One new pyrroline compound from Callistemon viminalis (Sol. Ex Gaertner) G.Don Ex Loudon

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One new pyrroline compound from *Callistemon viminalis* (Sol. Ex Gaertner) G.Don Ex Loudon

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One new compound: 3,4-dihydro-2-(hydroxymethyl)-4-methyl-2H-pyrrol-2-ol (5) was isolated from the fruits and bark of *Callistemon viminalis* along with the known compounds lupeol (1), octacosanol (2), β-sitosterol (3), betulin (4), betulinic acid (6), ursolic acid (7), corosolic acid (8), β-sitosterol-3-O-β-D-glucoside (9), methyl gallate (10), gallic acid (11), catechin (12), ellagic acid (13) and 3-O-acetylursolic acid (14) (compound 14 was isolated from the bark and not detected in the fruits). Structures of these compounds were elucidated on the basis of their spectroscopic data (NMR, MS, IR spectra and COSY and HR-MS for 5a). The antioxidant activity of the total extracts, petroleum ether, CH₂Cl₂ and EtOAc fractions together with the compounds 6, 7, 9, 10, 11, 12 and 13 was comparable with that of the standard antioxidant, ascorbic acid.

Keywords: *Callistemon viminalis*; Myrtaceae; pyrroline; triterpenes; sterols; tannins; antioxidant

1. Introduction

*Callistemon viminalis* (Sol. Ex Gaertner) G.Don Ex Loudon (Myrtaceae) is native to Australia but now it is widespread throughout the world. In Egypt, it is a common tree on roads and in many gardens. *Callistemon speciesus* D.E. and *Callistemon lanceolatus* DC were reported to contain betulin, betulinic acid, ursolic acid and ellagic acid (Ahmad, Omar, & Ali, 1999; Bhatia, Bhatia, Sharma, & Bajaj, 1972; Varma & Parthasarathy, 1975). Phytochemical investigation of *C. viminalis* W. revealed the presence of betulinic acid, corosolic acid, β-sitosterol-3-O-β-D-glucoside, methyl gallate, gallic acid and catechin (Djoukeng, 2005; El Dib & El-Shenawy, 2008). The crude extract of *C. lanceolatus* DC was reported to have molluscidial (Soliman & El-Ansary, 2007), antioxidant and hepatoprotective (Ahmed & Marzouk, 2002), antithrombin (Chistokhodova et al., 2002) and anti-inflammatory activities (Kumar, Kumar, & Prakash, 2011). Moreover, it is effective in Alzheimer’s disease (Park et al., 2010). The diversity and lack of presentation of the active constituents together with the scarce report on biological activities had attracted the author’s attention to investigate the local tree, *C. viminalis* (Sol. Ex Gaertner) G.Don Ex Loudon, from chemical and biological points of view. In this study, one new compound along with 13 known ones were isolated and identified from *C. viminalis* and the ABTS⁺(azino-bis-3-ethyl benzthiazoline-6-sulphonic acid) radical scavenging activities of different extracts and some of the isolated compounds were also carried out.

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2. Results and discussion

Chromatographic fractionation of the petroleum ether fraction of *C. viminalis* fruits on silica gel column afforded compounds 1–4, CH$_2$Cl$_2$ fraction compounds 5–9, and the EtOAc fraction compounds 10–13. The petroleum ether fraction of the bark afforded, in addition to compounds 1–4, compound 14 on fractionation using silica gel column chromatography (Figure 1). Compound 5 is a new one.

The CH$_2$Cl$_2$ sub-fraction (eluted with 5% EtOAc in petroleum ether) containing compound 5 was obtained in the form of oil and revealed the presence of two spots ($R_f = 0.49$ minor, 0.57 major, CHCl$_3$: petrol ether 6:4). This sub-fraction was purified by acetylation with anhydrous pyridine and acetic anhydride to afford compound 5a as colourless crystals, m.p. 112–113°C. Its molecular formula is C$_8$H$_{13}$O$_3$N established on the basis of HREIMS 171.0894 [M]$^+$, Caled 171.0895. $^{13}$C-NMR, APT experiment, spectrum revealed the presence of eight carbon signals, two of them for acetyl group at δ 175.1 and 36.4 (CO and CH$_3$ respectively). The carbon signal at δ 51.1 was assigned for the aliphatic oxygenated secondary carbon (CH$_2$–O) at C-2. Carbon signals at δ 162.9, 82.6, 31.4 and 22.7 suggested dihydropyrrolidine skeleton; C-5, C-2, C-4 and C-3, respectively (de Kimpe & Keppens, 1996). The other carbon signal at δ 25.7 was assigned for the methyl group at C-4. So, $^{13}$C-data suggested dihydropyrrolidine skeleton with methyl group at C-4, hydroxymethylene and hydroxyl functions at C-2. $^1$H-NMR confirmed the previous suggestion. It displayed two methyl signals at δ 1.64 (d, $J = 6.24$ Hz) and 2.73 (s), assigned for CH$_3$ at C-4 and CH$_3$ of the acetyl function, respectively, two protons of the oxygenated secondary carbon signal at 4.59 ppm (CH$_2$–O at C-2 of the dihydropyrrolidine skeleton) and proton signals at δ 2.07 (m), 2.99 (t, $J = 15, 7.68$ Hz, 2H) and 8.32 (d, $J = 9.16$ Hz) were assigned for H-4, 3 and 5 of the dihydropyrrolidine skeleton, respectively. The study of HMBC spectrum (Figure 2) confirmed the suggested structure since it revealed cross peaks between proton signal at δ 1.64 (CH$_3$–C-4) with carbon signals at δ 22.7 (C-3) and 162.9 (C-5), the proton signal at δ 2.07 (H-4) with carbon signals at δ 82.6 (C-2), the proton signal at δ 2.73 (CH$_3$–CO) with carbon signal at δ 175.1 (CO), the proton signal at δ 2.99 (H-3) with carbon signals at δ 36.4 (CH$_3$–C-4), 51.1 (CH$_2$–O) and 162.9 (C-5), the proton signal at δ 4.59 (C-2–CH$_2$) with carbon signals at δ 22.7 (C-3) and 175.1 (CO) and the proton signal at δ 8.32 (H-5) with carbon signals at δ 22.7 (C-3), 36.4 (CH$_3$–C-4) and 82.6 (C-2). COSY spectrum (Figure 1) displayed three cross peaks confirmed the suggested structure; H-5 (δ 8.32)–H-4 (δ 2.99), H-4–H-3 (δ 2.07) and H-4–CH$_3$ (δ 1.64). Deacetylation of 5a by boiling an aliquot with alcoholic KOH, extraction with CH$_2$Cl$_2$ and TLC chromatography (silica gel, CHCl$_3$: ether 6:4) revealed that 5a corresponds to the major spot $R_f$ 0.57. Thus compound 5 should be 3,4-dihydro-2-(hydroxymethyl)-4-methyl-2H-pyrrol-2-ol, new natural compound.

Additionally, 13 known compounds (Figure 1), namely lupeol (1), octacosanol (2), $\beta$-sitosterol (3), betulin (4), betulinic acid (6), ursolic acid (7), corosolic acid (8), $\beta$-sitosterol-3-$O$-$\beta$-d-glucoside (9), methyl gallate (10), gallic acid (11), catechin (12), ellagic acid (13) and 3-$O$-acetylsalicylic acid (14) were isolated and their structure was confirmed by the comparison of their different spectral data with those reported in the literature (An et al., 2005; Aguirre et al., 2006; Aher, Pal, Yadav, Patil, & Bhattacharya, 2009; Bano, Ahmed, Azhar, Ali, & Alam, 2002; Dat et al., 2005; Fan & He, 2006; Igoli & Gray, 2008; Jo, Suh, Shin, Jung, & Im, 2005; Lin & Lin, 1999; Lu & Foo, 1999; Mahato & Kundu, 1994; Masoodi, Ahmed, Khan, & Shah, 2010; Ramos, Ferreira, Lopes, & Vicente, 2003; Srivastava, Rao, & Shivanandappa, 2007; Volken, Sticher, Schaffner, & Heilmann, 1999; Wang, Yang, & Zhang, 2007; Witchuda & Orawan, 2007). Betulinic acid, corosolic acid, $\beta$-sitosterol-3-$O$-$\beta$-d-glucoside, methyl gallate, gallic acid and catechin were previously
Figure 1. Structure of the isolated compounds (1–14).
reported in *C. viminalis* (Djoukeng, 2005; El Dib & El-Shenawy, 2008). The other compounds; lupeol, octacosanol, β-sitosterol, betulin, ellagic acid and 3-O-acetylursolic acid in addition to the new compound 3,4-dihydro-2-(hydroxymethyl)-4-methyl-2H-pyrrol-2-ol are reported in *C. viminalis* plant for the first time.

2.1. Biological activity

Different plant extracts together with compounds 6, 7, 9, 10, 11, 12 and 13 were subjected to free-radical scavenging assay to test their antioxidant activity. The results of the antioxidant activity assay, of the different isolated compounds and extracts, are presented in Table 1. It revealed that the antioxidant activity is located in the methylene chloride and ethyl acetate extracts of both fruits and bark. Analysis of such extracts mainly afforded the phenolic compounds, flavonoids and tannins, having high antioxidant activity. The petroleum ether extract, mainly contains triterpenes, has low antioxidant activity. The activity of the phenolic compounds, the CH$_2$Cl$_2$ and EtOAc extracts are comparable to that of the standard antioxidant; ascorbic acid.

3. Experimental

NMR spectra were recorded with Jeol spectrophotometer (400 MHz for $^1$H and 100 MHz for $^{13}$C), Bruker DPX-400 spectrometer and Bruker DRX-500. Melting points was measured using Fisher–Johns Scientific Co., USA, apparatus. HREIMS: Micromass LCT spectrometer, EIMS and FABMS: JOEL JMS600 spectrometer, Japan. Infra-red spectrophotometer: Mattson 5000 FTIR (England). ABTS (Sigma Chemicals Co., St. Louis, USA); ascorbic acid (Cevarel®) tablets (Memphis Pharmaceutical Co., Cairo, Egypt). All used other chemicals were of analytical grade.

3.1. Extraction and isolation

3.1.1. Plant materials

*C. viminalis* fruits and bark were collected from the gardens of Mansoura University, Egypt. The plant was identified by Prof. Dr Ibrahim Mashaly, Systematic Botany Department, Faculty of Science, Mansoura University, Egypt. A voucher specimen (Myr-Cal-vim-2000-1) is kept in the Pharmacognosy Department, Faculty of Pharmacy, Mansoura University, Egypt.

Dried powder of both the fruits and bark (1 kg, each) were percolated with MeOH till exhaustion at room temperature. The combined extracts were collected and evaporated to dryness under reduced pressure at 40°C. The residues, (310 g, fruits) and (246 g, bark), were suspended in distilled water and extracted successively with petroleum ether, CH$_2$Cl$_2$
and EtOAc. The different fractions were evaporated under reduced pressure to obtain petroleum ether fraction; 9.3, 10.3 g, CH₂Cl₂ fraction; 20.3, 75.3 g and EtOAc fraction; 40.5, 20.5 g of fruits and bark, respectively.

Petroleum ether fraction of fruits (9.3 g) was subjected to silica gel column using petroleum ether–EtOAc (gradient elution). Similar fractions were pooled together, collected fraction were subjected to purification through re-chromatography and repeated crystallization to afford lupeol 1, eluted with 5% EtOAc in petroleum ether, octacosanol 2 (5% EtOAc–petroleum ether), β-sitosterol 3 (5% EtOAc–petroleum ether), betulin 4 (10% EtOAc–petroleum ether) and 3-O-acetylursolic acid 14 (10% EtOAc–petroleum ether).

Chromatography of the fruits CH₂Cl₂ fraction (20.3 g) on silica gel packed column and elution with petroleum ether–EtOAc, gradient, afforded different fractions. These fractions were monitored by TLC. Similar fractions were pooled together to collect five groups. Solvents were evaporated and the main components were purified by re-chromatography and repeated crystallization to afford 3,4-dihydro-2-(hydroxy-methyl)-4-methyl-2H-pyrrol-2-ol 5, betulinic acid 6, ursolic acid 7, corosolic acid 8 and β-sitosterol-3-O-β-D-glucoside 9. Different compounds were eluted with 5, 20, 50, 60 and 90% EtOAc in petroleum ether, respectively.

The fruits EtOAc fraction (40.5 g) was loaded onto silica gel column and elution was started using petroleum ether–EtOAc (0–100%). Eluted fractions were monitored by TLC. Similar fractions were pooled together. Collected fraction were subjected to purification through re-chromatography and repeated crystallization to afford methyl gallate 10 (30% EtOAc–petroleum ether), gallic acid 11 (80% EtOAc–petroleum ether), catechin 12 (80% EtOAc–petroleum ether) and ellagic acid 13 (100% EtOAc).

The bark petroleum ether, CH₂Cl₂ and EtOAc, extracts were subjected to column chromatography, applying the same sequence with the fruit extract. This afforded in addition to the compounds isolated from the fruits, 3-O-acetylursolic acid 14 from the petroleum ether fraction.

3.1.2. (3,4-Dihydro-2-hydroxy-4-methyl-2H-pyrrol-2-yl)methylacetate (5a)

Colourless needles; m.p. 112–113°C; HREIMS: m/z 171.0894 [M]+, Calcd 171.0985; ¹H-NMR (400 MHz, DMSO-d₆): δ 2.99 (t, J = 15, 7.68 Hz, 2H, H-3), 8.32 (d, J = 9.16 Hz, H-5), 2.07 (m, H-4), 4.59 (s, 2H, CH₂-O), 2.73 (s, CH₃) and 1.64 (d, J = 6.24, CH₃ at C-4); ¹³C-NMR (100 MHz, DMSO-d₆): δ 82.6 (C-2), 22.7 (C-3), 31.4 (C-4), 162.9 (C-5), 51.1 (CH₂-O), 175.1 (CO), 25.7 (CH₃-CO) and 36.4 (CH₃-C-4).

<table>
<thead>
<tr>
<th>Fractions</th>
<th>Percentage of inhibition</th>
<th>Compounds</th>
<th>Percentage of inhibition</th>
</tr>
</thead>
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<tr>
<td>Total fruits extract</td>
<td>84.45</td>
<td>6</td>
<td>48.82</td>
</tr>
<tr>
<td>Petroleum ether (fruits)</td>
<td>31.37</td>
<td>7</td>
<td>21.56</td>
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<tr>
<td>Methylene chloride (fruits)</td>
<td>83.52</td>
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<td>23.33</td>
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<tr>
<td>Ethyl acetate (fruits)</td>
<td>85.88</td>
<td>10</td>
<td>87.25</td>
</tr>
<tr>
<td>Total bark extract</td>
<td>87.45</td>
<td>11</td>
<td>87.84</td>
</tr>
<tr>
<td>Petroleum ether (bark)</td>
<td>23.52</td>
<td>12</td>
<td>86.07</td>
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<tr>
<td>Methylene chloride (bark)</td>
<td>85.68</td>
<td>13</td>
<td>85.88</td>
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<tr>
<td>Ethyl acetate (bark)</td>
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<tr>
<td>Ascorbic acid (standard)</td>
<td>88.03</td>
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</table>
4. Conclusions

3,4-dihydro-2-(hydroxymethyl)-4-methyl-2H-pyrrol-2-ol (new compound), lupeol, octacosanol, \( \beta \)-sitosterol, betulin, betulinic acid, ursolic acid, \( \beta \)-sitosterol-3-\( \alpha \)-D-glucoside, methyl gallate, gallic acid, catechin, ellagic acid and 3-\( \alpha \)-acetylursolic acid were isolated and identified from the fruits and bark of \( C. \) viminalis. The antioxidant activity of the total extracts, \( \text{CH}_2\text{Cl}_2 \) and \( \text{EtOAc} \) together with the compounds 10, 11, 12 and 13 was comparable with that of ascorbic acid.

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