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Mini review

Novel saponin and benzofuran isoflavonoid with *in vitro* anti-inflammatory and free radical scavenging activities from the stem bark of *Pterocarpus erinaceus* (Poir)

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ABSTRACT

The phytochemical study of the stem bark of *Pterocarpus erinaceus* led to the isolation of a new saponin (**1**) and a new benzofuran isoflavonoid (**2**) along with seven known compounds namely friedelin (**3**), triacontanoic acid (**4**), dotriacontanoic acid (**5**), 2,3-dihydroxypropyl hexacosanoate (**6**), octacosanoic acid (**7**), calycosin (**8**), stigmasterol glucoside (**9**). The structures of the new compounds were characterized on the basis of IR, UV, 1D and 2D NMR analyses in conjunction with EIMS, HRMS and literature review. The free radical scavenging activity and serum bovine albumin denaturation activity of the isolated compounds were evaluated. Compounds **2** (SC_{50}, 12.63 ± 0.86 μg/mL) and **8** (SC_{50}, 42.53 ± 1.77 μg/mL) showed antioxidant properties although lesser than that of the reference drug ascorbic acid (SC_{50}, 5.99 ± 0.59 μg/mL). Compound **3** (IC_{50}, 14.87 ± 1.51 μg/mL) was the most active against the denaturation of the protein followed by compounds **1** (IC_{50}, 28.60 ± 4.10 μg/mL) and **9** (IC_{50}, 35.94 ± 2.10 μg/mL). Sodium diclofenac (IC_{50}, 7.20 ± 0.97 μg/mL) was used as reference drug.

1. Introduction

Pterocarpus erinaceus is a deciduous tree, 15–25 m tall and belongs to the Fabaceae family. It is found in the savanna zone of West and Central Africa (Duvall, 2016). Its stem bark is used as a decoction to treat inflammatory disorders and has been scientifically proven to possess the activity (Noufou et al., 2012). The stem bark also demonstrated other significant biological activities including antioxidant (Toukam et al., 2016), antimalarial (Karou et al., 2003), antimycobacterial (Ibrahim et al., 2004), antianemic (Nadro and Modibo, 2014), anticancer (Noufou et al., 2016; Ngulde et al., 2015), anthelmintic (Chabi-China et al., 2014)and neuroprotective (Hage et al., 2015). Previous phytochemical studies on *P. erinaceus* reported the isolation of friedelin, lupeol, epicatechin, 3α-hydroxyfriedelan-2-one, α-sophoradiol, stigmasterol, maltol-6-*O*-apiofuranoside-glucopyranoside, 2,3-dihydroxypropyl octacosanoate, β-sitosteryl-β-D-glucopyranoside and a mixture of β-sitosterol, stigmasterol and campesterol (Noufou et al., 2012, 2017; Tittikpina et al., 2018).

The traditional use and the pharmacological activities exhibited by the stem bark of this plant, prompted us to carry out isolation, characterization, antioxidant and anti-inflammatory studies of its secondary metabolites. This paper reports the occurrence of a new benzofuran isoflavonoid and a new saponin alongside seven known compounds.

2. Results and discussion

2.1. Characterization of isolated compounds

The dried powder (5 kg) of *P. erinaceus* was successively extracted by percolation using hexane, ethyl acetate and the mixture methanol-dichloromethane (1:1). All extracts were subjected to column chromatography over silica gel and sephadex LH-20. Two compounds were

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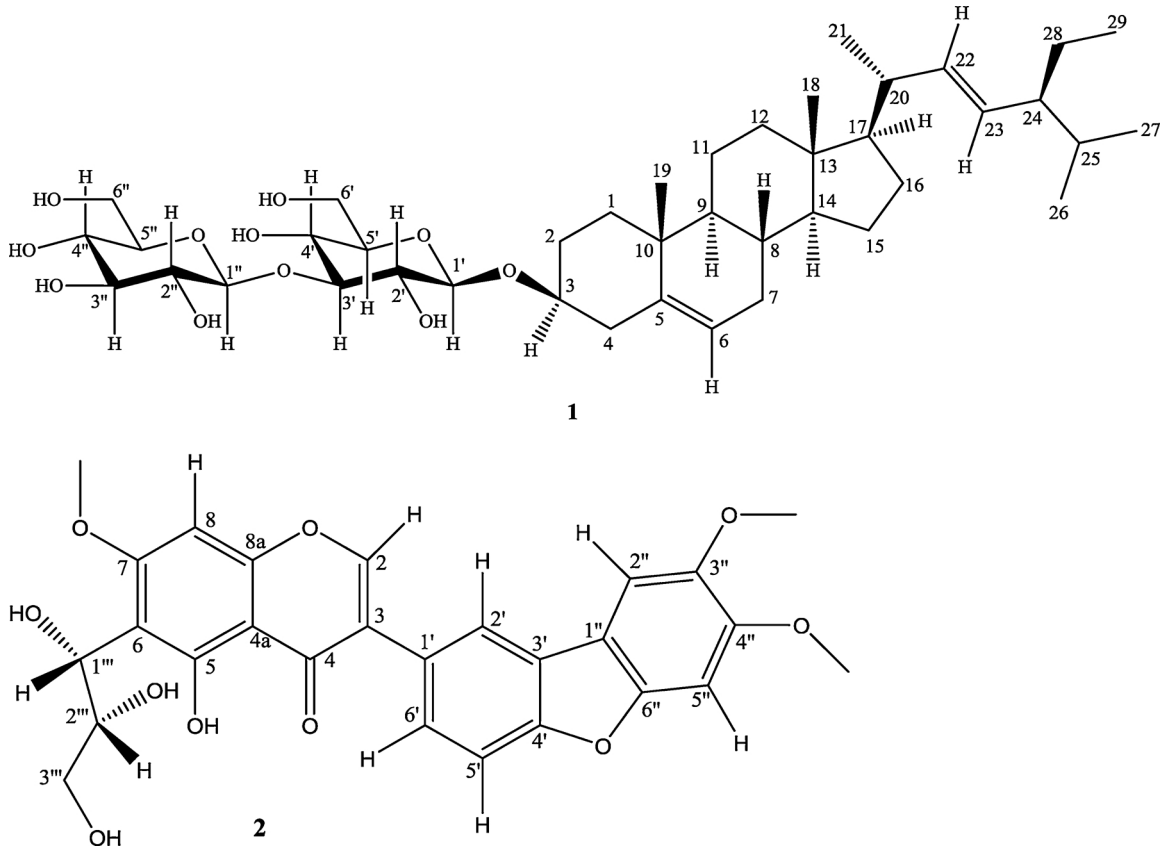

Fig. 1. Chemical structures of new compounds isolated from *P. erinaceus*.

isolated from the hexanic extract: friedelin (**3**) (Sousa et al., 2012) and triacontanoic acid (**4**) (Jameel et al., 2017). The ethyl acetate extract afforded one new compound (**2**) and five known compounds which were identified by spectroscopic analysis and comparison from literature data. These are one isoflavonoid: calycosin (**8**) (Martinez et al., 2017), two saturated fatty acids: octacosanoic acid (**7**) (Khan et al., 2012), dotriacontanoic acid (**5**), one monoester of glycerol: 2,3-dihydroxypropylhexacosanoate (**6**) (Prinsen et al., 2012) and stigmasterol glucoside (**9**) (Kang et al., 2003). From the n-butanol extract, another new compound (**1**) was isolated (Fig. 1).

Compound **1** was isolated as a pink amorphous solid from the n-butanol extract. Its molecular formula $C_{41}H_{68}O_{11}$ was deduced from the HRESI-MS which showed a pseudomolecular ion peak $[M + Na]^+$ at *m/z* 759.46402. The IR spectrum displayed characteristic absorption bands for hydroxyls (3309 cm^{-1}), aliphatic C–H (2931 cm^{-1}) and olefin (1468 cm^{-1}). The ^1H NMR spectrum displayed characteristic peaks of stigmasterol at δ_H 3.62 (m, H-3), 5.38 (m, H-6), 5.17 (dd, J = 15.1 and 6.1 Hz, H-22), 5.04 (dd, J = 15.2 and 6.3 Hz, H-23), two methyl singlets at δ_H 0.71 (s, H_3-18) and δ_H 1.06 (s, H_3-19). The APT spectrum displayed chemical shifts for stigmasterol as described in the literature (Chaturvedula et Prakash, 2012) with a clear difference in the chemical shift of C-3 which has shifted downfield.

Two doublets were observed at δ_H 4.53 (d, J = 7.8 Hz, H-1'') and 4.45 (d, J = 7.8 Hz, H-1'). Moreover, the sugar region was also covered with several signals. Two anomeric carbons (δ_C 103.1 and 106.6 ppm), two oxymethylenes (δ_C 63.1 and 64.1 ppm) and eight oxymethines (δ_C 71.3, 72.6, 75.3, 76.5, 78.4, 78.9, 79.2 and 89.9 ppm) were observed (Table 1). All the above data suggested a stigmasterol aglycone with two linked hexose sugar moieties identified as di- glucose by comparing their ^{13}C and ^1H chemical shifts with those of the literature (Tapondjou et al., 2005; Orsini et al., 1991). This was confirmed by HRESIMS which showed fragment ions at *m/z* 563.46362 and 413.26565 corresponding to the loss of one and two glucose units respectively. Both glucose units had the β configuration since the anomeric protons (H-1' and H-1'') have coupling constants (J = 7.8 Hz) out of the range of α configuration (J = 2.5–4.0 Hz) (Altona et Haasnoot, 1980). HMBC spectrum showed correlation between H-1' and C-3, H-3 and C-1' confirming linkage point of sugar to aglycone at C-3, shifted downfield to 81.4 ppm due to glycosylation (Fig. 2). Likewise, C-3' signal at 89.9 ppm was shifted downfield by + 11.8 ppm as compared to stigmasterol 3-*O*-β-D-glucoside (Kang et al., 2003) suggesting that C-3' is the linkage point with the second glucose unit; this is confirmed by the HMBC correlations observed between H-1'' and C-3' and conversely between H-3' and C-1'' (Fig. 2). Based on the above mentioned, the chemical name 3-*O*-[β-glucopyranosyl-(1→3)-*O*-β-glucopyranosyl]-stigmasterol was assigned to compound **1**.

Compound **2** was isolated as a yellow powder; its molecular formula $C_{27}H_{24}O_{10}$ accounting for 16 unsaturations was obtained from the HRESI-MS which showed a quasimolecular ion peak $[M+H]^+$ at *m/z* 509.1432. The UV spectrum showed a single absorption maximum around λ_{max} 300 nm indicating the presence of conjugated or aromatic system. The IR spectrum displayed absorption at 1623 and 1588 cm^{-1} which is consistent with the presence of conjugated carbonyl and aromatic functions respectively. Large absorption bands were observed at 2500 and 3200–3500 cm^{-1} corroborating the presence of chelated hydroxyphenol and free alkylhydroxyl functions respectively. The ^1H NMR spectrum showed a signal at δ_H 8.11 (s, 1 H) with several others in aromatic and non-aromatic region. The APT and HSQC spectra displayed characteristics of isoflavone type skeleton with a carbonyl group at δ_C 179.4 and the proton at δ_H 8.11 bore by the methine carbon at δ_C 155.5 (Table 2). A normal isoflavone has 11 unsaturations, the 5 unsaturations remaining may belong to a benzene ring and another ring which could account for the one unsaturation left. The signal displayed by the ^1H NMR spectrum at δ_H 6.74 (s, 1 H)