55: 45, 40:60, 35:65, 30:70, 25:75, 20:80, 15:85, 10:90, 5:95) then followed by ethyl acetate- methanol solvent mixture (95:5, 90:10, 85:15, 80:20, 75:25, 70:30, 65:35,60:40, 55: 45, 40:60, 35:65, 30:70, 25:75, 20:80, 15:85, 10:90, 5:95,100) collections was made on (50 ml each). Base on the TLC profiles, some of the fractions with the same R_f values were pooled together [25].

Spectroscopic Analysis

The spectroscopic and spectrometric analyses of the isolated compound were recorded on a Bruker AVANCE-600 (500MHz) for ^{1}HNMR and 150 MHz for ^{13}CNMR in deuterated chloroform with TMS as internal standard

RESULTS AND DISCUSSIONS

4.2 mg of Compound AGC-1 was isolated using column chromatography as a brownish yellow armorphous liquid. The column chromatography isolation was done using using gradient elution with different mobile phases, the fraction of chloroform / ethyl acetate (65:35) with R_f 0.69 derived from the chloroform fraction of the stem bark extract of *Ageratum Conyzoides* and identified as known Lupeol which has been isolated from many sources [26-27]. The structural elucidation of AGC-1 was principally achieved using NMR MS and IR, One dimensional NMR (^{1}H and ^{13}C) along with 2D NMR Spectra (COSY, HMBC and HSQC). The MS spectrum showed a molecular ion peak at m/z 426, which corresponded to a molecular formula of $C_{30}H_{50}O$. A double bond equivalence of six was calculated The FTIR spectrum showed an absorption band 3437cm^{-1} that was attributed to a hydroxyl group stretch

The ^{1}H NMR spectrum of compound ACG-1 showed the presence of seven methyl group proton resonance at δ 1.69, 0.77, 0.79, 0.83, 0.97, 0.98, 1.02, and 1.69 which corresponded to the carbon resonance at δ 19.5, 18.2, 14.7, 16.2, 16.3, 15.6 and 28.2 in the^{13}CNMR spectrum and HSQCDEPT spectrum. The ^{1}H NMR spectrum showed a proton resonance at δ3.18 ^{1}H, dd. *J* =11.35, *J* = 4.85 Hz) which was seen to correspond to a carbon resonance at δ 79.3 in the HSQCDEPT spectrum. This proton resonance and the H-5 proton resonance at δ 0.69 were seen to correlate in the HMBC spectrum with the 3H-23 and 3H-24 methyl group resonances at δ 0.97 and 0.77. The HMBC spectrum showed correlation between downfield methyl group resonance at δ 1.69 and the two non-equivalent methylene proton resonance at δ 4.68 and 4.56 which were ascribed to H-29A and H-29B. Compound ACG-1 was identified as the known 3β-hydroxylup-20(29)-ene, commonly known as the lupeol, which has been evaluated for its anti-inflammatory and anti-angiogenic activities. A literate search revealed that the ^{13}CNMR chemical shifts similar to those of compound ACG-1 had reported for lupeol.,

FTIR Spectrum revealed a very intense broad band at 34 37cm $^{-1}$ and medium intense band at 1215cm^{-1} were observed for the O-H bond vibration of hydroxyl group. The stretching and bending vibrations of methyl parts were noticed by the intense band 2928 cm^{-1} and medium intense band at 1463 cm^{-1}. The out of plane C-H vibration of the unsaturated part was observed at 897cm^{-1}. The corresponding C=C vibration was observed at 1635cm^{-1} was weakly intense.

Table 1: Comparison of ^{13} CNMR and ^{1}HNMR of compound AGC-1 with that of lupeol reported in the literature

No	^{13}C NMR (125MHz) ($CDCl_3$)	^{13}C NMR (100MHz) ($CDCl_3$)	^{1}HNMR (500 MHz) ($CDCl_3$)	^{1}HNMR (400 MHz) ($CDCl_3$)
1α	38.9 CH_2	38.7	1.65 m	0.90 d
1β			0.9` m	1.67 t
2α	27.6 CH_2	27.4	1.60 m	1.60 d
2β			1.56 m	1.56 q
3	79.2 CH	79.0	3.18 dd, *J* =11.35, 4.85 Hz	3.19 dd
4	38.9 C	38.8	-	-
5	55.5 CH	55.3	0.69 d, *J* =9.25 Hz	0.68 d
6α	18.5 CH_2	18.3	1.51 m	1.51 d
6β			1.39”	1.39 q
7α	34.5 CH_2	34.3	1.38”	1.39 m
7β			1.38”	1.39 m
8	40.1 C	40.8	-	-
9	50.7 CH	50.4	1.27 m	1.27 d
10	37.4 C	37.2	-	-
11α	21.2 CH_2	20.9	1.41 m	1.41 d
11β			1.21 m	1.23 q
12α	25.4 CH_2	25.2	1.06 m	1.07 q
12β			1.67”	1.67 d
13	38.3 CH	38.1	1.64”	1.66 t
14	43.1 C	42.9	-	
15α	27.6 CH_2	27.5	1.00 m	1.00 d
15β			1.67”	1.68 t
16α	35.8 CH_2	35.6	1.38”	1.37 t
16β			1.47 d	1.47
17	43.2 C	43	-	-
18	48.5 CH	48	1.35”	1.36 t
19	48.2 CH	47.9	2.38 m	2.39 m
20	151.1 C	151.0	-	-
21α	29.9 CH_2	29.9	1.32”	1.32 m
21β			1.92 m	1.92 m
22α	40.2 CH_2	40.0	1.19 m	1.19 m
22β			1.38”	1.38 m
23	28.2 CH_2	28.0	0.97 s	0.97 s
24	15.6 CH_2	15.4	0.77 s	0.77 s
25	16.3 CH_2	16.1	0.83 s	0.83 s
26	16.2 CH_2	16.0	1.02 s	1.03 s
27	14.7 CH_2	14.6	0.98 s	0.95 s
28	18.2 CH_2	18.0	0.79 s	0.79 s
29α	109.5 CH_2	109.0	4.56 br s	4.56 m
29β			4.68 br s	4.69 m
30	19.5 CH_2	19.3	1.69 s	1.68 s

Reference lupoel isolated by [26].

Table 2: FTIR Analysis of compound [1]

Absorption frequency (CM^{-1})	Functional Group	Stretching Vibration
3437	Alcohols	- OH
2928	Alkanes	CH
1635	Alkenes	C=C
1463	Alkanes	CH_3
1215	Alcohol	O-H bending
897	Out of plane C-H vibrations of unsaturated part	C= C

The proposed compound AGC-1 on comparison with reported data is lupeol (figure 1) a pentacyclic in terpenoid

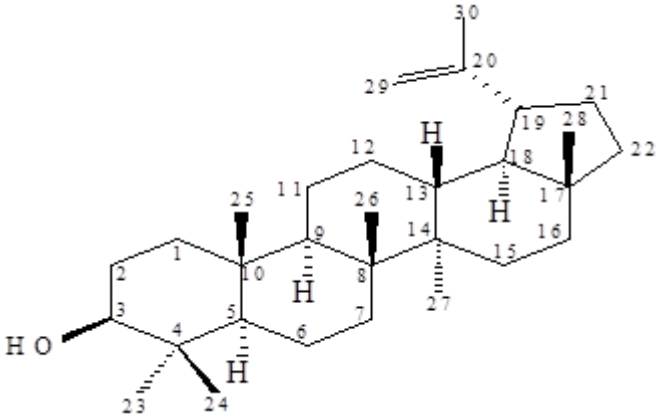

Figure 1: Proposed structure of Compound AGC-1

CONCLUSION

Spectroscopic analysis involving 1D and 2D NMR, MS and FTIR was used in structural elucidation of the compound. Chromatographic techniques were also employed in the isolation and purification of the compound. This is the first time of identifying this compound from *Ageratum conyzoides*.Lupeol is well known to have a wide range of biological activity.

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