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Chemical constituents from *Cordia myxa* L. (Boraginaceae) and their antibacterial activity

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ABSTRACT

Chemical investigation of *Cordia myxa* L. (Boraginaceae) resulted in the isolation of the following ten known compounds: 1-naphthaleneacetic-5-carboxy-1,2,3,4,4a,7,8,8a-octahydro-1,2,4a-trimethyl-[1*S*-(1*α*,2*β*, 4a,8a*α*)]-acid (**1**), hexacosanoate-1-glyceryl (**2**), 3*β*-urs-12,20(30)-diene-27,28-dioic acid (**3**), 3*β*-D-glucopyranosylurs-12,20(30)-diene-27,28-dioic acid (**4**), stigmasterol (**5**), stigmasterol-3-*O*-*β*-D-glucopyranoside (**6**), oleanolic-acid (**7**), 3-*O*-acetyl-oleanolic acid (**8**), betulin (**9**) and spinasterol-3*β*-*O*-D-glucopyranoside (**10**). The isolated compounds were characterised by using spectroscopic methods, 1D and 2D NMR, mass spectroscopy (ESI-MS) and by comparison with the literature data. To the best of our knowledge, compounds **1**, **3**, **4**, **8** and **10** were isolated for the first time from the *Cordia* genus. This result improves the chemotaxonomy knowledge of the *Cordia* genus. The antibacterial activities were performed by the Muller–Hinton agar diffusion method. The antibacterial activities were studied on *Salmonella typhi*, *Staphylococcus aureus*, *Vibrio cholerae*, *Pseudomonas aeruginosa* and *Escherichia coli* ATCC 25922. Compounds **8** and **9**, at 20.0 mg/mL resulted to be effective antimicrobial against *E. coli*, *V. cholerae* and *P. aeruginosa*.

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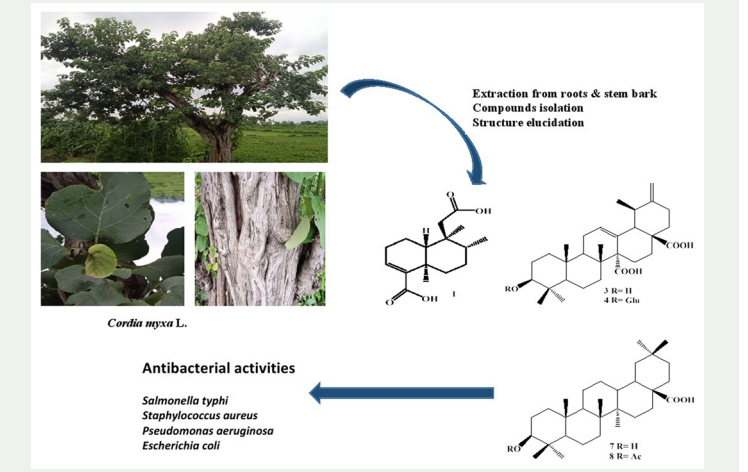

1. Introduction

Plant derived medicines are considered to be first in line of defense in maintaining health and combating diseases and even today plants are one of the principal source for new drugs with therapeutic properties (Samy et al. 2008). *Cordia* is a genus of deciduous flowering trees or shrubs belonging to the Boraginaceae family. It consists of more than 300 species distributed widely in the tropical region of both the hemispheres including East Africa, Mexico, West India, Central America, Central Africa, South America, Pakistan, West Africa, Nigeria, and Ghana (Manisha and Oza Yogesh 2016). *Cordia myxa L.* is a dioecious shrub or small tree up to 12 m tall. Its bole is tortuous or straight, the bark is grey and cracked, their branches spread and form a dense crown. Leaves are alternate, simple with the absence of stipules. Its petiole is 0.5–4.5 cm long. Flowers are unisexual, regular and white to creamy colour. The fruit is a globular to ovoid drupe 2–3.5 cm long, apiculate, enclosed at base by the accrescent calyx, yellow and apricot or blackish when ripe. The pulp is almost transparent, mucilaginous and sweet-tasting (Dubard 1915; Al-Snafi 2016).

The phytochemical screening carried out on the *Cordia* genus indicates the presence of different classes of natural products such as alkaloids, triterpenes, steroids, carbohydrates, flavonoids, coumarins, phenolic acids, saponins, together with resins, gums and mucilage (Al-Snafi 2016). The major secondary metabolites isolated from the genus include terpenoid hydroquinones, prenylated hydroquinones, meroterpenoid naphthoquinones, oleanane and ursane-type triterpenes, arylnaphthalene type lignins and dammarane-type triterpenes (Manisha and Oza Yogesh 2016).

The prenylated hydroquinone from the root bark of *Cordia alliodora* (Ruiz & Pev.) Oken exhibited antifungal and larvicidal activities against the phytopathogenic mould *C. cucumerinum* and the larvae of yellow fever transmitting mosquito, *Aedes aegypti*, respectively (Kaur et al. 2010). Triterpenoid acids isolated from *Cordia latifolia* Roxb. (syn. of *Cordia dichotoma* G.Forst.) showed nematicidal activities (Sabira et al. 2011). Flavonoid glycoside was isolated from the leaves of *C. dichotoma* G.Forst. (Rahman

and Akhtar 2016). A substituted furfuryl ester of stearic acid and aromatic compounds were isolated from the fruits and leaves of *C. latifolia*. (Siddiqui et al. 2010). The hexane extract and essential oil of *Cordia africana* Lam. exhibit potent cytotoxicity against breast cancer cell line most likely through apoptosis regulation (Abeer et al. 2020). Synthetic approaches have also been applied to obtain the potential bioactive methyl 2,4,5-trimethoxyphenylpropionate, a metabolite isolated from *C. alliodora* (Ruiz & Pav.) Oken (Sinha et al. 2003). The leaves of *C. myxa* possess aphrodisiac properties and are also useful in gonorrhoea and opthalmodynia treatment (Siddiqui et al. 2010). The stems extracts of *C. myxa* with dichloromethane, ethyl acetate, and methanol 80 % solvents by maceration method have weak drug ability to act as anti-HIV-1 agent (Rashed et al. 2014). Its mucilage, due to a high level of nutrients is a value added by-product in food and pharmaceutical systems (Keshani-Dokht et al. 2018). In Cameroon *C. myxa* is traditionally used to treat diarrhoea, stomachaches, fever, and urinary tract infections. The (23*R*)-campesta-9(11),24(30)-diene-18,23-diol-3-*O*-α-rham nopyranoside and alphitolic acid isolated from *C. myxa*, exhibited at 30 mg/mL effective antibacterial activity on *Salmonella typhi*, respectively 18.6 ± 0.6 mm and 14.5 ± 0.4 mm of inhibition zone (Dabolé et al. 2021), and this may suggest a chemical rationale for the traditional medicinal use of this plant species as antimicrobial. The objective of the present study is to isolate, characterise and assess the antibacterial effect of the phytocomponents obtained from the roots and the stem bark of *C. myxa*.

2. Results and discussion

2.1. Identification of the compounds

The structures of isolated compounds (Figure 1) were elucidated by means of spectroscopic and spectrometric evidences, mainly by 1D and 2D NMR, ESI-MS experiments and by comparison of experimental data with literature ones.

The phytochemical study of the ethyl acetate extract of the stem bark and roots of *C. myxa* led to the isolation of ten following known compounds: 1-naphthaleneacetic-5-carboxy-1,2,3,4,4a,7,8,8a-octahydro-1,2,4a-trimethyl-[1*S*-(*1α,2β*,4a,8a*α*)]-acid (**1**) previously isolated from *Jacaranda mimosifolia* D.Don (Sidjui et al. 2014), hexacosanoate-1-glyceryl (**2**) already detected in extract from *C. lutea* Lam. (Mayevych et al. 2015; Lainer et al. 2019), 3*β*-urs-12,20(30)-diene-27,28-dioic acid (**3**) previoulsy identified from *Crossopteryx febrifuga* Benth. (Chouna et al. 2015), 3*β*-D-glucopyranosylurs-12,20(30)-diene-27,28-dioic acid (**4**) isolated from *C. febrifuga* (Chouna et al. 2015), stigmasterol (**5**) already found in *Cordia exaltata* Lam (Nogueira et al. 2013; Mohammed et al. 2017; Nyemb et al. 2018), stigmasterol-3-*O*-*β*-D-glucopyranoside (**6**) previously observed in *Cordia millenii*, Baker (Ramaiarantsoa et al. 2008;), oleanolic-acid (**7**) identified in *Gardenia aqualla* Stapf & Hutch. (Seebacher et al. 2003; Ferreira et al. 2015; Nyemb et al. 2022), 3-*O*-acetyl-oleanolic acid (**8**), betulin (**9**) isolated from *Combretum glutinosum* Perr. ex DC (N'diaye et al. 2017; Ranjendra and Neha 2022) and spinasterol-3*β*-*O*-D-glucopyranoside (**10**) isolate from *Stewartia koreana* Nakai ex Rehder (syn. of *Stewartia pseudocamellia* Maxim.) (Lee et al. 2011). The occurrence of pentacyclic triterpenes of oleanane and ursane type with various functionalizations such as compounds **3**, **4**, **7** and **8** has been confirmed in *C. myxa* and already observed

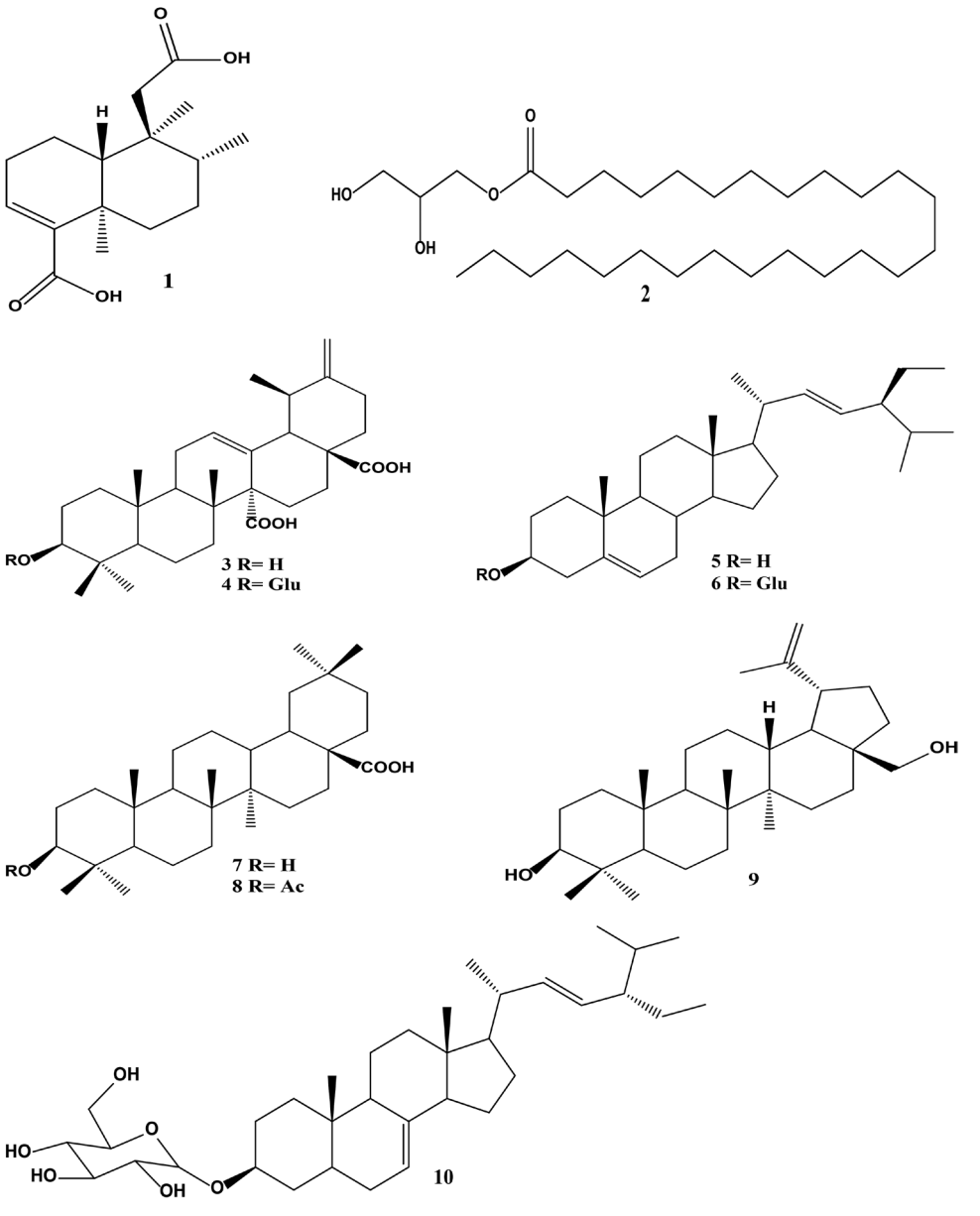

Figure 1. Chemical structures of isolated compounds **1**–**10** from *Cordia myxa L.*

in other *Cordia* species (Manisha and Oza Yogesh 2016) as well as the presence of saponins such as the glycosides of stigmasterol and spinasterol **6** and **8**. Pentacyclic triterpenes with ursane and oleanane skeleton, similar to phytosterols, have little chemotaxonomic relevance since their wider distribution in plant species (Venditti et al. 2015a, 2015b, 2016, 2017a; Frezza et al. 2020). Interestingly, compound **2** is one mono glyceride possibly involved in the metabolism of fats (mono-, di-, and tri-glycerides of fatty acids) in the studied organism. Compound **1** is formally one tetranorditerpene already observed in *Detarium microcarpum* Guill. & Perr. of Fabaceae

(formerly Leguminosae family) (Aquino et al. 1992), *Jacaranda mimosifolia* D. Don (Bignoniaceae) (Sidjui et al. 2014) and firstly isolated from Velloziaceae species (e.g. *Barbacenia bicolour* Mart., synonym of *Barbacenia brasiliensis* Willd.) (Barreiro et al. 1980). Compound **1** is possibly derived from one clerodane precursor but further studies on this aspect are necessary to confirm or not this hypothesis. If confirmed this could be of chemosystematic relevance because the clerodanes are not ubiquitous in plant species. In fact they are distributed in some specific plant families and in particular in Lamiaceae (Venditti et al. 2015c; Li et al. 2016; Venditti et al. 2017b; Frezza et al. 2018). Naphthaleneacetic acid in plants has auxin like function, stimulating plant growth and stress resistance (Zahedi et al. 2021) and therefore the analogous compound **1** recognised in the present study might have one specific function in the metabolism of this plant species. Also about these aspects, related to plant physiology and chemotaxonomy, further studies are auspicable in the near future.

2.2. Antibacterial activities

The antibacterial activities of compounds **1, 2, 3, 7, 8** and **9** was carried out by the Muller Hinton agar diffusion method, as proposed in the recommendations of the Antibiogram Committee of the French Society of Microbiology (CA-FSM/EUCAST 2016). The antibacterial activities were classified according to the DIZ (diameters of the inhibition zone, measured in millimeters) as follows: not sensitive (DIZ < 8.0 mm); moderately sensitive (8.0 < DIZ <14.0 mm); sensitive (14.0 < DIZ< 20.0 mm) and extremely sensitive (DIZ > 20.0 mm). The antibacterial activity of the compounds tested on these different strains, shows that activities depend on the concentrations of these compounds. At a high concentration of 20 mg/ml, compound **8** showed a moderate effectiveness on the three studied strains. The inhibition zone diameters recorded are 13.10 ± 0.3, 10.00 ± 0.1 mm and 12.00 ± 0.2 mm respectively for *Escherichia coli* ATCC 25922*, Vibrio cholerae* and *Pseudomonas aeruginosa* (Table S4). The colonies of *E. coli* ATCC 25922*, V. cholerae and P. aeruginosa* resulted to be moderately sensitive at the lower concentrations but sensitive at a highest concentration of 20 mg/mL, showing respectively 13.75 ± 0.2 mm, 15.00 ± 0.2 mm and 20.00 ± 0.2 mm (Table S4) of inhibition zone diameters. In addition, compound **1** resulted moderately effective on *Salmonella typhi*, *Staphylococcus aureus* and *P. aeruginosa* strains showing inhibition diameters of 10.50 ± 0.2 mm, 9.00 ± 0.2 mm and 12.00 ± 0.3 mm, respectively, at the concentration of 20 mg/mL (Table S3). At the same concentration, compounds **8** and **9** showed the same antibacterial potentials as the reference antibiotics such as chloramphenicol (C30) and nalidixic acid (NA30) which inhibition diameters are respectively 20.00 ± 0.4 mm and 20.00 ± 0.2 mm, on *P. aeruginosa* strains (Table S2). Furthermore, these two compounds showed the same effect as antibiotics such as amoxicillin + clavulanic acid (AMC30) and tetracyclin (TE30), respectively on *S. aureus* and *S. typhi* strains, obtaining inhibition zone of 20.00 ± 0.5 mm and 20.00 ± 0.2 mm of diameter. These results represent an effective antimicrobial activity as the inhibition diameters are between (14.0 < DIZ < 20.0 mm) (Table S2). However, *V. cholerae*, *E. coli* and *P. aeruginosa* strains resulted moderately sensitive when compound **8** was applied at a lower concentration (10 mg/mL), showing inhibition areas with 8.00 ± 0.1 mm, 11.00 ± 0.4 mm and 12.00 ± 0.1 mm of diameter, respectively. Compound **9** also inhibited moderately *V. cholerae* and *P. aeruginosa*

colonies at a dose of 10 mg/mL, showing inhibition diameters of 12.00 ± 0.5 mm and 11.00 ± 0.4 mm, respectively (Table S3). Similarly compound **1**, at a dose of 10 mg/mL, showed moderate effectiveness on *S. typhi* (8.30 ± 0.2 mm), *S. aureus* (8.00 ± 0.5 mm) and *P. aeruginosa* (11.50 ± 0.4 mm). At a concentration of 10 mg/mL compound **1** showed better performance than the antibiotics such as chloramphenicol (C30) (6.00 ± 0.1 mm), which resulted to be no effective antimicrobial against *S. typhi* strain (Table S2). Our results are similar to those obtained by Dabolé and colleagues (Dabolé et al. 2022), using the same method to evaluate the antibacterial activity of compounds isolated from *Gardenia ternifolia* (Schumach. & Thonn.). The antibacterial activity of compound **2** and **3** was also evaluated on the different strains, *S. typhi*, *S. aureus*, *P. aeruginosa* and *E. coli*, the results are presented in Table S3. Some of the identified compounds were not subjected to the antimicrobial test because these informations are already widely reported in the literature.

3. Conclusion

The phytochemical study of the ethyl acetate extract of the stem bark and root of *C. myxa* led to the isolation of ten known compounds (**1–10**). To the best of our knowledge, compounds **1**, **3**, **4**, **8** and **10** are reported here for the first time from species belonging to the *Cordia* genus. This result improves the knowledge about the phytochemistry of *Cordia* genus even if not so relevant to the chemotaxonomy standpoint due to the wide distribution of these natural products in the Plant Kingdom. In this context is worth of mention the possible role of compound **1** related to plant physiology and chemotaxonomy but further studies are necessary to confirm or not these hypothesis. The antibacterial activities of compounds **1, 2, 3, 7, 8** and **9** was evaluated on *E. coli*, *S. typhi*, *S. aureus, P. aeruginosa* and *V. cholerae* stains. Compounds **8** and **9**, at a concentration of 20.0 mg/mL, resulted active against *S. typhi*, *S. aureus* and *P. aeruginosa*.

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