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A new indole alkaloid, antioxidant and antibacterial activities of crude extracts from *Saccocalyx satureioides*

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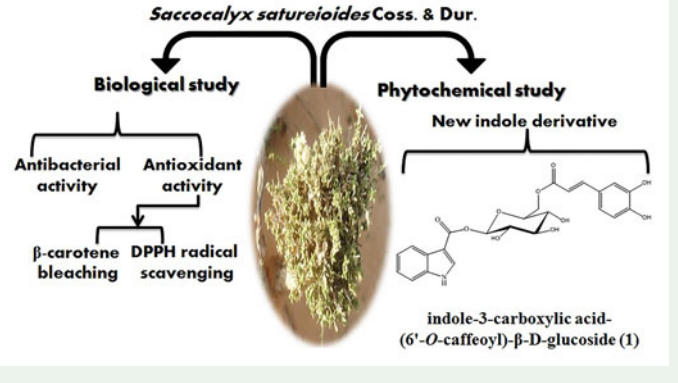
ABSTRACT

The new acylated indole alkaloid glucoside indole-3-carboxylic acid-(6′-*O*-caffeoyl)-β-D-glucoside **1** has been isolated from the ethyl acetate (EtOAC) extract of *Saccocalyx satureioides* Coss. & Dur. (Lamiaceae) together with eight known secondary metabolites **2**-**9**. Two indoles **2** and **3**, five methylated flavone aglycones **4-8** and one monoterpene glucoside **9** were reported for the first time in the genus *Saccocalyx*. The structural elucidation of these compounds was accomplished by spectroscopic methods including 1 D (^{1}H and ^{13}C) and 2 D (COSY, HSQC and HMBC) NMR techniques, and mass spectrometry, and by comparison with literature data. Light petroleum, EtOAc, chloroform and *n*-butanol (*n*-BuOH) extracts of *S. Satureioides* were screened for their antioxidant activity using DPPH radical scavenging and β-carotene bleaching methods. The antibacterial activity of these extracts indicates that *n*-BuOH and EtOAc extracts possess the strongest activity.



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1. Introduction

Saccocalyx satureioides Coss. & Dur., an endemic species of Algeria, is a small aromatic shrub growing in Sahara septentrional and belonging to the family Lamiaceae (Quezel and Santa 1963; Ozenda 2004). It has attracted a great attention due to its traditional medicinal usage for gastric disorders and spasms (Biondi et al. 2006). The essential oil of *S. satureioides* has been extensively investigated and revealed the presence of numerous oil components, which possess interesting biological activities (Biondi et al. 2006; Laouer et al. 2006; Bendahou et al. 2008). However, a literature survey reveals that there is only one study describing the isolation and structural elucidation of phytochemical components of this plant (Mohamadi et al. 2015).

The TLC profile of the different extracts (light petroleum, $CHCl_3$, EtOAc and *n*-BuOH) obtained from *S. satureioides* revealed the especial richness of EtOAc extract in phytochemicals compared to the other extracts, and this is the reason why our phytochemical study is focused on this extract.

This study led to the isolation and identification of one new acylated indole alkaloid glucoside **1** and eight known compounds, including two indoles **2** and **3**, five methylated flavone aglycones **4-8** and one monoterpene glucoside **9**. Since several isolated compounds such as indole derivatives and methylated flavones were reported to have an interesting antibacterial and antioxidant activities, we decided to assess these bioactivities against the light petroleum, $CHCl_3$, EtOAc and *n*-BuOH extracts obtained from this species.

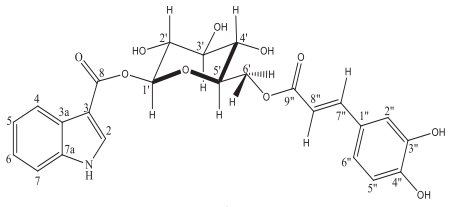
2. Results and discussion

2.1. Chemicals constituents of EtOAc extract

The multistep chromatographic separations and purifications (VLC, CC and TLC) were applied on EtOAc extract of the aerial parts of *S. satureioides* allowing the isolation of indole-3-carboxylic acid-(6′-*O*-caffeoyl)-β-D-glucoside **1**, a new indole alkaloid derivative (Figure 1), together with eight known secondary metabolites **2-9**. The structures of the isolated compounds **1-9** were elucidated using 1 D and 2 D NMR analysis, HRESI-MS spectroscopy and comparison with literature data. The known products were identified as: indole-3-carboxylic acid-β-D-glucoside **2**, indole-3-carboxylic acid **3** (Hagemeier et al. 2001), luteolin-7-methyl ether **4** (Saewan et al. 2011), velutin **5** (Kraut et al. 1995), conzalitosin **6** (Salan et al. 2001), 4′,5,6-trihydroxy-3′,7-dimethoxyflavones **7** (Intekhab and Aslam 2011), thymonin **8** (Broucke et al. 1982), α-terpinol-8-*O*-β-D-glucoside **9** (Yamahara et al. 1985). It should be noted that all the isolated compounds **1-9** were reported for the first time from this genus, and among them, the two indoles **2** and **3** are described herein for the first time in Lamiaceae family.

2.2. Structural elucidation of the new compound

Compound **1** was isolated as a brown wax. Its HR-ESI$^+$MS showed a pseudo-molecular ion peak at *m/z* 508.4301 $[M + Na]^+$ (calcd. for $C_{24}H_{23}NO_{10}Na$, 508.4293), consistent with the molecular formula $C_{24}H_{23}NO_{10}$, indicating the presence of 14 degrees of



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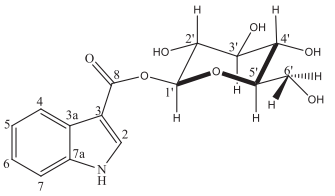

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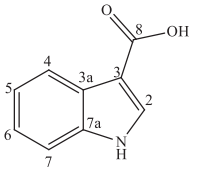

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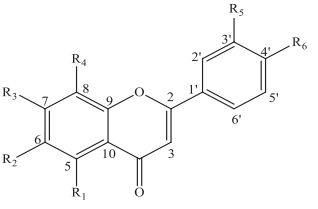

	R_1	R_2	R_3	R_4	R_5	R_6
4:	OH	H	OCH_3	H	OH	OH
5:	OH	H	OCH_3	H	OCH_3	OH
6:	OH	H	OCH_3	H	OCH_3	OCH_3
7:	OH	OH	OCH_3	H	OCH_3	OH
8:	OH	OH	OCH_3	OCH_3	OCH_3	OH

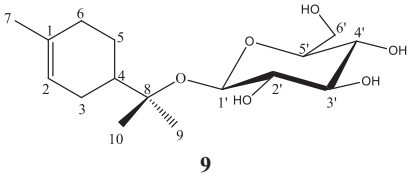

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Figure 1. Structures of isolated compounds **1**–**9**.

unsaturation. The ^{1}H NMR spectrum of compound **1** showed five downfield signals of an ABCD aromatic spin system at δ_H 8.02 (d, *J* 7.5 Hz, H-4), 7.18-7.22 (m, H-5), 7.20-7.24 (m, H-6) and 7.49 (d, *J* 7.7 Hz, H-7), and of a vinylic proton at δ_H 8.16 (s, H-2). These main characteristic features suggested the presence of an indole alkaloid skeleton (Levy et al. 2000). The absence of a coupling constant for H-2 proved the existence of a substituent at C-3 of the indole ring. Indeed, the HMBC spectrum confirmed this assumption with a ^3J cross-peak between H-2 and the quaternary carbon at δ_C 162.8, assigned to the carbonyl ester C-8, and a ^2J correlation of this proton (H-2) with the signal of C-3. The ^{13}C NMR spectrum of **1** exhibited 24 carbon resonances classified into eight quaternary carbons (δ_C 105.6, 125.4, 125.7, 136.4, 145.5, 148.4, 162.8 and 166.5), fifteen methine (δ_C 69.4, 72.5, 74.6, 76.3, 93.6, 112.4, 113.7, 114.9, 115.7, 120.5, 121.4, 121.5, 122.5, 133.5 and 145.4) and one methylene (δ_C 63.4) carbons. The carbon resonances at δ_C 105.6, 112.4, 120.5, 121.5, 122.5, 125.4, 1334.4 and 136.4 were attributed to the indole skeleton using HMBC and HSQC correlations. The downfield signals at δ_C 63.4, 69.4, 72.5, 74.6, 76.3 and 93.6 were assigned to a glucopyranosyl moiety based in the comparison with data reported in the literature (Hagemeier et al. 2001). In addition, the ^{1}H NMR spectrum supported the presence of the glucosyl moiety with the anomeric proton resonating at δ_H 5.63 (d, *J* 7.2 Hz, H-1′). The analysis of the COSY and HSQC spectra permitted the full assignment of the glucose proton signals. Furthermore, acid hydrolysis of **1** in refluxing MeOH and HCl (1N) solution provided (+)-D-glucose which was confirmed by TLC comparison with an authentic sample. The cross-peak of the anomeric proton H-1′ and carbonyl carbon C-8 (δ_C 162.8) allow us to establish the linkage point between the sugar moiety and the substituted indole skeleton (Figure S10, supplementary material). This linkage was confirmed by the chemical shift value of the glucosyl carbon C-1′ at δ_C 93.6, which would appear at higher than

100 ppm in 3-*O*-glucosylated derivatives (Meng et al. 2017). From these results, the partial structure of compound **1** was assigned as an indole-3-carboxylic acid-β-D-glucopyranosyl ester derivative (Hagemeier et al. 2001). However, the remaining part of the molecule it was identified as a $C_9H_7O_3$ moiety according to the ESI-MS spectrum. The ABX spin system of a caffeoyl moiety appear at δ_H 7.05 (s, H-2"), 6.75 (d, *J* 7.9 Hz, H-5") and 7.01 (d, *J* 7.9 Hz, H-6"), while their vinylic protons appear as two doublets at δ_H 7.46 (H-7") and 6.28 (H-8"), with a high ^3J 15.9 Hz characteristic of a *trans* configuration. The remaining proton and carbon assignments were accomplished by HSQC and HMBC spectra. The ^{13}C NMR spectrum of the glucose moiety also showed a downfield shift of C-6′ signal ($\Delta + 0.9$) relatively to the chemical shift values of an unlinked carbon C-6′ (Hagemeier et al. 2001, Meng et al. 2017). This shift is expected for a glucose C-6′ acylation (Hosokawa et al. 1997). This chemical bond was also confirmed by a ^3J HMBC cross-peak of H-6′ signals (δ_H 4.16, dd, *J* 11.7, 6.0 Hz and 4.41, d, *J* 11.7 Hz) with the caffeoyl carbonyl ester C-9" signal (δ_C 166.5) (Figure S10, supplementary material). These findings showed that caffeic acid was linked to C-6′ of glucosyl moiety. All the above-mentioned NMR spectral data established the new structure of compound **1** as indole-3-carboxylic acid-(6′-*O*-caffeoyl)-β-D-glucoside.

2.3. Spectral data

Indole-3-carboxylic acid-(6′-*O*-caffeoyl)-β-D-glucoside: Brown wax; HR-ESI$^+$-MS: *m/z* 508.4301 $[M + Na]^+$ (calcd. for $C_{24}H_{23}NO_{10}Na$, 508.4293) for formula $C_{24}H_{23}NO_{10}$. ^1H NMR (300 MHz, DMSO-d_6) δ_H (ppm): 12.06 (s, NH), 8.16 (s, H-2), 8.02 (d, *J* 7.5 Hz, H-4), 7.49 (d, *J* 7.7 Hz, H-7), 7.46 (d, *J* 15.9 Hz, H-7"), 7.20-7.24 (m, H-6), 7.18-7.22 (m, H-5), 7.05 (s, H-2"), 7.01 (d, *J* 7.9 Hz, H-6"), 6.75 (d, *J* 7.9 Hz, H-5"), 6.28 (d, *J* 15.9 Hz, H-8"), 5.63 (d, *J* 7.2 Hz, H-1′), 4.41 (d, *J* 11.7 Hz, H-6), 4.16 (dd, *J* 11.7 Hz, 6.0 Hz, H-6), 3.58-3.64 (m, H-5′), 3.36-3.42 (m, H-2′ and H-3′), 3.29 (t, *J* 8.1 Hz, H-4′). ^{13}C NMR (75 MHz, DMSO-d_6) δ_C (ppm): 162.8 (C-8), 166.5 (C-9"), 148.4 (C-4"), 145.5 (C-3"), 145.4 (C-7"), 136.4 (C-7a), 133.5 (C-2), 125.7 (C-1"), 125,4 (C-3a), 122.5 (C-6), 121.5 (C-5), 121.4 (C-6"), 120.5 (C-4), 115.7 (C-5"), 114.9 (C-2"), 113.7 (C-8"), 112.4 (C-7), 105.6 (C-3), 93.6 (C-1′), 76.3 (C-3′), 74.6 (C-5′), 72.5 (C-2′), 69.4 (C-4′), 63.4 (C-6′).

2.4. Evaluation of antioxidant activity

2.4.1. DPPH radical scavenging method

The antioxidant activity of the different extracts of *S. satureioides* was measured in terms of hydrogen donating or radical scavenging ability using the stable DPPH radical (Von Gadow et al. 1997). Quercetin was used as positive control. The antioxidant power of the different extracts (Table S1, supplementary material) decreased in the order of EtOAC (IC_{50} 20.81 ± 3.52 μg/mL) > $CHCl_3$ (IC_{50} 23.93 ± 4.15 μg/mL) > *n*-BuOH (IC_{50} 93.23 ± 6.43 μg/mL) > light petroleum (IC_{50} 537.02 ± 1.03 μg/mL). The EtOAc and $CHCl_3$ extracts showed the highest radical scavenging activity while the activity of light petroleum extract was much lower. It should be noted that the substrate polarity of extracts obtained from plants does not affect DPPH scavenging activity (Yamaguchi et al. 1998; Pekkarinen et al. 1999; Koleva et al. 2002). However, the lower

effectiveness of the $CHCl_3$ extract in comparison with EtOAc extract may be explained by the weak concentration of active components in $CHCl_3$ extract, having capacity to donate hydrogen atoms or electrons and to capture free radicals (Kulisic et al. 2004). Moreover, this study revealed that the major constituents of EtOAc extract are flavonoids known especially for their antioxidant activity.

2.4.2. β-Carotene bleaching method

This method is based on the loss of β-carotene orange colour due to its reaction with radicals formed by linoleic acid oxidation in an emulsion system. The β-carotene bleaching rate can be slow down in the presence of antioxidants (Pratt 1980; Mallet et al. 1994). Butylated hydroxyanisole (BHA) was used as standard. The IC_{50} values of the different extracts (Table S1, supplementary material) clearly indicated that chloroform extract possessed the highest antioxidant potential (IC_{50} 40.32 ± 5.32 μg/mL). The weakest radical scavenging activity was exhibited by the light petroleum extract with an IC_{50} 908.35 ± 0.90 μg/mL. The fact that the $CHCl_3$ extract being more effective as antioxidant than the semi-polar EtOAc extract (IC_{50} 57.12 ± 1.55 μg/mL), in comparison with the results obtained for the DPPH assay, suggests that the antioxidant activity of the $CHCl_3$ extract is probably due to the presence of lipophilic active compounds (Kulisic et al. 2004).

2.5. Evaluation of antibacterial activity

The results obtained from disc diffusion method (Bauer et al. 1996) indicated that *Staphylococcus aureus* and *Escherichia coli* are the most sensitive microorganisms. Gentamicin was used as positive control. As can be seen in Figure S1 (supplementary material), *n*-BuOH and EtOAc extracts were found to have a good activity against *Staphylococcus aureus*, *Escherichia coli, Bacillus cereus* and *Enterobacter aerogenes*, where the *n*-BuOH extract exhibited a strong antibacterial activity against *Staphylococcus aureus*. In general, the weaker activity was observed for the $CHCl_3$ and light petroleum extracts, while this latter indicated particularly no activity against *Streptococcus pneumonia* and *Bacillus cereus*. The results of the antibacterial activity of the different extracts obtained from *S. satureoides* showed differences in inhibiting microorganisms which could be explained by the nature and amount of active compounds in the extracts.

3. Conclusion

The phytochemical study of the EtOAc extract of *S. satureoides* afforded indole-3-carboxylic acid-(6′-*O*-caffeoyl)-β-D-glucoside **1**, a new indole derivative, together with eight know compounds **2-9** consisting of two indoles, five methylated flavone aglycones and one monoterpene glucoside, detected for the first time in *Saccocalyx* genus. The two isolated indoles **2** and **3** were identified for the first time from the family Lamiaceae. The antioxidant activity evaluation using two different methods indicated that *S. satureioides* extracts (EtOAc and $CHCl_3$) possess remarkable antioxidant

properties. The results of the antibacterial activity suggest that polar *n*-BuOH and semi-polar EtOAc extracts are the most effective in inhibiting of most tested microorganisms.

The results of this phytochemical and biological research support the folkloric use of this medicinal plant (Biondi et al. 2006) and indicate that it could be used as potential resource of natural antioxidants. On the other hand, the polar and semi-polar extracts of *S. satureioides* showed a strong antibacterial activity, which seems to suggest their use as effective herbal protecting against a wide spectrum of pathogenic bacteria.

Disclosure statement

No conflict of interest reported by authors.

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