An α-Sophoradiol Glycoside from the Root Wood of *Erythrina senegalensis* DC. (Fabaceae) with α-Amylase and α-Glucosidase Inhibitory Potential

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**Abstract**

Phytochemical study of the roots of *Erythrina senegalensis* led to the isolation of a new α-sophoradiol glycoside, erythrinoside (1), together with four known compounds, lupeol (2), α-sophoradiol (3), isoneorautenol (4) and D-mannitol (5). The structures of the compounds were elucidated using spectroscopic data including 1D and 2D NMR, mass spectrometry and by comparison made with some data reported previously; the samples (extracts and compounds) were also subjected to antidiabetic assay. Erythrinoside and isoneorautenol exhibited good α-amylase inhibitory potential of 54.6% and 53.3%, respectively, compared to acarbose (72.5%) at 400 µg/mL. With α-glucosidase, all samples showed promising inhibition percentages above 50% at 200 µg/mL. In the α-glucosidase assay, the ethyl acetate extract (65.5%), methanol extract (72.1%), erythrinoside (63.3%) and isoneorautenol (66.0%) had percentage inhibitions closer to that of acarbose (69.0%) at 200 µg/mL. The methanol extract (IC₅₀ = 81.2 ± 0.9 µg/mL) was more active than acarbose (IC₅₀ = 94.5 ± 0.7 µg/mL) in the α-glucosidase assay. The inhibition of α-amylase and α-glucosidase indicates that *E. senegalensis* extracts and compounds could be used to manage diabetic conditions.

**Keywords**

*Erythrina senegalensis*, erythrinoside, α-sophoradiol glycoside, α-amylase inhibition, α-glucosidase inhibition, antidiabetic potential

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**Introduction**

The Fabaceae is a large family comprising over 16 000 species, which are grouped into about 480 genera. *Erythrina senegalensis* DC, a member of this family, is a tree (6 to 7 meters high)¹ found in Cameroon among the savannah woods and shrubs of the Sudano-Guinean woodland of the Adamawa Region and is equally spread in many other tropical and subtropical zones.¹,² In African countries, *E. senegalensis* is a medicinal plant used as a general tonic and as remedy for several ailments such as cough, pneumonia, bronchial infection, malaria, snake bites, stomach ache, wound healing, normal fever and yellow fever, toothache, gastrointestinal disorders, jaundice, nose bleed, female infertility, gonorrhea and other venereal diseases, abdominal pain, and microbial and parasitic infections.²³⁻⁶

Previous scientific studies have shown biological activities possessed by this plant such as antimicrobial, antidiabetic, antioxidiant, analgesic, anti-inflammatory, antiviral, antimalarial and antitumor.²⁻⁴,⁷⁻¹⁰ The reported constituents of *E. senegalensis* are mainly phenolic compounds and prenylated flavonoids such as erythrininate, octacosyl (E)-fenilate, auriculatin, 2,3-dihydroaureculation, lonchocarpol A, scadenone, eryseagalensein D-M, 4',5,7-trihydroxy-6,8-diprenylisoflavone, 6,8-diprenylgenisten, alpinumisoflavone,²⁻³,¹¹,¹² and alkaloids such as erysocline, crysopine, crysotrine, erythrathidine,
11-hydroxyerysodine, 11-hydroxyerysuvine, 11-oxyerysodine and glucocyrosodine.2,3,4

There are many documented medicinal benefits of Erythrina senegalensis, and, thereby, it is necessary to investigate scientifically the chemical compounds contained in this plant, as well as their biological activities. In this study, the roots of Erythrina senegalensis were investigated for their chemical composition, and the extracts, together with the isolated compounds, were evaluated for their antidiabetic potential through α-amylase and α-glucosidase inhibitory activities. To the best of our knowledge, the chemical compounds from the roots of E. senegalensis have not been the subject of any phytochemical and pharmacological studies.

Results and Discussion

Column chromatographic separation on silica gel of the ethyl acetate extract (25 g) of the roots of E. senegalensis led to the isolation of a new α-sophoradiol glycoside, named erythrinoside (1, 10 mg), isolated for the first time from a natural source, and three known compounds, lupeol (2, 1000 mg), α-sophoradiol (3, 14 mg) and isoneorautenol (4, 15 mg). The methanol extract (25 g) yielded one known compound, D-mannititol (5, 3 mg). The structures of the isolated compounds (Figure 1) were established by extensive analysis of their spectroscopic data and comparison with some previously reported data.

Compound 1 was obtained as an amorphous beige solid soluble in methanol. Its HR-ESI-TOF MS + mass spectrum (see supplemental material) showed a pseudomolecular ion peak [M + Na]+ at m/z 597.4129 (Calcd. for 597.4131) corresponding to the molecular formula C33H38O16, accounting for seven degrees of unsaturation. The compound responded positively to the Lieberman-Burchard test characteristic of a triterpene skeleton. The 1H-NMR spectrum of 1 showed signals attributable to eight methyl groups at δH 0.80 (3H, s), 0.89 (3H, s), 0.93 (3H, s), 1.00 (3H, s), 1.19 (3H, s), 1.02 (3H, s), 1.05 (3H, s), 1.15 (3H, s); one olefinic proton as a triplet at δH 5.26 ppm (1H, J = 6.7 Hz) characteristic of H-2 of the olean-12-ene triterpene type; two oxymethine protons at δH 3.42 ppm (1H, dd; J = 8.0, 3.1 Hz) corresponding to H-3, and 3.18 ppm (1H, dd, J = 13.6, 4.2 Hz) corresponding to H-22. The proton spectrum of 1 presents some similarities to that of sophoradiol, which was previously isolated and described from the same plant.6 However, this spectrum showed additional signals corresponding to the presence of a sugar moiety. Several signals were observed between δH 3.15 and 4.20 ppm. The signal at δH 4.20 ppm (1H, d, J = 7.4 Hz) corresponds to an anomic proton (H-1′) of the sugar moiety. The coupling constant (J = 7.4 Hz) of the anomic proton indicates that the sugar moiety adopts the β-configuration on the aglycon. We also observed three oxymethine proton signals appearing at δH 3.17, 3.31 and 3.49 ppm, as well as two diastereotopic protons of an oxymethylene at δH 3.85 and 3.14 ppm, all belonging to the sugar moiety. The sugar residue was identified as D-xylene by comparison with reported data, which is the form that usually occurs endogenously in living things.6 The 13C-NMR spectrum of 1 showed signals at δC 122.4 and 143.8 ppm corresponding to C-12 and C-13 of an olean-12-ene triterpene type.15 A joint analysis of the 13C-NMR spectrum with DEPT and HSQC spectra indicated two oxymethine carbons at δC 81.9 ppm (C-3) and 78.3 ppm (C-22); one anomic carbon from the sugar moiety at δC 101.3 ppm (C-1′), three oxymethine carbons of the sugar moiety at δC 69.9 (C-4′), 73.5 (C-2′) and 76.5 (C-3′) and one oxymethylene carbon at δC 65.3 ppm (C-5′). The COSY spectrum of 1 showed cross peaks between H-3 at δH 3.42 ppm and two diastereotopic protons at δH 1.52 and 1.36 ppm corresponding to H-a2 and H-b2, respectively. The spectrum also showed important correlation between the proton at δH 3.18 ppm (H-22) (which overlaps with two other proton signals) and two diastereotopic protons at δH 1.63 ppm and 1.57 ppm corresponding to H-a21 and H-b21, respectively. The HMBC correlation spectrum of 1 enabled us to locate the sugar moiety on the aglycon. The spectrum showed correlation between the anomeric proton at δH 4.20 ppm (H-1′) and C-3 at δC 81.9 ppm of the aglycon moiety, suggesting that the sugar moiety is attached to the aglycon on C-3. All of these spectral data led to the identification of compound 1 as 3-O-β-D-xylopyranosyl-12-en-22-ol to which the trivial name erythrinoside was given. To the best of our knowledge, this compound is isolated for the first time from a natural source.

The known compounds have been previously described in many other plants, lupeol (2),18 α-sophoradiol (3),16 isoneorautenol (4),19 and D-mannititol (5).20 Their structures were determined by comparison with the data from the literature referenced above respectively.

The ethyl acetate and methanol extracts and the isolated compounds from Erythrina senegalensis were evaluated for their antidiabetic capacity by measuring their α-amylase and α-glucosidase inhibition (Table 1); the results are reported as inhibition percentages and IC50 values of α-amylase and α-glucosidase inhibition. The methanol extract showed higher inhibition (46.3%) of α-amylase at the dose of 400 µg/mL than the ethyl acetate extract (39.9%) at the same dose. Both extracts showed moderate activity on the inhibition of α-amylase. Likewise, among the tested compounds, erythrinoside (1) showed the best percentage inhibition (54.6%) of α-amylase at the dose of 400 µg/mL, followed by isoneorautenol (4) (53.3%) and sophoradiol (3) (48.2%) at the same dose. Lupeol (2) exhibited weak inhibition (19.2%) of α-amylase. For α-glucosidase, all samples showed promising inhibition above 50% at 200 µg/mL. The ethyl acetate extract (65.5%), erythrinoside (63.3%) and isoneorautenol (66.0%) had percentage inhibitions closer to that of acarbose at 200 µg/mL, while the methanol extract (72.1%) showed the highest inhibition among all tested samples, and acarbose as well. The methanol extract of E. senegalensis was more active than acarbose with an IC50 of 81.2 ± 0.9 µg/mL compared to 94.5 ± 0.7 µg/mL for acarbose in the α-glucosidase assay. Erythrinoside and sophoradiol have the same
aglycon. However, erythrinoside was more active than sophoradiol, which could be due to the presence of a sugar moiety in its structure which correlated positively with the antidiabetic activity. D-mannitol was not tested because it was obtained in small amount. All samples tested were less active than acarbose (72.5% inhibition) used as a reference drug. Compounds 1 and 4 and the extracts showed good inhibition percentages on α-amylase and α-glucosidase and could be applied in the management of diabetic conditions.

**Material and Methods**

**General Experimental Procedure**

Column chromatography (CC) was performed on silica gel 60 (70-230 mesh, Merck), and thin layer chromatography (TLC) on silica gel pre-coated plates F-254 Merck (20 × 20 cm). Compounds were visualized under UV light (254 and 365 nm), then sprayed with dilute sulfuric acid, and heated. The \(^1\)H and \(^13\)C NMR data were recorded on Bruker Avance AV-500 and 600 spectrometers, with trimethylsilane (TMS) as standard. Chemical shifts are given in ppm (δ) and coupling constants (J) in Hz. HR-TOF-MS LD\(^+\) spectra were registered on a QTOF Spectrometer (Bruker, Germany).

**Plant Material**

The roots wood of *Erythrina senegalensis* was collected in Ngaoundere in the Adamawa Region during July 2020. The plant was identified at the National Herbarium of Cameroon (NHC) with the voucher number: N° 50119 NHC.

**Extraction and Isolation Procedure**

The roots of *Erythrina senegalensis* were collected, dried at room temperature then ground into powder. Two Kg of the plant material was successively extracted by maceration at room
temperature with 10 L of ethyl acetate and 8 L of methanol. For each solvent, extraction was made three times at the rate of one extraction every 72 h before moving on to the next solvent. The resulting solutions were evaporated using a rotary evaporator to obtain 30 g of ethyl acetate crude extract and 33 g of methanol crude extract. Twenty-five g of each extract was separated through column chromatography on silica gel using a gradient system of n-hexane/ethyl acetate (0 → 100%) and ethyl acetate/methanol (0 → 100%). From the ethyl acetate extract, 352 fractions were obtained and grouped into sixteen sub-fractions (A–P), according to their TLC profile. Sub-fractions B, C, D and G crystallized and were filtered and washed to obtain compounds 2 (1000 mg), 4 (15 mg), 3 (14 mg) and 1 (10 mg), respectively. Likewise, from the methanol extract, 200 fractions were collected and grouped into ten sub-fractions (A–J), out of which only sub-fraction E could be studied; this afforded compound 5 (3 mg) by purification using column chromatography.

**Table 1.** α-Amylase and α-Glucosidase Inhibition by Extracts and Tested Compounds.

| Samples           | α-amylose (400 µg/mL) | α-glucosidase (200 µg/mL) |
|-------------------|-----------------------|---------------------------|
| Ethyl acetate extract | >400                  | 65.5                      |
| Methanol extract | 46.3                  | 72.1                      |
| Erythinoside (1) | 54.6 ± 0.5             | 63.3                      |
| Lupeol (2)       | 19.2 ± 0.5             | 53.0                      |
| α-Sophoradiol (3) | 48.2 ± 0.5             | 57.0                      |
| Isoeucoranol (4) | 53.3 ± 0.8 ± 1.0       | 66.0                      |
| Acarbose         | 72.5 ± 263.3 ± 2.5     | 69.0                      |

α-Sophoradiol (3). White powder; ESI-MS (+) m/z = 465.8 [M + Na]+ for C_{19}H_{18}O_{10}·H NMR (CD_{3}OD, 500 MHz) δ_{H} ppm: 5.27 (H-12, t, J = 7.5 Hz), 3.41 (H-3, dd, J = 8.0, 3.1 Hz), 3.17 (H-22, dd, J = 13.6, 4.2 Hz), 1.55 (H-9, m), 1.02 (H-16a, m), 0.99 (H-16b, m), 1.91 (2H, m, H-11a and 11b), 1.78 (H-15a, m), 1.04 (H-15b, m), 1.76 (2H, m, H-19a and 19b), 1.47 (H-21a, m), 1.35 (Hb-21, m), 1.58 (H-6, m, 1.45 (H-6b, m), 1.66 (H-1a, m), 1.00 (H-1b, m), 1.64 (2H, m, 1.57 (H-2b, m), 1.41 (2H, m, H-7a and 7b), 2.03 (H-18, m), 1.14 (H-27, m), 1.05 (H-23, m), 1.02 (H-26, m), 1.00 (H-25, m), 1.00 (H-28, m), 0.93 (H-29, m), 0.90 (H-30, m), 0.81 (H-24, m), 0.77 (H-5, m).

α-Glucosidase (4). Gray powder; ESI-MS (+) m/z = 345.5 [M + Na]+ for C_{19}H_{18}O_{10}. H NMR (CD_{3}OD, 500 MHz) δ_{H} ppm: 7.29 (H-1, d, J = 8.4 Hz), 6.25 (H-7, m), 6.51 (H-2, dd, J = 8.4, 2.4 Hz), 6.32 (H-4, d, J = 2.4 Hz), 6.30 (H-4', d, J = 9.8 Hz), 5.51 (H-3', d, J = 9.8 Hz), 5.43 (H-11a, d, J = 6.8 Hz), 4.22 (H-6eq, dd, J = 10.7, 4.8 Hz), 3.53 (H-6ax, m), 3.43 (H-6a, m), 1.39 (H-5', m), 1.37 (H-6', m). 13C NMR (CD_{3}OD, 125 MHz) δ_{C} ppm: 111.3 (C-1a), 131.7 (C-1), 109.3 (C-2), 158.7 (C-3), 153.5 (C-4), 143.8 (C-5), 131.7 (C-6), 131.7 (C-7), 126.7 (C-8), 123.2 (C-9), 153.5 (C-10), 122.4 (C-11), 143.8 (C-12), 143.8 (C-13), 141.8 (C-14), 25.4 (C-15), 27.3 (C-16), 25.4 (C-17), 45.4 (C-18), 46.1 (C-19), 29.8 (C-20), 38.6 (C-21), 78.2 (C-22), 27.5 (C-23), 14.7 (C-24), 14.9 (C-25), 16.2 (C-26), 24.2 (C-27), 27.3 (C-28), 31.2 (C-29), 19.7 (C-30), 101.3 (C-1'), 73.5 (C-2'), 76.5 (C-3'), 69.9 (C-4'), 65.3 (C-5').

**General NMR Data of Compounds 1 to 5**

Erythinoside (1)<PE: Please Check Heading Level.>. Amorphous beige solid; HR-ESI-MS (+) m/z = 597.4129 [M + Na]^+ (Calcd. for C_{35}H_{38}O_{10}·Na, 597.4131). 1H NMR (CD_{3}OD, 600 MHz) δ_{H} ppm: 5.26 (H-12, t, J = 7.6 Hz), 4.20 (H-1', d, J = 7.6 Hz), 3.49 (H-4', m), 3.42 (H-3, dd, J = 8.0, 3.1 Hz), 3.32 (H-3', m), 3.18 (H-22, dd, J = 13.6, 4.2 Hz), 3.17 (H-2', m), 3.14 (H-5'a, m), 3.85 (H-5'b, m), 2.11 (H-9, m), 2.03 (H-18, m), 1.90 (H-16a, m), 1.93 (H-16b, m), 1.80 (H-11a, m), 1.03 (H-11b, m), 1.78 (H-15a, m), 1.04 (H-15b, m), 1.76 (2H, m, H-19a and 19b), 1.47 (H-21a, m), 1.35 (Hb-21, m), 1.58 (H-6a, m), 1.45 (H-6b, m), 1.53 (H-1a, m), 1.36 (H-1b, m), 1.36 (H-2b, m), 1.39 (H-7, m), 1.15 (H-27, m), 1.05 (H-23, m), 1.02 (H-26, m), 1.00 (H-25, m), 1.00 (H-28, m), 0.93 (H-29, m), 0.90 (H-30, m), 0.81 (H-24, m), 0.77 (H-5, m).
102.7 (C-4), 156.6 (C-4a), 66.1 (C-6), 39.4 (C-6a), 119.5 (C-7a), 121.9 (C-7), 114.8 (C-8), 98.3 (C-10), 160.1 (C-10a), 78.6 (C-11a), 76.0 (C-2'), 127.0 (C-3'), 121.9 (C-4'), 26.8 (C-5'), 26.7 (C-6').

D-Mannitol (5). White solid; ESI-MS (+) m/z = 205,06 [M + Na]⁺ for C₉H₁₄O₆; ¹H NMR (DMSO-d₆, 600 MHz) δ_H ppm: 4.50 (2H, d, J = 5.8 Hz, HO-2 and 5), 4.40 (2H, t, J = 6.0 Hz, HO-1 and 6), 4.25 (2H, d, J = 7.3 Hz, HO-3 and 4), 3.60 (2H, m, H-1a and 6a), 3.55 (2H, t, J = 7.1 Hz, H-3 and 4), 3.45 (2H, m, H-2 and 5), 3.40 (2H, m, H-1b and 6b). ¹³C NMR (DMSO-d₆, 150 MHz) δ_C ppm: 64.3 (C-1 and 6), 71.7 (C-2 and 5), 70.0 (C-3 and 4).

**In Vitro α-Amylase Inhibition Assay**

The α-amylase inhibition assay was performed using the 3,5-dinitrosalicylic acid (DNSA) method.²¹ Extracts and compounds (1, 2, 3 and 4) isolated from *Erythrina senegalensis* were dissolved in a minimum amount of 10% DMSO and further dissolved in buffer (Na₂HPO₄/NaH₂PO₄ (0.02 M), NaCl dissolved in a minimum amount of 10% DMSO and further dissolved in water (w/v)) was added to each tube and incubated for 3 min. The reaction was terminated by the addition of 200 µL of starch solution (1% in water (w/v)) and mixed with 200 µL of the extract and incubated for 10 min at 30 °C. Thereafter 200 µL of starch solution (1% in water (w/v)) was added to each tube and incubated for 3 min. The reaction was terminated by the addition of 200 µL of sodium phosphate tetratetrahydrate buffer (pH 6.9) to give concentrations of 100, 200 and 400 µg/mL. Two hundred µL of α-amylase solution (2 units/mL) was mixed with 200 µL of the extract and incubated for 10 min at 37 °C. The mixture was cooled to ambient temperature and diluted with 5 mL of distilled water, the absorbance was measured at 540 nm using a UV-Visible spectrophotometer. The blank with 100% enzyme activity was prepared by replacing the plant extract or compound with 200 µL of buffer. A positive control sample was prepared using acarbose and the inhibition was expressed as percentage inhibition and was calculated using the equation given below:

\[
\text{% inhibition of } \alpha\text{-amylase} = 100 \times \left(1 - \frac{\text{Abs sample} - \text{Abs blank}}{\text{Abs control} - \text{Abs blank}}\right)
\]

**In Vitro β-Glucosidase Inhibition Assay**

β-Glucosidase inhibitory activity was determined as described elsewhere.²² Briefly, mixtures of 20 µL sodium phosphate buffer (pH 5.0), 20 µL p-nitrophenyl-β-D-glucopyranoside (Sigma Chemical Co., 1 mg/mL) and 10 µL of the sample at different concentrations (dissolved in DMSO) were incubated in a 96-well plate at 37 °C for 10 min, followed by the addition of 10 µL β-glucosidase solution from almonds (Sigma Chemical Co., 5 mg/mL) to each well, and incubation at 37 °C for 30 min. The reaction was terminated by adding 140 µL of sodium carbonate buffer, pH = 10. Absorbance was determined at 410 nm using a microplate reader (*width* Microplate). To the control and blank were added 10 µL DMSO instead of the sample solution. The system without β-glucosidase was used as blank, and acarbose was used as positive control. The β-glucosidase inhibitory activity was expressed as the percentage of inhibition and calculated by the following equation:

\[
\text{% inhibition of } \beta\text{-glucosidase} = 100 \times \left(1 - \frac{\text{ODsample} - \text{ODblank}}{\text{ODcontrol} - \text{ODblank}}\right)
\]

**Conclusion**

Investigation of the chemical constituents from the roots of *Erythrina senegalensis* resulted in the isolation and characterization of five compounds, including one new oleane-type triterpenoid glycoside (erythrinoside) and four known compounds. The antidiabetic potential of the extracts and the isolated compounds were evaluated by measuring the inhibition of α-amylase. Compounds 1 and 4 exhibited good α-amylase inhibitory potential, while the extracts exhibited moderate activities by showing appreciable percentage inhibition of the enzyme. Since α-amylase is a key enzyme involved in the breakdown of starch into glucose, inhibiting this enzyme can delay starch hydrolysis and reduce the amount of glucose in the system. This is a favorable phenomenon in diabetic conditions thus indicating that *Erythrina senegalensis* and its constituents could be potent candidates for the development of antidiabetic therapies.

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**Ethical Approval**

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Statement of Informed Consent
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Not applicable, because this article does not contain any clinical trials.

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Supplemental Material
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