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β – amyrin-3- acetate Detected in Methanolic Leaf Extract of *Chrysophyllum albidium*

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ABSTRACT

Polarity guided cold extraction method was used to extract the crude extracts of hexane, chloroform, ethyl acetate, acetone and methanol of leaves of *Chrysophyllum albidum.* The structural elucidation by spectroscopic methods (^{1}H and ^{13}C NMR) of a fraction of methanol extract of *C. albidum* yielded one new characterized as β – amyrin acetate. Based on the presence of β-amyrin acetate in *C. albidum*, the plant could be a viable source of antioxidant and cytotoxic agents in cancer chemotherapy in the near future. *C. albidum* may also be used for the treatment of malaria, acquired immunodeficiency syndrome, heart disease, stroke, arteriosclerosis, diabetestoothache, rhematic pain, and other inflammatory related disorders and cancer because of the β – amyrin acetate.

Introduction

Despite the great advances witnessed in modern medicine in recent decades, plants still make an important contribution to health care. Much interest, in medicinal plants however, stems from their long use in folk medicines as well as their prophylactic properties, especially in developing countries [1]. Plants have been used for alleviating human suffering from the very beginning of human civilization, and records of the use of plants are available since about 5000 years ago [2]. The active principles isolated, have provided leads in the development of several life saving drugs, which are in use today and different civilizations developed their own indigenous system of medicines [3]. it has been pointed out that a traditional medicine is a combination of knowledge and practice, whether applicable or not, used in diagnosing, preventing, or eliminating physical, mental or social disease and which may rely exclusively on past experience and observation handed down from generation to generation verbally or in written form, while bearing in mind the original concept of nature which includes the material world [4]. The use of plants as medicine to cure illness and to lubricate the wheels of social interaction at interpersonal level is a behaviour that predates civilization, it is found in every society irrespective of its level of development and sophistication [5].

Phytochemical screening of various plants has been reported by many workers [6] [7]. Scientific investigations of medicinal plants have been initiated in many countries because of their contributions to health care. The primary benefits of using plant-derived medicines are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment [8]. The active components of herbal remedies have the merit of being combined with many other substances that appear to be inactive [7].

From available literature there is no work which has been done on the plant *Chrysophyllum albidum* leaves, in Calabar

Muncipality, Local Government Area, of Cross River State Nigeria. The present work is designed to carry out the characterization and identification of bioactive compounds from *C. albidum* leaf extracts.

MATERIALS AND METHODS

Sample Collection and Preparation

Chrysophyllum albidum leaves were collected from their natural habitat of plain sandy soil of coastal plain sands in Calabar Municipality (04^o 15^oN; 08^o 25^oE), Nigeria and identified by Prof David A. Ogar of Department of Forestry, University of Calabar. The sample were air-dried for two weeks and then milled into fine powder using a milling machine.

Method of Extraction

The method of cold maceration was used in the extraction by serial exhaustive extraction method which involves successive extraction with solvents of increasing polarity from a non polar (hexane) to a more polar solvent (methanol) to ensure that a wide polarity range of compound could be extracted. The extracts of the leaves were prepared by soaking 100 g of each in 250 ml hexane for four days with frequent agitation until soluble matter was dissolved. The resulting mixture was filtered by gravity filtration and the filtrate was concentrated by evaporation using rotatory evaporator and weighed. The procedure was repeated on the residue using the following solvents: chloroform, ethyl acetate, acetone and methanol sequentially in order of polarity. The extracts were kept in a refrigerator under argon condition until required for analysis.

Isolation of the Compound

The methanol extract was isolated and purified by mixing equal volume of solvent (chloroform) and water in a separating funnel. The funnel was placed on the stand and allowed the two liquids to separate completely. The two layers were carefully collected into different beakers. Solvents were evaporated under reduced pressure to get dried fractions of chloroform extract. The isolation and purification of the component was achieved through the application concentrated chloroform extract (60 g) to a Sephadex LH-20 column (150x4.5 cm) and eluted continuously with chloroform until the green color runs down the column.

As the elution progressed, green colour eluted out the column and was collected in a conical flask. The green pigments were collected from the column are then concentrated by removing the solvents using a rotary evaporator. The pigments left behind in round bottomed flask after rotary evaporator were transferred into watch glasses using spatula. The dried elute was washed with hexane to remove hexane soluble pigments, after that wash the same dried elute with methanol to remove methanol soluble pigments. The sample was dissolved in chloroform and filtered before spotting the sample on the TLC plate and the plate was ran in a desired mobile phase and visualize the spot in UV light. The silica gel on the TLC plate is impregnated with a fluorescent material that glows under ultraviolet (UV) light. The mobile phase is chloroform: methanol (4:6) and the chamber saturation time was 30 minutes.

Structural Elucidation

The structural elucidation of the leaves isolate of the methanol extract from *Chrysophyllum albidum* was done by spectroscopic methods (IR, ^{1}HNMR, and ^{13}CNMR). Infrared spectrophotometer model Brunker IFS 66 V/S. NMR spectra were obtained with a Brunker AVANCE 400. Fourier transform NMR spectrometer with chemical shifts reported in parts per million (ppm) with respect to TMS.

RESULTS

Result of Structural Elucidation

The result of structural elucidation by the structures were elucidated by

spectroscopic methods (IR, ^{1}H NMR, ^{13}C and NMR) an the result are as follows;

FTIR Spectra for Isolated Methanol Fraction

The IR spectrum displayed C-H asymmetric stretching in -CH_3 at 2964 cm^{-1}, C-H symmetric stretching in –CH_2 and =CH at 2915 cm^{-1} and 2843 cm^{-1} respectively. It also showed C-H bending vibration in –CH_2 and –CH_3 at 1468 and 1413 respectively and C-C stretch in C-C at 997.28.

NMR Spectra for Isolated Methanol Fraction (MF)

NMR analysis of isolated methanol fraction of *C. albidum* leaves extract was carried out and the result is summarized in Table 2 below. The ^{1}H-NMR spectra showed the presence of eight tertiary methyl groups by exhibiting signals at δ 0.82, 0.84, 0.93, 0.95, 0.97, 1.0, 1.1 and 1.25. A proton oxygen residue by exhibiting a signal at δ 3.2 and is placed at C-3. An unsaturated proton at δ 5.5 suggesting the presence of a double bond, one of the carbon is tetrasubstituted and the other carbon is trisubstituted. The ^{13}C-NMR spectra confirms the presence of eight methyl groups by exhibiting signals at δ 29.7, 15.4, 15.4, 17.5, 21.32, 28.0, 33.7 and 25.9. The presence of an oxycarbon atom is exhibited at a signal at δ 79.1. While the presence of double bond carbon-atoms by exhibited signals at δ 116.8 and 142.7, Also, the presence of a carbonyl carbon by exhibiting a signal at δ 158.1 and a acyl carbon by exhibiting a signal at δ 29.8. A comparison with the signals of known triterpenoids it matches well with β – amyrin acetate and is presented in the Table 1.

Table 1: The ^{13}CNMR and ^{1}HNMR Spectral Data of Isolated Methanol Component

C positions	Carbon type or Group	Carbon Signal (δ)	Proton Signal (δ)
C-1	CH_2	38.7	1.31,d
C-2	CH_2	28.8	1.67,d
C-3	CH	79.1	3.2t
C-4	C	38.5	-
C-5	CH	55.5	0.86t
C-6	CH_2	18.8	1.58m
C-7	CH_2	33.1	1.28t
C-8	C	38.8	-
C-9	CH	49.2	1.65s
C-10	C	36.7	-
C-11	CH_2	22.7	1.96,m
C-12	CH	116.8	5.5,m
C-13	C	142.7	-
C-14	C	41.3	-
C-15	CH_2	27.4	1.99,m
C-16	CH_2	27.1	1.60,m
C-17	C	49.2	-
C-18	CH	33.3	2.04,m
C-19	CH_2	48.7	1.93, d
C-20	C	29.1	-
C-21	CH_2	35.1	1.31, s
C-22	CH_2	37.5	1.63,s
C-23	CH_3	29.7	0.82,s
C-24	CH_3	15.43	0.84,s
C-25	CH_3	15.4	0.93,s

C-26	CH_3	17.54	0.95,s
C-27	CH_3	21.32	0.97,s
C-28	CH_3	28.0	1.0,s
C-29	CH_3	33.7	1.1,s
C-30	CH_3	25.9	1.2,s
C=O	C	158.1	-
Me	CH_3	29.8	2.07s

Results and Discussion

Nuclear magnetic resonance (NMR) is a spectroscopic method that is important to organic chemists. The combination of Infrared (IR) and NMR data is often sufficient to determine the structure of an unknown molecule [11]. The structural elucidation was done by spectroscopic methods (IR, ^{1}H NMR and^{13}CNMR) and was carried out on the purified extracts. The results revealed one novel compound identified as β – amyrin-3- acetate. The isolated compound, β – amyrin-3- acetate was subjected to TLC monitoring. The silica gel on the TLC plate is impregnated with a fluorescent material (Anisaldehyde- H_2SO_4 reagent followed by warming) that glows under ultraviolet (UV) light. The mobile phase was chloroform: methanol (4:6 V/V) and chamber saturation time was 30 mins and the R_f value was 0.894. The structure of the compound, β – amyrin-3- acetate was elucidated by comparison with literature values. The β – amyrin-3- acetate was isolated as yellowish substance. The IR spectrum displayed C-H asymmetric stretching in -CH_3 at 2964 cm^{-1} C-H symmetric stretching in –CH_2 and =CH at 2915 cm^{-1} and 2843 cm^{-1} respectively. It also showed C-H bending vibration in –CH_2 and –CH_3 at 1468 and 1413 respectively and C-C stretch in C-C at 997.28.

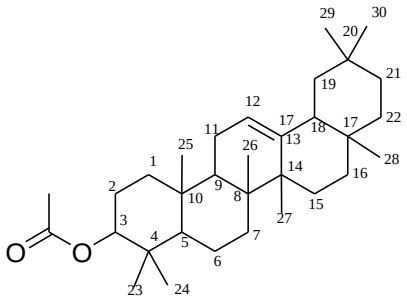
A large number of pentacyclic triterpenoids have been examined by ^{13}CNMR spectroscopy and considerable ^{13}C chemical shift data have been accumulated [12]. The ^{1}H NMR spectrum (400 MHz, $CDCl_3$) showed the presence of eight tertiary methyl group singlets at δ 0.82, 0.84, 0.93, 0.95, 0.97, 1.0, 1.1 and 1.25. The ^{13}C NMR spectrum (400 MHz, CDCl3) showed the compound, β – amyrin-3- acetate has a total of 32 carbon atoms with eight tertiary methyl groups by exhibiting signals at δ 29.7, 15.4, 15.4, 17.5, 21.32, 28.0, 33.7 and 25.9. The ^{13}CNMR spectrum displaced seven quaternary carbons at a δ of 55.53, 38.77, 36.77, 142.70, 41.30, 33.36 and 29.83. A proton exhibited a signal at δ 3.2 and is placed at C-3 and the presence of an oxycarbon atom exhibited a signal at δ 79.1. Compound 1 has the alkene protons (an unsaturated proton) at δ 5.5 suggesting the presence of a double bond. One of the carbons is tetrasubstituted and the other carbon is trisubstituted. Also, the presence of a double bond (carbon 12 and carbon 13) exhibited signals at δ 116.8 and 142.7. The presence of a carbonyl carbon is indicated by exhibiting a signal at δ 158.1. The presence of methyl carbon of the acyl group exhibited a signal at δ 29.8. The identity of compound 1 as β – amyrin acetate was confirmed by comparison with published values of [12], [11], [13], [14], and [15]. A comparison with the proton and carbon signals of known triterpenoids matches well with β – amyrin -3- acetate and is presented in the Table 1.

The β-amyrin acetate has been identified for the first time in the leaves of *C. albidum*. The data here suggests that the β-amyrin acetate fraction of the leaves of β-amyrin acetate can be extracted from *C. albidum*, which is used as anticancer, cytotoxic [16], antiparasitic, antiallergenic,

antispasmodic, antihyperglycemic and as herbicide, fungicide and antibiotic in medicines[17]. It has being pointed out that α, β-amyrin acetate has the potential to combat acute pancreatitis by acting as an anti-inflammatory and antioxidant agent [18]. *C. albidum* exhibited antioxidant activity [19], this could be attributed to the β-amyrin acetate. It may be stated that the result of the present study demonstrated new property of β-amyrin acetate as a potent lipid lowering agent. Based on the presence of β-amyrin acetate in C. *albidum* could be explored in biological profiling requiring antioxidant and cytotoxic dependent therapeutics as the plant could be a viable source of antioxidant and cytotoxic agents in cancer chemotherapy in the near future [20]. *Chrysophyllum albidum* may be used for the treatment of malaria, acquired immunodeficiency syndrome, heart disease, stroke, arteriosclerosis, diabetes and cancer because of the strong antioxidant activity of these extracts of the leaf [21]. Since β-amyrin acetate is isolated from *C. albidum* leaf extracts it may be used in the ethnomedicinal management of toothache, rheumatic pain, and other inflammatory related disorders.

A chromatographic purification of the crude methanol extract led to the isolation and structure elucidation of β-amyrin acetate and the study provided evidence of profound anti-inflammatory activity of β-amyrin acetate and α-amyrin acetate isolated from the *Alstonia boonei* stem bark [22]. The β-amyrin acetate can be explored in biological profiling requiring antioxidant and cytotoxic dependent therapeutics as the plant could be a viable source of antioxidant and cytotoxic agents in cancer chemotherapy in the near future [23]. β-amyrin acetate has cytotoxic properties which clearly indicates that it has potential cytotoxic properties and can be used as a source of antitumor agents [21]. Also, α, β-amyrin has the potential to combat acute pancreatitis by acting as an anti-inflammatory and antioxidant agent [24]**.** Antimicrobial screening reported from other natural products has also confirmed the microbial properties of β-amyrin acetate. It has been found that β-Amyrin was six times as active as aspirin in inhibiting platelets aggregation. β-amyrin was isolated for the first time from *Laurencia microcladia*, marine algae distributed widely in Egypt found to have antibacterial activity against *Staphylococcus aureus, Bacillus subtilis, Salmonella typhi, Escherichia coli,* and *Pseudomonas aeurginosa* [25].

Pentacyclic triterpenes are ubiquitously distributed throughout the plant kingdom, in a free form as aglycones or in combined forms, and have long been known to have a number of biological effects. α- and β-Amyrin are bioactive compounds commonly found in leaves, barks and resins [16]. β-amyrinhas a more potent than aspirin inhibiting collagen-induced platelet aggregation [26] and can be used as anti-irritant, anti-inflammatory action as well as enhancement of the sun protection factor of organic sunscreens and emollient effect [27] and [28]. β-amyrin has been found to exhibit antifungal and antimicrobial activity against some microbes. β-amyrin acetate were reported to possess anti-inflammatory activity [17]. β-amyrin acetate exhibited cytotoxic effects against MCF-7 cell line [16] and were also reported to exhibit sedative, anxiolytic and anticonvulsant properties [29]. β-AA and β-AP as a potent lipid lowering agent [30].



Conclusion

It can be concluded that the structural elucidation by spectroscopic methods (IR, ^{1}H NMR, ^{13}C and NMR) of *C. albidum* yielded a compound characterized as β – amyrin acetate. The beta-amyrin acetate has been identified for the first time in the leaves of *C. albidum*. The data here suggest that the beta-amyrin acetate fraction of the leave of β-amyrin can be extracted from *C. albidum*, which is used as anticancer, antiparasitic, antiallergenic, antispasmodic, antihyperglycemic and as herbicide, fungicide and antibiotic in medicines. α, β-amyrin has the potential to combat acute pancreatitis by acting as an anti-inflammatory, antioxidant agent cytotoxic and anticancer properties. The antioxidant activity of can be attributed to the beta-amyrin acetate (β-amyrin acetate). It may be stated that the result of the present study demonstrated new property of β-AA as a potent lipid lowering agent. The plant extracts could therefore be seen as a potential source for useful drug and this justifies the claims by the traditional healers that the *C. albidium* leaves are used to cure some illness. The continued traditional medicinal use of these plants is therefore encouraged.

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