



## Original Research Article

## Isolation and structural elucidation of new steroid from Stem Bark of *Boswellia papyrifera* (Del.) Hochst

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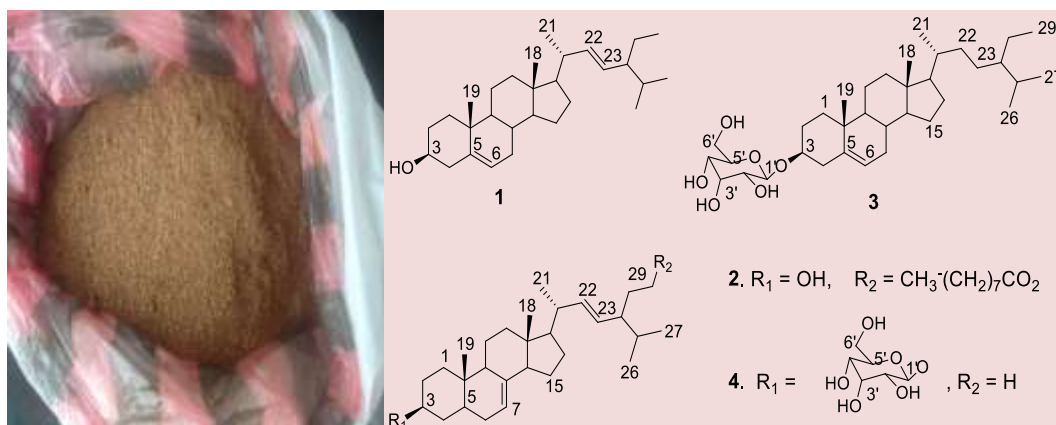
*Boswellia*

*papyrifera*

## ABSTRACT

Phytochemical investigation on Chemical Constituents of *Boswellia papyrifera* stem barks led to the isolation of new sterol (29-methyl, nonanoate-cholest-7, 22-dien-3 $\beta$ -ol **2**) along with three known related compounds, *Stigmasterol 1*,  *$\beta$ -sitosterol-3-O- $\beta$ -D-glucoside 3* and *Spinasterol 3-O- $\beta$ -D-glucopyranoside 4*. Their structures were defined by various spectroscopic studies including 1D, 2D-NMR, MS analysis and by comparison with those reported in the literature.

## GRAPHICAL ABSTRACT



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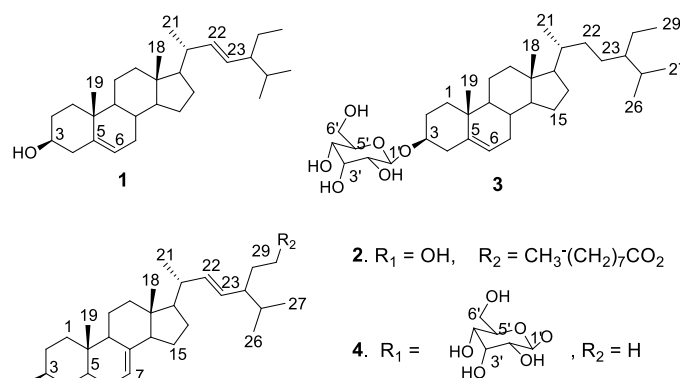
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## Introduction

*Boswellia papyrifera* (Del.) Hochst is an important Sudanese medicinal plant, belonging to (Burseraceae) family [1]. Widespread in tropical and subtropical regions; it grows in dry land region from East Africa to South Asia [2-5]. Its distribution area goes from Sudan, Eritrea, Ethiopia, and Somalia to Yemen Oman and India [6]. Members of this genus are trees or shrubs that are described as having outer barks that peel in parchment flakes, a greenish inner bark, watery aromatic resins, and wood with a milky latex [7, 8]. *B. papyrifera* in Sudan is a common savanna tree found in rocks or hill slopes, occurring in Bahr El-ghazal, Blue Nile, Nuba mountains and in Darfur, *B. papyrifera* occurs around Zalingei, Rodom and Jabel Marra [9]. It have been exploited for a years in traditional Sudanese medicine to alleviate pain and inflammation. It is used in medicinal preparations for the treatment of amenorrhoea, diarrhea, cough, asthma, and bronchitis as an ingredient of embalming fluid, a diuretic stimulant and an emmenagogue. However, its essential oil and absolute oil are used as fixatives in perfumes, soaps, creams, lotions and detergents [10-13].

A number of phytochemical studies on different organs of several *Boswellia* species have been reported, and many of these have identified as acetic triterpenes, diterpenes, highly aromatic essential oil, some of them showed anti-inflammatory, analgesic, antileukemic, immunosuppressant, and hepatoprotective activities [14, 15]. However, little is known regarding the phytochemistry of Sudanese *B. papyrifera*. Our previous investigations on this plant had led to identified and confirmed the structures of the different diterpenes [16]. In continuation of our studies on the chemical constituent of Sudanese *B. papyrifera*, we describe the isolation of four sterols derivatives from the bark of this plant.



**Figure 1:** Structures of compounds **1-4**

The structures of new compound **2** was identified through extensive spectroscopic detailed herein as *29-methyl, nonanoate-cholest-7, 22-dien-3 $\beta$ -ol*. **2** and three known compounds, *Stigmasterol* **1**,  *$\beta$ -sitosterol-3-O- $\beta$ -D-glucoside* **3** and *Spinasterol 3-O- $\beta$ -D-glucopyranoside* **4**.

## 2. Results and Discussion

The preliminary phytochemical screening of *Boswellia papyrifera* barks ethanolic extracts showed the presence of various secondary metabolites such as, Flavonoid Alkaloid, Triterpenes, Sterol, Saponins, Carbohydrate and Tannins, which are the bioactive principles responsible for medicinal values of the respective plants, were all present. These phytoconstituents were detected in varied concentrations in the methanol extracts presented in **Table 1**.

A 95% EtOH extract from the stem bark of *Boswellia papyrifera* was fractionated on a silica gel column chromatography using solvents such as chloroform, ethyl acetate and methanol.

The chloroform fraction was separated by repeated column chromatography silica gel and Thin Layer Chromatography (TLC), as well as recrystallization, to afford four sterols derivatives (**1-4**).

**Table 1:** Phytochemical screening of ethanol extract of *Boswellia papyrifera* barks

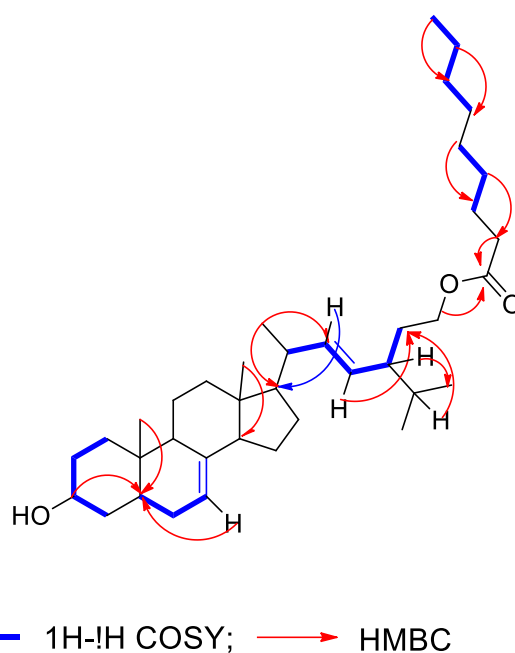
No	Constituents	Test	Results
1	Flavonoid	Alkaline reagent	++
2	Alkaloids	Mayer's,Wanger's reagent	+
3	Triterpen,Streol	Liberman	+++
4	Saponins	Forth	++
5	Carbohydrate	Molish's	+
6	Tannins	Ferric Chloride,/Alminum chloride	+++

Key: - (+++)-Heavy; (++)-Medium; (+)-Low ;(-) -indicates absent.

The structures of known compounds (**1**, **3-4**) were identified by detailed  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectroscopic data analysis and by direct comparison with those reported in the literature data [17-21] respectively. The structure of the new compound **2** was determined by various spectroscopic analyses.

Compound **2**, obtained as white powder, gave a quasi-molecular ion peak at  $m/z$  587.51  $[\text{M}+\text{H}_3\text{O}]^+$  in the APCI-MS spectrum which indicated that compound **2** have a molecular formula  $\text{C}_{38}\text{H}_{64}\text{O}_3$ . In the  $^1\text{H}$ -NMR spectrum, six methyls resonate at  $\delta$  1.25 ( $2\times 3\text{H}$ , s, H-18, H-19), 0.79 ( $3\text{H}$ , d,  $J = 5.9$  Hz, H-27), 0.86 ( $3\text{H}$ , d,  $J = 6.7$  Hz, H-26), 1.01 ( $3\text{H}$ , d,  $J = 6.2$  Hz, H-21), and 0.54 ( $2\text{H}$ , t,  $J = 6.5$  Hz, H-5'). Two double bond was supported by  $^1\text{H}$ -NMR signals at  $\delta$  5.14 ( $1\text{H}$ , dd,  $J = 18.0, 8.6$  Hz, H-22), 5.02 ( $1\text{H}$ , dd,  $J = 14.9, 7.0$  Hz, H-23) and 5.06 (dd,  $1\text{H}$ ,  $J = 10.7$ , Hz, H-7) and their corresponding  $^{13}\text{C}$ -NMR signals at  $\delta$  138.12 (C-22) 129.43 (C-23), 117.5 (C-7) and 139.53 (C-8). By comparing the  $^{13}\text{C}$ -NMR data of compound **2**, **Table 2** with those of spinasterol [22], it could be concluded that compound **2** is include sterol, possessing methyl, nonanoate connected with C-29.

Based on the HMBC correlation **Figure 2**, of H-29 ( $\delta$  4.12, dd,  $2\text{H}$ ), and H-2' ( $\delta$  2.33, t,  $2\text{H}$ ) with C-1' ( $\delta$  173.65) and H-5' ( $\delta$  0.54, t,  $3\text{H}$ ) with C-3' ( $\delta$  34.10), as well as correlations between H-19,  $\delta$  1.25(s,  $3\text{H}$ ), H-3  $\delta$  (3.65, dd,  $1\text{H}$ ) and H-7 ( $\delta$  5.15, t,  $1\text{H}$ ) with C-5 ( $\delta$  40.24) confirmed the presence of 7, 8-double bond.



**Figure 2.** The key  $^1\text{H}$ - $^1\text{H}$ -COSY and HMBC correlations observed in compound **2**

The other double bond could be assigned to C-22 and C-23 in view of cross peak between H-22 ( $\delta$  5.06, dd,  $1\text{H}$ ) and H-21 ( $\delta$  1.01) with C-17 ( $\delta$  55.9). The  $^1\text{H}$ - $^1\text{H}$  Correlated Spectroscopy (COSY) showed correlation between H-3 with H-2, H-5, H-7 with H-6, and H-21, H-23 with H-22 and H-22, H-24 with H-23. The  $^{13}\text{C}$ -NMR signal at  $\delta$  71.02 for C-3 suggested that 3-OH should be  $\beta$ -configured [23]. Thus, the structure of compound **2** was defined to be *29-methyl, nonanoate-cholest-7, 22-dien-3 $\beta$ -ol*.

**Table 2:**  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectral data of compound **2** in  $\text{CDCl}_3$ 

Position	$\delta_{\text{C}}/\text{ppm}$	$\delta_{\text{H}}/\text{ppm}$	Position	$\delta_{\text{C}}/\text{ppm}$	$\delta_{\text{H}}/\text{ppm}$
1	37.12		18	12.03	1.25 (3H, s)
2	31.42		19	12.22	1.24 (3H, s)
3	71.02	3.65 (1H, dd, $J = 9.2, 4.0$ )	20	40.88	
4	37.92		21	21.34	1.01 (3H, d, $J = 6.2$ Hz)
5	40.24		22	138.12	5.14 (1H, dd, $J = 18.0, 8.6$ )
6	29.65		23	129.43	5.02 (1H, dd, $J = 14.9, 7.0$ )
7	117.42	$\delta$ 5.06 (1H, t, $J = 10.7$ )	24	51.23	
8	139.53		25	31.87	
9	49.43		26	21.05	0.86 (3H, d, $J = 6.7$ Hz)
10	34.15		27	18.97	0.79 (3H, d, $J = 5.9$ Hz)
11	21.53		28	34.20	0.85 (3H, m, H-29)
12	39.44		29	63.04	4.12 (1H, dd, $J = 8.2, 4.9$ )
13	43.27		1'	173.65	
14	55.10	2.16 (1H, d, $J = 6.9$ Hz)	2'	34.10	2.33 (2H, t, $J = 6.4$ Hz)
15	23.00		3'-7'	29.33- 29.67	1.16-1.38 (10H, m)
16	28.47		8'	22.67	
17	55.84		9'	13.02	0.54 (3H, t, $J = 6.5$ Hz).

### 3. Materials and Methods

#### 3.1. Plant Material

The barks of *Boswellia papyrifera* were collected in May 2015 from Zalingei area, central Darfur state –Sudan, the plant was authenticated by prof: G, A, Yagoub, department of basic sciences, faculty of agriculture, University of Zalingei. Voucher

specimens (No. 20151014), have been deposited in the herbarium of author's laboratory.

#### 3.2. General Experimental Procedure

Melting points were recorded on an X-4 type micro-melting point apparatus, which was uncorrected. The NMR Spectrum were recorded on a BrukerAvance DRX-400-spectrometer ( $^1\text{H}$  at 400MHz and  $^{13}\text{C}$  at

100MHz) and chemical shift values are given on a  $\delta$  (ppm) scale with TMS as internal standard, 2D-NMR experiment were performed using standard Bruker micro-program (XWINNMR version 2.6 software), APCI-MS experiment was performed using micro-mass – OTOF micro instrument, with an atmospheric pressure chemical Ionization source. Column chromatography was carried out on silica gel (Merck kiesel gel 300-400 mech), TLC were carried out on silica gel GF<sub>254</sub> (Merck), all the chemicals and solvents were commercial grade and used after further purification.

### 3.3. Extraction and Isolation

#### 3.3.1. phytochemical analysis

The stem barks powder (2 kg) was macerated (cold extraction) three times with 95% EtOH at room temperature (Each 7 days  $\times$  4 L). Then filtered and evaporated in vacuum at 40°C using a rotary evaporator. The extract concentrated (400 g) was labeled. The dried ethanolic extract was subjected to qualitative chemical screening and identification of the secondary metabolites such as Flavonoids, Alkaloids, Sterols, Triterpenes, Saponins, Carbohydrate, Tannins, phenols, as described by Safowora and Evans [24-26].

#### 3.3.2. Isolation

The ethanol extract (350 g) was fractionated on a silica gel column chromatography using solvents such as chloroform, ethyl acetate and methanol. (2000 g.) silica gel 300-400 mesh was packed manually into a column (15 $\times$ 150 cm). The column was eluted with chloroform until no elute come out yield 45 g of dried extracts fraction (**F<sub>I</sub>**), and then the column was eluted with EtOAc yield 43g, fraction (**F<sub>II</sub>**), and in the last the column eluted with MeOH pure to yielding **60g**, fraction (**F<sub>III</sub>**).

**F<sub>I</sub>** (45g) was subjected to column chromatography (silica gel, (500 g) using a gradient solvent system of PE/EtOAc and Chloroform/EtOAc, to give three subfractions (**f<sub>1</sub>**- **f<sub>3</sub>**). **f<sub>1</sub>** (12g) subjected on CC using (silica gel (60g) eluted with Et<sub>2</sub>O/ Chloroform, 19:1-4:19), after repeated column chromatography yielded compound **1** (123.3 mg), **2** (112.2 mg mg). Fraction **f<sub>2</sub>** (9g) was subjected to column chromatography, silica gel (45g), eluent (PE/EtOAc, 4:1- 1:1) yielded compound **3** (132.1 mg). **f<sub>3</sub>** (19g) subjected on CC using silica gel (90g) eluted with Chloroform/EtOAc (10:1-4:10), after repeated column chromatography yielded compound **4** (141.2 mg).

**Stigmasterol 1**, obtained as a white powder, 123.3 mg, m.p 147-149°C, <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>),  $\delta$  0.68 (3H, s, H-18) 0.81(3H, d, H-27) 0.82(3H, d, H-26), 0.84 (3H, t, H-29), 0.93(3H, d, J= 5.9 Hz, H-21) 1.02 (3H, s, H-19), 3.53 (1H, d, J = 11.6 Hz, H-3), 5.02 (1H, dd, J = 15.0, 8.5 Hz, H-23), 5.15 (1H, dd, J = 15.2, 8.7 Hz, H-22), 5.35 (1H, br s, H-6). <sup>13</sup>C-NMR data (100 MHz, CDCl<sub>3</sub>),  $\delta$  37.24 (C1), 31.88 (C2), 71.79 (C3), 42.25 (C4), 140.73 (C5), 121.68 (C6), 31.65 (C7), 31.80 (C8) 50.13 (C9), 36.50 (C10), 21.24 (C11), 39.71 (C12), 42.25 (C13), 56.80 (C14), 24.32 (C15), 29.14 (C16), 56.75 (C17), 11.95 (C18), 21.20 (C19), 40.41 (C20), 21.31 (C21), 138.29 (C22), 129.25 (C23), 51.21 (C24), 29.69 (C25), 20.14 (C26), 19.81 (C27), 25.39 (C28), 12.24 (C29).

**29-methyl, nonanoate-cholest-7, 22-dien-3 $\beta$ -ol 2**, obtained as white powder, 112.2 mg mp (over 300°C) <sup>1</sup>H -NMR (400 MHz, CDCl<sub>3</sub>) and <sup>13</sup>C-NMR (100MHz, CDCl<sub>3</sub>) **Table: 1**, gave a quasi-molecular ion peak at m/z 587.51 [M+H<sub>3</sub>O]<sup>+</sup> in the APCI-MS corresponding to the molecular formula C<sub>38</sub> H<sub>64</sub> O<sub>3</sub>.

**$\beta$ -sitosterol-3-O- $\beta$ -D-glucoside 3**, obtained as white powder; 132.1 mg; mp ( 272-274°C); <sup>1</sup>H-NMR (400 MHz, CDCl),  $\delta$  0.65(3H, s,H-18), 0.78

(3H, d, H-27), 0.83 (3H, d, H-26), 0.85 (3H, t, H-29), 0.92 (3H, d, H-21) 1.02 (3H, s, H-19), 3.18 (1H, d,  $J = 6.8$  Hz, H-3), 3.28 (1H, m, H-2'), 3.37 (1H, m, H-4'), 3.70 (1H, m, H-3'), 3.81 (1H, dl,  $J = 10.0$  Hz, H-6'), 5.33 (1H, d, H-6). ( $^{13}\text{C}$ -NMR (100MHz,  $\text{CDCl}_3$ ),  $\delta$  38.46 (C1), 29.44 (C2), 78.87 (C3), 39.87 (C4), 140.10 (C5), 121.56 (C6), 31.79 (C7), 33.79 (C8) 50.12 (C9), 37.16 (C10), 20.92 (C11), 40.40 (C12), 42.17 (C13), 55.69 (C14), 24.11 (C15), 28.07 (C16), 55.89 (C17), 11.53 (C18), 19.39 (C19), 36.57 (C20), 18.62 (C21), 35.94 (C22), 25.90 (C23), 45.74 (C24), 29.01 (C25), 20.66 (C26), 19.00 (C27), 22.89 (C28), 11.53 (C29). 104.99 (C-1'), 77.46 (C-2'), 80.41 (C-3'), 74.14 (C-4'), 79.84 (C-5'), 65.64 (C-6').

**Spinasterol 3-O- $\beta$ -Dglucopyranoside 4**, obtained as white powder; 141.2 mg;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  5.30 (1H, br s, H-7), 5.12 (1H, dd,  $J = 16.9, 8.0$  Hz, H-22), 5.02 (1H, dd,  $J = 16.0, 7.0, 8.0$  Hz, H-23), 4.20 (1H, d,  $J = 7.6$  Hz, H-1'), 3.62 (1H, dd,  $J = 12.2, 6.0$  Hz, H-3), 3.53 (1H, d,  $J = 5.4$  Hz, H-6), 3.39 (1H, d,  $J = 5.4$  Hz, H-6), 3.10 (1H, m, H-5), 3.04 (1H, m, H-3), 3.00 (1H, m, H-4), 2.87 (1H, m, H-2). 2.34 (1H, d,  $J = 6.9$  Hz, H-14), 0.49, 0.93 (2 $\times$ 3H, s, H-18, H-19 respectively), 0.98 (3H, d,  $J = 6.4$  Hz, H-21), 0.72 - 0.81 (3 $\times$ 3H each, d, H-26, H-29, H-27).  $^{13}\text{C}$ -NMR (100MHz,  $\text{CDCl}_3$ ),  $\delta$  36.99 (C1), 31.77 (C2), 77.13 (C3), 37.26 (C4), 40.51 (C5), 29.53 (C6), 117.52 (C7), 139.53 (C8) 49.04 (C9), 34.15 (C10), 21.68 (C11), 39.20 (C12), 43.26 (C13), 54.10 (C14), 22.97 (C15), 28.56 (C16), 55.88 (C17), 12.32 (C18), 19.54 (C19), 40.76 (C20), 21.43 (C21), 138.39 (C22), 129.31 (C23), 51.01 (C24), 31.88 (C25), 21.44 (C26), 19.30 (C27), 25.32 (C28), 12.57 (C29). 101.06 (C-1'), 73.91 (C-2'), 76.75 (C-3'), 70.49 (C-4'), 77.19 (C-5'), 61.54 (C-6').

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