Peer-reviewed version available at *Molecules* **2018**, *23*, 1892; doi:10.3390/molecules23081892

Phytochemical and Biological Investigation of *Jatropha pelargoniifolia* Root Native to the Kingdom of Saudi Arabia

Hanan Y. Aati[1], Ali A. El-Gamal[1,2], Oliver Kayser[3]

[1]Department of Pharmacognosy, Faculty of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia

[2]Department of Pharmacognosy, College of Pharmacy, Mansoura University, El-Mansoura 35516, Egypt

[3]TU Dortmund University, Technical Biochemistry, Emil-Figge-Strasse 66, D-44227 Dortmund, Germany

Corresponding Author

Ali Ali El-Gamal

[1]Department of Pharmacognosy, Faculty of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia. Phone +966-569780176.

E-mail: aelgamel@ksu.edu.sa

Peer-reviewed version available at *Molecules* **2018**, *23*, 1892; doi:10.3390/molecules23081892

Abstract

Extensive phytochemical and chromatographic analysis of different root fractions of *Jatropha pelargoniifolia* Courb. (Euphorbiaceae) resulted in the isolation and identification of **22** distinct secondary metabolite compounds. Two new compounds, 6-hydroxy-8-methoxycoumarin-7-*O*-*β*-D-glycopyranoside and (3-(2-(methylamino) ethyl)-1H-indol-2-yl) methanol, were isolated and identified for the first time from a natural source. In addition, other known compounds, such as hovetricoside C and N-methyltryptamine were isolated from Euphorbiaceae, and hordenine, N-methyltyramine, their salts, cynaroside and linarin were identified in *Jatropha* spp. for the first time. Some isolated metabolites, such as β-sitosterol, β-sitosterol glucoside, curcusons D and C, naringenin, apigenin, cleomiscosins B and A, spruceanol, propacin, jatrophadiketone**,** and uracil were previously identified in various *Jatropha* species. The structures of the isolated compounds were determined using different spectroscopic techniques. The anti-inflammatory, antinociceptive, antipyretic, and antioxidant activities were evaluated for some adequately available isolated compounds. Compounds showed significant antinociceptive activity compared with the standard analgesic drug indomethacin. The edema size was significantly reduced ($p< 0.05$–0.001) in the animals treated with low doses (5 and 10 mg/kg) of the isolated compounds compared with those treated with a high dose (100 mg/kg) of standard anti-inflammatory drug (phenylbutazone). Furthermore, all tested compounds showed a significant ($p< 0.05$–0.001) reduction in the rectal temperature of hyper-thermic mice.

Key words: *Jatropha pelargoniifolia*; alkaloids; flavonoids; coumarinolignans; diterpenes; anti-inflammatory; antinociceptive; antipyretic; free radical scavenging.

Peer-reviewed version available at *Molecules* **2018**, *23*, 1892; doi:10.3390/molecules23081892

1. Introduction

The Euphorbiaceae family, which is considered as one of the largest families of flowering plants, includes approximately 7,800 species distributed among approximately 300 genera and five subfamilies worldwide. These species occur preferentially in tropical and subtropical regions [1, 2]. Among the main genera of this family, *Jatropha* L. belongs to the subfamily Crotonoideae (tribe Jatropheae) and is represented by approximately 200 species. This genus is widely distributed in tropical and subtropical regions of Africa and the Americas [2].

Jatropha is a Greek word derived from *"jatros"* meaning "physician" and "*trophe"* meaning "food" and is associated with its medicinal uses [3]. *Jatropha* species are used in folk medicine to treat various diseases, such as skin inflammation, eye infection, chest pain, stomach pain, itching, and as a vermifuge or as ornamental plants and energy crops in Latin America, Africa, and Asia [3]. Several known species from the genus *Jatropha*, such as *J. gossypiifolia*, *J. elliptica, J. curcas,* and *J. mollissima*, among others, have been reported for their chemical constituents, biological activities, and medicinal uses [4].

Jatropha glauca, *J. curcas*, *J. spinose*, and *J. pelargoniifolia* are the only four species distributed in Saudi Arabia and employed as traditional herbal medicines owing to their anti-inflammatory, antioxidant, antiseptic, and analgesic properties [5, 6].

Among these species, *J. pelargoniifolia* Courb. is discussed here. It is a shrub that can grow 20–200 cm tall and is widely known as "Obab" in Arabic. The plant is sometimes harvested from the wild for local medicinal use; especially, the sap of its petiole is applied to treat ulcers and severe skin inflammation and for wound healing [7]. It is widely distributed in East Tropical Africa (Sudan, Eritrea, Ethiopia, Somalia, and Kenya) and Arabian Peninsula (Yemen, Oman, and Saudi Arabia) [7].

Reviewing the current literature for the phytochemical importance of the plants belonging to the genus *Jatropha* revealed a broad range of isolated secondary metabolites, such as diterpenoids, triterpenoids, non-conventional coumarino-lignoids, alkaloids, coumarins, flavonoids, cyclic peptides, and steroids [8]. This is indeed a reflection of the versatility of the enzymatic system present in Euphorbiaceae plants, but nothing was reported regarding the *J. pelargoniifolia* species. Thus, it was of interest to explore the active constituents and biological importance of *J. pelargoniifolia.*

Peer-reviewed version available at Molecules 2018, 23, 1892; doi:10.3390/molecules23081892

2. Result and Discussion

2.1. Structure elucidation

A total of 22 compounds belonging to different chemical classes were isolated from the roots of *J. pelargoniifolia* growing in Saudi Arabia (Figure 1).

Compound **15** was obtained as white crystals. Its ^{1}H nuclear magnetic resonance (NMR) spectrum was measured in deuterated methanol (CD_3OD) (Table 1 and Figure 2). It showed a coumarin skeleton by a pair of ortho-coupled doublets that appeared at δ_H 6.26 and 7.88 with a large coupling constant (d, J = 9.5 Hz) and were assigned to H-3 and H-4 of the α-pyrone ring system, respectively, and a singlet aromatic proton that appeared at δ_H 7.00 and was assigned to H-5 in the coumarin ring. The aromatic methoxyl group resonated at 3.91 ppm (H-8). Moreover, the glycosidic nature of compound **15** was observed by the appearance of a doublet at δ_H 4.99 with a coupling constant of 7.8 Hz, confirming the β-configuration nature of the sugar. Additionally, the ^{13}C NMR exhibited signals assigned at δ_C 106.2 (C-1'), 75.5 (C-2'), 77.8 (C-3'), 71.0 (C-4'), 78.5 (C-5'), and 62.2 (C-6'), indicating the presence of a β-D-glucopyranosyl unit in compound **15**. Furthermore, the ^{13}C NMR spectrum displayed sixteen carbon signals; nine for the coumarin skeleton, six for β-glucopyranose unit, and one methoxylated carbon. In the ^{1}H-^{1}H correlated spectroscopy (COSY) spectrum of **15**, the proton signal at δ_H 6.26 (H-3) showed a cross-peak correlation with that of δ_H 7.88 (H-4). The glucose sugar moiety was located at C-8 from the heteronuclear multiple bond correlation (HMBC) spectrum; showing a clear three-bond correlation between the H-1' (anomeric proton) and C-8 of the coumarin ring. Besides, OCH_3 was unambiguously located at C-8 because of the presence of a three-bond correlation between oxymethyl protons and C-8 of aglycon. In addition, the hydroxyl group was attached to C-6 and neither C-5 and this is evidenced via HMBC, where H-5 exhibited clear long-range correlations with C-4 and C-8a (Figure 2).

From the above evidence, the structure of compound **15** was elucidated as a new compound isolated from a natural source and identified as 6-hydroxy-8-methoxy coumarin-7-*O*-*β*-D-glycopyranoside. It is worth mentioning that the closely related coumarin glycoside derivatives have been previously isolated from the leaves of *Daphne pseudomezereum* [9] and the gametophytes of *Tetraphis pellucida* [10].

The white needle crystals of compound **15** were isolated; they exhibited positive Dragendorff's test, indicating their alkaloid nature. The ^{1}H NMR spectrum displayed four aromatic signals for 2,3-disubstituted indole ring, which was proved by the presence of four coupled aromatic protons at δ_H 7.29, 7.07, 7.00, and 7.41 directly connected via one bond correlation to their carbon in heteronuclear single quantum coherence (HSQC) spectrum at H-4, H-5, H-7, and H-8, respectively. The singlet signal corresponding to the proton at position 2 of the indole ring (in compound **17**)

Peer-reviewed version available at *Molecules* **2018**, *23*, 1892; doi:10.3390/molecules23081892

was absent and instead appeared as a singlet signal resonating at 3.91 ppm in compound **18**. The disubstituted derivative was proved to be located at position 2 and 3 of the indole nucleus, and the substituents were identified as hydroxy methyl (δ_H 3.91) and N-methyl ethyl amine. The ^{13}C NMR spectrum was consistent with the structure and allowed the assignments of carbon resonances. Eight aromatic signals were observed: two of them downfield shifted corresponding to the quaternary carbons at positions 8 and 9 (δ_C 0.3-2.8) of the indole ring, the other four signals of the phenyl ring were observed at 122.4, 120.0, 118.6, and 112.60 ppm, and position 2 of the indole ring appeared at 128.0 ppm, which was more downfield compared to **17** (124.3 ppm), confirming that position 2 of the indole ring was substituted by CH_2OH, while the quaternary carbon at position 3 appeared at 107.2 ppm. In addition, the presence signal for the nitrogenated carbon (methyl) appeared at 44.9 ppm and for the aliphatic carbons (two methylene carbons of the side chain) appeared at 54.1 and 21.5 ppm. Finally, a signal appeared at 52.9 ppm and was assigned to the carbon of hydroxy methyl group (Table 2).

Additionally, the COSY spectrum showed proton-proton correlations between the aromatic indole ring protons H-6 and H-7 and between H-5 and H-4, and vice versa. As well, the side-chain α and β CH_2 groups exhibited clear COSY correlation. The HMBC spectrum showed the exact site of attachment of CH_2OH at position 2 of the indole ring based on the presence of obvious two- and three-bond correlations between the protons of CH_2OH and C-2 and C-3, respectively. Moreover, the amino methyl part in the side chain showed a three-bond correlation with α CH_2 neither β CH_2 and this is evidence for the exact location of attachment for CH_3 group (Figure 3).

On the basis of these evidences, the structure of compound **18** was assigned as **3-(2-(methylamino) ethyl)-1H-indol-2-yl) methanol**, a new indole alkaloid isolated here for the first time from a natural source, and it is a 2-hydroxymethyl derivative of N-methyltryptamine (compound **17**).

Furthermore, the obtained spectral data for the known isolated compounds were consistent with the reported literature; they were unambiguously identified as jatrophadiketone **(1)** [11], β.sitosterol **(2)** [12, 13], curcuson D **(3)** [14], curcuson C **(4)** [14], naringenin **(5)** [15], β.sitosterol glucoside **(6)** [16], spruceanol **(7)** [17], propacin **(8)** [18, 19], cleomiscosin B **(9)** [18, 19], cleomiscosin A **(10)** [18, 19], apigenin **(11)** [20], uracil **(12)** [21, 22, 23], cynaroside **(13)** [24], linarin **(14)** [25, 26], hovetricoside C **(16)** [27], N-methyltryptamine **(17)** [28], N-methyltyramine **(19)** [29], hordenine **(20)** [30], hordenine HCL **(21)** and N-methyltyramine HCL **(22)** [compounds **21** and **22** were identical to the standard samples (Sigma-Aldrich, USA), as shown by ^{1}H NMR and TLC.].

2.2. Biological activity

Our study proved that *J. pelargoniifolia* roots are a source of several biologically-active compounds such as hordenine, which exhibited various biological activities like

Peer-reviewed version available at *Molecules* **2018**, *23*, 1892; doi:10.3390/molecules23081892

inhibiting melanogenesis in human melanocytes, increasing the respiratory and heart rates [31], stimulation of gastrin release, inhibition of monoamine oxidase B, and antibacterial properties [32]. Furthermore, Chrisitine *et al.* (1979) reported that N-methyltyramine increases blood pressure in an anaesthetized rat, relaxes guinea pig ileum, and increases both the force and rate of contraction of guinea-pig right atrium by inducing the release of noradrenaline [33]. Additionally, naringenin has been reported to have several pharmacological properties, including anti-dyslipidemic, anti-obesity and antidiabetic, and antifibrotic [34]. Moreover, cleomiscosin A showed strong anti-inflammatory activity and has analgesic and antipyretic potencies [35]. Curcuson C has been reported to have antipyretic activity *in vivo* [36]. In the current study, the compounds isolated in good yields (**1–6**, **8–11**, **13–14**, **16,** and **21–22**) were evaluated for their analgesic, anti-inflammatory, antipyretic, and antioxidant activities by using *in vivo* and *in vitro* models.

The analgesic activities of the isolated compounds were assessed via acetic acid-induced writhing in mice, hot-plate, and tail-flick methods. In the acetic acid-induced writhing method, compounds **22, 5, 13, 6, 1, 4, 10, 16, 14, 9,** and **11** showed significant reduction in the number of writhings ($p < 0.05$–0.001) in the mice treated with a low dose of 5 mg/kg (33.33, 49.53, 41.20, 31.48, 32.40, 35.64, 26.38, 25.46, 14.53, 5.55, and 1.85%, respectively) as well as in the mice treated with a high dose of 10 mg/kg (65.74, 62.96, 58.33, 52.77, 49.53, 49.07, 32.87, 29.62, 23.61, 16.16, and 9.72%, respectively) compared with the standard antinociceptive drug (indomethacin), which showed 72.68% reduction in the number of writhings at a concentration of 4 mg/kg (Table S9). On the other hand, compounds **2**, **3**, **8,** and **21** showed either very low or no considerable antinociceptive effect. The thermal stimuli study using a hot plate revealed that the thermal responses in the mice treated with selected compounds after half, 1, and 2 hours were significantly reduced ($p < 0.001$). Especially, in the mice treated mice with 10 mg/kg of compounds **22**, **10**, **6**, **4**, **5**, **13**, **16,** and **14**, the antinociceptive effects were reduced by 78.57, 76.19, 74.46, 73.33, 65.95, 59.57, 53.48, and 34.88%, respectively (Table S10). While in the tail-flick method, the tested animals treated with 10 mg/kg of compounds **22**, **1**, **10**, **4**, **13**, **16**, **5**, **6**, **21**, **3,** and **9** showed significant ($p < 0.001$) reduction in antinociceptive activity (67.32, 67.21, 60.45, 57.59, 54.04, 53.59, 45.58, 40.30, 21.75, 20.39, and 10.87%, respectively) compared with indomethacin (95.61%), Table S11. The obtained results confirmed that the strong analgesic activity exhibited by the roots of *J. pelargoniifolia* could be due to its content of numerous bioactive compounds that have analgesic activities with different CNS mechanisms (peripheral and central). In future, studies with purified compounds could be conducted for further pharmacological and toxicological characterization, in order to elucidate the mechanisms involved in the central analgesic effect of these compounds.

The anti-inflammatory activities of some isolated compounds were evaluated by using the carrageenan-induced paw edema in rats. The size of edema was significantly reduced ($p < 0.05$–0.001) in the animals treated the low doses of 5 and 10 mg/kg

Peer-reviewed version available at *Molecules* **2018**, *23*, 1892; doi:10.3390/molecules23081892

compared with the standard anti-inflammatory drug (phenylbutazone) at a high dose of 100 mg/kg. The rats treated with compounds **10**, **16**, **1**, **5**, **6**, **22**, **4, 13, 3, 8, 9, 14,** and **21** exhibited a significant reduction in their hind paw edema. At a low dose of 5 mg/kg, the edema size reduced by 20.44, 47.23, 17.95, 45.85, 33.97, 44.47, 13.53, 26.51, 7.18, 2.20, 5.52, 6.35, and 6.35, respectively, while at 10 mg/kg the edema size reduced by 64.91, 55.24, 54.94, 51.38, 51.10, 50.27, 49.17, 48.61, 13.53, 12.98, 10.22, 10.22, and 8.01%, respectively in comparison with the high dose of phenylbytazone (100 mg/kg) that produced 69.06% reduction in the size of edema, which was almost similar to that produced by the low dose (10 mg/kg) of compound **10** (64.91%; Table S12). The anti-inflammatory results were almost compatible with the antinociceptive activity results for the tested compounds.

In addition, the isolated compounds from *J. pelargoniifolia* roots were tested for their antipyretic activity by using the mice with yeast-induced hyperthermia. All tested compounds administered at 5 and 10 mg/kg doses showed significant ($p < 0.05$–0.001) reduction in the rectal temperature of the hyper-thermic mice ranging between 36.73±0.13 °C and 38.56±0.16 °C as compared with the hypothermic effect (36.33±0.11 °C) resulting from indomethacin administration (Table S13).

The percentage inhibition ± SD of nitric oxide-scavenging activity was determined for the selected compounds that were isolated at the concentrations of 20, 40, 60, 80, and 100 µg/mL from the roots of *J. pelargoniifolia*, and the obtained results were compared with the standard antioxidant drug (ascorbic acid). Compounds **22**, **4**, **2**, **10**, **1**, **14**, **21**, **9**, **8**, **11**, **6**, **3**, **13**, **5**, and **16** exhibited significant free radical-scavenging potency when compared with the free radical-scavenging activity of a strong known antioxidant drug (ascorbic acid) 87.23±0.98. The ability of the tested compounds to produce antioxidant effects was found to be concentration-dependent. At 100 µg/mL dose, the % inhibition ± SD of the tested compounds were 77.60±4.22, 77.36±4.22, 76.83±5.01, 75.26±5.54, 71.66±0.70, 70.36±14.73, 67.67±5.75, 63.67±12.85, 63.67±12.85, 57.00±10.21, 56.54±6.03, 38.30±5.63, 33.06±1.86, 27.00±7.85 and 25.61±5.18, respectively (Table S14). The significant antioxidant activity associated with the administration of *J. pelargoniifolia* roots was perhaps due to its content of several phenolic and polyphenolic compounds, which play an important role in free radical-scavenging activity with less cytotoxicity.

It is important to mention here that in our previous study, which was carried out on the crude alcoholic extract of *J. pelargoniifolia* roots, we observed a significant anti-inflammatory activity and analgesic potency, likely resulting from the presence of cleomiscosin A, hovetricoside C, jatrophadiketone, naringenin, β.sitosterol glucoside, N-methyltyramine HCL, curcuson C, cynaroside, curcuson D, propacin, cleomiscosin B, linarin, and hordenine HCL in good yield. Undoubtedly, a synergistic effect between these bioactive constituents produces significant antinociceptive and anti-inflammatory effects. These results justify the use of this plant in folk medicine for the treatment of pain and several inflammatory conditions. Further study will be

Peer-reviewed version available at *Molecules* **2018**, *23*, 1892; doi:10.3390/molecules23081892

conducted on the pure isolated compounds to investigate the exact mechanisms underlying their promising biological activities.

3. Materials and methods

3.1. Chemicals and analytical instruments

The high-resolution electron spray ionization-mass spectrometry (HRESI-MS) analyses were carried out on an Agilent Triple Quadrupole 6410 QQQ LC-MS mass spectrometer (Central Lab. College of Pharmacy, King Saud University (KSU)). The infra-red spectra were generally recorded in the potassium bromide pellets unless otherwise specified using the FTIR spectrophotometer (Shimadzu). The melting points were recorded by using a Mettler FP 80 Central Processor supplied with a Mettler FP 81 MBC Cell Apparatus. The spectral data for proton and carbon were measured by using Bruker AVANCE 700, 500, and 600 (College of Pharmacy, KSU and Department of Chemistry in TU Dortmund), resonating at either 700, 500, and 600 MHz for proton or at 125 MHz for carbon. The chemical shift values were expressed in ppm with respect to the internal standard tetramethyl silane (TMS) or residual solvent peak, the coupling constants (*J*) were recorded in Hertz (Hz). The two-dimensional NMR experiments (COSY, HSQC, and HMBC) were performed using the standard Bruker program. The silica gel 60/230–400 mesh (EM Science), R_P C_{18} silica gel 40–63/230–400 mesh (Fluka)®, and sephadex LH-20 with particle size 18–111 µm (GE Healthcare, USA) were used for column chromatography, while the silica gel and reversed phase 60 F_{254} (Merck) were used for thin-layer chromatography (TLC). The detection was achieved by using 10% H_2SO_4 in ethanol or ceric sulfate followed by heating. Alkaloids were tested with Mayer’s reagent, Hager’s reagent, and Dragendorff’s reagent. All solvents for analytical purpose (HPLC- and analytical-grade) and drugs for biological investigation (sodium nitroprusside, sulphanilamide, λ-carrageenan, acetic acid, ascorbic acid, and phenylbutazone) were procured from Sigma Chemical Company (Sigma-Aldrich, USA), and the solvents were distilled prior to use. The preparative and semipreparative Shimadzu HPLC were performed; characterized by Rp-18 (ODS-80 TM, TSK, Japan), 10 µm PS, 30 cm L × 2.15 cm i.d. fitted with a guard column (10 µm PS, 7.5 cm L × 2.15 cm i.d.) (ODS-80 TM, TSK, Japan), and VP 250/10 NUCLEODUR C18 HTec, 6 µm PS, 25 cm L × 2 cm i.d., respectively both used PDA detector.

3.2. Plant material

The roots of *J. pelargoniifolia* were harvested from Wadi Mojasas, Jazan district (south of Saudi Arabia) in September 2015. The plant was authenticated by Dr. Jacob Thomas, a botanist of the Science College Herbarium, KSU, where a voucher specimen (#23064) was deposited.

Peer-reviewed version available at *Molecules* **2018**, *23*, 1892; doi:10.3390/molecules23081892

3.3. Animals

Male Wistar rats and white male Swiss albino mice with approximate body weights of 200 g and 20–25 g, respectively, were divided into groups of six animals. The animals were obtained from the Experimental Animal Care Center, College of Pharmacy, KSU. After a 7-day period in animal accommodation, they were divided into groups and maintained at 12 h:12 h light-dark conditions at 55% humidity. Purina chow rat diet (UAR-Panlab, Barcelona, Spain) and drinking water were supplied to the animals *ad libitum*. The protocols for the present study were based on the recommendations of the Ethical Committee of the Experimental Animal Care Center of KSU (approval number CPR-7569).

3.4. Extraction, Fractionation and Purification

The air-dried powder of *J. pelargoniifolia* roots (2.5 kg) was divided into two parts A and B. Two kilograms of the plant root powder (part A) was subjected to solvent extraction, while the remaining 500 g of the root powder (part B) was exposed to the acid-base treatment. Part A was extracted by maceration with 80% ethanol (3 L×5) for three successive days with occasional shaking. This process was repeated until complete exhaustion of the plant material [37]. The alcoholic extract was then concentrated to dryness under reduced pressure at 40 °C using a rotary evaporator to give 270 g of the dried alcoholic extract. The dried alcoholic extract was suspended in H_2O and successively partitioned with petroleum ether, dichloromethane, ethyl acetate (EtOAc) and *n*-butanol (*n*-BuOH) (600–700 ml × 3) of each to obtain 13.3, 10.3, 5.1, and 33.6 g, respectively.

A part of the petroleum ether fraction (12.8 g) was chromatographed over silica gel column (700 g, 140 cm × 5 cm i.d.) using a gradient of petroleum ether/EtOAc followed by methanol (MeOH). The 100-ml fractions of each were collected and screened by TLC, and similar fractions were combined together to give 14 fractions, six of which were promising based on the TLC screening. Fraction 53–83 eluted by 15% EtOAc in petroleum ether (609.8 mg) was further subjected to CC and eluted by petroleum ether/acetone gradient elution, fraction 31–62 (188.9 mg) was eluted by 6% acetone in petroleum ether and further purified by preparative HPLC gradient elution (4 ml/min., acetonitrile: H_2O: TFA) to yield 25.0 mg of compound **1**. The direct crystallization of fraction 120–139, eluted by 20% EtOAc in petroleum ether, yielded 302.7 mg of compound **2**. The fractions 167–170 and 189–196 eluted with 30 and 40% EtOAc in petroleum ether, respectively, were subjected to repeated crystallization with acetone to yield compounds **3** and **4** (14.3 and 25.4 mg, respectively). Additionally, fraction 218–248 (287.3 mg) eluted by 50% EtOAc was exposed to repeated crystallization with acetone to give 20.2 mg of compound **5**, while fraction 285–287 eluted with 40% MeOH in EtOAc yielded 330.4 mg of compound **6**, which was purified by crystallization with acetone.

Peer-reviewed version available at Molecules 2018, 23, 1892; doi:10.3390/molecules23081892

The dichloromethane (DCM) fraction (9.8 g) was subjected to column chromatography using a column (700 g silica gel; 1 m ×4.5 cm i.d.) packed by wet method with petroleum ether. The polarity of the column was gradually increased by treating it with DCM, followed by MeOH. The 75-mL fractions of each compound were collected. Among the collected fractions (142 fractions), similar fractions were pooled together depending on their TLC similarity and then dried under reduced pressure at 40 °C. Fraction 48–64, which was eluted by 10% MeOH in DCM, was concentrated (4.6 g) and then subjected to further silica column chromatography (CC; 250 g; 1 m ×4 cm i.d.). The elution carried out by using gradient DCM/MeOH at 12% MeOH in DCM afforded subfractions 38–51 (3.6 g), which were subjected to further purification by using silica gel CC (150 g; 80 cm × 3 cm i.d). Elution was carried out by using a mixture of petroleum ether/acetone, followed by MeOH. Fraction 116–143 was eluted at 50% petroleum ether/acetone and further purified through a preparative TLC using MeOH: H_2O (3: 1) as a solvent system (5 times), leading to the isolation of white crystals of compound **7** (7.5 mg). Moreover, three promising subfractions obtained by using 90% acetone in petroleum ether, 100% acetone, and 10% acetone in MeOH, followed by crystallization with MeOH afforded compounds **8** (15.1 mg), **9** (15.9 mg), and **10** (16.4 mg), respectively.

The EtOAC extract (4.6 g)_was subjected to silica gel CC (250 g, 1 m ×4 cm i.d) using a gradient of DCM/MeOH. TLC was used to monitor fractions (50 mL), followed by pooling of similar fractions. Fractions 34–39 eluted with 84% DCM afforded 17 mg of compound **11** after crystallization with MeOH. Fractions 55–57 eluted with 70% DCM afforded 8.6 mg of compound **12**. The fractions eluted with 35 and 40% MeOH in DCM were further purified by repeated acetone crystallization to give 14.9 and 13.2 mg of compounds **13** and **14**, respectively. Fractions 75–78 eluted with 45% MeOH and further subjected to CC using DCM/MeOH, followed by a semi-preparative HPLC (Rp-18) using gradient elution at 1 ml/min with MeOH: H_2O: TFA as solvent system afforded 8.6 mg white crystals of compound **15**. Finally, fractions 88–102 eluted with 50% MeOH in DCM were subjected to further purification over sephadex LH-20 (100 g, 1 m × 4 cm i.d), using water and methanol as an eluent in the gradient mode. The subfraction eluted by 20% H_2O/MeOH was further purified over a reversed-phase column to give a further subfraction eluted by 75% H_2O/MeOH, affording 12 mg of compound **16**.

Furthermore, the remaining 500 g of the root powder was subjected to an acid-base treatment according to the Stas-Otto method I described by Mandhumitha and Fowsiya [38]. The crude alkaloidal fraction was subjected to silica columns using gradient elution with solvent system DCM/MeOH: NH_4OH, resulting in five fractions. The first fraction was eluted by using 17% MeOH in DCM with an addition of 1% NH_4OH followed by purification through reversed-phase semipreparative HPLC using MeOH: H_2O: TFA to give the white crystals of compound **17** (6.3 mg). The second fraction was separated by 20% MeOH in DCM to afford a subfraction, which was further purified by a semipreparative HPLC (gradient elution 2 ml/min) using

Peer-reviewed version available at *Molecules* **2018**, *23*, 1892; doi:10.3390/molecules23081892

MeOH: H_2O: TFA as a solvent system to afford the white needle crystals of compound **18** (9.4 mg). The third and fourth fractions were eluted by 23 and 26% MeOH in DCM, followed by an addition of a few drops of NH_4OH, affording 6.8 and 8.8 mg of compounds **19** and **20**, respectively. The fifth fraction was eluted by 60% MeOH in DCM with an addition of a few NH_4OH drops to yield 86.7 mg of a compound, which was subjected to further purification using the reversed-phase semipreparative HPLC in gradient mode with MeOH:H_2O:TFA as the mobile phase, resulting in the production of the white crystals of compounds **21** and **22** (20.3 and 21.5 mg), respectively.

6-hydroxy-8-methoxycoumarin-7-*O*-*β*-D-glycopyranoside (compound **15**): White crystals; m.p. 219–220 °C; UV (MeOH) λ_{max} nm (log ε): 325 and 250; IR (KBr) ν_{max} (cm^{-1}): 2510–3349, 1719, 1625, 1520, 1465, 829; ^1H NMR, ^{13}C NMR, and HMBC data, see Table 1 and Figure 2; HRESIMS (positive) *m/z* 371 $[M+H]^+$ (calculated for $C_{16}H_{18}O_{10}$, 370).

3-(2-(methylamino)ethyl)-1H-indol-2-yl)methanol (compound **18**): White needle crystals; m.p. 179–189 °C; UV (MeOH) λ_{max} nm (log ε): 295, 287, 279, 230; IR (KBr) ν_{max} (cm^{-1}): 750, 855, 1011, 1105, 1120, 1140, 1055, 3309; ^1H NMR, ^{13}C NMR, and HMBC data, see Table 2 and Figure 3; HRESIMS (positive) *m/z* 205 $[M+H]^+$ (calculated for $C_{12}H_{16}N_2O$, 204).

3.5. Antinociceptive activity test

3.5.1. Hot-plate method

The hot-plate method described by Turner (1965) was used to determine the antinociceptive activity of the compounds isolated form *J. pelargoniifolia* root [39].

3.5.2. Acetic acid-induced writhing in mice test

The method of Koster *et al.* (1959) was used to evaluate the analgesic effect of pure compounds isolated from *J. pelargoniifolia* root [40].

3.5.3. Tail-flick method

Acute nociception was induced using the tail-flick apparatus (Tail flick Apparatus Harvard), following the method recommended by D'amour and Smith [41].

3.6. Anti-inflammatory activity test

3.6.1. Carrageenan-induced edema in rat paw method

The method described by Winter *et al.*, 1962 was used to evaluate the anti-inflammatory potency of the isolated compounds [42].

Peer-reviewed version available at *Molecules* **2018**, *23*, 1892; doi:10.3390/molecules23081892

3.7. Antipyretic activity screening

3.7.1. Yeast-Induced Hyperthermia in Rats

Hyperthermia was induced in mice followed by the administration of the isolated compounds, and their hypothermic activity was determined by applying the method described by Loux (1972) [43].

3.8. Antioxidant effect

3.8.1. Nitric oxide radical-scavenging assay

This assay was carried out according to the procedure described by Green *et al*. (1982) [44].

Statistical analysis

The values in the tables are given as mean ± SE. The data were analyzed by using one-way analysis of variance (ANOVA) followed by the Student's *t*-test. Values with $P < 0.05$ were considered significant.

4. Conclusion

This is the first report on the phytochemical and biological investigation of *J. pelargoniifolia* roots resulting in the isolation of 22 secondary metabolite compounds (two steroids, five flavonoids, three coumarinolignans, seven alkaloids, four diterpenes, and one coumarin glucoside). This work has enhanced our understanding about the constituents of the selected plant. Hovetricoside C and N-methyltryptamine were isolated from the Euphorbiaceae family for the first time, while hordenine, N-methyltyramine, their salts, cynaroside, and linarin were characterized in the genus *Jatropha* for the first time. Compounds **15** and **18** were isolated for the first time from a biological source. Compound **18** can be used as a starting material for the semi-synthesis of related analogues aiming to produce new drug leads used for the treatment of contemporary diseases such as depression, anxiety, pain, obesity and many more. Furthermore, the roots of *J. pelargoniifolia* showed significant antinociceptive, anti-inflammatory, antipyretic, and free radical-scavenging activities probably because of its content of many bioactive compounds belonging to various chemical classes that have analgesic and anti-inflammatory activities as well antipyretic and antioxidant activities that work together to produce synergistic effects. These results support the efficient use of this plant in Saudi Traditional Medicine as a remedy for pain and several inflammatory conditions.

Peer-reviewed version available at *Molecules* **2018**, *23*, 1892; doi:10.3390/molecules23081892

Acknowledgments

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding the work through the Research Group, Project no. RG-1437-021.

Our thank is continuing for Professor. Attallah F. Ahmad, Professor of Pharmacognosy and Chemistry of Natural Products in KSU, for the assistant in the structure elucidation of compounds 19-22.

Author Contributions

H.A, A.E.-G. and O.K. conceived and designed the experiments; HA performed the experiments; H.A., A.E.-G. analyzed the data; H.A. contributed reagents/materials/analysis tools; H.A. wrote the paper; A.E.-G participated in the experiments design and coordination and helped to draft the manuscript; O.K. participated in the experiment design and supervised.

Conflicts of Interest

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data associated with ^{1}H NMR, ^{13}C NMR, COSY and HMBC of compounds **15** and **18** are available in Supplementary Information. In addition, Tables for all biological tests results are provided.

Peer-reviewed version available at *Molecules* **2018**, *23*, 1892; doi:10.3390/molecules23081892

References

1. Alves, M.V. Checklist das espècies de Euphorbiaceae Juss. Ocorrentes no semi-áridopernambucano, Brasil. *Acta Bot. Bras*. 1998, 12 (3), 485–495.

2. Webster, G.L. Classification of the Euphorbiaceae. *Ann. Mo. Bot. Gard*. 1994, 81, 3–143.

3. Sabandar, C.W.; Ahmat, N.; Jaafar, F.M.; Sahidin, I. Medicinal property, phytochemistry and pharmacology of several *Jatropha* species (Euphorbiaceae): a review. *Phytochemistry* 2013, 85, 7–29.

4. Félix-Silva, J.; Giordani, R.B.; Silva-Jr, A.A.; Zucolotto, S.M.; Fernandes-Pedrosa, M.F. *Jatropha gossypiifolia* L. (Euphorbiaceae): A review of traditional uses, phytochemistry, pharmacology, and toxicology of this medicinal plant. *Evid.-Based Complement. Altern. Med*. 2014, 1–32.

5. Oskoueian, E.N.; Saad, W.Z.; Omar, A.; Ahmad, S.; Kuan, W.B.; Zolkifli, N.A.; Hendra, R.; Ho, Y.W. Antioxidant, anti-inflammatory and anticancer activities of methanolic extracts from *Jatropha curcas* Linn. *J. Med. Plants Res*. 2011, 5 (1), 49–57.

6. Yusuf, S.O.; Maxwell, I.E. Analgesic activity of the methanolic leaf extract of *Jatropha Curcas* (Linn). *Afr. J. Biomed. Res*. 2010, 13, 149–152.

7. Schmelzer, G.H.; Fakim, G.A. *Jatropha pelargoniifolia*. Record from Prota4u. PROTA (Plant Resources of Tropical Africa/Ressources Végétales de l'Afrique Tropicale), Wageningen, Netherlands. 2007.

8. Zhang, X-P.; Zhang, M-L.; Sua X-H.; Huoa C-H.; Gub Y-C.; Shi Q-W. Chemical constituents of the plants from genus *Jatropha*. *Chem. Biodivers*. 2009, 6, 2166–2183.

9. Konishi, T.; Wada, S.; Kiyosawa, S. Constituents of the leaves of *Daphne pseudo-mezereum*. *YAKUGAKU ZASSHI*. 1993, 113 (9), 670–675.

10. Jung, M.; Geiger, H.; Zinsmeister, H.D. Tri-and tetrahydroxycoumarin derivatives from *Tetraphis pellucida*. *Phytochemistry* 1995, 39 (2), 379–381.

11. Liu, J-Q.; Yang, Y-F.; Wang, C-F.; Li, Y.; Qiu, M-H. Three new diterpenes from *Jatropha curcas*. *Tetrahedron* 2012, 68, 972–976.

12. Habib, M.R.; Nikkon, F.; Rahman, M.; Haque, M.E.; Karim, M.R. Isolation of stigmasterol and β-sitosterol from methanolic extract of root bark of *Calotropis gigantean* (Linn). *Pak. J. Biol. Sci*. 2007, 10, 4174–4176.

13. Jamal, A.K.; Yaacob, W.A.; Din, L.B. A chemical study on *Phyllanthus columnaris*. *Eur. J. Sci. Res*. 2009, 28,76–81.

14. Naengchomnong, W.; Thebtaranonth, Y. Isolation and structure determination of four novel diterpenes from *Jatropha curcus*. *Tetrahedron Lett*. 1986, 27 (22), 2439–2442.

Peer-reviewed version available at *Molecules* **2018**, *23*, 1892; doi:10.3390/molecules23081892

15. Maltese, F.; Erkelens, C.; Kooy, F.V.D.; Choi, Y.H. Identification of natural epimeric flavanone glycosides by NMR spectroscopy. *Food Chem*. 2009, 116, 575–579.

16. Wei, S.; Liang, H.; Zhao, Y.Y.; Zhang, R.Y. Isolation and determination of compounds from Radix *Achyranthis Bidentatae*. *Chin. J. Chin. Mater. Med*. 1997, 22 (5), 293–296.

17. Alimboyoguen, A.B.; De Castro-Cruz, K.A.; Shen, C.C.; Li, W-T; Ragasa, C.Y. Chemical constituents of the bark of *Aleurites moluccana* L. Willd. *J. Chem. Pharm. Res*. 2014, 6 (5), 1318–1320.

18. Akbar, E.; Sadiq, Z. Coumarinolignoid rare natural product: A review. *Asian J. Chem*. 2012, 24 (11), 4831–4842.

19. Begum, S.A.; Sahai, M.; Ray A.B. Non-conventional lignans: coumarinolignans, flavonolignans, and stilbenolignans. *Prog. Chem. Org. Nat. Prod*. 2010, 93, 1–70.

20. Lee, D.G.; Mok, S-Y.; Choi, C.; Cho, E.J.; Kim, H.Y.; Lee, S. Analysis of apigenin in *Blumea balsamifera* Linn DC. and its inhibitory activity against aldose reductase in rat lens. *J. Agric. Chem. Environ*. 2012, 1 (1), 28–33.

21. Lee, J.M.; Lee, D.G.; Lee, K.H.; Cho, S.H.; Nam, K-W.; Lee, S. Isolation and identification of phytochemical constituents from the fruits of *Acanthopanax senticosus*. *Afr. J. Pharm. Pharmacol*. 2013, 7 (6), 294–301.

22. Hurd, R.E.; Reid, B.R. NMR spectroscopy of the ring nitrogen protons of uracil and substituted uracils; relevance to a base pairing in the solution structure of transfer RNA. *Nucleic Acids Res*. 1977, 4 (8), 2747–2756.

23. Staubmann, R.; Schubert-Zsilavecz, M.; Hiermann, A.; Kartnig, T. A complex of 5-hydroxypyrrolidin-2-one and pyrimidine2,4-dione isolated from *Jatropha curcas*. *Phytochemistry* 1999, 50, 337–338.

24. Elhawary, S.S.; Eltantawy, M.E.; Sleem, A.A.; Abdallah, H.M.; Mohamed N.M. Investigation of phenolic content and biological activities of *Scabiosa atropurpurea* L. *World Appl. Sci. J.* 2011, 3, 311–317.

25. Zhang, T.; Zhou, J.; Wang Q. Flavonoids from aerial part of *Bupleurum chinense* DC. *Biochem. Syst. Ecol*. 2007, 35, 801–804.

26. Fernández, S.; Wasowski, C.; Paladini, A.C.; Marder M. Sedative and sleep-enhancing properties of linarin, a flavonoid-isolated from *Valeriana officinalis*. *Pharmacol. Biochem. Behav*. 2004, 77, 399–404.

27. Thuy, T.T.; Kamperdick, C.; Ninh P.T.; Lien T.P.; Thao, T.T.P.; Sung, T.V. Immunosuppressive auronol glycosides from *Artocarpus tonkinensis*. *Pharmazie* 2004, 59, 297–300.

28. Grina, J.A.; Ratcliff, M.R.; Stermitz, F.R. Old and new alkaloids from *Zanthoxylum arborescens*. *J. Org. Chem*. 1982, 47, 2648–2651.

29. Yokoo, Y.; Kohda, H.; Kusumoto, A.; Naoki, H.; Matsumoto, N.; Amachi, T.; Suwa, Y.; Fukazawa, H.; Ishida, H.; Tsuji, K.; Nukaya, H. Isolation from beer and structural determination of a potent stimulant of gastrin release. *Alcohol Alcohol*. 1999, 34 (2), 161–168.

30. Abdel-Kader, M. S.; Kassem, F. F.; Abdallah, R. M. Two alkaloids from *Ephedra aphylla* growing in Egypt. *Nat. Prod. Sci*. 2003, 9 (2), 1–4.

31. Fana, Y.; Li, X.; Zhang, L.; Duana, P.; Li, F.; Zhao, D.; Wang, Y.; Wub, H. Ether-functionalized ionic liquids: Highly efficient extractants for hordenine. *Chem. Eng. Res. Des*. 2017, 124, 66–73.

32. Ma, J.; Wang, S.; Huang, X.; Geng, P.; Wen, C.; Zhou, Y.; Yu, L.; Wang, X. Validated UPLC–MS/MS method for determination of hordenine in rat plasma and its application to pharmacokinetic study. *J. Pharm. Biomed. Anal.* 2015, 111, 131–137.

33. Christine, S.; Bell, A.; Stewart-Johnsox, E. N-methyltyramine, a biologically active amine in *Acacia* seeds. *Phytochemistry* 1979, 18, 2022–2023.

34. Jadeja, R. N.; Devkar, R. V. Polyphenols and flavonoids in controlling non-alcoholic steatohepatitis. In *Polyphenols in Human Health and Disease*, 1st ed.; Watson, R.R., Preedy, V.R., Zibadi, S., Eds.; Academic Press: San Diego, USA, 2014; pp. 615–623.

35. Begum, S.; Saxena, B.; Goyal, M.; Ranjan, R.; Joshi, V. B.; Rao, V.; Krishnamurthy, S.; Sahai, M. Study of anti-inflammatory, analgesic and antipyretic activities of seeds of *Hyoscyamus niger* and isolation of a new coumarinolignan. Fitoterapia 2010, 81(3), 178–84.

36. Picha, P.; Naengchomnong, W.; Promratanapongse, P.; Kano, E.; Hayashi, S.; Ohtsubo, T.; Zhang, S.W.; Shioura, H.; Kitai, R.; Matsumoto, H.; Kawahara, K.; Puribhat, S.; Phanthumachinda, P. Effect of natural pure compounds curcusones A and C from tropical herbal plant *Jatropha curcus* on thermosensitivity and development of thermotolerance in Chinese hamster V-79 cells *in vitro*. *J. Exp. Clin. Cancer Res*. 1996, 15, (2), 177–183.

37. Skoog, D.; Holler, F.J.; Nieman, T.A. An introduction to chromatographic separations. In *Principles of Instrumental Analysis*:, 5th ed.; Skoog, D., Holler, F.J., Nieman, T.A., Eds.; Saunders College Publishing: Philadelphia, USA, 1992, pp. 674–700.

38. Madhumitha, G.; Fowsiya, J. *A Hand Book on: Semi Micro Technique for Extraction of Alkaloids*. International E-Publication: Indore, India, 2015, pp. 9.

39. Turner, R.A. Analgesics. In *Screening Methods in Pharmacology*; Academic Press: London, UK, 1965, pp. 100.

40. Koster, R.; Anderson, M.; De Beer, E.J. Acetic acid for analgesic screening. *Fed. Proc*. 1959, 18, 412–417.

Peer-reviewed version available at *Molecules* **2018**, *23*, 1892; doi:10.3390/molecules23081892

41. D'amour, F.E.; Smith, D.L. A method for determining loss of pain sensation. *J. Pharmacol. Exp. Ther*. 1941, 72 (1), 74–79.

42. Winter, C.A.; Risley, E.A.; Nuss, G.W. Carregeenan-induced oedema in hind paw of the rats as an assay for anti-inflammatory drugs. *Exp. Biol. Med.* 1962, 111, 544–547.

43. Loux, J.J.; Depalma, D.D.; Yankell, S.L. Antipyretic testing of aspirin in rats. *Toxicol. Appl. Pharmacol*. 1972, 22, 672–675.

44. Green, L.C.; Wagner, D.A.; Glogowski, J.; Skipper, P.L.; Wishnok, J.S.; Tannenbaum, S.R. Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. *Anal. Biochem*. 1982, 126 (1), 131–138.

Peer-reviewed version available at *Molecules* **2018**, *23*, 1892; doi:10.3390/molecules23081892

Tables

Table 1. The ^{1}H (600 MHz, δ in ppm, *J* in Hz) and ^{13}C NMR (125 MHz, δ in ppm) spectral data for compound **15** in deuterated methanol (CD_3OD).

Position	**δH**	**δC**
1	-	-
2	-	163.5
3	6.26 (d, *J* = 9.5 Hz, 1H)	116.2
4	7.88 (d, *J* = 9.5 Hz, 1H)	146.5
5	7.00 (s, 1H)	106.1
6	-	145.7
7	-	133.2
8	-	147.5
4a	-	112.7
8a	-	144.4
1'	4.99 (d, *J* = 7.8 Hz, 1H)	106.2
2'	3.57 (dd, *J* = 9.4, 9.4, 1H)	75.5
3'	3.46 (d, *J* = 1.9, 1H)	77.8
*4'	3.47 (brs, 1H)	71.0
*5'	3.30 (brs, 1H)	78.5
6'	3.72 (d, *J* = 4.9, 1H) 3.80 (d, *J* = 2.4, 1H)	62.2
OCH_3-8	3.91 (s, 3H)	57.0
OH-6	10.53	-

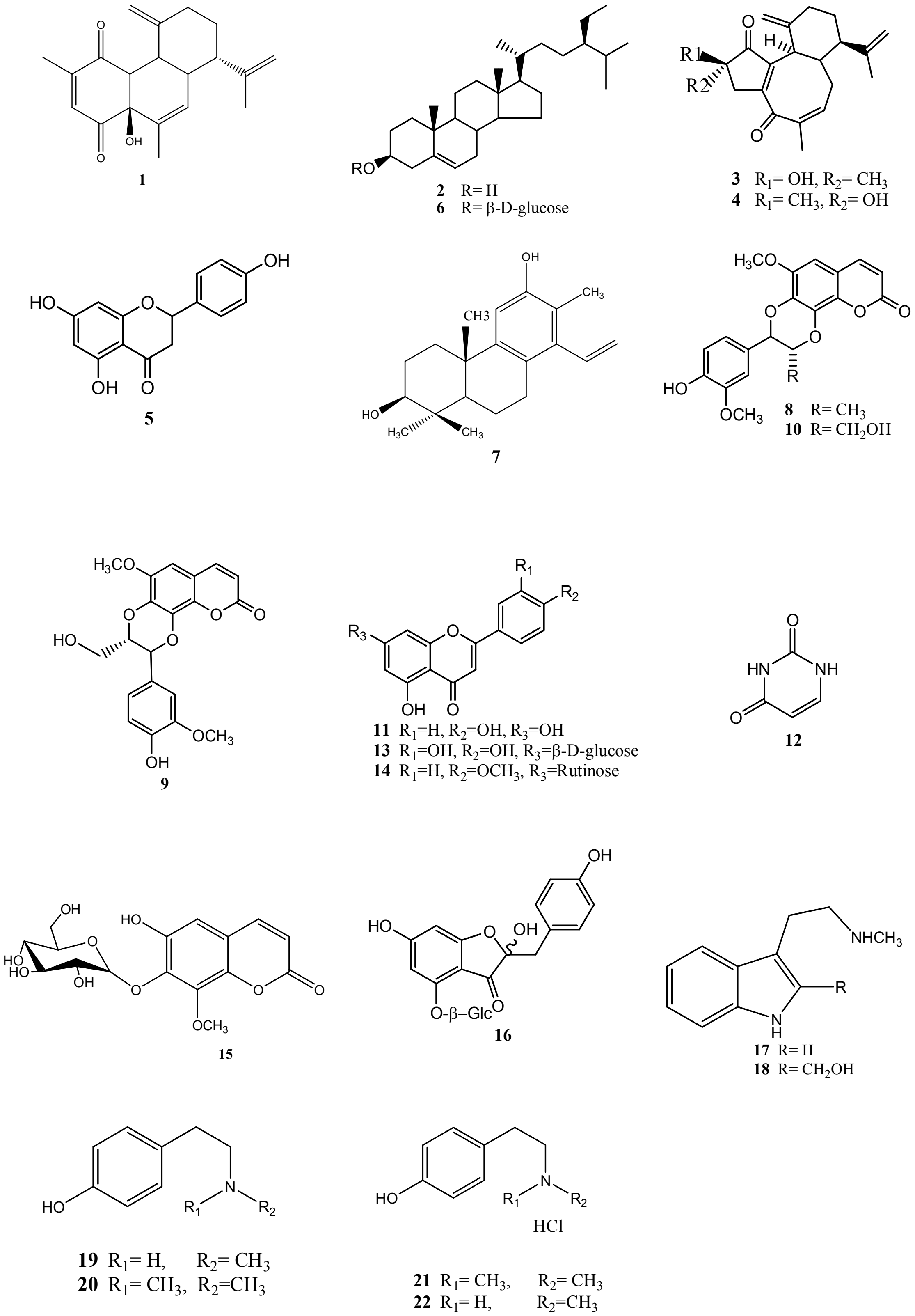
* Overlapped with solvent signal

Table 2. ^{1}H (700 MHz, δ in ppm, *J* in Hz) and ^{13}C NMR (125 MHz, δ in ppm) spectral data for compound **18** in deuterated methanol (CD_3OD).

Position	**δH**	**δC**
1	-	-
2	-	128.0
3	-	107.2
4	7.29 (d, *J* = 8.1 Hz, 1H)	112.0
5	7.07 (t, *J* = 7.8 Hz, 1H)	122.4
6	7.00 (t, *J* = 7.6 Hz, 1H)	120.0
7	7.41 (d, *J* = 7.8 Hz, 1H)	118.6
8	-	138.1
9	-	130.6
αCH_2	3.10 (t, *J* = 5.8 Hz, 2H)	54.1
βCH_2	3.00 (t, *J* = 5.8 Hz, 2H)	21.5
CH_3	2.69 (s, 3H)	44.9
CH_2OH	3.91 (s, 2H)	52.9

Figures

Figure 1. Chemical structures of the compounds isolated from the roots of *Jatropha pelargoniifolia*.



1

2 R= H
6 R= β-D-glucose

3 R_1= OH, R_2= CH_3
4 R_1= CH_3, R_2= OH

5

7

8 R= CH_3
10 R= CH_2OH

9

11 R_1=H, R_2=OH, R_3=OH
13 R_1=OH, R_2=OH, R_3=β-D-glucose
14 R_1=H, R_2=OCH_3, R_3=Rutinose

12

15

16

17 R= H
18 R= CH_2OH

19 R_1= H, R_2= CH_3
20 R_1= CH_3, R_2=CH_3

HCl

21 R_1= CH_3, R_2= CH_3
22 R_1= H, R_2=CH_3

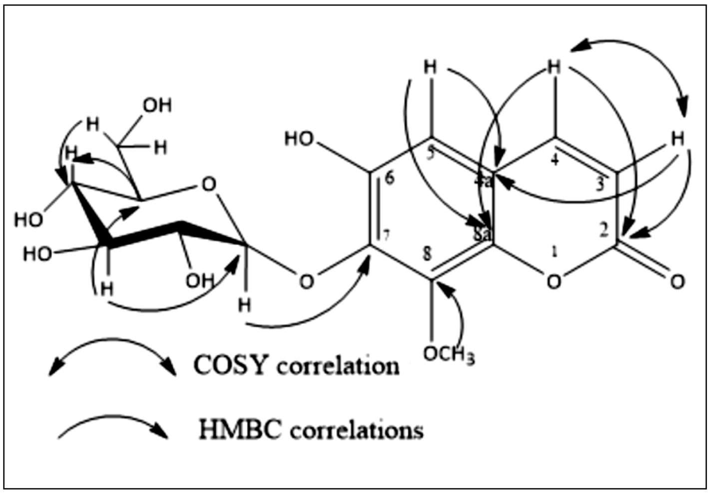


Figure 2. Selected heteronuclear multiple bond correlation (HMBC; H→C) and correlation spectroscopy (COSY; H↔H) correlations of compound **15**.

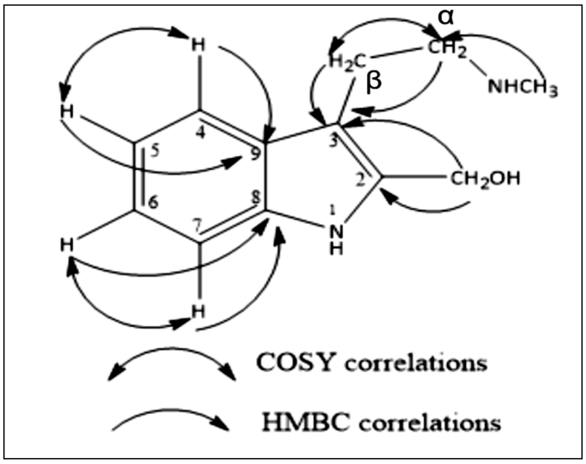


Figure 3. Selected heteronuclear multiple bond correlation (HMBC; H→C) and correlation spectroscopy (COSY; H↔H) correlations of compound **18**.