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Phytochemical analysis and biological evaluation of three selected *Cordia* species from Panama

Georgia Marini[a], Konstantia Graikou[a], Gökhan Zengin[b], George A. Karikas[c], Mahabir P. Gupta[d], Ioanna Chinou[a,*]

[a] Division of Pharmacognosy and Chemistry of Natural Products, Department of Pharmacy, National & Kapodistrian University of Athens, Greece
[b] Department of Biology, Science Faculty, Selcuk University, University Campus, Konya, Turkey
[c] Department of Medical Laboratories Technology, Technological and Educational Institute of Athens, Greece
[d] Center for Pharmacognostic Study of Panamanian Flora, Faculty of Pharmacy, University of Panama, Panama

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ABSTRACT

The aim of the present study was the phytochemical analysis of the leaves from three not previously studied tropical species *Cordia bicolor*, *Cordia megalantha* and *Cordia dentata* (Boraginaceae) collected from Panama. The genus *Cordia* is one of the major and most important of the family and involves a wide range of therapeutic uses in traditional medicine. Eleven known compounds allantoin **(1)**, rosmarinic acid **(2)**, caffeic acid **(3)**, isoquercetin **(4)**, rutin **(5)**, quercetin-3-*O*-β-D-neohesperidoside **(6)**, kaempferol 3-*O*-β-D-neohesperidoside **(7)**, helichrysoside **(8)**, kaempferol 3-*O*-(2″-*O*-α-L-rhamnosyl-6″*trans*-p-coumaroyl)-β-D-glucoside **(9)**, quercetin 3-*O*-(6″*trans*-p-coumaroyl)-β-D-galactoside **(10)**, 4-hydroxyphenyl lactic acid **(11)**, have been isolated and structurally elucidated. Compounds **6** and **7** have been isolated for the first time in *Cordia* genus, compounds **8-10** are reported for the first time in the Boraginaceae family, while secondary metabolite **9** is isolated as natural product for the second time. The methanolic extracts of the plants have been assayed for their antioxidant properties by free radical scavenging, reducing power, phosphomolybdenum and metal chelating assay. Enzyme inhibitory activity has been also evaluated against cholinesterases, α-amylase and α-glucosidase. *C. megalantha* exhibited the strongest antioxidant activity compared to the other studied *Cordia* species and a high inhibitory activity against α-glucosidase, which suggests that this herbal material could be used for further studies as a potential source for therapeutic applications.

1. Introduction

Over the past decades, plants and theirs biologically-active compounds have proved to be potent natural agents (De Monte et al., 2015; Mocan et al., 2017; Zengin et al., 2017). The *Cordia* genus (Boraginaceae) comprises more than 3000 species, mostly evergreen trees and shrubs distributed widely in the tropical regions (Oza and Kulkarni, 2017). Plants from this genus have been widely studied with regard to the various ethnobotanical and ethnopharmacological aspects. Leaves, fruit, bark and seed of a majority of the species are extensively used in traditional medicine for antimicrobial, anti-inflammatory, anthelmintic, analgesic and diuretic purposes and for treating digestive system, respiratory, urogenital, cardiac, vascular and blood disorders (Kumari et al., 2016; Matias et al., 2015; Oza and Kulkarni, 2017). Moreover, compounds isolated from *Cordia* species have displayed a broad range of biological activities, including anti-inflammatory, antimicrobial (Menezes et al., 2001), antifungal (Nariya et al., 2011) and analgesic activity (Ficarra et al., 1995).

Various secondary metabolites like flavonoids, triterpenes, sesquiterpenes, tannins, naphtoquinones, alkaloids and fatty acids have been isolated from different parts of *Cordia* plants (Jasiem et al., 2016; Matias et al., 2015; Oza and Kulkarni, 2017).

To the best of our knowledge, this is the first study of *Cordia bicolor, Cordia megalantha* and *Cordia dentata* leaves, while traditionally a decoction of *Cordia dentata* flowers has been used to treat bronchitis (Grandtner and Chevrette, 2013), and its extract has previously been studied yielding rosmarinic acid and the flavonoids rutin and quercetin 3-*O*-rhamnosyl-(1 → 6)-galactoside (Ferrari et al., 1997).

In the framework of our research on Boraginaceae species (Damianakos et al., 2016, 2014, 2013, 2012; Fouseki et al., 2016), we report the isolation and structural elucidation of secondary metabolites from the methanolic leaf extracts, which were found to be used most frequently to treat many ailments (Oza and Kulkarni, 2017), of three *Cordia* species from Panama as well as the biological evaluation of their

* Corresponding author.
E-mail address: ichinou@pharm.uoa.gr (I. Chinou).

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antioxidant and enzyme inhibitory activity. Different chemical assays were employed to detect antioxidant effects, including free radical scavenging, reducing power, phosphomolybdenum and metal chelating. Cholinesterases, α-amylase and α-glucosidase were selected as targets to evaluate enzyme inhibitor effects, which linked to global health problems.

2. Material and methods

2.1. General

High resolution mass spectra (ESI) were recorded on a Thermo Scientific LTQ Orbitrap Discovery mass spectrometer. ^{1}H NMR, 2D-NMR (400 MHz) and ^{13}C NMR (50 MHz) were recorded on Bruker DRX400 and Bruker AC200 spectrometers, respectively, with TMS as an internal standard. The stationary phases used for column chromatography were silica gel (Merck), Sephadex LH-20 (25–100 µm, Pharmacia), microcrystalline cellulose (20–160 µm, Merck) and XAD-4 resin (0.90–0.30 mm, Supelco). Kieselgel 60 F254, 0.2 mm layer thickness and cellulose 20 × 20 cm precoated plates (Merck) were used for thin layer chromatography (TLC). Zones on TLC plates were detected under UV light (254 and 366 nm) and sprayed with a methanolic solution of 2.5% H_2SO_4 and 2.5% vanillin regarding the silica plates or with Naturstoff reagent (1% complex solution of 2-aminoethyl diphenyl borate acid in MeOH) regarding the cellulose plates, followed by heating. The solvents used were HPLC grade.

2.2. Plant material

Cordia megalantha leaves (voucher specimen no. 8226) were gathered at La Messa, El Valle de Anton at 20/3/2009, *C. bicolor* leaves (voucher specimen no. 7077) were collected in Cerro Azul, Finca Casa Club at 31/05/2007 and *C. dentata* leaves (voucher specimen no. 2331) were collected in Sarigua, Herrea at 26/11/2005.

Plant species were botanically identified by Mireya Correa, Director of the Herbarium of Panama University (PMA), where voucher specimens are deposited.

Plant parts were air dried and pulverized in a Wiley Mill and stored in zip lock bags in dark until further analysis.

2.3. Extraction and isolation

The air-dried and powdered leaves of *C. bicolor* (250 g) were successively extracted with MeOH, by immersion in 2.5 L of the solvent for 24 h, three times at room temperature (r.t.). The methanolic extract (12.4 g) was first fractionated by XAD-4 resin column chromatography and eluted with H_2O, H_2O/methanol (MeOH) 50:50 and MeOH, affording three fractions (Frs. A1-3). Part of Fr. A1 (0.8 g) was further purified by Sephadex LH-20 column chromatography eluted with MeOH, to yield pure compound **1** (10.0 mg). Part of Fr. A2 (1.0 g) was subjected to microcrystalline cellulose column chromatography, eluted with gradients of cyclohexane/ethyl acetate (EtOAc) (100:0 to 0:100) and EtOAc/MeOH (100:0 to 85:15) solvent systems, to obtain 20 fractions (Frs. A2.1-20). Fr. A2.13, FrA2.14 and FrA2.15 were further purified via cellulose prep TLC (development with H_2O/acetic acid (AcOH) 85:15, extraction of the scraped-off zone with 90/10 MeOH/H_2O) and the compounds **5** (6.5 mg), **6** (5.0 mg) and **7** (3.2 mg) were isolated respectively. Part of Fr. A3 (1.1 g) was also fractionated by silica gel column chromatography eluting with gradients of cyclohexane/dichloromethane (CH_2Cl_2) (100:0 to 0:100) and CH_2Cl_2/MeOH (98:2 to 50:50) solvent systems. Eighteen fractions (Frs. A3.1-18) were obtained. Fr. A3.3 and Fr. A3.5 were further purified with cellulose prep TLC (development with H_2O/AcOH 80:20, extraction of the scraped-off zone with 90:10 MeOH/H_2O) to afford compounds **2** (6.2 mg), **3** (4.0 mg) and **4** (4.5 mg). Fr. A3.4 was further separated by silica gel column chromatography eluting with gradients of CH_2Cl_2/MeOH (100:0 to 50:50) solvent systems and the compound **8** (6.0 mg) was isolated. Fr. A3.6 afforded compound **9** (9 mg).

The air-dried and powdered leaves of *C. megalantha* (250 g) were extracted as described above. The methanolic extract (4.8 g) was fractionated by silica gel column chromatography (CH_2Cl_2/MeOH 100:0 to 85:15) solvent systems to yield 10 fractions (Frs M1-10). Fraction M6 (200 mg) was subjected to Sephadex LH-20 column chromatography, eluted with MeOH to yield the pure compound **10** (25.5 mg). The fraction M9 with cellulose preparative-TLC (development with MeOH: AcOH 80:20, extraction of the scraped-off zone with 90:10 MeOH/H_2O) led to the isolation of compound **2** (4.0 mg).

The air-dried and powdered *C. dentata* leaves (80 g) were extracted as described above. The methanolic extract (3.9 g) was fractionated by Sephadex LH-20 column chromatography, eluted with MeOH and 22 fractions were obtained (Frs. D1-22). Fr D13 and Fr D20 afforded pure compounds **11** (15 mg) and **2** (6 mg), while Frs D12 and D14 were further purified by cellulose prep TLC (solvent system H_2O/AcOH 85:15) in order to yield compounds **3** (3.5 mg) and **5** (4.5 mg), respectively.

2.4. Determination of total bioactive components

2.4.1. Total phenolic content

The total phenolic content was determined by the method of Slinkard and Singleton (1977) with slight modifications. Sample solution (0.25 mL) was mixed with diluted Folin-Ciocalteu reagent (1 mL, 1:9) and shaken vigorously. After 3 min, Na_2CO_3 solution (0.75 mL, 1%) was added and the sample absorbance was read at 760 nm after 2 h incubation in dark at r.t. The total phenolic content was expressed as equivalents of gallic acid (mgGAE/g extract).

2.4.2. Total flavonoid content

The total flavonoid content was determined using the Dowd method as adapted by Berk et al. (2011). Briefly, sample solution (1 mL) was mixed with the same volume of aluminium trichloride (2%) in methanol. Similarly, a blank was prepared by adding sample solution (1 mL) to methanol (1 mL) without $AlCl_3$. The absorbances were read at 415 nm, after 10 min incubation at r.t. The total flavonoid content was expressed as equivalents of rutin (mgRE/g extract).

2.4.3. Phosphomolybdenum method

The total antioxidant activity of the samples was evaluated by phosphomolybdenum method according to literature (Zengin et al., 2015) with slight modification. Sample solution (0.3 mL) was combined with 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The sample absorbance was read at 695 nm, after 90 min incubation at 95 °C. Trolox was used as a positive control and the total antioxidant capacity was expressed as Trolox equivalent (mmolTE/g extract).

2.4.4. 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

The effect of the samples on DPPH radical scavenging activity was estimated according to Zengin et al. (2015). The nitrogen-centered DPPH radical is often used as an indicator to measure the radical scavenging capacity of antioxidants. Sample solution (1 mL) was added to a 4 mL of a 0.004% methanol solution of DPPH. The sample absorbance was read at 517 nm after 30 min incubation in dark at r.t. Trolox was used as a positive control and DPPH radical scavenging activity was expressed as Trolox equivalent (mgTE/g extract).

2.4.5. 2,2 Azino-bis (3-ethylbenzothiazloine-6-sulfonic acid) (ABTS) radical scavenging activity

The scavenging activity against ABTS radical cation was measured according to the method of Zengin et al. (2015). $ABTS^{+}$ radical is soluble in both aqueous and organic solvent media, so it enables the simultaneous determination of hydrophilic and lipophilic antioxidants.