SHORT REPORT

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Bioactive Natural Products from Two Sudanese Medicinal Plants *Diospyros mespiliformis* and *Croton zambesicus*

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Abstract: Phytochemical investigations were performed in two plant species used in Sudanese traditional medicines to treat different illnesses, *Diospyros mespiliformis* and *Croton zambesicus*. The investigations revealed compounds of triterpenes (lupane series), one trihydroxyflavone and one diterpene. The compounds have been isolated and identified using various chromatographic and spectroscopic techniques. These were lupeol (**1**), betulinic acid (**2**), betulin (**3**) and lupenone (**4**) from *Diospyros mespiliformis.* Compounds **1**, **2, 3** in addition to diterpene ent -kaurane-3β, 16β, 17-triol (**5**) and vitexin (**6**) were re-isolated from *Croton zambesicus*. However, compound **5** and **6** were isolated for the first time from this source. The pure isolated compounds and semi-synthesized acetates **1Ac**, **2Ac** and **3Ac,** which were prepared from compounds **1**, **2** and **3** respectively, were subjected to two bioassays: α- glucosidase enzyme inhibition assay and antioxidant activity. Compounds, **1**, **1Ac**, **3** and **4** showed a marked α-glucosidase inhibitory potential, while compound **6** exhibited strong antioxidant activity.

Keywords*: Croton zambiescus; Diospyros mespiliformis;* antioxidant; *α*-glucosidase; triterpenes; vitexin; ent – kaurane.

1. Plant Source

Diospyros mespiliformis (Hochst. ex A. DC) (Ebenaceae) is confined to tropical and sub tropical regions notably in central Africa [1, 2]. Several ethnopharmacological applications have been reported for *Diospyros mespiliformis* (Hochst. ex A. DC), which include the use of leaf decoction as extraordinary remedy for fever, whooping cough and for wounds [1, 3]. Barks and roots are used for serious infections such as malaria, pneumonia, syphilis, leprosy, and dermatomycoses, as an

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anthelmintic and to facilitate delivery [4]. Different parts of the tree are used against diarrhea, skin infections, headache, toothache and similar pains and as a psycho-pharmacological drug [2].

Croton zambesicus Muell. Arg. (Syn. Name: *C. amabilis* Muell. Arg.), (Euphorbiaceace). It is a Guineo-Congolese species widely spread in tropical Africa. Ethnobotanically, the roots are used in Sudan for menstrual pain [5] and as aperients [6]. The root is also used in some regions of Nigeria as antimalarial and antidiabetic [7]. The leaf decoction is used in Benin as a wash for fevers, dysentery, convulsions, antihypertensive and as antimicrobial for urinary infections [8] and in parts of Nigeria as antidiabetic and malarial remedy [9, 10]. The seed decoction is commonly used to treat cough, malaria and to relieve menstrual pain [11].

2. Previous Studies

Previous research on the phytochemistry of the stem barks or wood of *Diospyros mespiliformis* has revealed the presence of triterpenes: ***a-***amyrin-baurenol, trihydroxy-triterpenoid acid, α-amyrin, β -sitosterol, lupeol, betulin and Betulinic acid [12, 13, 14, 15] and naphthoquinones e. g. diospyrin, Isodiospyrin, Diosquinone and Plumbagin [13, 14]. The cytotoxicity and antibacterial activity have been reported for the isolated compounds and crude extracts of the plant [14], but no reports on the present biological investigations.

Croton zambesicus is known for its diterpenoid content and different types of diterpenes; clerodane, labdane, kaurane, trachylobane, isopimarane etc. have been reported [6, 16, 17, 18, 19] in addition to sitosterol, lupeol and sitosterol glycoside [6]. The most isolated compounds from *C. zambesicus* have been screened for antimicrobial, cytoxicity and anti-plasmodial activity; include diterpenoids, quinines, triterpenoids and flavonoids [7, 9, 19]. The leaf extract of *C. zambesicus* demonstrates an antioxidant activity in the testes as was reported [20].

3. Present Study

1. *Diospyros mespiliformis:* the dried grounded stem barks of about one kg. were extracted successively using; petroleum ether (P.E.) b. p. (40-60), (3x3L); dichloromethane (CH_2Cl_2) (3x3 L); ethyl acetate (EtOAc) (3x3L) and methanol (MeOH) (3x3 L). All extracts were concentrated using Rota vap., 11g of yellow residue for the P.E extract was obtained while those of the CH_2Cl_2, EtOAc and MeOH gave 9.6, 7.5 and 13 g gummy brown residue respectively. All extracts were then initially analyzed by TLC.

EtOAc crude extract was subjected to flash column chromatography (CC) on silica gel (70-230 mesh) using stepwise gradient elution of n-hexane to CH_2Cl_2, CH_2Cl_2 to EtOAc and EtOAc to MeOH. 12 fractions (100 ml portion) were collected, concentrated and combined according to their similarity in behaviour on TLC. Fractions three, four and seven were rechromatographed to remove impurities over small column of flash silica to obtain major pure compounds **1** (70 mg) **2** (75mg), **3** (70mg) and **4** (50 mg).

2- *Croton zambesicus:* dried fruits about 500 g. were grounded to fine particles and were extracted with MeOH after percolation at room temperature for 5-7 days. The MeOH extract was evaporated under reduced pressure, furnishing a yellowish-green thick residue weighing about 20g. The residue was re-dissolved in distilled water (2L) followed by defatting with P.E. (2x2L), to afford about 4 g extract. The defatted aqueous extract was further fractionated with CH_2Cl_2 (2x2L), EtOAc (2x2L) and then with n-BuOH (2x2L). On the evaporation of the three fractions, 4.3, 2.2 and 5 g of extracts were obtained, respectively.

EtOAc extract was subjected to sephadex CC which, led to separation of four fractions; water fraction (fraction one) was subjected to another sephadex column, 20 sub-fractions were obtained. Of them sub-fraction 13 was subjected to flash silica column chromatography to afford **1** (7 mg), while sub-fraction 14 gave a mixture of two compounds. This mixture was subjected to CC over silica gel to give compounds **2** (25 mg) and **3** (20); flash CC over silica gel of fraction two was carried out using

EtOAc: MeOH gradient; 45 yellowish sub-fractions (100 ml portion) were obtained. Repeated flash column of sub-fraction two resulted in a pure compound **5** (5 mg).

A yellow precipitate was obtained from the n-BuOH fraction which was filtered, washed with MeOH several times and was found to be a pure compound **6**.

Compounds **1**, **2** and **3** (5 mg from each) were dissolved in C_5H_5N (1 ml) and then 2 ml of Ac_2O were added, and the reaction mixture was left overnight at room temperature; before it was poured into iced H_2O and extracted with EtOAc. The EtOAc extract was concentrated in vacuum followed by its purification using Si gel CC to obtain **1Ac**, **2Ac** and **3Ac** respectively.

The isolated compounds were identified by physical and spectroscopic methods and comparing the values with reported literature.

α-Glucosidase Enzyme Inhibition Assay: The α-glucosidase (E. C. 3. 2. 1. 20) enzyme inhibition assay was performed according to the slightly modified method of Oki *et al*. [21].

Antioxidant Activity: Percentages of 1, 1 diphenyl-2-picryl-hydrazil DPPH free radicals scavenging activity by samples were determined comparable to propyl gallate and 3-t-butyl-4-hydroxy anisole as controls, using the method of Lee *et al.* [22].

The ^{1}H –NMR spectra were recorded on Bruker AM 300,400 and AMX 500 MHZ NMR spectrometers. ^{13}C-NMR, COSY 45°, HMQC and HMBC spectra were recorded on a Bruker AM 400 and AMX 500 MHZ NMR spectrometers. Ultraviolet (UV) spectra were recorded in methanol on a Hitachi U-3200 spectrophotometer. Infrared (IR) spectra were taken as KBr pellets and/or as a solution in chloroform on a Jasco A-302 Infrared spectrophotometer. Electron impact mass spectra (EI-MS) were recorded on a Finnigan MAT 311 mass spectrometer with a MASPECO Data System. High –resolution electron impact mass (HREI-MS) measurements were carried out on a Jeol JMS HX 110 mass spectrometer.

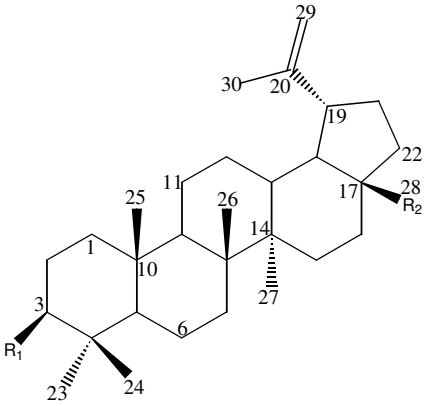
Comparison of obtained spectroscopic data, ^{1}H-NMR, MS, IR together with the previously reported ones [23-26], led to the identification of compounds **1**, **2**, **3** and **4** as well known lupane type triterpenes; lupeol (20(29) lupene-3-ol, 3β-form), betulinic acid *3β- Hydroxylup-20(29)-en-28-oic acid,* betulin *lup- 20(29)-ene-3β, 28-diol* and lupenone *lup -20(29) -ene-3β –one*, respectively. compound **5** was deduced to be ent-kaurane -3β, 16β, 17-triol, previously isolated from *Croton lacciferus* [27]. Compound **6** was identified as apigenin-8-C-β-D-glucopyranoside, vitexin, on the basis of FT-IR, ^{1}HNMR, and UV spectral data and the previously reported data [28, 29]. It is noteworthy, that this first time to report ent-kaurane -3β, 16β, 17-triol and vitexin from *C. zambesicus.*

Compound **1**, **3**, **4** and **1Ac**, out of all tested compounds, showed potent α-glucosidase inhibitory activity (Table 1) comparable to standard compound deoxynojirimycin (positive control). Inhibition of these compounds was measured at different concentrations (0.5, 0.25, 0.125 and 0.06). A lupane- type triterpenes have been reported to possess α-glucosidase inhibitory activity (IC_{50} = 42.5 μM) [30] which correlates well with our results. Vitexin (**6**) possessed the strongest activity with DPPH radical scavenging activity 80.5% at 1 mM. The remaining compounds were revealed mild activity or inactive. Free radical scavenging activity of vitexin has been reported about 60% at a concentration of 100 μg/mL. [31], which seems to be in accordance with our results.

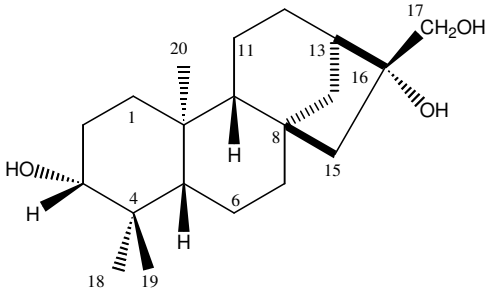
Table 1. α-glucosidase enzyme inhibitory activity of isolated compounds.

Compound code	Conc. mM	% Inhibition	(IC_{50}±SEM) mM
1	0.006	64.4	0.002±0.004
2	0.5	> 20	Nd
3	0.003	75	0.46±0.002
4	0.125	100	0.0624±0.002
5	Nd	Nd	Nd
6	0.5	>10	Nd
1Ac	1.00	74.8	0.668±0.025
2Ac	0.5	>20	Nd
3Ac	0.5	>20	Nd
Deoxynojirimycin	0.425		0.425± 0.00814

SEM = standard error of the mean. Nd = not determined.



	R_1	R_2
1	OH	CH_3
2	OH	COOH
3	OH	CH_2OH
4	= O	CH_3
1Ac	CH_3COOH	CH_3
2Ac	CH_3COOH	COOH
3Ac	CH_3COOH	$COOCH_3$



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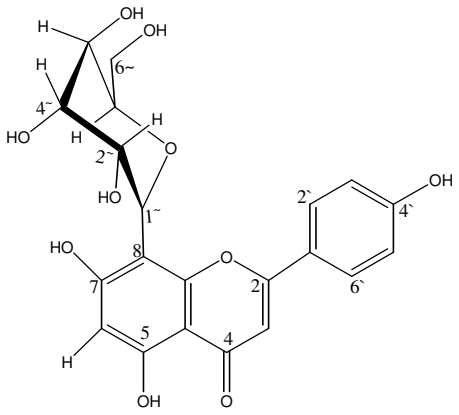

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Figure 1. Isolated compounds from *Diospyros mespiliformis* and *Croton zambesicus*.

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