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A new sesquiterpene, prosoterpene, from *Prosopis africana* (Guill. & Perr.) Taub

Raza Ali^a, Kayode Muritala Salawu^b, Muhammad Aamer^a, Humera Jahan^c, Priya Tufail^c, Rimsha Irshad^a, Farooq-Ahmad Khan^{a,d}, Bilge Sener^{a,e}, M. Iqbal Choudhary^{a,d}  and Yan Wang^a 

^aH. E. J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi, Pakistan; ^bDepartment of Pharmacognosy and Drug Development, University of Ilorin, Ilorin, Nigeria; ^cDr. Panjwani Center for Molecular Medicine and Drug Research, International Center for Chemical and Biological Sciences, University of Karachi, Karachi, Pakistan; ^dThird World Center (TWC) for Chemical Sciences, International Center for Chemical & Biological Sciences, University of Karachi, Karachi, Pakistan; ^eDepartment of Pharmacognosy, Faculty of Pharmacy, Gazi University, Ankara, Turkey

ABSTRACT

A new sesquiterpene (Prosoterpene, **1**) and eleven reported compounds (**2–12**) of several classes, such as flavonoids, alkaloids, phenolic acids, and long-chain alcohols, were isolated from the BuOH extract of *Prosopis africana* (Guill. & Perr.) Taub. Compounds **2–10** were reported for the first time from this plant. Isomers **11** and **12** were separated for the first time. Extensive spectroscopic techniques and literature comparisons were used to characterise their structures. Furthermore, compounds **3**, **5–8**, and **10–12** were performed for anti-glycation and cytotoxicity activities. Compound **3** (quercetin-3-O- α -L-rhamnoside) exhibited moderate anti-glycation activity. All tested compounds were non-cytotoxic against MCF-7 (breast cancer), NCI-H460 (lung cancer), Hela (cervical cancer), and BJ (normal human fibroblast) cell lines.

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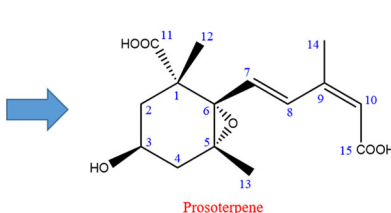
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KEYWORDS

Prosopis africana; sesquiterpene; prosoterpene; quercetin-3-O- α -L-rhamnoside; anti-glycation



Prosopis africana (Guill. & Perr.) Taub.



1. Introduction

The genus *Prosopis* belongs to the family *Leguminosae*, generally known as the legume, bean, or pea family. In 1976, this genus was fully described taxonomically (Pedersen 1980). Till now, 63 plants have been recognised as accepted species in this

genus (<http://www.worldfloraonline.org/>). Most species of *Prosopis* genus are naturalised in African countries like Sudan, Senegal, Ethiopia, Mali, Guinea-Bissau, and Nigeria, growing as trees or shrubs (Choge et al. 2007; Sharifi-Rad et al. 2019).

Prosopis africana (Guill. & Perr.) Taub. is a woody tree growing in semi-arid/arid places (Achi & Okolo 2004). Traditionally, *P. africana* has been used for treating various diseases. The extract of the bark and leaves are used for tooth pain, ophthalmias, skin infections, intestinal worms, dysentery, malaria, rheumatism, vertigo, migraine, wounds, and burn-related problems (Ayanwuyi et al. 2010; Diarra et al. 2015; Catarino et al. 2016). Besides, *P. africana* also has great economical values, for instance, it is used for tanning leather as it contains red dyes. It is also a source of low-cost protein, and its fermented seeds are used for food seasoning. With respect to phytochemistry, till now, only eight compounds have been reported from this plant, including a dihydro flavonoid (Elmezughi et al. 2013), and seven alkaloids (Khuong-Huu et al. 1972a) (Khuong-Huu et al. 1972b). From the pharmacological aspect, methanol extract of *P. africana* stem bark showed analgesic and anti-inflammatory effects (Ayanwuyi et al. 2010). Ethyl acetate extract of the stem bark displayed antimicrobial activity against *Mycobacterium aurum* and *Staphylococcus aureus*, and anti-cancer activity against ZR 75 (Breast cancer cell) (Elmezughi et al. 2013). Due to the limited research progress of this species, it was selected to perform phytochemistry investigation and biological evaluation. Finally, a new sesquiterpene (**1**), named Prosoterpene, as well as eleven known compounds (**2–12**) were isolated and elucidated. Besides, quercetin-3-O- α -L-rhamnoside (**3**) was found to be active against the glycation, and all tested compounds were non-cytotoxic.

2. Results and discussion

Compound **1** (Figure 1) was obtained as an oily liquid. HR-FAB-MS showed $[M - H]^-$ at m/z 295.1182 (calcd. for $C_{15}H_{19}O_6^-$ 295.1182), indicating the molecular formula is $C_{15}H_{20}O_6$, with six degrees of unsaturation. In the 1H NMR spectrum (CD_3OD , 500 MHz), three methyl groups were observed at δ_H 2.01, 1.33, and 1.06, three olefinic protons were shown at δ_H 7.87, 6.26, and 5.87, and an oxymethine signal at δ_H 3.83 also existed. The presence of fifteen carbons was identified from the ^{13}C NMR spectrum (CD_3OD , 500 MHz). They can be categorised into four quaternary carbons, four methines, two methylenes, three methyl, and two carbonyl carbons based on the DEPT spectrum. All signals of **1** resemble monaspilosuslin (Figure 1 and Table S1) (Cheng et al. 2010), except for one more carbonyl carbon (δ_C 181.0) appearing in **1**, and a hydroxymethyl (δ_C 77.3, δ_H 3.70, 3.81) is only present in monaspilosuslin. In HMBC spectrum of **1**, CH_3 -12 (δ_H 1.06) showed correlations with C-1 (δ_C 53.5), C-2 (δ_C 41.0), C-6 (δ_C 82.9), and the carbonyl carbon at δ_C 181.0. Simultaneously, H-2 (δ_H 1.73) displayed a correlation with the carbon at δ_C 181.0. This evidence supported the presence of a carboxylic group (δ_C 181.0) at C-1 (Figure S1). Moreover, another methyl at δ_H 1.33 exhibited correlations with C-4 (δ_C 42.3), C-5 (δ_C 90.0), and C-6 (δ_C 82.9), and its carbon at δ_C 18.5 found in HSQC also correlated with H-4 (δ_H 2.25) in HMBC, indicating the location of this methyl (δ_H 1.33) at C-5 (Figure S1). Therefore, the different signals compared with monaspilosuslin were assigned.

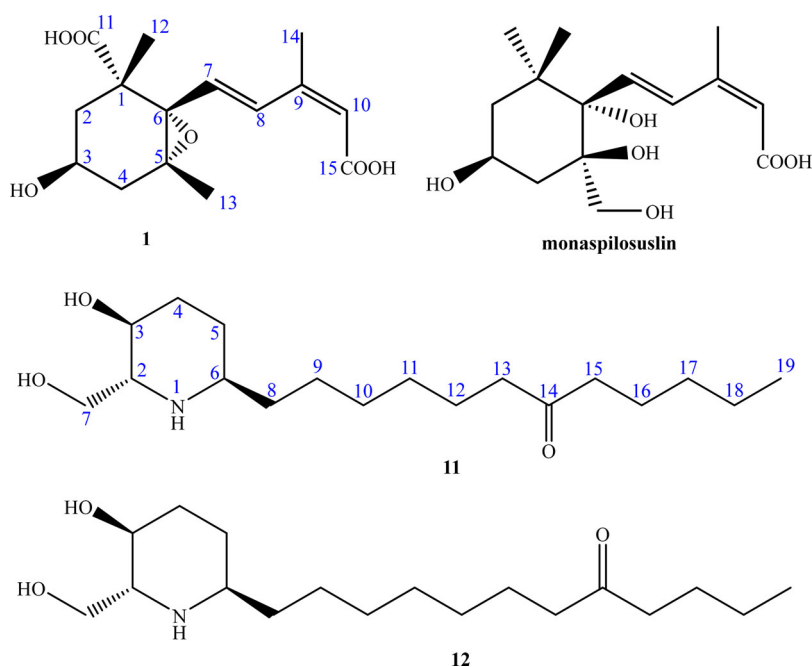


Figure 1. Structures of compounds **1**, monaspilosuslin, **11**, and **12**.

In the COSY spectrum, correlations were observed between H-2 (δ_{H} 1.89 and 1.73), H-3 (δ_{H} 3.83), and H-4 (δ_{H} 2.25 and 1.85). Meanwhile, the olefinic protons at H-7 (δ_{H} 6.26) and H-8 (δ_{H} 7.87) also correlated with each other. In HMBC spectrum, long-range correlations of CH₃ (δ_{H} 2.01) with C-8 (δ_{C} 134.1), C-9 (δ_{C} 144.0), and C-10 (δ_{C} 126.7) were observed. All these correlations proved the similarity of the remaining part of the structure with monaspilosuslin. Moreover, the presence of an ether bond (C-O-C) between C-5 and C-6, which is different from monaspilosuslin, was confirmed by the molecular weight, unsaturation degree, along with the typical peak at 1267 cm⁻¹ in the IR spectrum. Another carbonyl carbon at C-15 (δ_{C} 171.8) was very weak in the ¹³C NMR spectrum, and the long-range correlation between H-10 (δ_{H} 5.87) and C-15 was also not clearly visible in the HMBC spectrum. However, the conjugated system including the α,β -unsaturated acid at C-9, C-10, and C-15, plus one more double bond at C-7 and C-8 have contributed the maximum UV absorption at 253 nm, indicating the presence of the carboxyl at C-15.

The coupling constant of H-7 (δ_{H} 6.26, d, J = 16.0 Hz) and H-8 (δ_{H} 7.87, d, J = 16.0 Hz) revealed the transposition of the double bond. The coupling constant of H-3 (δ_{H} 3.83, tt, J = 10.5, 7.0 Hz) indicated its axial configuration. H-2 (δ_{H} 1.73, dd, J = 13.5, 11.0 Hz) and δ_{H} 1.89 (ddd, J = 13.5, 7.5, 1.0 Hz) indicated the axial and equatorial configurations of them, respectively. Similarly, H-4 (δ_{H} 1.85, dd, J = 14.0, 10.0 Hz) and δ_{H} 2.25 (ddd, J = 14.0, 7.0, 1.0 Hz) confirmed the axial and equatorial configuration these two germinal protons, respectively. In the NOESY spectrum, H-7 (δ_{H} 6.26) showed correlations with H_a-4 (δ_{H} 1.85) and H_a-2 (δ_{H} 1.73), indicating the axial orientation of the side chain at C-6. CH₃-13 (δ_{H} 1.33) correlated with H_a-4 (δ_{H} 1.85) and H_e-4 protons (δ_{H} 2.25), but not H-3 (δ_{H} 3.83), showing the equatorial-orientation of CH₃-13.

Similarly, CH₃-12 (δ_{H} 1.06) at C-1 correlated with H_a-2 (δ_{H} 1.73) and H_e-2 protons (δ_{H} 1.89), but not H-3 (δ_{H} 3.83), confirming the equatorial-orientation of CH₃-12 and axial-orientation of the carboxylic group at C-1 (Figure S2). As a result, the structure of compound **1** was identified as shown in Figure 1 and given the name Prosoterpene.

Compounds **2–12** were elucidated by comparison with reported data. They are quercetin (**2**) (Leena and Aleykutty 2016), quercetin-3-O- α -L-rhamnoside (**3**) (Ma et al. 2005), *p*-coumaric acid (**4**) (Lee et al. 2013), protocatechuic acid (**5**) (Szewczyk et al. 2019), vanillic acid (**6**) (Liu et al. 2015), 2S,3S,4S-trihydroxypentanoic acid (**7**) (Agnihotri et al. 2008), pinitol (**8**) (Duquesnoy et al. 2008), stearyl alcohol (**9**) (Kanya et al. 2007), elaidyl alcohol (**10**) (Glazer & Goddard 1950), isoprosopinine A (**11**), and isoprosopinine B (**12**) (Khuong-Huu et al. 1972b). Compounds **2–10** were obtained and elucidated from this species for the first time. Having similar structures with the same molecular weight, compounds **11** and **12** were identified as a mixture in 1972 from this plant. In this study, two compounds were successfully separated and purified as pure components for the first time. Therefore, the NMR and EI-MS data are also provided (S 3.3.1 and S 3.3.2)(Khuong-Huu et al. 1972b).

The Anti-glycation activity of compounds **3**, **5–8**, and **10–12** was evaluated by the *in vitro* BSA (Bovine serum albumin)-glucose glycation model. Compound **3** (quercetin-3-O- α -L-rhamnoside) showed a moderate anti-glycation activity with IC₅₀ 433 $\pm$ 0.013 μ M, while standard reference rutin (quercetin-3-O-rutinoside) had IC₅₀ as 31 $\pm$ 0.1 μ M. Flavonoids have been shown therapeutic potential for diabetes (Johari et al. 2014). Having a similar structure to the standard (rutin), quercetin-3-O- α -L-rhamnoside has been reported to possess moderate inhibition on α -glucosidase (Lee et al. 2019) and the Maillard reaction (Iida et al. 2007). This is the first time to further prove its anti-glycation activity by the BSA-glucose glycation model.

Compounds **3**, **5–8**, and **10–12** were also performed for anti-cancer activity against MCF-7 cell (breast cancer), NCI-H460 (lung cancer), Hela (cervical cancer), and cytotoxicity against normal human cell line BJ, which were obtained from a cell culture biobank (PCMD, ICCBS) of American Type Culture Collection (ATCC), previously described MTT assay was used for this activity (S 3.5) (Choudhary et al. 2017). All of them were observed to be non-cytotoxic with inhibition < 50% at 50 μ M.

3. Experiment

3.1. General experiment procedures

The Bruker AMS-400 and AMX-500 were used to record NMR spectra. For LR-FAB-MS and LR-EI-MS, JEOL MS Route JMS 600H mass spectrometer was used, and for HR-FAB-MS, JEOL JMS-HX110 mass spectrometer was used. A UV/Visible spectrophotometer was used to accomplish the UV/Visible method. The optical rotations were done on the p-2000 Polarimeter, and the IR (Infrared) spectra were recorded on the FTIR8900 (Fourier Transform Infrared Spectrophotometer) in the KBr disc. For fractionation by column chromatography, normal silica gel (E. Merck, type 70–230 Mesh) was utilised. C18 (Wakogel, 38–63 Mesh), Sephadex LH-20, and normal and reverse-phase HPLC was employed for purification. The purity of the sample was confirmed using normal and reverse-phase pre-coated TLC. UV light at 254 nm was used to evaluate TLC plates.

Dragendroff, ceric sulfate, and vanillin were among the chemicals employed to visualise the spots on TLC plates.

3.2. Plant material

The clean and dry fruits of *Prosopis africana* (Guill. & Perr.) Taub. were brought to Pakistan by the collaboration of Mr. Kayode Muritala Salawu, Lecturer grade I, Department of pharmacognosy and Drug Development, University of Ilorin, Nigeria. The plant components were taken at the University of Ilorin in Kwara State, Nigeria, in July 2017. Then the materials were identified and authenticated at the Forest Herbarium Ibadan (FHI), which is part of the Forestry Research Institute of Nigeria (FRIN), where voucher specimens were deposited, and a voucher number (FHI 111954) was provided.

3.3. Extraction, isolation, and purification

Fruits of *P. africana* were dried and crushed into powder (5.2 kg), then extracted with 80% MeOH (3×20 L) under reflux. Extracts were combined and concentrated using a high-vacuum rotary, yielding a dark brown color residue (580 g). The residue was suspended in water and extracted with *n*-hexane (3×4 L), dichloromethane (3×4 L), EtOAc (3×4 L), and *n*-BuOH (3×4 L), sequentially. The *n*-BuOH extract (102 g) was obtained and processed to silica gel column chromatography (CC) (EtOAc: EtOH 100:0–0:100) to obtain 14 fractions (F_1 – F_{14}). F_1 (4.2 g) was again performed by silica gel CC (Hexane: EtOAc, gradient) to get subfractions F_{1-1} – F_{1-24} . Subfraction F_{1-5} and F_{1-6} were purified by NP-HPLC (Hexane: EtOAc 88:12, $R_t = 38$ min) to get compounds **9** (3.0 mg) and **10** (3.4 mg), respectively. Subfractions F_{1-17} and F_{1-18} were combined to run on Sephadex LH-20 (DCM: MeOH 50:50), and compound **2** (4.4 mg) was obtained. Subfraction F_{1-19} and F_{1-20} were combined to run on Sephadex LH-20 (DCM: MeOH 30:70) to get further three subfractions, among which the second subfraction was subjected to C18 (Water: MeOH, gradient) to get compound **4** (3.1 mg). Similarly, F_{1-21} was run on Sephadex LH-20 (Water: MeOH 30:70) to get four further subfractions, among which the second and third fractions were subjected to RP-HPLC (MeOH: Water = 35:65) to get three compounds **7** (2.7 mg, $R_t = 25$ min) and **5** (4.0 mg, $R_t = 33$ min) from the same injection, and **6** (5.0 mg, $R_t = 39$ min), respectively. Fraction F_2 (4.2 g) was performed on a silica gel CC (DCM: MeOH, gradient) to obtain subfractions F_{2-1} – F_{2-8} . Subfraction F_{2-7} (590 mg) was purified by Sephadex LH-20 (DCM: MeOH 40:60) to get six subfractions (F_{2-7-1} – F_{2-7-6}), among which F_{2-7-2} (114 mg) was performed on a pencil silica gel CC and then further purified by NP-HPLC (DCM: MeOH 92:8, $R_t = 40$ min) to get new compound (**1**) (2.5 mg). Similarly, by series of sequential steps on subfraction F_{2-7-6} (430 mg) and RP-HPLC (MeOH: Water 57:43, $R_t = 36$ min) was used to purify compound **3** (3.2 mg). Compound **11** (7.0 mg) was obtained from fraction F_8 (951 mg) using a gradient system (MeOH: Water) by the C-18 column. Compound **8** (8.0 mg) was obtained as pure crystals from F_9 using silica gel CC (DCM/MeOH Gradient). While compound **12** (7.0 mg) was obtained from a subfraction F_{9-4} (88.4 mg) by using silica gel pencil CC (DCM: MeOH gradient)

3.3.1. Prosoterpene (1)

An oil (2.5 mg); Molecular formula: $C_{15}H_{20}O_6$, $[\alpha]_D^{24}$: -1.14 (c 0.08, MeOH). UV λ_{max} nm. IR ν_{max} 3331, 2959, 1721, 1639, 1538, 1267 cm^{-1} . CD [nm (mdeg)]: 432 (-1.27), 350 (1.49), 312 (-0.52), 288 (4.21), 270 (0.46), 208 (17.3); 1H NMR (CD_3OD , 500 MHz): δ_H 1.73 (1H, dd, $J_{2a-2e} = 13.5$ Hz, $J_{2a-3} = 11.0$ Hz, H-2a), 1.89 (1H, ddd, $J_{2e-2a} = 13.5$ Hz, $J_{2e-3} = 7.0$ Hz, $J_{2e-4e} = 1.0$ Hz, H-2e), 3.83 (1H, tt, $J_{3-2a-4a} = 10.0$ Hz, $J_{3-2e-4e} = 7.0$ Hz, H-3), 1.85 (1H, dd, $J_{4a-4e} = 14.0$ Hz, $J_{4a-3} = 10.0$ Hz, H-4a), 2.25 (1H, ddd, $J_{4e-4a} = 14.0$ Hz, $J_{4e-3} = 7.0$ Hz, $J_{4e-2e} = 1.0$ Hz, H-4e), 6.26 (1H, d, $J_{7-8} = 16.0$ Hz, H-7), 7.87 (1H, d, $J_{7-8} = 16.0$ Hz, H-8), 5.87 (1H, s, H-10), 1.06 (3H, s, H-12), 1.34 (3H, s, H-13), 2.01 (3H, s, H-14); ^{13}C NMR (CD_3OD , 125 MHz) δ_C 53.5 (C-1), 41.0 (C-2), 65.3 (C-3), 42.3 (C-4), 90.0 (C-5), 82.9 (C-6), 129.1 (C-7), 134.1 (C-8), 144.0 (C-9), 126.7 (C-10), 181.3 (C-11), 14.6 (C-12), 18.5 (C-13), 20.6 (C-14), 171.8 (C-15). FAB-MS $[M - H]^-$ m/z 295.1; HR-FAB-MS $[M - H]^-$ m/z 295.1182 (calcd. for $C_{15}H_{19}O_6^-$ 295.1182).

3.4. Anti-glycation activity

The anti-glycation effect was performed by BSA (Bovine serum albumin)-glucose glycation model *in vitro*. Glucose (0.5 M), BSA (10 mg/mL), and sodium azide (NaN_3) (0.1 mM) were dissolved in sodium phosphate buffer (pH 7.4) as the reaction reagent to perform with or without tested sample. The test compounds, and rutin as a standard reference, were dissolved in 10% DMSO. BSA without a glycating agent was used as a negative control. All samples were initially screened at 1 mM concentration. The assay was performed in triplicates, and a final reaction volume of 200 μ L was maintained in each well of a 96-well plate. The reaction mixture was incubated at 37 °C for 7 days in a dark sterile condition. The fluorescence was measured (excitation 340 nm, and emission 440 nm) by using Varioskan Lux microtitre plate reader (Thermo Fisher Scientific, Waltham, Massachusetts, USA).

The percent (%) inhibition was calculated by using the following formula:

$$\text{Inhibition (\%)} \text{ of fluorescence} = (1 - \text{Fluorescence of test compound} / \text{Fluorescence of glycated BSA}) \times 100.$$

The compounds that exhibited more than 50% inhibition were processed for IC_{50} . The two-fold dilution was made, and multiple concentrations (1, 0.5, 0.25, 0.125, 0.06, and 0.03 mM) of compound **3** and standard rutin were incubated with BSA-glucose at 37 °C for 7 days. The fluorescence was measured, and the IC_{50} (μ M) was calculated by using EZ-FIT Enzyme Kinetics Program (Perrella Scientific Inc., Amherst, USA).

4. Conclusion

An undescribed sesquiterpene, named Prosoterpene (**1**), was discovered from the BuOH fraction of *P. Africana*. Nine known compounds (**2–10**) were obtained for the first time from this plant. Besides, two isomer compounds (**11** and **12**) were successfully separated from each other. Among compounds **3**, **5–8**, **10–12**, only compound **3** (quercetin-3-*O*- α -L-rhamnoside) showed anti-glycation activity. All tested compounds were non-cytotoxic against MCF-7, NCI-H460, Hela cancer cell lines, and normal human cell line BJ. Based on the limited research progress on this species, this study provided

a valuable reference and more knowledge for the phytochemistry and pharmacology properties of the *Prosopis* genus and *P. africana*. According to the medicinal uses of this plant, further study is also required to discover more bioactive components from it.

Disclosure statement

No potential conflict of interest was reported by the authors.

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ORCID

M. Iqbal Choudhary  <http://orcid.org/0000-0001-5356-3585>

Yan Wang  <http://orcid.org/0000-0001-9786-7492>

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