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COMPARATIVE ANTIOXIDANT ACTIVITY OF TWO ALGERIAN *Reseda* SPECIES

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The *Reseda* genus is found in the Mediterranean and the Southwestern Asian areas [1]. In Algeria, there are twenty two species and subspecies [2, 3]. Eight flavones [4–9], sixteen flavonols [7, 8, 10–16], and one isoflavone [13] have been reported from the genus *Reseda*.

R. villosa (Resedaceae) is an endemic species [1] collected from Ghardaia (Septentrional Algerian Sahara) in March 2002 and authenticated by Prof. Gerard De Belair (Annaba, Algeria).

R. duriaeana (Resedaceae) is an endemic species [2] collected from Djebel El-Ouach-Constantine (Eastern Algeria) in May 2004 and authenticated by Prof. Gerard De Belair (Annaba, Algeria).

Voucher specimen were deposited in the herbarium of the Laboratory of Therapeutic Substances (LOST) at Mentouri University (LOST/ZKAK Rv/03/02 and LOST/ZKAK Rd/05/04).

Air-dried and powdered aerial parts (1 kg) of *R. duriaeana* and *R. villosa* (1 kg) were macerated in a methanolic solution (70%). The extract of each plant was successively concentrated to dryness (under low pressure); the residue was dissolved in boiling water and extracted with petroleum ether, dichloromethane, ethyl acetate, and *n*-butanol successively.

The butanolic extract was column chromatographed on polyamid SC6 and eluted with toluene–methanol with increasing polarity. Whatman 3MM paper chromatography using 15% AcOH and BAW (*n*-BuOH–AcOH–H_2O, 4:1:5 upper phase) and TLC on polyamid DC6, eluted with H_2O–MeOH–methyl–ethyl ketone–acetylacetone (13:3:3:1) followed by column flash chromatography over Sephadex LH-20 in MeOH led to five pure flavonoid glycosides from *R. duriaeana* (**1–5**) and six flavonoid glycosides (**5–10**) from *R. villosa,* which were identified by the usual physicochemical techniques: R_f, fluorescence, UV, NMR spectroscopies, and acid hydrolysis [17–19].

Acid Hydrolysis. The pure compounds were treated with 2 M HCl at 100°C for 1 h. The hydrolysates were extracted with EtOAc, and the aglycones were identified by their UV spectra in methanol and by comparison of their R_f with authentic samples.

Sugars in the aqueous residue were identified by comparison with authentic samples on silica gel TLC impregnated with 0.2 M NaH_2PO_4, solvent Me_2CO–H_2O (9:1), and revealed with aniline malonate.

Compound 1, $C_{21}H_{19}O_{12}$; mp 176–177°C; UV (MeOH, λ_{max}, nm): 264.5, 339; NaOH: 273, 322, 389; $AlCl_3$: 273, 394; $AlCl_3$ + HCl: 274, 393. NaOAc: 277, 350; NaOAc + H_3BO_3: 278, 345.

^{1}H NMR (250 MHz, CD_3OD, δ, ppm, J/Hz): 7.80 (2H, d, J = 8.8, H-2′, H-6′), 6.90 (2H, d, J = 8.8, H-3′, H-5′), 6.40 (1H, d, J = 2.0, H-8), 6.20 (1H, d, J = 2.0, H-6), 5.40 (1H, d, J = 1.7, H-1″, 3-*O*-Rha), 0.90 (3H, d, J = 6.0, CH_3, 3-*O*-Rha). Identified as kaempferol 3-*O*-α-rhamnoside.

Compound 2, $C_{21}H_{20}O_{10}$; mp 194–196°C (methanol); UV (MeOH, λ_{max}, nm): 265.5, 366; NaOH: 292.5, 434; $AlCl_3$: 273, 420; $AlCl_3$ + HCl: 274, 419. NaOAc: 267, 373; NaOAc + H_3BO_3: 268, 375.

^{1}H NMR (250 MHz, CD_3OD, δ, ppm, J/Hz): 7.80 (2H, d, J = 8.7, H-2′, H-6′), 6.95 (2H, d, J = 8.7, H-3′, H-5′), 6.70 (1H, d, J = 2.0, H-8), 6.47 (1H, d, J = 2.0, H-6), 5.41 (1H, d, J = 6.0, H-1″, 7-*O*-Glu). Characterized as kaempferol 7-*O*-β-glucoside.

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TABLE 1. Free Radical Scavenging Capacity in the DPPH System (IC_{50}, μg/mL) of the Samples[a]

Extract	IC_{50}, μg/mL	Extract	IC_{50}, μg/mL
MERD	76.3±2.29	BERV	209±0.6
BERD	99±3.6	Quercetin[b]	12±0.5
MERV	100.1±1.7	Pycnogenol[b]	25±1

[a]Values expressed are means ±S.D. of three parallel measurements. ($P<0.05$).
[b]Reference compound.

Compound 3, $C_{21}H_{19}O_{12}$; mp 180–184°C; UV (MeOH, λ_{max}, nm): 256, 348; NaOH: 272, 325, 400.5; $AlCl_3$: 274.5, 426.5; $AlCl_3$ + HCl: 272.5, 395.5. NaOAc: 272, 390; NaOAc + H_3BO_3: 273, 372. ^{1}H NMR (250 MHz, CD_3OD, δ, ppm, J/Hz): 8.50 (1H, d, J = 2.0, H-2′), 7.40 (1H, dd, J = 8.3; 2.0, H-6′), 6.90 (1H, d, J = 8.3, H-5′), 6.40 (1H, d, J = 2.0, H-8), 6.20 (1H, d, J = 2.0, H-6), 5.30 (1H, d, J = 1.3, H-1″, 3-*O*-Rha), 0.90 (3H, d, J = 6.1, CH_3, Rha). Identified as quercetin 3-*O*-α-rhamnoside.

Compound 4, $C_{28}H_{32}O_{16}$; UV (MeOH, λ_{max}, nm): 264.5, 341; NaOH: 270.5, 384; $AlCl_3$: 273.5, 399; $AlCl_3$ + HCl: 273.5, 397. NaOAc: 267, 380; NaOAc + H_3BO_3: 267, 350.

^{1}H NMR (250 MHz, CD_3OD, δ, ppm, J/Hz): 7.80 (2H, d, J = 8.8, H-2′, H-6′), 6.80 (2H, d, J = 8.8, H-3′, H-5′), 6.70 (1H, d, J = 2.1, H-8), 6.40 (1H, d, J = 2.1, H-6), 5.40 (1H, d, J = 2.0, H-1″, 7-*O*-Rha), 5.10 (1H, d, J = 7.3, H-1″, 3-*O*-Glu), 0.89 (3H, d, J = 6.0, CH_3, Rha), 3.60 (3H, s, O-CH_3). Based on the reported NMR data [18], this compound was characterized as 4′-*O*-methylkaempferol 3-*O*-β-glucosyl-7-*O*-α-rhamnoside.

Compound 5, $C_{27}H_{30}O_{15}$; mp 248–248.2°C; UV (MeOH, λ_{max}, nm): 266.2, 349.8; NaOH: 273, 389.4; $AlCl_3$: 274.4, 396; $AlCl_3$ + HCl: 275, 396.8; NaOAc: 266, 352.6; NaOAc + H_3BO_3: 266, 351.4.

^{1}H NMR (250 MHz, CD_3OD, δ, ppm, J/Hz): 8.08 (2H, d, J = 8.7, H-2′, H-6′), 6.89 (2H, d, J = 8.7, H-3′, H-5′), 6.84 (1H, d, J = 2.0, H-8), 6.45 (1H, d, J = 2.0, H-6), 5.50 (1H, d, J = 2.0, H-1″, 7-*O*-Rha), 5.42 (1H, d, J = 7.1, H-1″, 3-*O*-Glu), 1.10 (3H, d, J = 5.8, CH_3, Rha). Based on the reported NMR data [18], this compound was identified as kaempferol 3-*O*-β-glucosyl-7-*O*-α-rhamnoside.

Compound 6, $C_{21}H_{20}O_{10}$; mp 221–223°C; UV (MeOH, λ_{max}, nm): 266, 368.6; NaOH: 286.2, 437.6; $AlCl_3$: 267.2, 426.4; $AlCl_3$ + HCl: 266.2, 425.2; NaOAc: 267, 372; NaOAc + H_3BO_3: 268, 367.6.

^{1}H NMR (250 MHz, CD_3OD, δ, ppm, J/Hz): 8.15 (2H, d, J = 8.9, H-2′, H-6′), 6.93 (2H, d, J = 8.9, H-3′, H-5′), 6.77 (1H, d, J = 2.0, H-8), 6.44 (1H, d, J = 2.0, H-6), 5.59 (1H, d, J = 2.0, H-1″), 1.23 (3H, d, J = 6.0, CH_3, Rha). Characterized as kaempferol 7-*O*-α-rhamnoside.

Compound 7, $C_{27}H_{30}O_{16}$; mp 248.2–248.4°C; UV (MeOH, λ_{max}, nm): 257, 355.4; NaOH: 269.6, 410.6; $AlCl_3$: 274.6, 423.8; $AlCl_3$ + HCl: 269.6, 403.2; NaOAc: 261.8, 359; NaOAc + H_3BO_3: 262, 375.8.

^{1}H NMR (250 MHz, CD_3OD, δ, ppm, J/Hz): 7.74 (1H, d, J = 2.1, H-2′), 7.63 (1H, dd, J = 8.0; 2.1, H-6′), 6.88 (1H, d, J = 8.0, H-5′), 6.75 (1H, d, J = 2.0, H-8), 6.46 (1H, d, J = 2.0, H-6), 5.59 (1H, d, J = 2.0, H-1″, 7-*O*-Rha), 5.35 (1H, d, J = 7.4, H-1″, 3-*O*-Glu) 1.21 (3H, d, J = 6.0, CH_3, Rha). Based on the reported NMR data [18], this compound was identified as quercetin-7-*O*-α-rhamnosyl-3-*O*-β-glucoside.

Compound 8, $C_{28}H_{32}O_{16}$; mp 222–224°C; UV (MeOH, λ_{max}, nm): 268, 353.3; NaOH: 271.8, 407.6; $AlCl_3$: 272.5, 403.1; $AlCl_3$ + HCl: 271.6, 401.8; NaOAc: 268.4, 357.6; NaOAc + H_3BO_3: 268.1, 355.8.

^{1}H NMR (250 MHz, CD_3OD, δ, ppm, J/Hz): 7.98 (1H, d, J = 2.0, H-2′), 7.65 (1H, dd, J = 8; 2.0, H-6′), 6.94 (1H, d, J = 8.0, H-5′), 6.78 (1H, d, J = 2.0, H-8), 6.48 (1H, d, J = 2.0, H-6), 5.60 (1H, d, J = 2.0, H-1″, 7-*O*-Rha), 5.52 (1H, d, J = 6.0, H-1″, 3-*O*-Glu), 3.97 (3H, s, O-CH_3), 0.89 (3H, d, J = 6.0, CH_3, Rha), 3.97 (3H, s, O-CH_3). Based on the reported NMR data [18], this compound was characterized as isorhamnetin 3-*O*-β-glucosyl-7-*O*-α-rhamnoside.

Compound 9, $C_{28}H_{32}O_{16}$; UV (MeOH, λ_{max}, nm): 272, 358; NaOH: 272, 405.85; $AlCl_3$: 278.2, 413.1; $AlCl_3$ + HCl: 278.2, 414.4; NaOAc: 271.8, 364.2; NaOAc + H_3BO_3: 271.4, 362.2.

^{1}H NMR (250 MHz, CD_3OD, δ, ppm, J/Hz): 8.18 (2H, d, J = 9.0, H-2′, H-6′), 6.93 (2H, d, J = 9.0, H-3′, H-5′), 6.70 (1H, s, H-6), 5.61 (1H, d, J = 2.0, H-1″, 7-*O*-Rha), 5.39 (1H, d, J = 7.5, H-1″, 3-*O*-Glu), 1.30 (3H, d, J = 6.1, CH_3, Rha), 3.94 (3H, s, O-CH_3). Based on the reported NMR data [18], this compound was characterized as 8-*O*-methylkaempferol 3-*O*-β-glucosyl-7-*O*-α-rhamnoside.

Compound 10, $C_{27}H_{30}O_{14}$; mp 219–221°C (methanol); UV (MeOH, λ_{max}, nm): 265, 344; NaOH: 267.6, 384.5; $AlCl_3$: 274, 396; $AlCl_3$ + HCl: 274.6, 396; NaOAc: 264.8, 348.6; NaOAc + H_3BO_3: 264.8, 345.2.

^{1}H NMR (250 MHz, CD_3OD, δ, ppm, J/Hz): 7.83 (2H, d, J = 8.8, H-2′, H-6′), 6.97 (2H, d, J = 8.8, H-3′, H-5′), 6.76 (1H, d, J = 2.2, H-8), 6.50 (1H, d, J = 2.2, H-6), 5.58 (1H, d, J = 1.7, H-1″, 7-*O*-Rha), 5.42 (1H, d, J = 1.7, H-1″, 3-*O*-Rha), 1.29 (3H, d, J = 6, CH_3, 7-*O*-Rha), 0.96 (3H, d, J = 6, CH_3, 3-*O*-Rha). Based on the reported NMR data [18], this compound was identified as kaempferol-3-*O*-α-rhamnosyl-7-*O*-α-rhamnoside.

Flavonoids **1**, **2**, **5**, **6**, **7**, and **8** have been isolated from other species of *Reseda* [5, 6, 12–14, 16], while compounds **3**, **4**, **9**, and **10** are reported for the first time from the genus *Reseda*.

Antioxidant Activity. The methanolic (MERD) and *n*-butanolic (BERD) extracts of *R. duriaeana* and the methanolic (MERV) and *n*-butanolic (BERV) extracts of *R. villosa* were investigated for their antioxidant activity using the model of scavenging the stable DPPH radical in a relatively short time compared with other methods [20]. As shown in Table 1, the methanolic and *n*-butanolic extracts of *R. duriaeana* exhibited better antioxidant activity than the respective extracts of *R. villosa*. This may be explained by the presence of more quercetin derivatives in *R. duriaeana.*

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