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CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF *Oldenlandia corymbosa* L.

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Abstract

Oldenlandia corymbosa (family Rubiaceae) has been long used in traditional medicine for the treatment of various ailments such as fever, hepatitis, gastrointestinal disorders, cancer, and other inflammatory conditions. Numerous compounds isolated from this species have been reported to exhibit notable pharmacological activities, including anti-inflammatory, antioxidant, anticancer, immunomodulatory, and neuroprotective effects. In the present study, the antibacterial activity of different solvent fractions of O. corymbosa was evaluated to identify promising extracts for further phytochemical investigation. The ethyl acetate fraction showed the strongest inhibitions against Vibrio sp. and Aeromonas caviae, with particularly high inhibition against Vibrio sp. (86.78 ± 0.24 mm at 5.000 mg/mL). Chemical analysis of the ethyl acetate fraction was performed using silica gel column chromatography, and the chemical structures were elucidated by NMR spectroscopy through comparison with reference data, leading to the identification of three compounds: geniposide (1), rutin (2), and quercetin (3). These findings highlight the potential of O. corymbosa as a promising natural source of antibacterial agents.

Keywords: *antbacterial activity, chemical constituents, Oldenlandia corymbosa* L.

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NGHIÊN CỨU THÀNH PHẦN HÓA HỌC VÀ KHẢ NĂNG KHÁNG KHUẨN CỦA CÂY LƯỠI RẮN (*Oldenlandia corymbosa* L.)

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Tóm tắt

Cây Lưỡi rắn (Oldenlandia corymbosa), thuộc họ Cà phê (Rubiaceae), từ lâu đã được sử dụng trong y học cổ truyền để điều trị nhiều bệnh như sốt, viêm gan, rối loạn đường ruột, ung thư và các bệnh viêm nhiễm khác. Nhiều hợp chất phân lập từ loài này đã được ghi nhận với các hoạt tính dược lý nổi bật, bao gồm chống viêm, chống oxy hóa, chống ung thư, điều hòa miễn dịch và bảo vệ thần kinh.Trong nghiên cứu này, hoạt tính kháng khuẩn của các cao chiết phân đoạn từ cây Lưỡi rắn đã được thực hiện nhằm sàng lọc cao chiết tiềm năng để định hướng nghiên cứu sâu hơn về thành phần hóa học. Kết quả cho thấy cao chiết ethyl acetate thể hiện tác dụng kháng khuẩn đáng kể đối với hai loài vi khuẩn gây bệnh thường gặp trong nuôi trồng thủy sản là Vibrio sp. và Aeromonas caviae, đặc biệt hiệu quả đối với Vibrio sp. với đường kính vòng ức chế đạt 86,78 ± 0,24 mm ở nồng độ 5.000 mg/mL. Phân tích cấu trúc hóa học của các hợp chất phân lập được bằng phổ cộng hưởng từ hạt nhân (NMR), kết hợp với đối chiếu dữ liệu từ tài liệu tham khảo, đã xác định được ba hợp chất: geniposide (1), rutin (2) và quercetin (3). Những kết quả thu được góp phần khẳng định tiềm năng của cây Lưỡi rắn như một nguồn dược liệu tự nhiên triển vọng với hoạt tính kháng khuẩn đáng chú ý.

Từ khóa: *cây lưỡi rắn, kháng khuẩn, thành phần hóa học.*

1. Introduction

Antibiotic resistance among pathogenic microorganisms has emerged as a critical global health threat. Natural products are considered promising, safe, and sustainable sources of novel antibacterial agents. *Oldenlandia corymbosa* L. (*O. corymbose*) contains flavonoids, iridoid glycosides, and other secondary metabolites (Chen, R. et al., 2016). Several compounds, including iridoids, lignans, and flavonol glycosides, have been isolated and shown to possess diverse biological activities (Noiarsa P. et al., 2008; Zhitao L. et al., 2008). A recent review further emphasized the pharmacological potential of *Oldenlandia* species, highlighting antibacterial, anti-inflammatory, and antioxidant effects (Al-Shuhaib, M.B.S. & Al-Shuhaib, J.M.B., 2024). However, the chemical profile and antibacterial properties of *O. corymbosa* in Vietnam remain poorly investigated. This study aimed to isolate and characterize compounds from *O. corymbosa* and to evaluate their antibacterial activity, thereby providing scientific evidence for its potential as a medicinal resource.

2. Experimental

2.1. Chemicals and reagents

NMR spectra were obtained on a Bruker AM500 FT-NMR spectrometer (Bruker, Karlsruhe, Germany) using deuterated solvents at the Institute of Chemistry, Vietnam Academy of Science and Technology (Hanoi, Vietnam). TLC analyses were carried out on silica gel 60 F-254 plates (0.063–0.200 mm, Merck, Germany), and spots were visualized under UV light (254 or 365 nm) or by spraying with $FeCl_3$/EtOH or H_2SO_4/EtOH. Column chromatography was performed on silica gel (240–430 mesh, Merck, Germany). Analytical-grade solvents, including *n*-hexane, dichlormethane (DCM), ethyl acetate (EtOAc), methanol (MeOH), and 96% ethanol, were purchased from Chemsol (Vietnam).

A clean bench (Class II BSC, Esco, Indonesia), an autoclave (HVE-50, Hirayama, Japan), a centrifuge (Mikro 12-24, Hettich, Germany), and a vortex mixer (ZX3, Velp, Italy) were used in the antibacterial activity assay.

2.2. Sample treatment and preparation

The aerial parts of *O. corymbosa* were collected in November 2024 from Can Tho City and taxonomically identified by Dr. Nguyen Thi Kim Hue. A voucher specimen (Oc251124) has been deposited at the Department of Biology, College of Natural Sciences, Can Tho University. The plant material was cleaned to remove soil and debris, with any deteriorated parts discarded. The samples were air-dried at ambient temperature for several days and subsequently oven-dried at 50 °C until completely dried.

2.3. Extraction and isolation

The aerial parts of *O. corymbosa* (50 kg) were thoroughly washed with water and shade-dried in a clean, dust-free environment. The dried material was ground into a fine powder, of which 5.0 kg was extracted with ethanol at room temperature. The extract was concentrated under reduced pressure to yield a dark crude ethanol extract (850 g). This crude extract was suspended in water (1:1, v/v) and successively partitioned with *n*-hexane and EtOAc. Each fraction was concentrated under reduced pressure, affording the *n*-hexane (195 g), EtOAc (45 g), and aqueous (50 g) fractions. The distribution of yields among the solvent fractions suggests an efficient extraction and solvent partitioning process based on compound polarity.

The EtOAc fraction (45.0 g) was subjected to silica gel column chromatography with gradient elution (*n*-hexane–EtOAc, 80:20 → 0:100, v/v; followed by EtOAc–MeOH, 95:5 →

0:100, v/v), affording 15 subfractions (EA1–EA15). Subfraction EA14 (2.5 g) was further chromatographed on silica gel (EtOAc–MeOH, 95:5 → 0:100, v/v) to give eight subfractions (EA14.1−EA14.8). Subsequent purification of EA14.5 by silica gel column chromatography using DCM−MeOH (95:5 → 0:100, v/v) and $CHCl_3$−EtOAc (10:1, v/v) yielded five subfractions (EA14.5.1−EA14.5.5) and compound **1** (15 mg) from EA14.5.2.

Similarly, fraction EA15 was chromatographed on silica gel with *n*-hexane−EtOAc (50:50 → 0:100, v/v) to afford ten subfractions (EA15.1−EA15.10). Further purification of EA15.5 by silica gel column chromatography using DCM−MeOH (10:1 → 5:1, v/v) provided compound **2** (16 mg) from EA15.5.2.

In the same manner, fraction EA11 (2.9 g) was separated by silica gel column chromatography into seven subfractions, eluted with *n*-hexane−EtOAc (70:30→ 0:100, v/v), followed by EtOAc−MeOH (97:3 → 0:100, v/v). Successive purification of subfraction EA11.6 (130 mg) afforded compound **3** (13 mg).

2.4. Antibacterial activity

In this study, the antibacterial potential of extracts from *O. corymbosa* was determined by the agar well diffusion method through measurement of inhibition zone diameters and minimal inhibitory concentrations (MICs). Antimicrobial tests were performed in triplicate against three standard bacterial strains obtained from the Research Institute for Aquaculture No. 2, Vietnam, namely *Vibrio sp*. and *Aeromonas caviae* (*A. caviae*), which are important aquaculture pathogens. The bacteria were cultured on tryptic soy agar (TSA, Acumedia, USA) for 24 h at 37 °C. Cell suspensions were prepared in 0.85% NaCl solution and adjusted to 0.5 McFarland standard. The bacterial lawns were then established by swabbing onto Müeller–Hinton agar (Merck, Germany) plates (95 × 15 mm). Subsequently, 50 µL of each test sample (dissolved in DMSO) was introduced into 9 mm wells bored into the agar. Plates were incubated at 37 °C for 24 h, after which samples producing inhibition zones were considered active. Ampicillin (Sigma, USA) and DMSO served as positive and negative controls, respectively.

The minimal inhibitory concentration (MIC) was determined by the broth microdilution method using Müeller–Hinton broth (MHB, Biolife, Italy) with standardized bacterial inocula (1.5×10^6 CFU/mL). Two-fold serial dilutions were prepared. After incubation at 37 °C for 24 h, wells showing no visible growth were subcultured on TSA. The MIC was defined as the lowest concentration that completely inhibited bacterial growth (Yeo, Y.L. et al., 2014).

2.5. Statistical analysis

One-way ANOVA was employed to assess the variability among data sets. All results are expressed as the mean ± standard deviation (SD) of three independent experiments, with statistical significance determined at $p < 0.05$.

3. Results and discussion

3.1. *In vitro* antibacterial activity results

The extracts of *O. corymbosa* from Can Tho city exhibited antibacterial activity against both *Vibrio sp.* and *A. caviae* in a dose-dependent manner, with significant effects observed at 5.000 mg/mL. At this concentration, the ethyl acetate extract showed the strongest inhibition against *Vibrio sp*. (86.78 ± 0.24 mm), which was higher than that against *A. caviae* (68.31 ± 0.37 mm; $p < 0.05$). Notably, both bacterial strains were more sensitive to the ethyl acetate extract than to ampicillin, which showed no inhibition at concentrations below 500 mg/mL.

Therefore, direct comparisons between plant extracts and the reference antibiotic should be interpreted with caution due to differences in concentration and formulation.

The unusually large inhibition zones observed at higher extract concentrations may reflect the combined effects of compound diffusibility in agar, concentration-dependent antibacterial activity, and the sensitivity of the tested bacterial strains.

Although no report has directly examined the antibacterial activity of *O. corymbosa* against *A. caviae* and *Vibrio sp*., several studies have demonstrated its broad-spectrum efficacy against Gram-negative bacteria. For instance, Divya et al. (2023) showed that methanolic extracts of *O. corymbosa* inhibited *Escherichia coli* and *Klebsiella pneumoniae*, while Hussain et al. (2014) confirmed notable antimicrobial activity of its GC–MS characterized fractions. Such findings suggest that the rich phenolic and flavonoid content of *O. corymbosa* may contribute to cell membrane disruption and enzyme inhibition in Gram-negative pathogens. Supporting this, Vazquez-Armenta et al. (2024) reported that plant-derived phenolic compounds effectively attenuate virulence and biofilm formation in *V. parahaemolyticus*, providing a mechanistic rationale for potential activity of *O. corymbosa* against this species. Furthermore, since *A. caviae* has emerged as a major etiological agent of human intestinal infections and has been increasingly implicated in diverse extra-intestinal infections over the past few years (Song et al., 2023), investigating the efficacy of *O. corymbosa* extracts against this bacterium is both scientifically and therapeutically important. More recently, Al Bashera et al. (2025) reinforced the antimicrobial potential of *O. corymbosa* through integrated antioxidant, docking, and GC–MS analyses. Collectively, these findings warrant targeted assays against *A.caviae* and *Vibrio sp.* to validate the plant's antibacterial potential.

Table 1. Zone of inhibition and MIC of extracts

Samples		**Diameter of growth inhibition zones at different concentrations**					**MIC (mg/mL)**
Extracts (mg/mL)		**0.3125**	**0.625**	**1.250**	**2.500**	**5.000**	
Crude extract	*A. caviae*	$17.85^{e}\pm0.15$	$22.43^{d}\pm0.36$	$28.42^{c}\pm0.42$	$39.51^{b}\pm0.28$	$44.16^{a}\pm0.22$	MIC≤0.3125
	Vibrio sp.	$17.44^{e}\pm0.16$	$26.46^{d}\pm0.24$	$34.41^{c}\pm0.22$	$42.61^{a}\pm0.19$	$51.80^{b}\pm0.50$	MIC≤0.3125
***n*-Hexane extract**	*A. caviae*	$8.23^{e}\pm0.12$	$12.29^{d}\pm0.16$	$19.43^{c}\pm0.21$	$27.63^{b}\pm0.33$	$39.48^{a}\pm0.34$	MIC≤0.3125
	Vibrio sp.	$8.93^{c}\pm0.26$	$14.24^{d}\pm0.13$	$21.33^{b}\pm0.23$	$29.83^{a}\pm0.33$	$35.29^{d}\pm0.24$	MIC≤0.3125
Ethyl acetate extract	*A. caviae*	**$31.93^{d}\pm0.33$**	**$44.73^{c}\pm0.42$**	**$60.60^{b}\pm0.36$**	**$68.43^{a}\pm0.45$**	**$68.31^{a}\pm0.37$**	**MIC≤0.3125**
	Vibrio sp.	**$40.17^{e}\pm0.29$**	**$51.57^{d}\pm0.35$**	**$63.85^{c}\pm0.25$**	**$77.45^{b}\pm0.37$**	**$86.78^{a}\pm0.24$**	**MIC≤0.3125**
Positive control: Ampicillin (500 mg/mL)	*A. caviae*	28.00 ± 0.33					
	Vibrio sp.	36.56 ± 0.56					
Negative control: DMSO 10%	*A. caviae*	-	-	-	-	-	
	Vibrio sp.	-	-	-	-	-	

Note: Values are presented as means ± SE (n=3). Different letters (a, b, c, d, e) in the same row show significant difference at the level of 0.05. The results were statistically analyzed using Minitab 16 software (ANOVA-Turkey's).

3.2. Structure elucidation

The structures of the isolated compounds were elucidated based on NMR spectral data and comparison with reported literature.

*3.3.1. Compound **1***

Compound **1** was obtained as needle-shaped crystals, white-pink in color, m.p. 245-247°C.

^{1}H-NMR (600 MHz, DMSO-d_6) δ_H (ppm): 7.46 (1H, *s,* H-3); 5.68 (1H, *s*, H-7); 5.12 (1H, *d*, *J* = 6.6 Hz, H-1); 4.53 (1H, *d*, *J* = 7.8 Hz, H-1′); 4.13 (1H, *d*, *J* = 15.0 Hz, H-10a); 3.98 (1H, *d*, *J* = 14.4 Hz, H-10b); 3.64 (3H, *s,* H-12); 3.67 (1H, *brd, J* = 4.2 Hz*,* H-6′a); 3.42 (1H, *m,* H-6′b); 3.06 (1H, *m,* H-5); 3.16 (1H, *m,* H-3′); 3.12 (1H, *m,* H-5′); 3.06 (1H, *m,* H-4′); 2.99 (1H, *m,* H-2′); 2.04 (1H, *m,* H-6a); 2.69 (1H, *dd*, *J* = 8.4 and *J* = 6.6 Hz, H-6b); 2.64 (1H, *t*, *J* = 7.2 Hz ,H-9).

^{13}C-NMR (150 MHz, DMSO-d_6) δ_C (ppm): 166.9 (C-11); 151.6 (C-3); 144.1 (C-8); 125.5 (C-7); 111.0 (C-4); 98.6 (C-1′); 95.8 (C-1); 77.2 (C-5′); 76.6 (C-3′); 73.1 (C-2′); 70.0 (C-4′); 61.0 (C-6′); 59.3 (C-10); 51.0 (C-12); 45.9 (C-9); 38.0 (C-6); 34.5 (C-5).

The ^{1}H-NMR spectrum (600 MHz, DMSO-d_6) of **1** showed characteristic signals in both downfield and upfield regions. In the downfield region, two singlets at δ_H 7.46 (1H, *s*) and 5.68 (1H, *s*) were assigned to olefinic protons adjacent to electronegative atoms or electron-withdrawing groups. A doublet at δ_H 5.12 (1H, *d*, *J* = 6.6 Hz) corresponded to a methine proton flanked by two oxygen atoms, typical of the genipin skeleton.

Signals at δ_H 4.13 (1H, *d*, *J* = 15 Hz), 3.98 (1H, *d*, *J* = 14.4 Hz), and 3.64 (3H, *s*) were attributed to protons on carbons bonded to electronegative atoms. The non-equivalent methylene protons at δ_H 4.13/3.98, 2.04/2.69, and 3.67/3.42 suggested attachment to chiral centers. Methine resonances appeared at δ_H 2.99, 3.06, 3.12, and 3.16 (all *m*). Additional signals at δ_H 3.67 (1H, *br d*, *J* = 4.2 Hz) and 3.42 (1H, *m*), together with an anomeric proton at δ_H 4.53 (1H, *d*, *J* = 7.8 Hz), confirmed the presence of a *β*-D-glucopyranosyl moiety. Upfield multiplets between δ_H 2.02–2.71 were attributed to methylene and methine groups.

The ^{13}C-NMR spectrum (150 MHz, DMSO-d_6), supported by HSQC, revealed 17 carbons. A resonance at δ_C 166.9 indicated an ester carbon, while δ_C 151.6, 144.1, 125.5, and 111.0 corresponded to olefinic carbons. A distinct signal at δ_C 95.8 was assigned to a methine carbon adjacent to two oxygen atoms, consistent with the genipin core. Five oxymethine carbons appeared at δ_C 77.2, 76.6, 73.3, 70.0, and 61.0, while δ_C 98.6 correlated with the anomeric proton (δ_H 4.53, *J* = 7.8 Hz), confirming a *β*-D-glucopyranoside. Additional resonances at δ_C 59.3 and 51.0 represented deshielded methylene groups, while δ_C 45.9, 38.0, and 34.5 were assigned to aliphatic carbons.

HMBC correlations, notably between the anomeric proton (δ_H 4.53) and δ_C 95.8, established the sugar linkage at C-1 of the genipin skeleton (**Figure 1**).

Taken together, the ^{1}H-NMR, ^{13}C-NMR, HSQC, and HMBC data, in agreement with literature (Tran & cs, 2018), identified compound **1** as genipin 1-*O*-*β*-D-glucopyranoside (geniposide).

*3.3.2. Compound **2***

Compound **2** was characterized as yellow powder, m.p. 260-262°C.

^{1}H-NMR (600 MHz, DMSO-d_6) δ_H (ppm): 12.58 (1H, *s,* 5–OH); 7.55 (1H, *d*, *J* = 2.4 Hz, H-2′); 7.53 (1H, *dd*, *J* = 2.4 and 8.4 Hz, H-6′); 6.84 (1H, *d*, *J* = 8.4 Hz, H-5′); 6.38 (1H, *d*, *J* = 2.4 Hz, H-8); 6.19 (1H, *d*, *J* = 1.8 Hz, H-6); 5.34 (1H, *d*, *J* = 7.8 Hz, H-1′′); 4.39 (1H, *d, J* = 1.2 Hz, H-1′′′); 3.71 (1H, *d*, *J* = 10.2 Hz, H-6′′b); 3.40 (1H, *m*, H-2′′′); 3.29 (1H, *m*, H-6′′a); 3.28 (1H, *m*, H-3′′′); 3.26 (1H, *m*, H-5′′′); 3.25 (1H, *m*, H-5′′); 3.23 (1H, *m*, H-2′′); 3.22 (1H, *m*, H-3′′); 3.07 (1H, *m*, H-4′′′); 3.06 (1H, *m*, H-4′′); 1.00 (3H, *d*, *J* = 6.6 Hz, H_3-6′′′).

^{13}C-NMR (150 MHz, DMSO-d_6) δ_C (ppm): 177.3 (C-4); 164.3 (C-7); 161.2 (C-5); 156.5 (C-2); 156.4 (C-9); 148.4 (C-4′); 144.7 (C-3'); 133.3 (C-3); 121.6 (C-6′); 121.1 (C-1′); 116.2 (C-2′); 115.2 (C-5′); 103.8 (C-10); 101.2 (C-1′′); 101.7 (C-1′′′); 98.7 (C-6); 93.6 (C-8); 76.4 (C-3′′); 75.9 (C-5′′); 74.1 (C-2′′); 71.8 (C-4′′′); 70.5 (C-3′′′); 70.3 (C-2′′′); 70.0 (C-4′′; 68.2 (C-5′′′); 67.0 (C-6′′); 17.7 (C-6′′′).

The ^{1}H-NMR spectrum (600 MHz, DMSO-d_6) exhibited five characteristic aromatic signals at δ_H 6.19 (1H, *d*, *J* = 1.8 Hz), 6.38 (1H, *d*, *J* = 2.4 Hz), 6.84 (1H, *d*, *J* = 8.4 Hz), 7.53 (1H, *dd*, *J* = 8.4 and 2.4 Hz), and 7.55 (1H, *d*, *J* = 2.4 Hz). Additionally, resonances attributable to two sugar residues were observed: *β*-glucose, indicated by multiple signals between δ_H 3.06–3.40 and an anomeric proton at δ_H 5.34 (1H, *d*, *J* = 7.8 Hz, H-1′′), and *α*-rhamnose, evidenced by an anomeric proton at δ_H 4.39 (1H, *d*, *J* = 1.2 Hz, H-1′′′) together with a methyl doublet at δ_H 1.00 (3H, *d*, *J* = 6.6 Hz, H-6′′′).

The ^{13}C-NMR and DEPT spectra revealed 27 carbon signals, including 15 carbons attributed to the aglycone moiety and 12 carbons assigned to the sugar unit. Among them, six oxygenated quaternary carbons (δ_C 156.5, 164.3, 161.2, 144.7, 148.4, 133.3) correspond to the flavonol skeleton, consistent with hydroxyl substitution on both A and B rings. Five aromatic methine carbons were observed at δ_C 93.6 (C-8), 98.7 (C-6), 115.2 (C-5′), 116.2 (C-2′), and 121.6 (C-6′). The carbohydrate region displayed two anomeric carbons at δ_C 101.2 (C-1′′, glucose) and 100.7 (C-1′′′, rhamnose), a methyl carbon at δ_C 17.7 (C-6′′′), a –CH_2OH at δ_C 67.0 (C-6′′), and multiple oxygenated methines between δ_C 68.2–76.4, further confirming the presence of *β*-glucose and *α*-rhamnose.

In the HMBC spectrum, the rhamnose anomeric proton (δ_H 4.39, H-1′′′) showed a correlation with C-6′′ of glucose (δ_C 67.0), indicating a rutinosyl linkage. Moreover, the glucose anomeric proton (δ_H 5.34, H-1′′) correlated with C-3 of the flavonol core (δ_C 133.3), confirming substitution at this position.

Taken together, the NMR data closely matched reported values for rutin (quercetin 3-*O*-rutinoside) (Olszewska, M.; 2005). HSQC and HMBC correlations, in agreement with literature, definitively established the structure of compound **2** as rutin.

*3.3.3 Compound **3***

Compound **3** was obtained as pale yellow powder, m.p. 251-253ºC

^{1}H-NMR (500 MHz, DMSO**)**, δ_H (ppm): 7.68 (1H, *s*, H-2′); 7.54 (1H, *d*, 9.0 Hz, H-6′); 6.88 (1H, *d*, 9.0 Hz; H-5′); 6.41 (1H, *s*, H-8); 6.19 (1H, *s*, H-6).

^{13}C-NMR (125 MHz, DMSO), δ_C (ppm): 176.3 (C-4); 164.4 (C-7); 161.2 (C-5); 156.6 (C-9); 148.2 (C-2); 147.3 (C-4′); 145.5 (C-3′); 136.2 (C-3); 122.4 (C-1′); 120.4 (C-6′); 116.1 (C-5′); 115.5 (C-2′); 103.5 (C-10); 98.7 (C-6); 93.8 (C-8).

The ^{1}H-NMR spectrum of compound **3** displayed five aromatic proton signals in a range of δ_H 6.19 to 7.68. ^{13}C-NMR and DEPT spectra also showed signals of total 15 carbons of a flavone backbone. Thus, one carbonyl carbon at δ_C 176.3; five aromatic methine carbons at δ_C 93.8, 98.7, 115.5, 116.1, 120.4; two quaternary carbons at δ_C 103.5, 122.4 and seven oxygenated aromatic carbons with δ_C from 136.2 to 164.4. The mentioned ^{1}D-NMR spectral data and those of quercetin (Liu, 2010) are the same. In this way, compound **3** was identified as 3,3′,4′,5,7-pentahydroxyflavone or quercetin.

Three compounds **1**-**3** were isolated and identified from the aerial parts of *O. corymbosa*, including geniposide (**1**), rutin (**2**), and quercetin (**3**) by analysis of their NMR spectra and comparison with literature data (**Figure 2**).

Although no new compounds were identified, the present study provides original experimental data on the antibacterial activity of *Oldenlandia corymbosa* collected in Vietnam. To the best of our knowledge, this is the first report demonstrating inhibitory effects of Vietnamese *O. corymbosa* extracts against *Vibrio sp*. and *Aeromonas caviae*. This regional and biological specificity adds incremental yet valuable knowledge, particularly in relation to the exploration of locally available medicinal plants as potential antimicrobial agents for aquaculture applications.

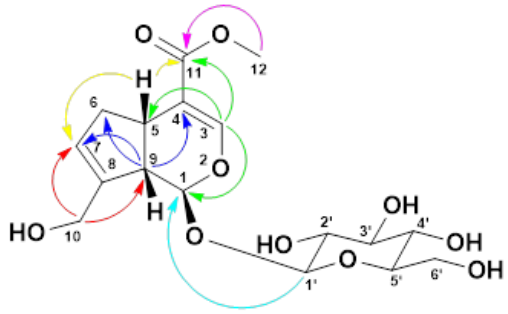
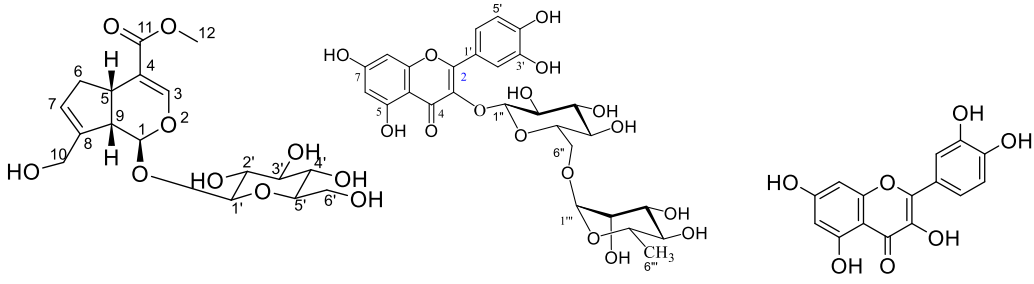


Figure 1. Some typical HMBC correlations of compound (1)

Note: ↷ *show typical H-C heteronuclear multiple bond correlation*



1 (geniposide) **2 (rutin)** **3 (quercetin)**

Figure 2. Chemical structures of compounds 1–3 isolated from *O. corymbosa*

4. Conclusion

Three compounds - geniposide, rutin, and quercetin - were successfully isolated from the ethyl acetate extract of *O. corymbosa*, which exhibited the most pronounced antibacterial activity among the tested fractions. This finding underscores the ethyl acetate extract as a valuable reservoir of bioactive secondary metabolites. Comprehensive evaluation of the antibacterial properties of these isolated compounds is warranted to elucidate their mechanisms of action and therapeutic relevance. Ultimately, such investigations may contribute to the discovery of novel antibacterial agents and support their potential applications in pharmaceutical development and aquaculture, where natural alternatives to conventional antibiotics are increasingly needed to address antimicrobial resistance.

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