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COMPONENTS AND ANTIMICROBIAL ACTIVITY OF *Lamium amplexicaule* FROM ALGERIA

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Few flavonoids have been reported from the genus *Lamium* (Lamiaceae) [1-3]. We've isolated, for the first time from the species *Lamium amplexicaule* (L), two flavonoids from the aerial parts and two sterols (β-stigmasterol and γ-sitosterol) from the roots of the plant collected in Algeria.

Tests using the disk diffusion method [4, 5] carried out in petroleum ether, ethyl acetate, butanolic, and roots extracts showed a great antimicrobial activity of the petroleum ether extract against the major microorganisms in the list comprising *Pseudomonas aeruginosa ATCC 27853*, *Escherichia coli ATCC 25922*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Staphylococcus aureus ATCC 25923*, *Streptococcus* α*-hemolytic, Serratia, Enterobacter*, and *Bacillus subtilis.*

Lamium amplexicaule (L) was collected in may 2000 from Djebel El-Ouahch at an altitude of 800 m in Constantine, Algeria. The plant was authentified by Professor M. Kaabeche, Faculty of Sciences, University Ferhat Abbas, Setif. The air-dried aerial parts (500 g) of *Lamium amplexicaule* (L) were extracted with methanol (80%) at room temperature. The extract was concentrated under low pressure. The condensed solution was diluted with water and successively treated with petroleum ether, dichloromethane, ethyl acetate, and *n*-butanol.

The butanolic extract was column chromatographed on polyamide SC6 eluted with toluene-methanol with increasing polarity. Preparative TLC plates on silica gel eluted with ethyl acetate-methanol-acetic acid (8:1:1) led to compound **1**.

The ethyl acetate extract was column chromatographed on silica gel (70-230 mesh) eluted with dichloromethane-methanol with increasing polarity to afford compound **2**.

The air-dried and powdered roots (380 g) were continuously extracted on a Soxhlet apparatus with boiling acetone. The extract was concentrated and then subjected to silica gel column (70-230 mesh) chromatography eluted with cyclohexane–ethyl acetate with increasing polarity then with methanol. Preparative TLC silica gel plates led to compounds **3-4.**

These compounds were identified using ^{1}H NMR, EI/MS, GC, and GC/MS analysis and UV analytical methods.

Compound 1 (7-*O*-glucosyl-3-methylkaempferol), $C_{22}H_{22}O_{11}$, *m/z*: 642 $[M]^+$, mp 336-337°C, UV spectrum (MeOH, λ_{max}, nm): 251, 340; +$ALCl_3$/HCl: 252, 267, 358; + NaOH: 268, 263, 406; +NaOAc: 252, 275, 346.

^{1}H NMR data (250 MHz, DMSO-d_6, δ, ppm, J/Hz): proton signals at 3.2-3.9 (7H, Glu), 3.8 (3H, s, OCH_3), 5.1 (1H, d, J = 6, H″ [7-*O*-Glu]), 6.4 (1H, d, J = 2, H-6), 6.8 (1H, d, J = 2, H-8), 6.9 (2H, d, J = 9.1, H-3′, H-5′), 7.5 (2H, d, J = 9.1, H-2′, H-6′), 9.5 (1H, br.s, 4′-OH), 12.8 (1H, s, 5-OH) [6, 7].

Compound 2 (5,7,4-trihydroxy-3-methoxyflavone, chrysoeriol), $C_{16}H_{12}O_6$, *m/z*: 300 $[M]^+$, mp 336-337°C, UV spectrum (MeOH, λ_{max}, nm): 254, 270, 346; +$ALCl_3$/HCl: 257, 277, 375; + NaOH: 268, 265, 406; +NaOAc: 268, 275, 369.

^{1}H NMR data (250 MHz, DMSO-d_6, δ, ppm, J/Hz): proton signals at 3.8 (3H, s, OCH_3), 5.9 (1H, d, J = 2, H-6), 6.2 (1H, d, J = 2, H-8), 6.6 (1H, s, H-3), 6.8 (1H, d, J = 8, H-5′), 7.5 (2H, dd, J = 2 and J = 8, H-2′ and H-6′), 9.5 (1H, br.s, 4′-OH), 10.3 (1H; br.s, 7-OH), 12.8 (1H, s, 5-OH) [6, 7].

Compound 3 (stigmasterol), $C_{29}H_{48}O$, *m/z*: 412 $[M]^+$. The IR spectrum of compound **3** exhibited absorption bands of hydroxyls (3560 cm^{-1}) and aromatic C=C bonds (806-1469 cm^{-1}).

^{13}C NMR data of compound **3** were identical with those published in the literature [8].

GC/MS with Wiley literature confirmed the structure of stigmasterol.

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TABLE 1. Antimicrobial Activity of the Petroleum Ether Extract of *Lamium amplexicaule*

Microorganisms	CMI, µg/mL	Inhibition Zone diameters, mm				
		Dilution				
		1	1/2	1/4	1/8	1/16
S. aureus	32	16	12	-	-	-
B. subtilis	4	24	20	20	18	16
S. α-hemolytic	32	22	20	18	18	16
E. coli	0.32	20	18	16	16	14
P. aeruginosa	0.32	22	16	20	18	18
K. pneumoniae	0.32	16	16	14	12	12
Serratia	0.016	16	16	16	14	12
Enterobacter	8	18	16	16	14	14

Compound 4 (sitosterol), $C_{29}H_{50}O$, *m/z*: 414 $[M]^+$. The IR spectrum of compound **4** exhibited absorption bands of hydroxyls (3560 cm^{-1}) and aromatic C=C bonds (806-1469 cm^{-1}).

^{13}C NMR data of compound **4** were identical with those published in the literature [8].

GC/MS with Wiley literature confirmed the structure of sitosterol.

Antimicrobial Activity. As shown in Table 1, the petroleum ether extract inhibited remarkably the growth of the microorganisms *Serratia*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus subtilis*, *Enterobacter*, *S. α-hemolytic,* and *Staphylococcus aureus* at the concentration levels of 0.016 µg/mL, 0.032 µg/mL, 0.032 µg/mL, 0.032 µg/mL, 4 µg/mL, 8 µg/mL and 32 µg/mL with 16 mm, 22 mm, 20 mm, 16 mm, 24 mm, 18 mm, 22 mm, and 16 mm inhibition zone diameters, respectively.

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