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Isolation Characterization of phytosterol (3(β) Lup -20(29)en-3yl Benzoate) from the Leaf of Gongronema Latifolium Benth

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Abstract
Plants secondary metabolites tend to be of immense health benefit to man, the extraction and characterization of these compounds are of profound importance. Isolation and characterization of 3(β) Lup -20(29) en-3yl benzonate from the leaf of Gongronema latifolium Benth was done with the aid of column chromatography, thin layer chromatography IR spectrophotometer, ^{1}H and ^{13}C NMR and mass spectrometer. IR Spectrum was obtained from a thermo Nicolet Nexus 470 FR-IR spectrometer. The ^{I}H and ^{13}C NMR spectra were obtained on a Bruker Avance 400 FT NMR Spectrometer using tetra methyl silane as internal standard. Chemical shifts are expressed in ծ values. LC - ESIMS spectra where determined in the positive ion mode on a PE. Biosystem APl 156 single quadruple instrument. HRESIMS (positive ion mode) spectra were recorded on a thermo Finniga MAT 95 x L. 3(β) Lup -20(29) en-3yl benzonate was identified having m/z 530. Lupeol and its derivatives are phytosterols which acts as therapeutic and chemo-preventive agents for the treatment of inflammation and cancer.

Keywords: Chemical Shift, Phytosterol, Lupeol, Spectrophotometer, Metabolites

Introduction
Gongronema latifolium Benth is a common plant found in The eastern Nigeraia, it belongs to the family Asclepiadaceae. [1] *Gongronema latifolium* leaves possess moderate concentrations of carbohydrate, protein, saponins, tannins and least levels of alkaloids and fat. The plant is good for consumption as food. It has been [2], revealed that the plant contains appreciable levels of phytochemicals, nutrients, essential elements and vitamin. [2] The antioxidant property of the plant was studied and reports have it that methanol extract of *Gongronema latifolium* revealed a higher antioxidant potential compared to aqueous extract. They concluded that *Gongronema latifolium* leaf extract is a good source of natural antioxidant due to its high antioxidant potentials [3].

It has been revealed that treating albino rats with the plant extract lowers the mean bodyweight significantly, while the mean weight of the stomach, liver and small intestine to body weight ratio was increased significant. the extract reduced the blood glucose level similar to the non-diabetic level [4]. Gongronema latifolium leaves contains flavonoids, tannins, saponins, alkaloids, terpenoids as well as minerals such as calcium magnesium, potassium, sodium, phosphate, iron, zinc and chromium. The presence of bioactive agents, minerals and free radical scavenging potentials of Gongronema latifolium leaves makes it a beneficial medicinal plant. G. latifolium extracts exerted a hypoglycemic effect, improved the lipid profile in diabetic rats and positively affected integrity and function of both liver and pancreas [5]. Reports has indicated that the twig and root bark extracts of the plant may be a good source of medically active phytochemicals and micronutrients relevant in human and animal nutrition [6].

The ethnomedical importance of G. latifolium has been studied and it was reported that It is enriched with verities of flavonoids, saponins, alkaloids and steroidal phytochemicals which exhibit prominent pharmacological actions such as hypoglycaemic, hypolipidemic, cytotoxic, antioxidant, and antimicrobial in-vitro and in-vivo. He indicated that one of the bioactive compounds, iloneoside, showed potent antileukemic activity [7]. Recent studies revealed that the plant extract has moderately high transfer factors and capacity to accumulate copper, mercury and arsenic [8]. The fruits of G. latifolium are even more potent than its leaves due to the higher concentration of most of its phytochemical constituents [9].

Anti-nutritional substances like phytate and oxalate found in the plant were not regarded as harmful for human consumption because of the traditional method of processing the vegetable [10]. The proximate composition of the leaf extract of G. latifolia shows high composition of protein and the plant is used in treatment of certain diseases such as diabetes hypercholesterolemia and liver problems [10, 11]. Oral administration of the G. latifolium extracts is said to led to the elevated level of serum urea, while the serum triglycerides and creatinine levels were reduced. it has been suggested that G. latifolium extracts at the doses tested had minimal renal toxicity [12]. With all these works already done on the plant, its full constituents and applications have not been fully elucidated. Our research was aimed at discovering and characterizing compound in the leaves of the plant which has not yet been elucidate.

Materials and Method

Sample Collection and Preparation

Leaves of *Gongronema latifolium* was collected from the farm in Ntibunka, Umudagu, Mbieri in Mbaitolu Local Government Area of Imo State of Nigeria. They were identified by Prof. Mbagwu in department of Plant Science and Biotech, School of Agriculture And Agricultural Technology, Federal University of Science and Technology, Owerri. The leaves were washed and air dried at room temperature and pulverized [13].

Extraction Procedures

About 250 g of the sample was percolated in 500 ml of ethanol for 48 hours and filtered. The filtrates were dewaxed with chloroform and concentrated with rotary evaporator. The concentrated crude extract was partitioned with ethanol and water mixture and separated.

Chromatographic Purification of Bioactive Compound

The bioactive compounds were isolated from the ethanol extracts according works by eailer research [14]. Column chromatographic isolation was performed to separate the different components. The crude extracts were partitioned in chloroform/ethanol mixture (ratio 1:1) and filtered to remove the soluble hydrophilic components. The extract was separated into different components using silica gel (stationary phase) packed in a chromatographic column. Slurry of finely powdered silica gel dispersed in diethyl ether were packed into the glass column to a height of about 12m and loaded with 10ml of the extracts dissolved in 100% diethyl ether. By varying concentrations of diethyl/chloroform and chloroform mixtures, different fractions were collected at intervals of 30ml each.

Thin-Layer Chromatographic

Thin-layer chromatographic was conducted to determine the pure compounds present in the fractions. Using TLC plates (coated with silica gel), the samples were then placed on the plates with a microcapillary tube The plate were placed in a glass tank containing the mobile phase (diethyether/chloroform/methanol; ratio 1:1:1) and allowed to run until the mobile phase reached the highest point on the plate, after which the plate was removed, dried in air and developed in iodine tank. The Rf value of the component were determined.

Infra Red Spectroscopic Analysis

Infra-Red analyses of the selected pure chromatographic fraction were performed at central Research Laboratory, University of Ibadan, Nigeria using FTIR Spectrophotometer BX-model manufactured by Perkin Eimer.

^{1}H-NMR And ^{13}C-NMR Analysis

The ^{1}H and ^{13}C NMR spectra were obtained on a Bruker Avance 400 FT NMR Spectrometer using tetra methyl silane as internal standard. Chemical shifts are expressed in ð values.

Mass Spectroscopy

LC – ESIMS spectra where determined in the positive ion mode on a PE. Biosystem APl 156 single quadruple instrument. HRESIMS (positive ion mode) spectra were recorded on a thermo Finniga MAT 95 x L.

Results and Discussion

Analysis of infra-red (IR) spectrum Table 2 showed absorptions bonds at 719.376 cm^{-1} and 875.924cm^{-1} showing the presence of out of plane aromatic and aromatic bending respectively. Presence of ethers (C-O) was observed at 1088.383 cm^{-1}. Aromatic bonds (C=C) were seen at 1461.116 cm^{-1}, 1576.664 cm^{-1}. Carbonyl bonds (C=O) showed its absorption peak at 1740.667 cm^{-1} and Aliphatic peaks were observed at 2851.413 cm^{-1} and 2918.503cm^{-1}. From the IR spectrum, the presence of alcohol was depicted at 3373.241cm^{-1}.

Table 2: (IR) Analysis of compound

IR Absorption (cm^{-1})	Bond Type	Functional group
719.376	C=C	Aromatic
875.924	C=C	Aromatic
1088.383	C-O	Ether
1461.116	C=C	Aromatic
1576.664	C=C	Aromatic
1740.667	C=C	Carbonyl
2851.413	C-H	Aliphatic
2918.505	C-H	Aliphatic
3373.241	OH	Alcohol

Table 3: ^{1}HNMR and ^{13}CNMR analyses of compound

Position	^{13}C Chemical Shift (ð)	Types of carbon	^{1}H chemical shift (ð)	Type of proton
1	15.110	CH_2	1.201	CH_2
2	15.690	CH_2	1.217	CH_2
3	75.453	HC-O	4.600	HC-O
4	30.100	C	----	----
5	26.830	CH	1.621	CH
6	16.400	CH_2	1.340	CH_2
7	16.750	CH_2	1.377	CH_2
8	31.461	C	----	----
9	27.111	CH	1.645	CH
10	32.070	C	----	----
11	17.010	CH_2	1.396	CH_2
12	18.770	CH_2	1.400	CH_2
13	28.060	CH	1.660	CH

14	33.600	C	----	----
15	19.461	CH_2	1.415	CH_2
16	19.716	CH_2	1.440	CH_2
17	24.101	C	----	----
18	29.312	CH	1.722	CH
19	29.910	CH	1.810	CH
20	35.690	C	----	----
21	20.110	CH_2	1.510	CH_2
22	20.361	CH_2	1.563	CH_2
23	9.100	CH_3	0.832	CH_3
24	10.515	CH_3	0.912	CH_3
25	10.890	CH_3	0.986	CH_3
26	11.890	CH_3	1.100	CH_3
27	12.716	CH_3	1.116	CH_3
28	12.913	CH_3	1.124	CH_3
29	36.090	C	----	----
30	13.620	CH_3	1.201	CH_3
1’	172.600	C=O	----	----
2’	37.800	C	----	----
3’	112.016	=CH	6.150	=CH
4’	123.417	=CH	6.893	=CH
5’	123.713	=CH	7.215	=CH
6’	140.0=20	=CH	7.365	=CH
7’	146.901	=CH	7.875	=CH

Proton Nuclear Magnetic Resonance (^{1}HNMR) of Compound

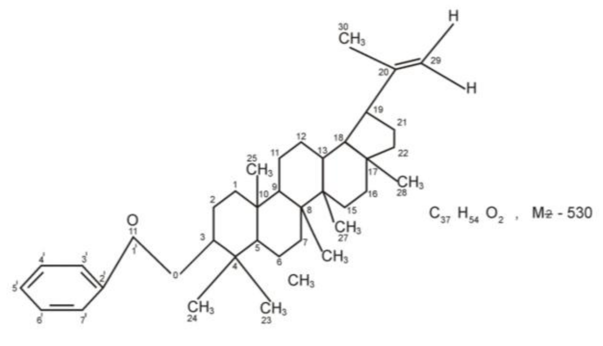
The spectrum of HNMR was analyzed in Table 3, from the spectrum, presence of methyl protons were seen at σ0.832, σ0.912, σ0.986, σ1.100, σ1.116, σ1.124, and σ1.201 labeled H-23, H-24, H-25, H-26, H-27, H-28 and H-30 respectively. Methylene protons showed their peaks at σ1.201, σ1.217, σ1.340, σ1.377, σ1.396, σ1.400, σ1.415, σ1.440, σ1.570 and σ1.563 labeled H-1, H-2, H-6, H-7, H-11, H-12, H-15, H-16, H-21 and H-22 respectively. Presence of methine protons in the spectrum were seen at σ1.621, σ1.645, σ1.660, σ1.722 and σ1.810 and were labeled H-5, H-9, H-13, H-18 and H-19 respectively. Aromatic protons were seen at σ6.150, σ6.893, σ7.215, σ7.365 and σ7.875 labeled H-3’, H-4’, H-5’, H-6’, and H-7’, respectively.

Proton Nuclear Magnetic Resonance (^{1}HNMR) of Compound

The spectrum of HNMR was analyzed in Table 3. From the spectrum presence of methyl protons were seen at σ0.832, σ0.912, σ0.986, σ1.100, σ1.116, σ1.124, and σ1.201 labeled H-23, H-24, H-25, H-26, H-27, H-28 and H-30 respectively. Methylene protons showed their peaks at σ1.201, σ1.217, σ1.340, σ1.377, σ1.396, σ1.400, σ1.415, σ1.440, σ1.570 and σ1.563 labeled H-1, H-2, H-6, H-7, H-11, H-12, H-15, H-16, H-21 and H-22 respectively. Presence of methine protons in the spectrum were seen at σ1.621, σ1.645, σ1.660, σ1.722 and σ1.810 and were labeled H-5, H-9, H-13, H-18 and H-19 respectively. Aromatic protons were seen at σ6.150, σ6.893, σ7.215, σ7.365 and σ7.875 labeled H-3’, H-4’, H-5’, H-6’, and H-7’, respectively.

^{13}CNMR Nuclear Magnetic Resonance of Compound

Analysis of ^{13}CNMR spectrum was shown in Table 3. From the spectrum, methyl carbons were seen at ð 9.100, ð 10.515, ð 10.890, ð 11.600, ð 12.716, ð 12.913, and ð 13.620 labeled C-23, C-24, C-25, C-26, C-27, C-28 and C-30 respectively. Methylene carbons showed their absorption peaks at ð 15.110, ð 15.690, ð 16.400, ð 16.750, ð 17.010, ð 18.770, ð 19.461, ð 20.110, and ð 20.361 labeled C-1, C-2, C-6, C-7, C-11, C-12, C-15, C-16, C-21, and C-22 respectively. Peaks indicating the presence of methine carbons were seen at ð 26.830, ð 27.111, ð 28.060, ð 29.312, and ð 29.910 labeled C-5,C-9,C-13,C-18 and C-19. The presence of carbonyl (C=O) in the spectrum was depicted at σ172.600, labeled C-1’. The carbon bearing hydroxyl (OH) group was seen at σ 75.453. Quaternary carbons were observed at ð 30.100, ð 31.461, ð 32.070, ð 33.600, ð 34.101, ð 35.690, ð 36.090 and ð 37.800 labeled C-4, C-8, C-10, C-14, C-17, C-20, C-29, C-2' respectively. Aromatic carbons absorption peaks were depicted at ð 112.016, ð 123.417, ð 123.713, ð 140.020, ð 146.901 labeled C-3’, C-4’, C-5’, C-6’, C-7’ respectively.



3 (β) Lup - 20 (29) en - 3yl benzoate

Fig 1: Structure of compound

Table 1: Result of R_F value of compound

Plant Compounds	R_F Values
Gongronema latifolium (Compound	0.25

The Compound Fig 1 was isolated as yellow oil, with chloroform and diethyl ether_at the ratio 1:1. Thin layer chromatography (TLC) carried on the compound showed one spot on iodine vapour (Rf value = 0.25). Table 1. Using infra-red (IR), proton nuclear magnetic resonance (^{1}HNMR) and carbon – 13 nuclear magnetic resonance (^{13}CNMR) spectra and mass spectrum. The compound was characterized and proposed as 3(β) lup -20(29)en-3yl benzoate with molecular formula $C_{37}H_{54}O_2$. mz = 530.

Lupeol is a natural triterpenoid found in edible fruits and vegetables and in medicinal plants. lupeol and its derivatives have been reported as therapeutic and chemo-preventive agents for the treatment of inflammation and cancer [15]. Natural triterpenoids called phytosterols are known to possess wide biological activities. Lupeol has been reported to be an antioxidant and anti-neuroinflammatory agent. It has been used in the treatment of Alzheimer’s disease that leads to memory weaknesses [16]. 3(β) Lup-20 (29) en-3yl acetate is a derivative of lupeol and has been isolated from maytenus acanthophylla Reissek, a medicinal plant from Bahia state, Brazil. The presence of this compound may be the reasons why Gongronema latifolium (utazi leaves) are used to treat many illnesses traditionally [17]. The compound is known for its biological properties such as anti-inflammatory, antioxidants, anti-cancer, antivenom, antimicrobial [18] and antiarthritic activities [19]. Lupeol is a triterpene active in both food and medicine having cholesterol-lowering properties. Fagarsterol another name for the chemical lupeol is found in plants like *Brassica oleracea var, capitata, Capsicum annuum Group, Fragaria, Olea europaea, Mangifera indica, Vitis vinifera, Aloe* *barbandesismiller*, Semaltree etc. Lupeol is an efficient curative and protective constituent for a wide range of disorders. It is an anti-carcinogenic and curative compound. Lupeol with their linoleate and palmitate esters, are non-competitive as well as competitive suppressions of serine protease trypsin and chymotrypsin [20, 21].

Conclusion

The identification of lupeol derivative in the plant may be the reason the plant is used traditionally for the treatment of various ailments such as a inflammatory, cancer, arthritics and wide of health disorders.

Conflict of Interest

None.

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