Industrial Crops & Products 120 (2018) 84–89

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop

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

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ARTICLE INFO

Keywords:
Cordia
Boraginaceae
Phenolics
Flavonol glucosides
Antioxidant activity
Enzyme inhibitory activity

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

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https://doi.org/10.1016/j.indcrop.2018.04.037
Received 8 February 2018; Received in revised form 11 April 2018; Accepted 15 April 2018

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.

Briefly, $ABTS^{+}$ radical cation was produced directly by reacting 7 mM ABTS solution with 2.45 mM potassium persulfate and allowing the mixture to stand for 12–16 min in dark at r.t. ABTS solution was diluted with methanol to an absorbance of 0.700 ± 0.02 at 734 nm. Sample solution (1 mL) was added to ABTS solution (2 mL) and mixed. The sample absorbance was read at 734 nm after 30 min incubation at r.t. Trolox was used as a positive control and ABTS radical cation scavenging activity was expressed as Trolox equivalent (mgTE/g extract).

2.4.6. Cupric ion reducing antioxidant capacity (CUPRAC)

The cupric ion reducing activity (CUPRAC) was determined according to the method of Zengin et al. (2015). During the CUPRAC method the copper(II)-neocuproine(2,9-dimethyl-1,10-phenanthroline) reagent can oxidize antioxidant compounds that are soluble in water or oil, enabling the simultaneous determination of hydrophilic and lipophilic antioxidants, itself being reduced to the colored copper(I)-neocuproine chelate complex.

Sample solution (0.5 mL) was added to premixed reaction mixture containing $CuCl_2$ (1 mL, 10 mM), neocuproine (1 mL, 7.5 mM) and NH_4Ac buffer (1 mL, 1 M, pH 7.0). Similarly, a blank was prepared by adding sample solution (0.5 mL) to premixed reaction mixture (3 mL) without $CuCl_2$. The absorbances were read at 450 nm, after 30 min incubation at r.t. Trolox was used as a positive control and CUPRAC activity was expressed as Trolox equivalents (mgTE/g extract).

2.4.7. Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was carried out as described by Zengin et al. (2015). The ferric-reducing antioxidant power (FRAP) method is based on the reduction of the Fe(III)-complex of 2,4,6-tripyridyl-*s*-triazine (TPTZ) by antioxidants to the intensely colored Fe(II)-TPTZ chelate. Sample solution (0.1 mL) was added to premixed FRAP reagent (2 mL) containing acetate buffer (0.3 M, pH 3.6), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) (10 mM) in 40 mM HCl and ferric chloride (20 mM) in a ratio of 10:1:1 (v/v/v). The sample absorbance was read at 593 nm after 30 min incubation at r.t. Trolox was used as a positive control and FRAP activity was expressed as Trolox equivalents (mgTE/g extract).

2.4.8. Metal chelating activity on ferrous ions

The metal chelating activity on ferrous ions was determined by the method described by Zengin et al. (2015). Briefly, sample solution (2 mL) was added to $FeCl_2$ solution (0.05 mL, 2 mM). The reaction was initiated by the addition of 5 mM ferrozine (0.2 mL). Similarly, a blank as prepared by adding sample solution (2 mL) to $FeCl_2$ solution (0.05 mL, 2 mM) and water (0.2 mL) without ferrozine. The absorbances were read at 562 nm after 10 min incubation at r.t. EDTA was used as a positive control and the metal chelating activity was expressed as equivalents of EDTA (mg EDTAE/g extract).

2.5. Enzyme inhibitory activity

2.5.1. Cholinesterase inhibition

Cholinesterase (ChE) inhibitory activity was measured using Ellman's method, as previously reported (Zengin, 2016) .Briefly, sample solution (50 µL) was mixed with DTNB (125 µL) and acetylcholinesterase (AChE) (or butyrlcholinesterase (BChE)) solution (25 µL) in Tris-HCl buffer (pH 8.0) in a 96-well microplate and incubated for 15 min at r.t. The reaction was then initiated with the addition of acetylthiocholine iodide (ATCI) or butyrylthiocholine chloride (BTCl) (25 µL). Similarly, a blank was prepared by adding sample solution to all reaction reagents without enzyme (AChE or BChE) solution. The absorbances were read at 405 nm after 10 min incubation at r.t. The absorbance of the blank was subtracted from that of the sample. Galanthamine was used as a positive control and the cholinesterase inhibitory activity was expressed as equivalents of galanthamine (mgGALAE/g extract).

2.5.2. α-Amylase inhibition

α-amylase inhibitory activity was performed using Caraway-Somogyi iodine/potassium iodide (IKI) method (Zengin, 2016). Briefly, sample solution (25 µL) was mixed with α-amylase solution (50 µL) in phosphate buffer (pH 6.9 with 6 mM sodium chloride) in a 96-well microplate and incubated for 10 min at 37 °C. After pre-incubation, the reaction was initiated with the addition of starch solution (50 µL, 0.05%). Similarly, a blank was prepared by adding sample solution to all reaction reagents without enzyme (α-amylase) solution. The reaction mixture was incubated 10 min at 37 °C. The reaction was then stopped with the addition of HCl (25 µL, 1 M). This was followed by addition of the iodine-potassium iodide solution (100 µL). The absorbances were read at 630 nm. The absorbance of the blank was subtracted from that of the sample. Acarbose was used as positive control and the α-amylase inhibitory activity was expressed as equivalents of acarbose (mmolACAE/g extract).

2.5.3. α-Glucosidase inhibition

α-glucosidase inhibitory activity was performed using 4-Nitrophenyl-β-D-glucopyranoside (PNPG) as a substrate (Zengin, 2016). Briefly, sample solution (50 µL) was mixed with glutathione (50 µL), α-glucosidase solution (50 µL) in phosphate buffer (pH 6.8) and PNPG (50 µL) in 96-well microplate and incubated for 15 min at 37 °C. Similarly, a blank was prepared by adding sample solution to all reaction reagents without enzyme (α-glucosidase) solution. The reaction was then stopped with the addition of sodium carbonate (50 µL, 0.2 M). The absorbances were read at 400 nm. The absorbance of the blank was subtracted from that of the sample. Acarbose was used as positive control and the α-glucosidase inhibitory activity was expressed as equivalents of acarbose (mmolACAE/g extract).

2.6. Statistical analysis

All the assays were carried out in triplicate. The results were expressed as mean value and standard deviation (mean ± SD). Statistical differences between the extracts were analyzed by using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference post hoc test ($\alpha = 0.05$). All the analysis was carried out using SPSS v22.0 software.

3. Results and discussion

3.1. Chemical composition of extracts

The methanolic extract of *C. bicolor* was subjected to chromatographic separations to yield nine compounds. The ureide allantoin **(1)**, which was obtained as white solid, was identified after comparison of its spectroscopic data with those reported in the literature (Damianakos et al., 2013). Allantoin occurs often in the Boraginaceae family (Fell and Peck, 1968; Romussi et al., 1979) as well as in the *Cordia* genus (Chauhan and Srivastava, 1978; Fouseki et al., 2016; Menezes et al., 2001). The rosmarinic acid **(2)**, which was identified after comparison of its spectroscopic data with those reported in the literature (Ly et al., 2006), has been reported several times in *Cordia* species like *C. americana* (Fernández et al., 2013), *C. latifolia* (Begum et al., 2011), *C. sebestena* (Dai et al., 2010), *C. spinescens* (Lim et al., 1997) and *C. verbenacea* (Ticli et al., 2005). Caffeic acid **(3)**, which was confirmed through spectroscopic data (Jeong et al., 2011), has been previously reported from the Boraginaceae family, from the species *Tournefortia sarmentosa* (Chen et al., 2013), *Borago officinalis* (Thanina et al., 2014) as well as from the *Cordia* genus (*C. martinicensis*, *C. myxa*, *C. polycephala*, *C. serratifolia*) (Ficarra et al., 1995).

Six flavonoids have been also isolated: isoquercetin **(4)** (Kuruüzüm-Uz et al., 2013), that has been previously reported from *C. sinensis* (Al-Musayeib et al., 2011), rutin **(5)** (Kazuma et al., 2003) that has been previously reported from *C. dichotoma*, (Sharma et al., 2015) *C.*

verbenacea (Matias et al., 2013) and *C. americana* (Geller et al., 2010), quercetin-3-*O*-β-D-neohesperidoside **(6)** and kaempferol 3-*O*-β-D-neohesperidoside **(7)** (Kazuma et al., 2003; Norbaek and Kondo, 1999), which are reported for the first time from the *Cordia* genus, while they have been previously isolated from Boraginaceae species such as *Echium plantagineum* (Moita et al., 2014). Helichrysoside **(8)** (Alves et al., 2012; Barosso et al., 2014) which has been isolated for the first time from Boraginaceae family but it has been previously reported from Asteraceae (Barosso et al., 2014), Agavaceae (Fouedjou et al., 2016), Iridaceae (Duke, 1992), and Lamiaceae families (Karioti et al., 2007) and kaempferol 3-*O*-(2″-*O*-α-L-rhamnosyl-6″*trans*-p-coumaroyl)-β-D-glucoside **(9)**, which has been previously reported only once from the plant *Gaura longiflora* of the Onagraceae family (Xu et al., 2009).

The methanolic extract of *C. megalantha* leaves afforded rosmarinic acid (**2**) and quercetin 3-*O*-(6″*trans*-p-coumaroyl)-β-D-galactopyranoside **(10)** (Jin et al., 1999; Teponno et al., 2006). This flavonol glucoside is reported for the first time at the Boraginaceae family but it has been previously reported from species of the families Ericaceae (Jin et al., 1999), Rosaceae (Qing et al., 2012) and Dioscoreaceae (Teponno et al., 2006).

The methanolic extract of *C. dentata* yielded compounds **2**, **3**, **5** as well as 4- hydroxyphenyl lactic acid (**11**), which has been previously reported in *C. rothii*, *C. sinensis* (Firdous et al., 2014) and *C. macleodii* (Thirupathi et al., 2008).

3.2. Phenolic content

Total phenolic content of the methanolic extracts was determined using the Folin-Ciocalteu colorimetric method and expressed as gallic acid equivalents (GAE/g extract). The results are presented in Table 1. The highest phenolic content was found in *C. megalantha* (93.10 mgGAEs/g extract), followed by *C. bicolor* (72.13 mgGAEs/g extract) and *C. dentata* (40.52 mgGAEs/g extract). The obtained values are comparable to the values reported in the literature for other *Cordia* species such as *Cordia evolutior* (25.40 mgGAE mg/g extract, reported by Arunachalam and Parimelazhagan (2014); *C. boissieri* (230 mgGAE/100 g extract, reported by Viveros-Valdez et al. (2016); *C. perrottettii* (145 mgGAE/g extract, reported by Marwah et al. (2007). In this context, the *Cordia* genus could be considered as a promising source in terms of phenolics.

3.3. Flavonoid content

Total flavonoid content was determined $AlCl_3$ method and expressed as rutin equivalents (RE/g extract). Flavonoid content of the extracts ranged from 40.81 mg REs/g extract for *C. bicolor* to 21.56 mgREs/g extract for *C. dentata* (Table 1). Through these results, we can conclude that flavonoids constitute a major part of phenolics in the methanolic extract of *C. bicolor* extract, which is in accordance with the phytochemical analysis, as the majority of substances isolated from this extract belong to the chemical category of flavonoids. In accordance with our results, several flavonoids have also been isolated from various *Cordia* species such as *C. dichotoma* (Wang et al., 1996), *C. exaltata* (Nogueira et al., 2013), *C. macleodii* (El-Sayed et al., 1998), *C. polycephala, C. serratifolia* (Ficarra et al., 1995) and *C. sinensis* (Al-Musayeib et al., 2011).

Table 1
Antioxidant properties of *Cordia* methanolic extracts.*

Antioxidant assays	*C. dentata*	*C. megalantha*	*C. bicolor*
Total phenolic content (mgGAE/g extract)	40.52 ± 0.62^c	93.10 ± 1.54^a	72.13 ± 1.23^b
Total flavonoid content (mgRE/g extract)	21.56 ± 0.64^c	34.68 ± 0.24^b	40.81 ± 0.45^a
Phosphomolybdenum assay (mmolTE/g extract)	1.35 ± 0.09^c	3.28 ± 0.22^a	2.08 ± 0.15^b
DPPH (mgTE/g extract)	101.42 ± 2.13^c	195.68 ± 0.30^a	166.83 ± 1.59^b
ABTS (mgTE/g extract)	152.45 ± 1.06^c	476.78 ± 1.26^a	268.70 ± 10.65^b
CUPRAC (mgTE/g extract)	229.82 ± 5.30^c	661.82 ± 8.13^a	378.88 ± 8.12^b
FRAP (mgTE/g extract)	129.20 ± 4.68^c	370.46 ± 9.06^a	242.36 ± 9.67^b
Chelating activity (mgEDTAE/g extract)	14.31 ± 0.86^a	14.98 ± 0.04^a	4.15 ± 0.09^b

GAE: Gallic acid equivalents; RE: Rutin equivalents; TE: Trolox equivalents; EDTAE: EDTA equivalents. *Data from three repetitions, with mean ± standard deviation; means with different superscript letters (a, b and c) in the same line were significantly ($p < 0.05$) different.

3.4. Antioxidant activity

Antioxidant properties of *Cordia* extracts were evaluated using different assays including free radical scavenging (ABTS and DPPH), reducing power (CUPRAC and FRAP), phosphomolybdenum and ferrous ion chelating tests.

The DPPH scavenging ability showed the highest value for *C. megalantha* (195.68 ± 0.30 mgTEs/g extract), followed by *C. bicolor* (166.83 mgTEs/g extract) and *C. dentata* (101.42 mgTEs/g extract). Similarly to DPPH assay, the ABTS scavenging activity of the *Cordia* extracts followed the same descending order: *C. megalantha*, *C. bicolor*, *C. dentata* (Table 1) showing that the radical scavenging activity of the methanolic extracts follows the similar trend with total phenolic and flavonoid content.

The reducing ability of the *Cordia* extracts were assayed via FRAP and CUPRAC assays as illustrated in Table 1. The *C. megalantha* extract had the highest effectiveness in both the assays followed by the *C. bicolor* and *C. dentata* extracts demonstrated the least reducing power. This is not surprising since these extracts contained lower phenolic content.

The total antioxidant effect of the *Cordia* extracts was also evaluated by phosphomolybdenum method. In a descending order they could be ranked as *C. megalantha* (3.28 mmolTEs/g extract), *C. bicolor* (2.08 mmolTEs/g extract), *C. dentata* (1.35 mmolTEs/g extract) (Table 1). These results are also in accordance with the total phenolic content.

Moreover, the ferrous ion chelating abilities of the extracts were evaluated showing that *C. megalantha* and *C. dentata* possessed the best metal chelating ability (14.98 and 14.31 mg EDTAEs/g extract, respectively) (Table 1). Contrast to other antioxidant assays, these results may be explained with the presence of non-phenolic chelators and synergetic or antagonistic action of phytochemicals (Kalogeropoulos et al., 2013; Sarikurkcu et al., 2015).

These results are in accordance with reported data of other Cordia species, as *C. sinensis* (methanolic extract) and *C. macleodii* (alcoholic extract) have showed significant antioxidant (Matias et al., 2015) and hepatoprotective activities respectively (Qureshi et al., 2009). Moreover, C. verbenacea's extract, has been reported that can provide protection against lipid peroxidation and damage caused by free radicals (Michielin et al., 2011).

3.5. Enzyme inhibitory activity

The enzyme inhibitory activity of *Cordia* methanolic extracts was determined by spectrophotometric methods against acetylcholinesterase (AChE), butyrylcholinesterase (BChE), α-amylase and α-glucosidase using the already published methodology (Zengin, 2016).

Cholinesterase inhibitors are clinically used in the first line of treatment for Alzheimer's diseases (Howes et al., 2003). The results are demonstrated in Table 2. In the AChE inhibitory assay *C. megalantha* exhibited the strongest activity (2.12 mgGALAEs/g extract) followed by *C. dentata* (1.96 mgGALAEs/g extract) and *C. bicolor* (1.65 mgGALAEs/g extract). In the BChE inhibitory assay the highest inhibitory activity was demonstrated by C. *bicolor* followed by *C. dentata* while *C.*

Table 2
Enzyme inhibitory activity of *Cordia* methanolic extracts.*

Enzyme Inhibition assays	*C. dentata*	*C. megalantha*	*C. bicolor*
AChE Inhibition (mgGALAE/g extract)	1.96 ± 0.09[b]	2.12 ± 0.06[a]	1.65 ± 0.04[c]
BChE Inhibition (mgGALAE/g extract)	1.10 ± 0.08[b]	na	3.63 ± 0.47[a]
Amylase Inhibition (mmolACAE/g extract	3.27 ± 0.08[b]	3.46 ± 0.11[a]	3.17 ± 0.09[b]
Glucosidase Inhibition (mmolACAE/g extract)	11.89 ± 2.07[c]	32.16 ± 0.70[a]	15.40 ± 1.23[b]

GALAE: galantamine equivalents; ACAE: acarbose equivalents; na: not active.
*Data from three repetitions, with mean ± standard deviation; means with different superscript letters (a, b and c) in the same line were significantly ($p < 0.05$) different.

megalantha was inactive.

Hyperglycemia can be characterized by high circulating blood glucose levels particularly after fasting and consumption of carbohydrate meals. Pancreatic glucosidase and amylase play very important roles due to their hydrolytic effect on starch and oligosaccharides (Mocan et al., 2017; Zengin et al., 2017). Thus, an important strategy for managing hyperglycemia is to inhibit glucosidase and amylase. The studied extracts exhibited a moderate activity to these enzyme inhibition assays excluding *C. megalantha,* which showed a high inhibitory activity against α-glycosidase (32.16 mmolACAE/g extract).

4. Conclusion

The phytochemical analysis of the leaf extracts from three *Cordia* species led to the isolation of eleven secondary metabolites from which two were isolated for the first time from *Cordia* genus (**6** and **7**) and three of them (**8-10**) for the first time from the Boraginaceae family, while secondary metabolite **9** is not a trivial flavonoid derivative, isolated as a natural product for the second time.

The three methanolic extracts of the plants were evaluated for their phenolic and flavonoid content and their antioxidant capacity. Moreover, the anticholinesterase and antidiabetic effect of the extracts was determined by evaluating their inhibitory activities on linked key enzymes.

C. megalantha showed the highest phenolic content as well as the highest antioxidant activity in all assays, suggesting the direct correlation of the phenolic content with the antioxidant activity. Furthermore, this species showed a high inhibitory activity against α-glucosidase, which could further confirm that, the antioxidant activity and the phenolic content contribute to the enzyme inhibitory activity. In this concept, the present study could provide valuable informations on the phytochemical pool for the genus *Cordia* and it can stand a bridge for future bioassays and potential further uses in food supplements' area and phytotherapeutical applications of this important botanical species.

Acknowledgments

Prof. M.P. Gupta wishes to acknowledge SNI program of SENACYT, Panama and Ministry of Environment for authorizing plant collections

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