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Research Article

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Isolation and Characterisation of *β*-Amyrin from Stem Bark of *Dacryodes edulis*

Nna, Prince Joe

Medicinal and Phytochemistry Research Unit, Department of Chemistry, Ignatius Ajuru University of Education, Port Harcourt, Rivers State, Nigeria
Corresponding author: agaraprince@yahoo.com

Abstract *D. edulis is widely applied in Nigeria for treatment body pains, cough, diarrhea and fever. The study aims at isolating and characterizing compounds from the ethyl acetate and methanol extract of the plant stem bark. Healthy stem bark of the plant was collected, washed with distilled water, air dried, soxhlet extracted sequentially using ethyl acetate and methanol and concentrated to obtain the ethyl acetate and methanol extracts respectively. Column and thin layer chromatography were used for isolation and purification of the respective solvent extracts.* ^{1}*HNMRand* ^{13}*CNMR were employed for structural elucidation of the isolates. The result from the methanolic extract revealed a white crystal solids PDES-16 whose* ^{1}*HNMR and* ^{13}*CNMR spectra data agrees reasonably with literature for the compound β-amyrin. From the result, this is the first report of the isolation of β-amyrin from the methanolic extract of D. edulis.*

Keywords Unbranded Soymilk, Bacteria, Public Health, Good Quality, Beverage, Coliforms, Antibiotics

Introduction

The use of plants by man is appreciable to their effectiveness in the manufacturing of drugs, foods, as basis of oxygen for man and animals, raw materials for many industries. Plants have been ascertained to be most useful in curing of infections and showvibrant roles in pharmaceuticals industries as a result of the chemical substances present in them. These chemical substances include steroids, terpenes, flavonoids, alkaloids, tannins, glycosides, phenols, anthraquinones, carotenoids and saponins and are called phytochemicals or secondary metabolites [1]. They safeguard plants from numerous infections and contribute to plants scent, flavour and colour. The phytochemicals in plants serve as a defencing tool against environmental risks such as pathogenic violence, any kind of contamination, drought, UV contact and improve their usefulness in ethnomedicine [2].

It is consequently easy to see some animals and snakes eaten plants every time they are not healthy. It is likely that first man through exposure as ascertained by numerous herbalists revealed herbal drugs. Plants through the distinct power given to them by God are capable of synthesizing a wide variety of organic compounds of possible structural class. Owing to this, plants serve as valuable starting materials for chemical industries. Herbal treatment serves as a major characteristic of traditional medical practice in Nigeria [3]. The efficacy of traditional medical practice is a function of the chemical constituents of the plants.

Medicinal plants are bases of numerous significant drugs thus, the specific interaction of drugs with biomolecules such as nucleic acids and proteins in the body enhances physiological effects of drugs in the body. Thus, different drugs are required or useful for different purposes making plants useful precursors for drug development [4]. Plants

thus, play animportant position in traditional or herbal medicine, homeopathy and aromatherapy. For instance, spices and foods obtained from medicinal plants are used in the meals of women to boost their immune system [5]. A wide range of knowledge has been acquired concerning the efficacy of plants as diet and remedy for man as a result of some biological and pharmacological activities such as anti-inflammatory, diuretics, laxative, anti-plasmodics, antihypertensive and antimicrobial potencies of these plants [5].

The plant under investigation is believed to have originated from Central Africa and the Gulf of Guinea area [7]. It grows in nearly all the western coast of Africa across to Uganda. It is an evergreen tree growing to a height of 10-15m in the forest but not exceeding 12m in plantations [8]. The bark is pale gray and rough with droplets of resin. The upper surface of the leaves is glossy. The flowers are yellow and about 5mm across. They are arranged in a large inflorescence. The fruit of the plant, *Dacryodes edulis* are ellipsoidal and their size varies approximately from 4-9 cm long and from 2-5 cm wide [9, 10].

Sample collection

The stem bark of *Dacryodes edulis* was obtained from Horo Tai, Tai Local Government Area, Rivers State in Nigeria. The plant was identified and authenticated by Dr. M.G. Ajuru, a Plant Scientist at the Rivers State University, Port Harcourt. Sample was given Voucher specimen RSU/2019/DE-105 and saved in the herbarium. The sample was air-dried for two weeks and crushed to powder with the aid of a mortar and pistle. Later, it was deposited in a glass container and moved to Strathclyde Institute of Pharmacy and Biomedical Sciences laboratory, University of Strathclyde, Glasgow, United Kingdom for extraction and further analysis.

Extraction of Sample

About 300g of ground stem bark of *Dacryodes edulis* was positioned in soxhlet apparatus and sequentially extracted for two days each continuously with ethyl acetate and methanol. The extracts were evaporated to dryness in a rotary evaporator at 50 ºC. All dried extracts were combined together and kept in different sample bottles and labelled DE/Ea-001, and DE/ Me-001 for ethyl acetate and Methanol extracts respectively. About 20g of each crude extract was first subjected to qualitative phytochemical analysis while the remaining was dissolved in silica gel and loaded for column chromatography.

Isolation and Characterisation

About 7 g of dried extract of *Dacryodes edulis* was dissolved in dichloromethane and moved into a small beaker. 12 g of silica gel was transferred to the extract and stirred then allowed to dry in a fume cupboard. The silica gel was introduced to adsorb the extract. About 500 mL of ethyl acetate was added to 150 g of silica gel and stirred continuously until slurry was formed. The column was hung on a retort stand and rinsed three times with hexane to avoid impurity. At about 10 cm of solvent above silica packing, tap was closed and adsorbed extract slurry was transferred unto the column and then the tap was opened for excess solvent above loaded extract to run out of the column and allowed to settle on the silica. 5 % ethylacetate mixed with hexane was poured unto the column and the tap was allowed to run at about 10-15 drops per minute. About 25-30 mL fractions were uniformly collected using serially numbered vials. The eluates were allowed to dry and kept in a dust free fume hood [9]. Similar column fractions were combined after TLC analysis [10]. Fractions 001,002 and 003 elute with hexane: ethyl acetate gave similar TLC profile single creamy spot when charred with concentrated sulphuric acid, R_f value found to be 0.67. The combined fraction 001-003 were recrystallized in ethyl acetate to yield a compound labelled PJZZ-01. The compound was subjected to spectroscopic analysis (NMR-Spectroscopy).

Results

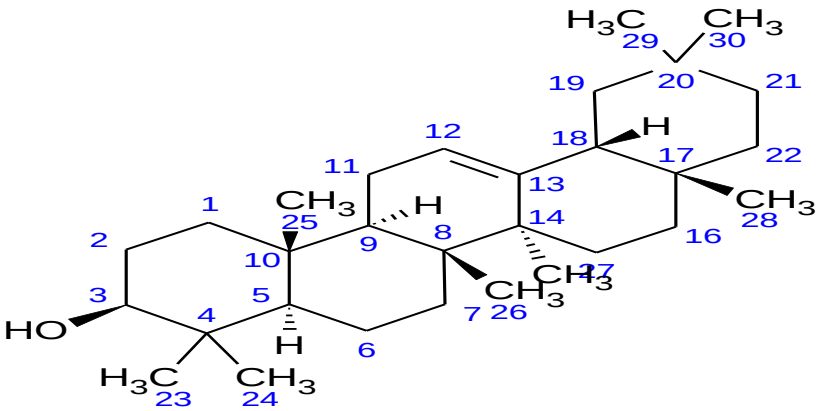
The soxhlet extraction of the plant material yielded a percentage of 7.1%. Isolate PDES-16 was a whitish needle-like crystal solid which was soluble in chloroform.

Table 1: ^{1}HNMR and ^{13}C NMR chemical shifts for PDES-16

Spectroscopic technique	Data
^{1}H NMR (Acetone)	δ 1.6, 1.6, 3.3, 1.7, 5.1, 0.9, 0.8, 1.0, 1.0, 1.1, 0.8, 0.8, 0.9 ppm
^{13}C NMR (Acetone)	δ 38.64, 27.25, 79.08, 38.64, 55.13, 18.35, 32.83, 40.04, 47.77, 36.9, 23.41, 124.4, 139.69, 42.17, 26.22, 28.24, 33.59, 58.98, 39.59, 39.43, 31.09, 41.41, 28.04, 15.59, 15.64, 16.81, 23.25, 28.73, 17.62, 21.55 ppm

Table 2: NMR data for PDES-16

	Experimental data		Literature data [11]	
Position	**^{1}H(δ)**	**^{13}(δ)**	**^{1}H(δ)**	**^{13}C**
1	1.6	38.64		38.79
2	1.6	27.25		27.29
3	3.3	79.08	3.2	79.05
4	1.7	38.64		38.79
5		55.13		55.19
6		18.35		18.36
7		32.83		32.94
8		40.04		40.02
9		47.77		47.73
10		36.9		36.91
11		23.41		23.37
12	5.1	124.4	5.12	124.42
13		139.69		139.58
14		42.17		42.09
15		26.22		26.63
16		28.24		28.11
17		33.59		33.76
18		58.98		59.08
19		39.59		39.66
20		39.43		39.61
21		31.09		31.26
22		41.41		41.53
23	0.9	28.04	0.99	28.11
24	0.8	15.59	0.79	15.61
25	1.0	15.64	0.95	15.68
26	1.0	16.81	1.01	16.87
27	1.1	23.25	1.07	23.27
28	0.8	28.73	0.80	28.75
29	0.8	17.62	0.79	17.46
30	0.9	21.55	0.91	21.39



Compound PDES-16: Beta amyrin

Characterization of PDES-16 as Beta-amyrin

PDES-16 was isolated as white needles from stem bark extract of *D. edulis*. The crystalline solid PDES-16 (150.0 mg) on examination by TLC and NMR was found to be a pure compound and was thus fully characterized. ^{1}H NMR spectrum showed signals observed at δ_H 3.15 (dd) and δ_H 5.18 (t). The signal at δ_H 3.15 is a characteristic of methine proton at C-3 of triterpenoids [11]. Similarly, the signal at δ_H5.18 is characteristic of vinyl proton attached at C-12 of amyrin (Table 1). The relatively deshielding signal is an indication of substitution of the hydroxyl group at C-3. From ^{13}C-NMR spectrum, eight methyl signals at 37.23, 28.05, 15.54, 15.64, 16.80, 25.9, 38.36, 33.3 and 23.66 and an oxygenated carbon at 78.97 [11]. These signals are typical of a sterol triterpenoid. From the ^{13}C NMR spectrum, two distinguishable signals were also observed atδc121.0 and 145.34 which are comparable to those of the olefinic carbons at C-12 and C-13, respectively published for amyrins by [11]. The positions of carbons and protons were confirmed on the basis of 2D NMR analyses such as HMQC, COSY and HMBC. Notable COSY signals were those between the vinylic proton δ_H 5.18 (H-12) and methylene protons δ_H 1.87 (H-11). COSY was also observed between

the alcoholic proton, 3.15 (H-3) and methylene protons, 1.60 (H-2) of alpha and beta amyrins. The presence of ten methylene groups and eight methyl groups were confirmed from HMQC spectrum. The attachment of the alcoholic proton, 3.15 (H-3) to an oxygenated carbon, 78.97 (C-3) and the olefinic proton 5.18 (H-12) to an olefinic carbon, 145.34 (C-12). Again, two sets of methyl protons at 1.0 and 0.7 (H-23 and H-24) respectively correlated to an oxygenic carbon 78.93 (C-3) through three bonds. These correlations are characteristic of alpha and beta-amyrins but integration of the proton signal at 0.87 6H singlet indicated that PDES-16 contain beta-amyrin. The structure was simulated using DEPT/NMR program to obtain the chemical shifts of both proton and carbon [12].

Conclusion

β-amyrin is a bioactive compound generally found in all parts of plants used in tradomedicine and their widespread pharmacological actions have been established. The fact that the compound is isolated from the stem bark of the plant supports some of the trado-medical claims of the plant.

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