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SHORT COMMUNICATION

Chemical constituents with antimicrobial and antioxidant activity from the aerial parts of *Callistemon lanceolatus* (Sm.) Sweet

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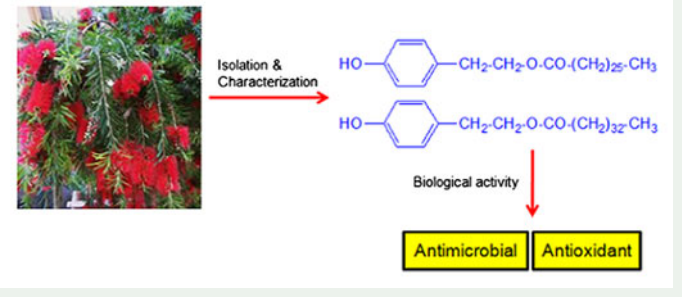
ABSTRACT

Callistemon lanceolatus (Sm.) Sweet grows all over the world and used to treat cough and bronchitis. The air-dried powder of the aerial parts was exhaustively extracted with methanol and the concentrated extract was adsorbed on silica gel for preparation of slurry. It was dried and subjected to silica gel column packed in petroleum ether. The column was eluted with organic solvents in order of increasing polarity to isolate 1-triacosanol (**1**), *n*-eicosanyl palmitate (**2**), *n*-heptadecanyl arachidate (**3**), *n*-tricosanyl palmitate, (**4**), 4-hydroxyphenethyl carbocerate (**5**), 4-hydroxyphenethyl gheddate (**6**), urs-12-en-3α-acetoxy-18β-H-28-oic acid (**7**) and stigmast-5-en-3β-ol-3β-D-glucuronopyranoside (**8**). Among them, compound **5** and **6** were new fatty acid ester isolated from this plant. Compound **7** showed MIC 32 μg/mL against *E. coli* which was comparable to amoxicillin having same MIC 32 μg/mL. Compound **5** and **6** showed significant antioxidant activity by inhibiting DPPH due to the presence of phenolic groups.



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1. Introduction

Callistemon lanceolatus (Sm.) Sweet, syn. *C. citrinus* (Curtis) Skeels, lemon bottle brush, red bottle brush, is originally from Australia and widely planted as an ornamental plant all over the world including India (Anonymous 1992). The leaves are used as a tea substitute with a delightfully refreshing flavor. Egyptians utilized the plant volatile oils as antimicrobial and insecticidal agents and for the treatment of cough and bronchitis (Shinde et al. 2012; Das and Singh, 2012). The alcoholic extracts of the leaves exhibited good antimicrobial effect against *Salmonella typhi*, *Bacillus ereus, Streptococcus epidermidi* and *B. anthracis* which was comparable with antibiotics (Seyydnejad et al. 2010). The ethanol extract of *C. lanceolatus* showed strong elastase inhibition and DPPH radical scavenging activities (Kim et al. 2009). We have carried out the antimicrobial and antioxidant activities of the compounds to know the responsible phytoconstituents which are imparting such activity to this plant.

In our earlier study, we have reported new flavones, phenolic esters, α-amyrin, betulinic acid, oleanolic acid and kaempferol from the chloroform fractions of *Callistemon lanceolatus* (Nazreen et al. 2012, 2014). In continuation of our work on the isolation of chemical constituents, we now report herein the isolation and characterization of chemical constituents with their antimicrobial and antioxidant activity.

2. Results and Discussion

Compound **1** was an aliphatic alcohol identified as 1-triacosanol (myricyl alcohol) (Jaybhay et al. 2010). Compounds **2**, **3** and **4** were the known fatty ester characterized as *n*-eicosanyl palmitate (eicosanyl-hexacosanoate), *n*-heptadecanyl arachidate (*n*-heptadecanyl eicosanoate) and *n*-tricosanyl palmitate, respectively (Ribechini et al. 2008; Alam et al. 2011). Compound **7** and **8** were characterized as urs-12-en-3α-acetoxy-18β-H-28-oic acid and stigmast-5-en-3β-ol-3β-D-glucuronoopyranoside, respectively. (Ali, 2001) (Supplementary data)

Compound **5**, designated as 4-hydroxyphenethyl carbocerate, responded positive phenolic tests and exhibited UV absorption maximum at 281 nm for aromatic compound. The IR spectrum revealed absorption bands 3450 cm^{-1} (OH), 1725 cm^{-1} (ester) and 725 cm^{-1} (aliphatic chain). Its mass spectrum exhibited a molecular ion peak at *m/z* 530 ($C_{35}H_{62}O_3$). The ^{1}H NMR spectrum showed two doublets at δ 7.08 ($J = 8.4$ Hz) and δ 6.76 ($J = 8.4$ Hz) integrating for two-protons each assigned to aromatic H-2, H-6 and H-3, H-5 protons, respectively, three two-proton triplets at δ 4.23 ($J = 7.0$ Hz), 2.85 ($J = 7.0$ Hz), 2.27 ($J = 7.4$ Hz) ascribed correspondingly oxymethylene H_2-8, methylene H_2-7 linked to the aromatic ring and methylene H_2-2′ adjacent to the ester function. The other methylene protons appeared as multiplets at δ 1.57 (2H) and 1.29 (4H) and as a broad singlet at δ 1.23 (42 H). A triplet at δ 0.87 ($J = 6.3$ Hz, 3H, Me-27′) was accounted to terminal C-27′ primary methyl protons. The ^{13}C NMR spectrum of **5** displayed signals for ester carbon at δ 176.23 (C-1′), aromatic carbons between δ 156.98 and 115.32, oxymethylene carbon at δ 63.94 (C-8), other methylene carbons from δ 35.09 to 21.33 and methyl carbon at δ 13.73 (C-27′). From the HSQC correlations (Figure S2), the aromatic protons at H-2 and H-6 (δ 7.08) showed correlation with C-2 and C-6 (δ 129.39) and H-3 and H-5 (δ 6.76) showed interactions with C-3 and C-5 (δ

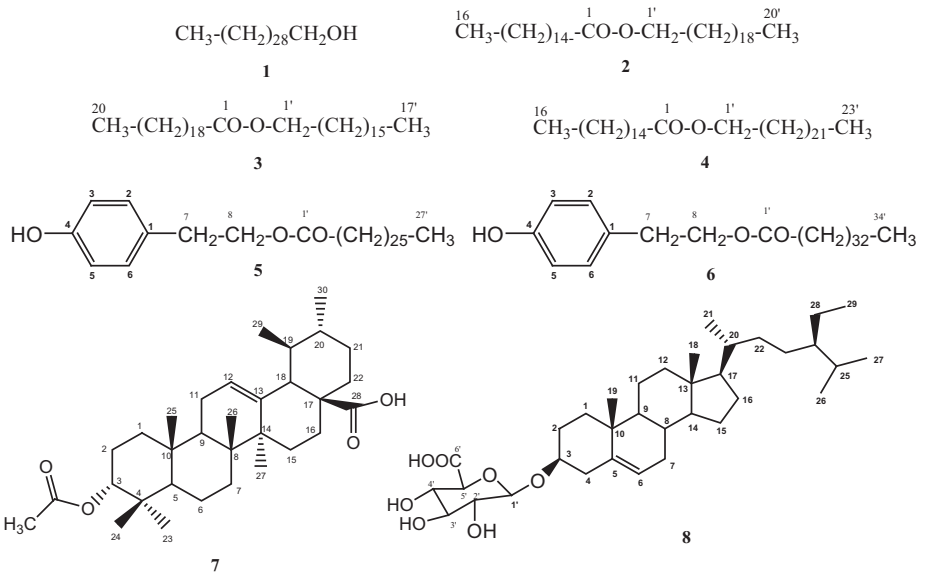

Figure 1. Structure of compounds (**1–8**) isolated from *Callistemon lanceolatus*.

115.32). Benzylic protons H_2-7 (δ 2.85) correlated with C-7 (δ 35.09); oxymethylene protons H_2-8 (δ 4.23) with C-8 (δ 63.94); methylene H_2-2′ (δ 2.27) with C-2′ (δ 35.89); other methylene protons (δ 1.57, 1.29 and 1.23) with C-3′ to C-25′ (δ 32.79–21.33) and methyl protons H_3-27′ (δ 0.87) with C-27′ (δ 13.73). From HMBC, 4-OH showed correlation (δ 9.27) with C-3 and C-5 (δ 115.32); The attachment of long alkyl side chain could be deduced from long-range ^1H–^{13}C correlations observed between H_2-7 (δ 2.85) with C-2 and C-6 (δ 129.39);H_2-8 (δ 4.23) with C-1 (δ 130.09), C-1′ (δ 176.23). (Figure S2). Acid hydrolysis of **5** yielded 4-hydroxyphenethyl alcohol (tyrosol), m.p. 90–92 °C, $[M]^+$ *m/z* at 138 ($C_8H_{10}O_2$) and carboceric acid (heptacosanoic acid), m.p. 88–89 °C; $[M]^+$ *m/z* at 410 ($C_{27}H_{54}O_2$). Analysis of the spectral data and chemical reactions led to formulate the structure of **5** as 4-hydroxyphenethyl heptacosanoate, a new fatty acid ester (Figure 1).

Compound **6**, named 4-hydroxyphenethyl gheddate, responded positively to phenolic tests and showed UV absorption maximum at 277 nm for an aromatic compound, It also showed IR absorption bands for hydroxyl group (3503 cm^{-1}), ester function (1735 cm^{-1}), aromatic ring (1647, 1541 cm^{-1}) and a long aliphatic chain (727 cm^{-1}). Its mass spectrum exhibited a molecular ion peak at *m/z* 628 consistent with a molecular formula of a fatty acid ester with aromatic alcohol, $C_{42}H_{76}O_3$. The ion peaks arising at *m/z* 507 [C_8–O fission, $OCO(CH_2)_{32}CH_3]^+$, 491[$C_{1'}$–O fission, $CO(CH_2)_{32}CH_3]^+$ and 137 $[M–491]^+$ indicated that gheddic acid (tetratriacontanoic acid) was esterified with 4-hydroxyphenethyl alcohol (tyrosol). The ^1H NMR spectrum of **6** displayed two two-proton doublets δ 7.08 ($J = 8.4$ Hz) and 6.76 ($J = 8.0$ Hz) assigned to aromatic H-2, H-6 and H-3, H-5 protons, respectively, three two-proton triplets at δ 4.23 ($J = 7.1$ Hz), 2.87 ($J = 7.7$ Hz) and 2.27 ($J = 7.4$ Hz) ascribed correspondingly oxymethylene H_2-8 and methylene H_2-7 and H_2-2′ protons, other methylene protons as multiplets at δ 1.56 (2H) and 1.28 (4H) and as a broad singlet at δ 1.25 (56 H) and a three-proton triplet at

δ 0.87 ($J = 6.3$ Hz) accounted to terminal primary C-34′ methyl protons. The ^{13}C NMR spectrum of **6** exhibited signals for ester carbon at δ 171.80 (C-1′), aromatic carbons between δ 159.32 and 115.39, oxymethylene carbon at δ 64.95 (C-8), other methylene carbons in the range of δ 34.57–22.70 and methyl carbon at δ 14.13 (Me-34′). From the HSQC spectrum (Figure S2), the aromatic protons at H-2 and H-6 (δ 7.08) showed correlations with C-2 and C-6 (δ 115.39), the aromatic protons H-3 and H-5 (δ 6.76) interacted with C-5 and C-6 (δ 130.05), benzylic protons H_2-7 (δ 2.87) correlated with C-7 (δ 34.57); oxygenated methylene protons H_2-8 (δ 4.23) with C-8 (δ 64.95); methylene protons H_2-2′ (δ 2.27) with C-2′ (δ 34.28); other methylene protons with C-3′ to C-33′ (δ 31.94 - 22.70) and methyl protons H_3-34′ (δ 0.87) with C-34′ (δ 14.13). The attachment of a hydroxyl group at C-4 was based on the long-range ^{1}H–^{13}C correlations (HMBC) observed between 4-OH (δ 10.94) with C-3 and C-5 (δ 115.39). The attachment of long alkyl side chain could be deduced from HMBC correlations observed between H_2-7 (δ 2.87) with C-2 and C-6 (δ 115.39); H_2-8 (δ 4.23) with C-1 (δ 138.41), C-1′ (δ 171.80). (Figure S2). Acid hydrolysis of **6** yielded 4-hydroxyphenethyl alcohol (tyrosol), m. p. 90-92 °C, $[M]^+$ *m/z* at 138 ($C_8H_{10}O_2$) and gheddic acid (tetratriacontanoic acid), m. p. 93-94° C; $[M]^+$ *m/z* at 508 ($C_{34}H_{68}O_2$). On the basis of spectral evidences and chemical reactions, the structure of **6** has been elucidated as 4-hydroxyphenethyl tetratriacontanoate, a new fatty acid ester (Figure 1).

Compound **7** showed good antibacterial activity against *S. aureus*, *E.coli* and *K. pneumoniae* with zone of inhibition 28, 28, 26 respectively at concentration 200 μg/mL comparable to standard drug Amoxycillin (30, 28, 29) respectively at the same concentration. Compounds **5**, **6**, and **8** showed moderate antimicrobial activity. Compound **7** showed MIC 32 μg/mL against *E. coli* which was comparable to amoxicillin having same MIC 32 μg/mL (Table S1 and S2).

Compounds **5** and **6** due to presence of phenolic group exhibited good antioxidant activity by inhibiting DPPH. These compounds strongly scavenged DPPH radical, with IC_{50} values of 3.8μM and 3.2 μM, respectively. Vitamin C, used as positive controls, showed IC_{50} values of 1.6 μM (Table S3). Compound **7** and **8** was found to be inactive.

Conclusion

This study led to the isolation of eight compounds (**1–8**) from *Callistemon lanceolatus*. Compound **7** was potent against bacterial strain *E. coli*. Compounds **5** and **6** showed good antioxidant activity. This work has enhanced understanding about the phytoconstituents of the undertaken plants. These secondary metabolites can be used as analytical markers for quality control of the aforementioned herbal drugs. All these phytoconstituents are reported for the first time from this plant and can be used for quality control of the plant.

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Disclosure statement

No conflict of interest was reported by the authors.

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