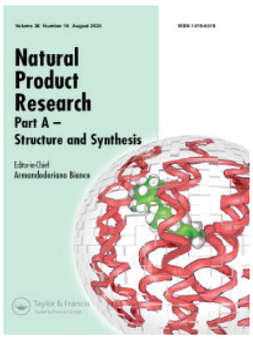

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Myxaoside A, a new triterpenoid saponin from *Cordia myxa* L. (Boraginaceae)

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ABSTRACT

The phytochemical study of *Cordia myxa* L. led to the isolation, through chromatographic techniques, of a new triterpenoid saponin, 3-*O*-[*α*-*L*-rhamnopyranosyl-(1→3)-(6-*O*-acetyl-*β*-*D*-glucopyranosyl)]-22*β*-hydroxyolean-12-ene (**3**) namely Myxaoside A, together with three known compounds, Soyasaponine I (**1**), oleanolic acid (**2**), and 3-*O*-acetyl-oleanolic acid (**4**). All structures were established, based on 1 & 2D-NMR spectroscopic analysis and comparison with previous published reports. Compound **1**-**4** were evaluated for their antibacterial activity on various strains of bacteria including *Salmonella typhi, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Vibrio cholerae*. It appears that compounds **1** and **3** were active on all the tested microbial species, while compounds **2** and **4**, shown no significant effect on *S. aureus* and *K. pneumoniae* at low concentrations 6.5 mg/mL and 3.0 mg/mL.

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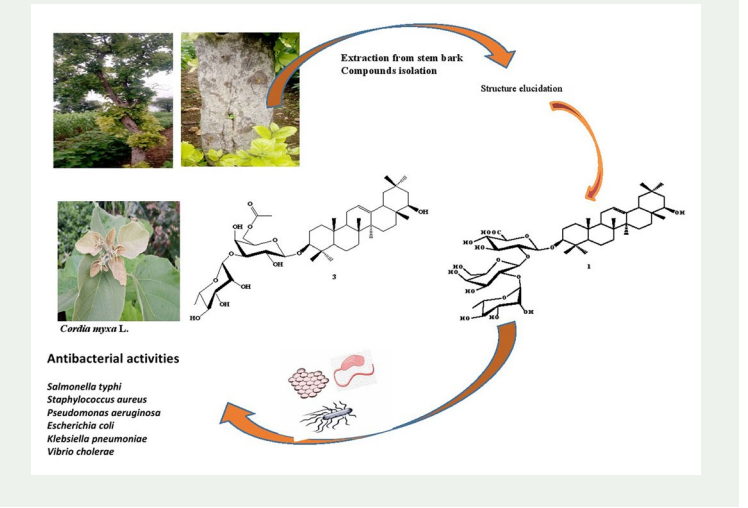

1. Introduction

Herbal medicines have gained popularity throughout world as alternative therapeutics for controlling common infections (de Albuquerque 2006; Prihanto et al. 2012). The emergence of multiple drug resistant strains of bacteria and high cost of synthetic compounds has directed researchers to look for new therapeutic agents in medicinal plants (Arshad et al. 2017). The genus *Cordia* belonging to the Boraginaceae family, with about 300 species, mostly in the warmer regions of the world (Thirupathi et al. 2008). The plant parts like fruits, leaves, stem bark, seeds and roots of most *Cordia* species, especially *C. dichotoma*, *C. myxa*, *C. oblique*, *C. verbenacea*, *C. martinicensis*, *C. salicifolia*, *C. spinescens*, *C. latifolia*, *C. ulmifolia*, among others, has long been used in traditional medicine for cicatrising, as astringent, antiinflammatory, anthelminthic, antimalarial, diuretic, febrifuge, appetite suppressant, cough suppressant, to treat urinary infections, lung diseases and leprosy (Thamer Mouhi et al. 2016). Traditionally, *Cordia myxa* has several virtues. Plant parts of *C. myxa* are reputed as diuretic, laxative, as a cure in diseases of lungs and spleen, coughs, helminthiasis, leprosy, skin diseases, dyspepsia, fever and diarrhoea (Pahang 2002; Sabira et al. 2011). Its fruits are very mucilaginous and the mucilage is highly esteemed for the treatment of coughs, as well as in diseases of the chest, the uterus and the urethra (Joshi 2000). Leaves are applied to ulcers and used for the treatment of headaches (Joshi 2000). *Cordia myxa* has high nutrient value and positive influence on human health. Our previous study on this plant led to the isolation of ten known compounds from the ethyl acetate extract of the stem bark and roots of the plant (Matcheme et al. 2023). This richness and diversity in secondary metabolites as well as the interesting antimicrobial activities obtained for some of these compounds, pushed us to further explore this plant with a view to identify new compounds with even more interesting activities, which could justify the use of this plant against microbial diseases. Therefore, the objective of the present study was to isolate, characterise and evaluate the antibacterial effects of the isolated compounds from the roots and the stem bark of *Cordia myxa*.

2. Results and discussion

2.1. Phytochemical investigation

The methanol extract of the root of *C. myxa* was submitted to column chromatography (CC) on silica gel leading to the isolation of four compounds, among which a new triterpenoid saponin.

Compound **3**, obtained as a white powder soluble in MeOH, $[\alpha]_D^{25}$ **= – 4.5** (c 0.13, MeOH), m.p. 535-536 °C, and showed positive Libermann-Burchard and Molish reactions, suggesting that **3** might be a triterpenoid glycoside or a steroidal glycoside. The IR spectrum showed absorption bands due to a hydroxyl (3438 cm^{-1}), a carbonyl ester (1735 cm^{-1}), and an alkenyl (1628 cm^{-1}) (Figure S1). The mass spectrum HR-TOF-MS-ESI in positive ion mode give a sodium adduct ion $[M+Na]^+$ at *m/z* 815.5023 uma, corresponding to the ion formula $C_{44}H_{72}O_{12}Na^+$, with nine degrees of unsaturation. The ^1H and ^{13}C NMR, COSY and HMBC spectra of compound **3** were quite similar with those of Kaikasaponin III (Sakamoto et al. 1992; Miyao et al. 1996). These spectra (Table S1) showed typical signals for an oleanene-type sapogenin, with eight angular methyl signals at δ_H 0.86 (3H, s), 0.91(3H, s), 0.94 (3H, s), 1.00 (3H, s), 1.02 (3H, s), 1.03 (3H, s), 1.13 (3H, s) and 1.14 (3H, s), an olefinic proton at δ_H 5.22 (1H, t, J=3,77 Hz) and two olefinic carbons at δ_C 122.5 (C-12) and 144.5 (C-13) corresponding to the C-12/C-13 double bond. The oximethine at C-3 was determined by signals at δ_H 3.20 (1H, sl) and δ_C 92.0 (Baltayev et al. 1997; Lehbili Meryem 2018; Dabolé et al. 2022). An additional signal of oximethine proton was visible at δ_H 3.42 (1H, *m*) and linked to the carbon at *δc* 76.7 (C-22) according to the HSQC spectrum. The HMBC spectrum shows a long-range coupling between the proton at δ_H 0.86 (3H, s, H-28) and the carbons at *δc* 76.7 (C-22), 28.8 (C-16), 37.9 (C-17) and 46.8 (C-19) which confirmed the position of hydroxyl functionalization at C-22. The carbohydrate chain consisted of two monosaccharide residues, deduced from the signals of two anomeric carbons (*δc* 102.1 and 105.7), which correlated with the corresponding signals of anomeric protons (δ_H 5.22, d, J=1,8 Hz and 4.47, t, J=8.45 Hz) by the HSQC spectrum. The deep analysis of COSY and HSQC correlations allow the identification of the sugar moieties as glucose and rhamnose. The glucose is represented by the anomeric proton at δ_H 4.47 (1H, t, J=8.45 Hz) which is linked to the carbon at *δc* 105.7 according to HSQC spectrum. The high value of coupling constant (J=8.45 Hz), corresponding to two *trans* diaxial protons, confirms the *β*-configuration of the glucoside unit (Nyemb, Tchinda et al. 2018). The rhamnose sugar is represented by the anomeric proton at δ_H 5.22 (H-1′, d, J=1.8), the oximethine protons at δ_H 3.96 (H-2′, t, J=3.39), 3.80 (H-3′, m), 3.44 (H-4′, m), 4.18 (H-5′, m) and the methyl proton at δ_H 1.27 (3H, d, J=3.34 Hz). The chemical shifts and coupling constants of the anomeric proton of this sugar unit indicated an *α*-rhamnose residue. The unshielding of the C-3 carbons of genin and C-3′ of glucose allow to suppose a diglycosidic chain [*α-L*-rham (1→3)-*β-D*-Glc] attached to the hydroxyl in 3 position of the genin skeleton. The saccharide composition of this compound was confirmed as glucose and rhamnose by TLC after acid hydrolysis, and their absolute configuration was determined to be *D* for glucose, and *L* for rhamnose, after preparative TLC and measurement of the optical rotation of each sugar. The signals at *δc* 180.4 and 23.9 were assigned to an acetyl group. This unusual

value for the carbonyl carbon of the acetyl group has sometimes been observed in experimental spectra. The deshielding (downfield) of ^{13}C-NMR chemical shift of carbonyl carbon is a phenomenon observed when the carbonyl is involved in H-bonding (Jena et al. 2022). The ^{1}H and ^{13}C NMR data of **3** were in good agreement with those of Kaikasaponin III (Sakamoto et al. 1992; Miyao et al. 1996), except for the glucoside moiety with additional acetyl group. The position ester linkage of the acetyl group was confirmed by HMBC (Figure S7e) spectrum of compound **3**, where the correlation between H-6′ protons (δ_H 3.66, 3.77) and acetyl group (δc 180.4) was observed, indicating that the acetyl group was attached at the oxygen atom of C-6′ of the *β-D*-glucopyranosyl moiety. The presence of an acetyl-glucopyranosyl and a rhamnopyranosyl group constituting the carbohydrate moiety was also confirmed by the following important fragment ion peaks at *m/z* 749.5 [M – C_2H_3O]$^+$, 585.1 [M – $C_6H_{11}O_5$ – C_2H_3O – H]$^+$ and 440.7 [M – $C_{14}H_{23}O_{10}$ – H]$^+$ (Figure S3). Further loss of 18 amu [–H_2O] from it produced a fragment ion peak at *m/z* 423.5, giving confirmation of the presence of the hydroxyl group at C-28. The fragment at *m/z* 585.1 [M – $C_6H_{11}O_5$ – C_2H_3O – H]$^+$ also confirmed the terminal position of the rhamnose moiety. Hence, the structure of **3** is determined to be 3-*O*-[*α-L*-rhamnopyranosyl]-(1→3)-(6-*O*-acetyl-*β-D*-glucopyranosyl)-22*β*-hydroxyolean-12-ene. To the best of our knowledge, this compound has not been reported previously and it has been trivially named Myxaoside A.

The other compounds were identified as 3-*O*-[*α-L*-rhamnopyranosyl-(1→2)-*β-D*-galactopyranosyl-(1→2)-*β-D*-glucuronopyranosyl]-3*β*,22*β*,24-trihydroxyolean-12-ene (Soyasaponine I, **1**) (Rao et al. 1985), oleanolic acid (**2**) (Nyemb et al. 2022), and 3-*O*-acetyl oleanolic acid (**4**) (Seebacher et al. 2003) (Figure 1).

Acid hydrolysis: The saponin sample (5.0 mg) was added to 5.0 mL of 2 M HCl in a flask and heated under magnetic stirring to 100 °C for 3 h. The aglycone was extracted

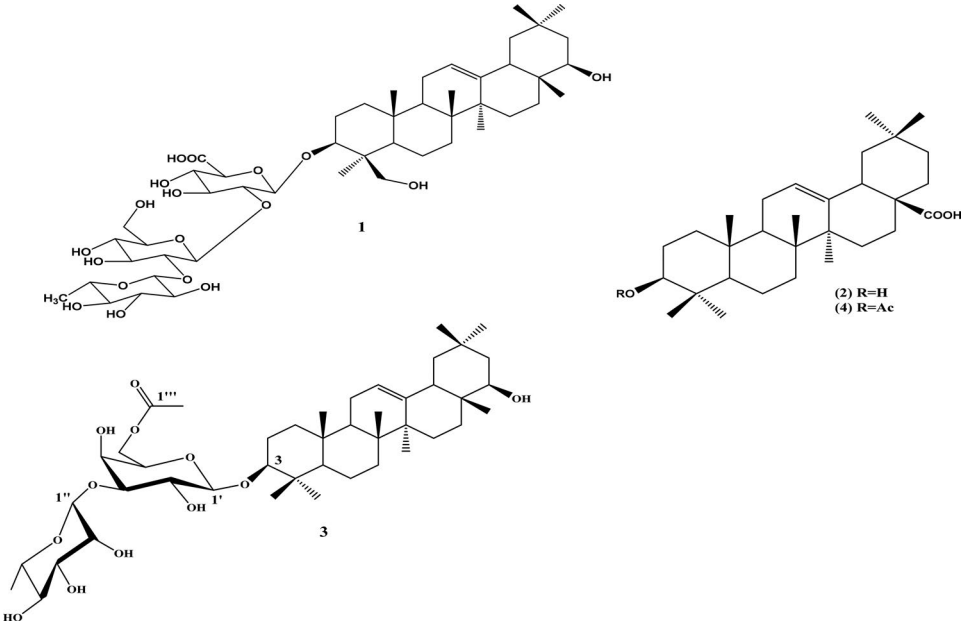

Figure 1. Chemical structures of isolated compounds **1-4** from ***cordia myxa*** L.

with CH_2Cl_2 thrice. The aqueous residue was concentrated under vacuum. The sugar mixture was then compared with authentic samples by analytical TLC eluted with $CHCl_3$-MeOH-H_2O [80-20-2].

2.2. Antibacterial test

Evaluation of the antimicrobial activities of compound **3** was performed by the Muller-Hinton agar diffusion method. At the concentration of 14 mg/mL, compound **3** showed a moderate sensitivity (8.0 < DIZ < 14.0 mm) effect on *S. typhi, S. aureus* and *P. aeruginosa*, with DIZ of 12.5 ± 0.2 mm, 09.0 ± 0.2 mm and 11.0 ± 0.2 mm respectively, comparatively to the reference antibiotics PB50 (Polymixin B, 15 ± 0.2 mm) (Table S2).

3. Experimental

See Supplementary materials.

4. Conclusion

The isolation of secondary metabolites from methanol extract of roots and ethyl acetate of stem bark of *C. myxa* L. led to a new terpenoid saponin, 3-*O*-[*α*-*L*-rhamnopyranosyl-(1→3)-(6-*O*-acetyl-*β*-*D*-glucopyranosyl)]-22*β*-hydroxyolean-12-ene (**3**), namely Myxaoside A. The antibacterial activities of compounds **1**, **2**, **3** and **4** was evaluated on *Escherichia coli, Salmonella typhi*, *Staphylococcus aureus*, *Pseudomonas aeruginosa, Klebsiella pneumoniae and Vibrio cholerae*. Compounds **1** and **3** exhibit moderate sensitive effect at 14 mg/mL on all the tested bacterial strains.

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Disclosure statement

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