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Phytochemical Constituents of the Leaves of *Landolphia owariensis*

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ABSTRACT

Landolphia owariensis is a plant common in West Africa with many ethnomedicinal uses. The leaves of the plant were investigated for phytochemical constituents. Successive extraction and chromatographic purification led to the isolation of two flavonoid glycosides myricitrin and quercitrin, and the triterpenoid and sterol glucoside, α-amyrin and daucosterol, respectively. The compounds were identified by their NMR chemical shifts and comparison with literature reports.

Keywords: *Landolphia owariensis*, Apocynaceae, myricitrin, quercitrin, α-amyrin, daucosterol.

Introduction

Landolphia owariensis P. Beauv (Apocynaceae) is a shrub or climbing plant commonly known as vine rubber. It is an important economic and ethnomedicinal plant widely distributed in the tropical, subtropical and coastal lowlands of West Africa and extensively used among the rural societies in the region. Different parts of the plant are used as a purgative and vermifuge, for the treatment of fever pains, malaria, venereal infections, and as an ingredient of arrow poison.[1-4] The plant is also used in the production of a native beer and beverage in Senegal and upper Nile respectively.[5] Pharmacological activities reported for the plant include analgesic, anti-inflammatory, anti-ulcer and gastric anti-secretory effects.[6, 7] The plant was also found to have anti-microbial effects.[8-10] The phytochemistry of *L. owariensis* stringy seed pulp led to the isolation of four compounds, (E)-chlorogenic acid, (E)-chlorogenic acid methyl ester, protocatechuic acid and 3*β*-sitosterol.[11] There are no previous reports on the phytochemical constituents of the leaves of *L. owariensis*, hence this study to isolate compounds that are responsible for its pharmacological activities and useful in its chemotaxonomy. The compounds could also be used for the standardization of any crude drugs produced from the plant. We hereby report the isolation of two flavonoid glycosides, myricitrin and quercetrin, and the commonly isolated triterpenoids α-amyrin, and daucosterol from the leaves of *L. owariensis*.

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Materials and Methods

General Experimental Procedures

NMR spectra were acquired on a Bruker AV400 (400 MHz spectrophotometer) using $CDCl_3$ or DMSO-d_6 as solvents and tetramethylsilane (TMS) as internal standard. Chemical shifts are in parts per million (δ ppm) and the coupling constants are in Hz. Column chromatography were performed on glass columns (2.2 × 28 cm) using silica gel (230–400 µm mesh, Merck, Germany). TLC analyses were carried out using pre-coated silica 60 F_{254} on glass backed plates (0.25 mm, 2.5 × 7.5 cm; Merck, Darmstadt, Germany). Spots were detected using vanillin-sulphuric acid reagent, followed by heating at 110°C for 5 minutes. Solvents and chemicals were obtained from commercially available sources and reagents prepared according to standard procedures.

Sample collection and preparation

The fresh leaves of *L. owariensis* were collected at Chaza, Suleja LGA of Niger State, Nigeria in September 2018 by Mr. Muazzam Ibrahim. The plant was identified and authenticated by Mr. Akeem Lateef at the herbarium of the National Institute for Pharmaceutical Research and development, Abuja, where a voucher specimen (NIPRD/7030) was deposited.

Extraction and Isolation

The air-dried leaves were pulverized and 200 g of the powdered material was successively extracted by maceration with 600 mL each of n-hexane, ethyl acetate, and methanol at room temperature for 48 h. The extracts were filtered and concentrated using a rotary evaporator. The hexane extract was subjected to column chromatography on silica gel and eluted using gradient amounts of ethyl acetate in hexane (20% increments of ethyl acetate) to obtain 20 fractions (LH1 – LH20). Based on TLC, six major fractions were obtained. Fraction LH5 eluted with 20% ethyl acetate in hexane, was recrystallized with heptane to yield **1** (58.6 mg), a white crystalline solid. The ethyl acetate extract was similarly subjected to column chromatography on silica gel eluting with gradient amounts of n-hexane, ethyl acetate and methanol

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to obtain 15 fractions (LE1 – LE15). Fraction LE7 eluted with 80% ethyl acetate in hexane yielded **2** (34.3 mg), as a cream coloured solid and was further purified by washing with ethyl acetate. Fraction LE9 eluted with 100% ethyl acetate yielded **3** and **4** (70.4 mg) as an amorphous yellow solid.

Daucosterol (**1**): ^{1}H NMR (DMSO-d_6): δ 5.33 (1H, d, *J* = 4.9 Hz, H-6), 1.95 (1H, t, *J* = 15.2 Hz, H-8), 1.51 (1H, dd, *J* = 15.1, 10.8 Hz, H-7), 0.96 (3H, s, H-19), 0.91 (3H, d, *J* = 6.3, H-21), 0.66 (3H, d, H-18), Glc H; 4.23 (1H, d, *J* = 7.7, H-1′), 3.65 (1H, ddd, *J* = 11.6, 5.6, 1.9 Hz, Hb-6′), 3.44 (1H, dd, *J* = 17.5, 11.6, 5.2 Hz, Ha-6′), 3.10-2.91 (m, other Glc H).

α-amyrin (**2**): ^{1}H NMR ($CDCl_3$): δ 5.21 (1H, t, *J* = 3.7 Hz, H-12), 3.25 (1H, dd, *J* = 11.2, 4.7 Hz, H-3), 1.16, 1.02, 0.99, 0.96, 0.90, 0.86, 0.82, 0.77 (3H, s, 8 × CH_3). Rest of the protons were overlapped.

Myricitrin (**3**) and Quercitrin (**4**): see Table 1.

Results and Discussion

Evaporation of the solvents yielded 7.20 g of hexane, 3.29 g of ethyl acetate and 9.50 g of methanol extracts. Column chromatography of the hexane and ethyl acetate extracts of *L. owariensis* yielded compounds **1**-**4**. Compounds **1** and **2** were identified as daucosterol (*β*-sitosterol 3-*O*-*β*-D-glucopyranoside) and α-amyrin (12-ursen-3*β*-ol), respectively based on literature comparisons.[12, 13] Compounds **3** and **4** were obtained as a mixture in the ratio 6:1 but the distinct NMR signals were clear enough to identify the compounds and assign their respective chemical shifts. The ^{1}H-NMR spectrum of compound **3** showed a H-bonded –OH proton at δ_H 12.68 ppm typical of a 5-OH of flavonoids. It also showed two meta-coupled doublets at 6.20 (H-6) and 6.37 (H-8), typical of a 5, 7 di-substituted pattern for ring A of a flavonoid. A singlet proton at 6.89 integrated for two protons indicated the presence of a 3′, 4′, 5′- trisubstituted ring B of the flavonoid. An anomeric proton signal at 5.20 ppm with other gylcosidic protons between 3.15 and 3.98 indicated the presence of a sugar moiety attached to the flavonoid. The presence of a deoxyhexose methyl group at 0.84 confirmed an L-rhamnose moiety. The ^{13}C Dept-Q spectrum revealed 21 carbon signals including a carbonyl at δ_C 177.8, seven phenolic carbons (157.5, 134.3, 161.3, 156.4, 145.8, 136.5, 145.8), an anomeric carbon at 101.9, a methyl carbon at 17.5 and four other glycosidic carbons (70.0, 70.4, 71.3, 70.6). From the HMBC spectrum, a long range correlation from the anomeric proton and C-3 (134.3) confirmed the site of glycosidation to be at C-3. Other correlations confirmed the structure as myricitrin (myricetin 3-*O*-α-rhamnopyranoside). The full chemical shift assignments are given in Table 1 and were in agreement with literature reports.[14] The ^{1}H-NMR spectrum of compound **4** also showed a H-bonded –OH signal at δ_H 12.66 (5-OH, s), a 3,4-disubstituted ring B with an ABX spin system with signals at 7.31 (H-2′, d, *J* = 2.2 Hz), 6.87 (H-5′, d, *J* = 8.6 Hz) and 7.26 (H-6′, dd, *J* = 8.6, 2.4 Hz). The usual meta coupled protons of ring A were observed at 6.21 (H-6, d, 2.0) and 6.40 (H-8, d, 2.0) while the anomeric proton H-1″ was observed at 5.26 (d, *J* = 1.6) and H-6″ at 0.82 (d, *J* = 6.0). The rest of the proton signals were overlapped under the major signals of myricitrin. The data obtained which were distinct for the compound from the ^{13}C and 2D spectra compared well with those of literature (Table 1) and compound 4 was identified as quercitrin (quercetin 3-*O*-α-rhamnopyranoside).[15]

Table 1: ^{1}H- and ^{13}C-NMR spectral data for myricetin 3-*O*-α-rhamnopyranoside (compound **3**) and quercetin 3-*O*-α-rhamnopyranoside **(**compound **4)**

Position	3		4	
	^{1}H δ ppm (mult, *J* in Hz)	**^{13}C δ ppm (mult)**	**^{1}H δ ppm (mult, *J* in Hz)**	**^{13}C δ ppm (mult)**
2	-	157.5 (C)	-	157.3 (C)
3	-	134.3 (C)	-	134.2 (C)
4	-	177.8 (C)	-	177.8 (C)
5	-	161.3 (C)	-	161.3 (C)
6	6.20 (d, 2.1)	98.7 (CH)	6.20 (d, 2.0)	*
7	-	164.2 (C)	-	*
8	6.37 (d, 2.1)	93.5 (CH)	6.40 (d, 2.0)	*
9	-	156.4 (C)	-	*
10	-	104.5 (C)	-	104.1 (C)
1′	-	119.6 (C)	-	120.7 (C)
2′	6.89 (s)	107.9 (CH)	7.31 (d, 2.2)	116.3 (CH)
3′	-	145.8 (C)	-	145.2 (C)
4′	-	136.5 (C)	-	148.4 (C)
5′	-	145.8 (C)	6.87 (d, 8.6)	115.5 (CH)
6′	6.89 (s)	107.9 (CH)	7.26 (dd, 8.6, 2.4)	121.1 (CH)
1″	5.20 (d, 1.5)	101.9 (CH)	5.26 (d, 1.6)	102.3 (CH)
2″	3.98 (m)	70.0 (CH)	*	*
3″	3.55 (m)	70.4 (CH)	*	*
4″	3.15 (m)	71.3 (CH)	*	*
5″	3.37 (m)	70.6 (CH)	*	*
6″	0.84 (d, 6.2)	17.5 (CH_3)	0.82 (d, 6.0)	17.8 (CH_3)
3′-OH	9.25 (s)	-	9.33 (s)	-
4′-OH	8.85 (s)	-	9.69 (s)	-
5-OH	12.68 (s)	-	12.66 (s)	-
5′-OH	9.25 (s)	-	-	-
7-OH	10.86 (s)	-	*	-

* Overlapped with signals for compound **3**

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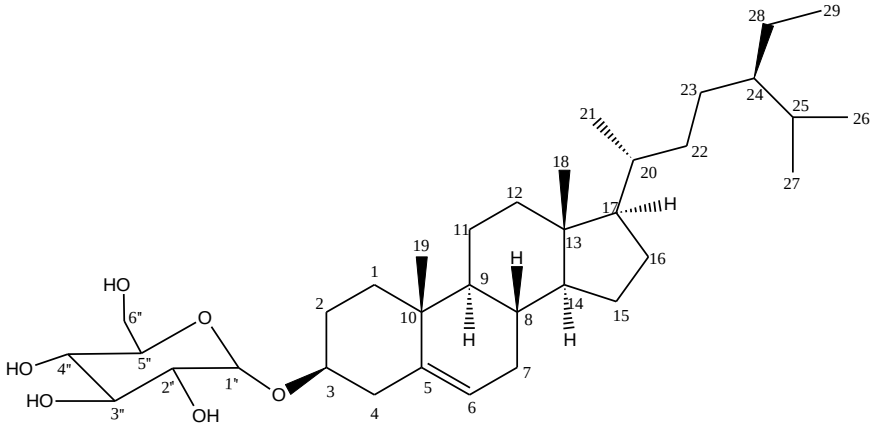

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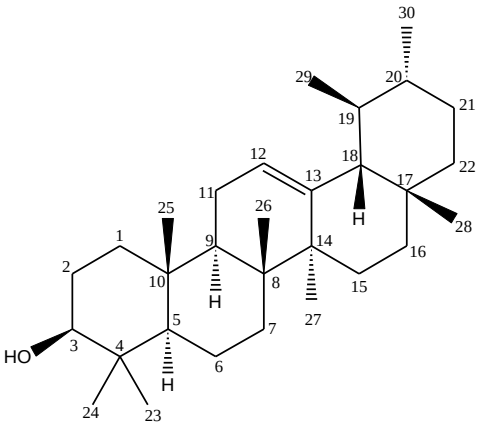

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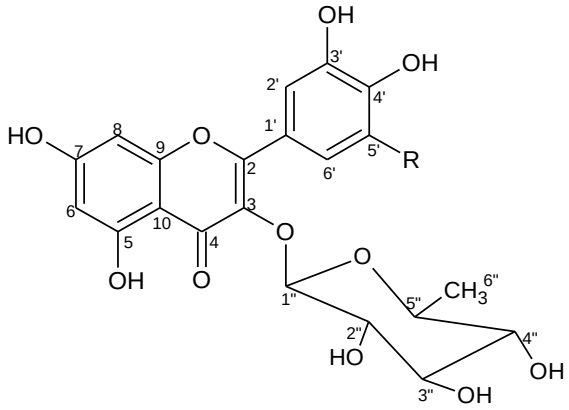

3 R= OH
4 R= H

Figure 1: Chemical structures of compounds **1**-**4** isolated from the leaves of *L. owariensis*

Although no biological tests were carried out in this study, literature survey of the isolated compounds showed that they possessed a number of bioactivities. Studies have shown that daucosterol is an immunomodulatory agent, plays important roles in suppressing inflammation, and increases the proliferative capacity of neural stem cells.[16,17,18] Daucosterol also inhibited the proliferation, migration, and invasion of hepatocellular carcinoma (HCC) and colorectal cancer cells.[19,20] A study revealed the oral efficacy of α, β-amyrin in reducing hyperglycemia and hyperlipidemia in the experimental models of STZ-induced diabetes and diet induced hyperlipidemia.[21] A review article by Vasquez *et al* also reported α-amyrin to have antimicrobial and anti-inflammatory activities.[22] Myricitrin was shown to possess a considerable antioxidant activity, with stronger free radical scavenging activity than other flavonol rhamnosides or quercetin.[23] *In vivo*, myricitrin has been reported as a nitric oxide (NO) and protein kinase C inhibitor that exerts antinociceptive effect .[24] The anti-inflammatory potential of myricitrin was demonstrated by Shimosaki *et al*. who found inhibitory effect of myricitrin against TNF-α production in RAW264.7 macrophages.[25] Another study demonstrated that myricitrin was a novel hepatoprotective agent, with stronger hepatoprotective activity than a reference compound silymarin.[26] Quercitrin showed antidiarrhoeic activity at doses of 50 mg kg^{-1}, against castor oil- and PGE_2-induced diarrhoea in mice.[27] The antioxidant and intestinal anti-inflammatory activities of quercitrin are also documented.[28-30]

Conclusion

Phytochemical studies of the leaves of *L. owariensis* led to the isolation of four compounds; α-amyrin, daucosterol, myricitrin and quercitrin. This is the first report of the chemical constituents of the leaves of the plant. The pharmacological activities of the isolated compounds lend credence to the reported studies and provide justification for some of the ethnomedicinal uses of the plant. Myricitrin can also be useful in chemotaxonomic applications and as a marker compound for the standardization of *L. owariensis* as herbal medicine.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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