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Antioxidant and Cytotoxicity of β-Amyrin acetate fraction from *Bridelia ferruginea* Leaves

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ABSTRACT

Objective: The objective of this work was to determine the beta-amyrin acetate fraction in leave extract of Bridelia ferruginea and evaluate for its antioxidant and cytotoxicity potentials. **Methods:** The dried and pulverized leaves of Bridelia ferruginea was extracted with hexane and then with ethyl acetate. The concentrated ethylacetate extract subjected to silica gel column chromatography and eluted with a mixture of equal volume of hexane and dichloromethane afforded two major fractions. The more polar fraction was concentrated and subjected to GCMS analysis which afforded the steroid, 12-Oleanen-3yl acetate commonly known as beta-amyrin acetate (66.14%). Its ability to act as a scavenger of DPPH radical and its cytotoxicity potential based on brine shrimp assay were investigated. **Results:** The DPPH antioxidant assay revealed that the fraction had a higher antioxidant potential with an IC_{50} value of 158.2 μg/mL relative to gallic acid which had IC_{50} of 201.1 μg/mL. The cytotoxicity assay using the brine shrimp a gave LC_{50} values of 319 and 5.86 μg/mL for acute and lethal doses respectively indicating extreme toxicity when compared to reference drug, cyclophosphamide which had LC_{50} value of 2506 μg/mL. **Conclusions:** Thus, the beta-amyrin acetate has been identified for the first time in the leave of Bridelia ferruginea. The data here suggest that the beta-amyrin acetate fraction of the leave of Bridelia ferruginea could be further 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.

1. Introduction

Bridelia ferruginea Benth (Euphorbiaceae) is a medicinal plant that is widely used in African folkloric medicine. The stem bark decoction is used in African traditional medicine to treat diarrhea, dysentery and gynaecological disorders (including sterility). A decoction of the leaves is used to treat diabetes. It has even been evaluated for antimalarial [1], antimicrobial [2], analgesic [3] and antidiabetic [4] activities. B. ferruginea bark is used for treatment of bacterial infections on wounds [5]. A decoction of the leaves is used as a purgative and also in the treatment of diabetes [6]. Roots and leaves extracts are used to cure piles, diarrhea and dysentery [7] and also confirmed for anti-inflammation activities [8]. Galocatechin has been isolated from the bark [9]. The chemical constituents of *Bridelia ferruginea* has not been thoroughly investigated, therefore this research was carried out in order to have an insight into the chemical basis for some of the pharmacological properties reported for the plant in traditional medical system.

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2. Materials and methods

2.1 Chemicals

α – Tocopherol, Sigma-Aldrich (US), Gallic acid Sigma-Aldrich (Germany) were obtained in analytical grade while solvents (redistilled), silica gel 60 F254 for Thin Layer

Chromatography, TLC, silica gel 60 with mesh 100 − 120 μm for gravity column chromatography, and vanillin spray reagent were obtained from the Department of Chemistry, University of Ilorin, Nigeria.

2.2 Instruments

A Gas Chromatography− Mass Spectroscopy (GCMS−QP 2010) PLUS (Shimadzu Japan) system coupled with a finigan MAT ion trap detector was used with the column being an RTX5MS column packed with 100% grade dimethylpolysiloxane. The GC−MS was operated under the following conditions; column temperature was initially held at 60°C for 5 min with injection volume of 1 μL and then programmed to rise at the rate of 5°C per minute to 250°C. The injector temperature was set at 200°C while the detector (mass spectrophotometer) temperature was maintained at 250°C. Helium was used as the carrier gas at a linear velocity of 46.3 cm sec−1 and pressure of 100.2 Kpa. Ionization mode was electron impact (EI) at a voltage of 70 eV. Identification of the components was carried out using the peak enrichment technique of reference compounds and as final confirmation of the peak identification by GC−MS, their spectral were compared with those of NIST library mass spectra.

2.3 Samples and sample preparation

Bridelia ferruginea leaves were obtained from Bode Saadu village in Kwara state Nigeria and taxonomically authenticated by Prof. F.A. Oladele of Plant Biology Department of the University of Ilorin, Ilorin, Nigeria. They were air dried at room temperature (27 0C) for two months after which they were pulverised using the laboratory mill (Christy and Norris Ltd Machine type 8). 1 Kg of the pulverised material was extracted successively (cold) with n−hexane and ethyl acetate for five days each. The ethyl acetate extract was filtered and concentrated using a rotary evaporator under vacuum to obtain the crude extract, BRDF/EtOAc. 30 g of the crude extract, was subjected to open column chromatography on silica gel using a glass column 10cm diameter 50cm long. The extract was eluted with petroleum ether and then with a mixture of equal volume of petroleum ether and dichloromethane. Thirty−five fractions of about 50 ml each were collected and later combined into two major fractions A and B based on the TLC profile. The concentrated fraction B was later subjected to GCMS analysis as well as antioxidant and cytotoxicity assays.

2.4 Tests for steroid

Salkowski reaction: A small quantity of fraction B was dissolved in chloroform and a few drops of concentrated sulfuric acid were added to the solution. A reddish color was seen in the upper chloroform layer [10].

2.5 DPPH antioxidant assay

The DPPH assay used for the determination of antioxidant power of a sample is a standard procedure based on the spectroscopic measurement of the free radical scavenging ability of test substances towards the stable radical [11, 12]. The free radical scavenging activity of the extract was examined in vitro using the DPPH assay. This spectrophotometric assay was carried out according to the method previously described with minor modifications [10, 13]. The DPPH free radical was prepared at a 0.1 mM concentration in methanol and protected from light after preparation. Stock solutions of the analytes (1mg/mL) was prepared and diluted to final concentrations of 500, 250, 200, 100 and 50 μg/mL in methanol. 1 mL of 0.1 mM DPPH methanol solution was added to solutions of the sample or standards (α −tocopherol and gallic acid separately) and incubated for 30 minutes in the dark. The absorbance was determined at 518 nm. Blank experiment was also carried out to determine the absorbance of DPPH before interacting with the sample. The antioxidant activity, AA was calculated using the equation given below. The IC50 was determined on graphpad prism 3 software through a non−regression analysis. The IC50 was taken as the concentration that scavenged 50% of the radicals.

$$AA = 100\times [(Abscontrol-Abssample)]/(Abscontrol)$$

2.6 Artemia Salina Lethality Test

Bioactive compounds are usually very toxic at high doses. The brine shrimp larva Artemia salina is highly sensitive to a variety of chemical substances and therefore used for the preceding determination of cytotoxicity. The method described by Hossain et al., [14] was adopted with slight modifications. The sample was dissolved in DMSO and made up with natural seawater to 5mL in four different concentrations (1000, 200, 20 and 2 μg/ml). The test was carried out in replicate and included a control with only DMSO in seawater. Eggs were sown in natural sea water and nauplii were obtained after 48 hours and inoculated with the test samples. After one hour and 20 hours of incubation at room temperature in the light, the number of survivors in each test tube was calculated for acute and lethal toxicity respectively. Cyclophosphamide was used as a standard cytotoxic test drug. Using the dose response curve, the LC50 was determined on graphpad prism 3 software through a non−regression analysis.

3.1 RESULTS

Table 1 shows the GCMS result of fraction B. It revealed 12−Oleanen−3yl acetate (Fig. 1, 2 and 3) also known as beta−amyrin acetate (66.14 %) as the major component. Its isomer, alpha amyrin acetate occurred in a less significant amount (8.53 %) with it. The antioxidant assay showed an IC_{50} value of 158.2 μg/mL which is quite significant compared with that of the gallic acid, 201 μg/mL. Artemia salina test showed an acute

Table 2

Brine Shrimp lethality test and DPPH antioxidant test of β -Amyrin acetate fraction from *Bridelia ferruginea* Leaves

Samples Tested	Brine Shrimp lethality test		DPPH antioxidant assay	
	LC_{50} μg/mL (Lethal)	LC_{50} μg/mL (Acute)	%DPPH (1mg/mL)	IC_{50} μg/mL
β -Amyrin acetate fraction	5.862±18.60	319.0±4.79	50.8	158.2±4.02
α - Tocopherol	ND	ND	45.45	4.577±2.38
Gallic Acid	ND	ND	61.81	201.1±1.65
Cyclophosphamide	0.0	2506±4.73	ND	ND

The results are presented as LC_{50} and IC_{50} values (μg/ml) at 95% Confidence Intervals (CI). ND means not determined.

toxicity with LC_{50} value of 319 μg/mL and lethal dose with LC_{50} value of 5.86 μg/mL (Table 2) indicating that B. ferruginea could be a source of cytotoxic and antioxidant agents.

Table 1

Percentage composition of the β -Amyrin acetate fraction.

Peak no	RI	%	Name of Compound
1	28.562	0.91	2-Pentadecanone,6,10,14-timethyl
2	29.371	0.47	5-9,13-Pentadecatrien-2-one,6,10,14-trimethyl
3	29.980	3.04	n-Hexadecanoic acid
4	30.314	0.66	Octadecanoic acid, ethyl ester
5	31.594	3.41	2-Hexadecen-1-ol,3,7,11,15-trimethyl
6	31.783	2.33	delta(sup9)-cis-oleic acid
7	32.027	2.30	Octadecanoic acid
8	32.412	1.11	Octadecane,1-(ethenyloxy)
9	33.146	1.16	Octadecanoic acid,2-hydroxy-1,3-propanol
10	33.667	1.24	4,8,12,16-Tetramethylheptadecan-4-olide
11	33.927	0.30	5,9,13-Pentadecatrien-2-one,6,10,14-trimethyl
12	34.867	0.90	1-Eicosanol
13	36.510	8.53	Alpha-amyrin acetate
14	38.816	1.52	Tetratetracontane
15	39.718	66.14	Beta-amyrin acetate
16	40.352	5.96	Stearyl aldehyde

RI, Retention index identical to bibliography; MS, identification based on comparison of mass spectra.

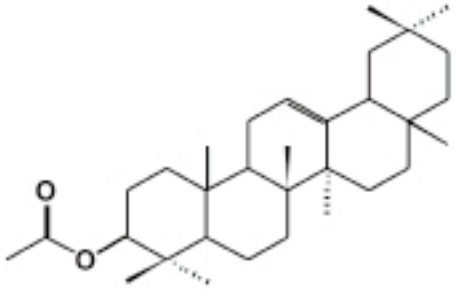

Fig. 1: Structure of β - amyrin acetate. Molecular Formula: $C_{32}H_{52}O_2$ Formula Weight: 468.761.

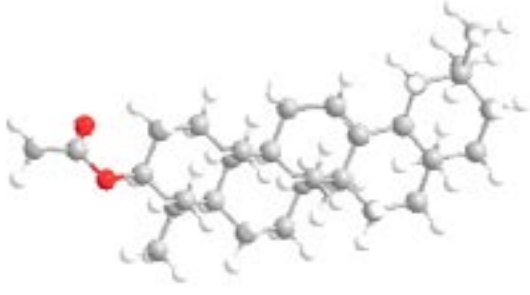

Fig. 2: Simulated 3D-structure of β - amyrin acetate

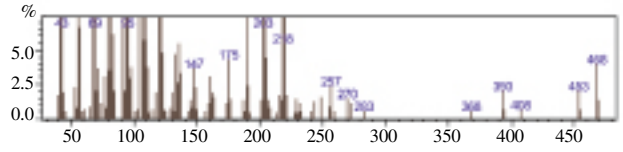

Fig. 3: Mass Spectrum obtained for 12-Oleanen-3yl acetate from *Bridelia ferruginea*

3.2 DISCUSSION

The cytotocity and antioxidant activities of the fraction indicated that the plant leaves could be a source of cytotoxic and antioxidant agents. The compound, β -amyrin acetate has been identified in very few plant sources and for the first time from *Bridelia ferruginea*. It has been identified as a very minor component in Centaurea ensiformis [15], Onorpodum caricum [16] and Allium sativum [17]. It has also been isolated from Tabernaemontana dichotoma and tested for its antidiabetic potential [18]. β -amyrin acetate is commonly found to exist in it alcohol form, i.e β -amyrin. Both β -amyrin and β -amyrin acetate have been found in various part of Calendula officinalis [19]. The triterpene, β -amyrin was discovered in Ligustrum Species Leaves and found to possess anti-inflammatory activity in an in vivo experiment [20]. α and β -amyrins are isomers differing only in the position of a methyl substituent. Both of them have been found in (Rosmarinus officinalis L [21]. A mixture of α and β -amyrins was isolated from protium heptaphyllum in the ration 2:1 respectively and found to inhibit platelet aggregation induced by collagen, adenosine diphosphate and arachidoic acid with IC_{50} values of 38.4 μg/mL, 50. 3 μg/mL and 77.4 μg/mL respectively [22]. Both isomers were also found in this study with the α -amyrin occurring as a minor constituent (8.53%). Interestingly, β -amyrin from Ardisia elliptica Thunb have been found to be six times more potent than aspirin in inhibiting collagen-induced platelet aggregation [22]. We presumed that the observed antioxidant and cytotoxicity activities reported must have been due to the presence of the major compound being β -amyrin acetate.

4. Conclusion

The potential antioxidant and cytotoxic activities of 12-Oleanen-3yl acetate fraction from *Bridelia ferruginea* have been assessed for the first time. There seems to be positive correlations between antioxidant capacity and cytotoxicity. The strong antioxidant activity, and extreme toxicity demonstrated by *B. Ferruginea* suggests that the plant may be promising source of natural antioxidants and other bioactives compounds for food and pharmaceutical industries

Conflict of interest statement

The authors affirm that there is no conflict of interest.

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