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Antioxidant, antibacterial activities and flavonoids of *Reseda phyteuma* L.

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ABSTRACT

*Six flavonoids were isolated for the first time from the aerial parts of Reseda phyteuma (Resedaceae): Apigenin (**1**), Luteolin (**2**), Apigenin 7-O-β-glucoside (**3**), Luteolin 7-O-β-glucoside (**4**), Luteolin 7-O-β-galactoside (**5**), Luteolin 3'-O-β-glucoside (**6**), A significant phenolic content (>5.44 g/100 g of dry extracts) were found for the butanolic extract which exhibited a good antioxidant activity (IC_{50} 15 µg/ml) compared with the reference (rutin IC_{50} 3.01 ± 0.2 µg/ml). The best antibacterial activity of the chloroformic extract of R. phyteuma was observed against Proteus mirabilis, Staphylococcus aureus, Staphylococcus aureus ATCC 43300, Pseudomonas aeruginosa ATCC 27853, Pseudomonas aeruginosa, with 13, 12, 11, 11, 11mm, inhibition zone diameters, respectively.*

Keywords: *Reseda phyteuma* L. Flavonoids. Antioxidant. Antibacterial.

INTRODUCTION

The Resedaceae are represented by six genus which are *Reseda, Caylusea, Oligomeris, Astrocarpus and Randonia*. The genus *Reseda* is found in the Mediterranean and the South Western Asian areas. There are twenty two species and subspecies in flora of Algeria from which *R. villosa*, *R. duriaeana* and *R. arabica* are endemic [1-3]. Flavonoids and phenolic acids [4-11], non-protein amino acids [12], glucosinolates [13] and alkaloids [14] were reported from *Reseda* genus. Pharmacological studies of extracts of various *Reseda* species showed anti-inflammatory [15,16] and antimicrobial [17], antioxidant [10,18], antibacterial [16-18] activities. In continuation of our works on *Reseda* species, we report here the antioxidant, antibacterial activities and flavonoids of the algerian species *Reseda phyteuma* L.

MATERIALS AND METHODS

Plant extract

Reseda phyteuma L. was collected from Constantine (North Eastern Algerian) in May 2009, during the flowering stage and authenticated by Mr. K. Kabouche. A voucher specimen has been deposited in the Herbarium of the laboratory of Therapeutic Substances (LOST), University of Constantine 1 (LOST Rp/05/09).

Extraction

The aerial parts of *R. phyteuma* (1.5 kg) were macerated in a methanolic solution (70%). After filtration and concentration, the residue was dissolved in boiling water (800 ml). The resulting solution was extracted successively with $CHCl_3$, EtOAc and *n*-butanol. Concentration in *vacuo* led to the following extracts: $CHCl_3$ (2.6g), EtOAc (4.3) and *n*-butanol (48.8g).

The butanolic extract (17 g) was column chromatographed on polyamid SC6, eluted with a gradient of MeOH - Toluene with increasing polarity to give twelve fractions (1-12). Fraction F5 obtained from toluene 88%, was subjected to a silica gel column chromatography eluted with ($CHCl_3$:EtOAc, 7:3) affording compound **1,** identified as Apigenin. Fraction F6 obtained from toluene 85%, was subjected to a silica gel column chromatography eluted with ($CHCl_3$:EtOAc: 7:3) and (AcOEt:MeOH:H_2O, 10:1:0.5) leading to seven **s**ubfractions: The subfraction f3 which was obtained as a yellow precipitate (compound **2)**, was identified as Luteolin. The subfraction f5 was separated by silica gel column chromatography being eluted with (AcOEt:MeOH:H_2O, 10:1:0.5) affording compound **3** which was identified as Apigenin 7-O-β-glucoside. Fraction F7, obtained from toluene 80% as a yellow precipitate (compound **4)**, was identified as Luteolin 7-O-β-glucoside.

Fraction F9, obtained from toluene 85%, was subjected to a silica gel column chromatography eluted with (AcOEt:MeOH:H_2O, 10:1:0.5) leading to two **s**ubfractions: The subfraction f1 afforded compound **5**, identified as Luteolin 7-O-β-galactoside and subfraction f2 led to compound **6**, identified as Luteolin 3'-O-β-glucoside

Phenols quantification

Total phenolic was quantified according to the Folin–Ciocalteu method, using pyrogallol as a standard [19]. Absorbance was measured at 760 nm with a Uvikon 930 UV/vis spectrophotometer (Kontron instruments), and the results were expressed as pyrogallol equivalents in grams per 100 g of dry material [20-21].

Antioxidant activity

The radical scavenging activity of the butanolic extract was measured by the slightly modified method of Hatano [22-24]. One milliliter of a 0.2 mM DPPH methanol solution was added to 4 mL of various concentrations of the extract in methanol. The mixture was shaken vigorously and left to stand at room temperature. After 30 min, the absorbance of the solution was measured at 517 nm and the antioxidant activity calculated, using the following equation:

Scavenging capacity % = [(Ab of sample – Ab of blank) × 100/ Ab of sample].

Methanol (1 mL) plus plant extract solution (4 mL) were used as a blank, while DPPH solution plus methanol was used as a negative control. The positive control was DPPH solution plus 1 mM rutin. Extract concentration providing 50% inhibition (IC50) was calculated from the plot of inhibition percentage against extract concentration.

Antibacterial activity

The antibacterial activity of the chloroformic extract was tested against a range of microorganisms, namely *Escherichia coli ATCC 25922*, *Escherichia coli*, *Staphylococcus aureus* ATCC 43300, *Staphylococcus aureus*, *Pseudomonas aeruginosa* ATCC 27853, *Pseudomonas aeruginosa*, *Klebsiella pneumonia Enterobacter sp* and *Proteus mirabilis Strep.* The reference strains were obtained from the Pasteur Institute (Algiers). The other strains were obtained from the laboratory of bacteriology, Benbadis Hospital, Constantine, using conventional methods (clinical isolation) [25].

RESULTS

Identification of components

Compound 1. $C_{15}H_{10}O_5$, mp 272°C, UV (λ_{max}, nm): 268, 336 ; +NaOH: 269, 325, 395, +$AlCl_3$: 276, 386; +$AlCl_3$/HCl: 277, 384; NaOAc: 275, 383. +H_3BO_3: 269, 345. ^{1}H NMR (250 MHz, DMSO-d_6, δ, ppm, J/Hz): 7.88 (2H, d, J = 9.0, H-6', H-2'), 6.95 (2H, d, J = 9.0, H-5', H-3'), 6.62 (1H, s, H-3), 6.48 (1H, d, J = 2.5, H-8), 6.22 (1H, d, J = 2.5, H-6). This compound was characterized as Apigenin.

Compound 2. $C_{15}H_{20}O_{10}$, mp 272°C, UV (DMSO-d_6, λ_{max}, nm): 253, 349; +NaOH: 270, 326, 408, +$AlCl_3$: 273, 425; +$AlCl_3$/HCl: 274, 404; NaOAc: 271, 397. +H_3BO_3: 261, 381. ^{1}H NMR (250 MHz, DMSO-d_6, δ, ppm, J/Hz): 7.51 (1H, dd, J = 8.9-2.2, H-6'), 7.02 (1H, d, J = 8.9, H-5'), 6.70 (1H, s, H-3), 6.56 (1H, d, J = 2.1, H-6), 6.30 (1H, d, J = 2.1, H-6). This compound was characterized as Luteolin

Compound 3. $C_{21}H_{30}O_{10}$, mp 272°C, UV (DMSO-d_6, λ_{max}, nm): 268, 333 ; +NaOH: 270, , 384, +$AlCl_3$: 275, 386; +$AlCl_3$/HCl: 276, 385; NaOAc: 267, 396. +H_3BO_3: 269, 333. ^{1}H NMR (250 MHz, DMSO-d_6, δ, ppm, J/Hz): 7.97 (2H, d, J = 8.9, H-6', H-2'), 6.94 (2H, d, J = 8.9, H-5', H-3'), 6.89 (1H, s, H-3), 6.84 (1H, d, J =2.1, H-8), 6.45 (1H, d, J = 2.1, H-6), 5.08 (1H, d, J = 7.2, H-1" glucose), 3.20-4.00 (sugar protons).

Acid hydrolysis of compound **3** produced apigénine and glucose. This compound was characterized as. Apigenin 7-O-*β*-glucoside

Compound 4. $C_{11}H_{20}O_{11}$, mp 272°C, UV (DMSO-d_6, λ_{max}, nm): 260, 343 ; +NaOH: 266, , 400, +$AlCl_3$: 273, 427; +$AlCl_3$/HCl: 273, 396; NaOAc: 273, 402. +H_3BO_3: 261, 369. ^{1}H NMR (250 MHz, CD_3DO, δ, ppm, J/Hz): 7.46 (1H, dd, J = 9.1-2.2, H-6'), 7.43 (1H, d, J = 2.2, H-3'), 6.90 (1H, d, J = 8.9, H-5'), 6.77 (1H, s, H-3), 6.82 (1H, d, J = 2.1, H-6), 6.45 (1H, d, J = 2.1, H-6). This compound was characterized as Luteolin, 5.07 (1H, d, J = 7.1, H-1" glucose), 3.20-4.00 (sugar protons).

Acid hydrolysis of compound **3** produced luteolin and glucose. This compound was characterized as Luteolin 7-O-*β*-glucoside.

Compound 5. $CH_{2730}O_{16}$, mp 272°C, UV (DMSO-d_6, λ_{max}, nm): 261, 342 ; +NaOH: 2264, , 402, +$AlCl_3$: 275, 426; +$AlCl_3$/HCl: 276, 396; NaOAc: 272, 400. +H_3BO_3: 263, 367. ^{1}H NMR (250 MHz, DMSO-d_6, δ, ppm, J/Hz): 7.40 (2H, d, J = 8.9, H-6', H-2'), 6.94 (2H, d, J = 8.9, H-5', H-3'), 6.89 (1H, s, H-3), 6.84 (1H, d, J =2.1, H-8), 6.45 (1H, d, J = 2.1, H-6), 5.08 (1H, d, J = 7.2, H-1" galactoside), 3.20-4.00 (sugar protons).

Acid hydrolysis of compound **5** produced apigénine and galactoside. This compound was characterized as. Luteolin 7-O-*β*-galactoside.

Compound 6. $C_{21}H_{20}O_{10}$, mp 272°C, UV (CD_3DO, λ_{max}, nm): 269, 339 ; +NaOH: 275, 324, 394, +$AlCl_3$: 274, 354; +$AlCl_3$/HCl: 279, 347; NaOAc: 274, 396. +H_3BO_3: 269, 379. ^{1}H NMR (400 MHz, CD_3DO, δ, ppm, J/Hz): 7.61 (1H, dd, J = 9.1-2.2, H-6'), 7.72 (1H, d, J = 2.2, H-3'), 6.97 (1H, d, J = 8.9, H-5'), 6.63 (1H, s, H-3), 6.74 (1H, d, J = 2.1, H-8), 6.19 (1H, d, J = 2.1, H-6), 4.8 (1H, d, J = 7.1, H-1" glucose), 3.20-4.00 . ^{13}C NMR (400 MHZ, DMSO-d_6, δ) 185.5 (C-4), 164.5 (C-7), 159.5 (C-5), 155.5 (C-4'), 159.0 (C-2), 161.5 (C-9), 105 (C-3), 114 (C-2)', 124(C-6'), 122.5 (C-1'), 145 (C-3'), 116.5(C-5'), 105.0 (C-10), 99.0 (C-6), 93.5 (C-8), 103.5 (C-1"), 77.15 (C-3"), 77.15 (C-5"), 72.5 (C-2"), 72.23 (C-4"), 63.0 (C-6").

HMBC experiment established a correlation between C-3' at δ 145.60 with H-1" at δ 4.80 (sugar protons). This compound was characterized as. Luteolin 3'-O-*β*-glucoside.

The known flavonoids: Apigenin (**1**), Luteolin (**2**), Apigenin 7-O-*β*-glucoside (**3**), Luteolin 7-O-*β*-glucoside (**4**), Luteolin 7-O-*β*-galactoside (**5**), Luteolin 3'-O-*β*-glucoside (**6**) were identified by extensive UV analyses and NMR spectroscopic analyses as well as by comparing their spectroscopic data with those reported in the literature comparison of their IR and ^{13}C NMR spectra with literature data [26-29].

The compounds (**1-6**) are isolated for the first time from the species *R. phyteuma.* It appears that the flavonoid contents of this species is different from our previously reported Resedaceae which were characterized by the presence of flavonol glycosides [8-11, 30].

Compared with rutin (IC_{50} : 3.01 µg/ml), the butanolic extract exhibited a good antioxidant activity (IC_{50} : 15.02 µg/ml) **(Table 1).**

A significant phenolic content (5.44 g/100 g of dry extract) and good radical scavenging activity were found for the butanolic extract (IC_{50} : 15.02 µg/ml). In general, the higher the free radical scavenging activity was, the higher the phenolic content was [31] (**Table 1**).

Table 1. Phenols quantification and Antioxidant activity of the butanolic extract

	Phenolic compounds (g/100 g equiv. Pyrogallol)	IC_{50} DPPH (µg/ml)
The butanolic extract	5.44 ± 0.29	15.02
Picnogenol	30.7	12
rutin		3.01

The chloroformic extract inhibited the growth of the tested miroorganisms. The best antibacterial activity was observed against *Proteus mirabilis, Staphylococcus aureus ATCC, Staphylococcus aureus 43300, Pseudomonas aeruginosa ATCC 27853, Pseudomonas aeruginosa,* with 13, 12, 11, 11, 11 mm, inhibition zone diameters, respectively, with 80 µg/ml MIC value **(Table 2).**

Table 2. Antibacterial activity of the chloroformic extract (Inhibition zones and MIC values)

Microorganism	Inhibition Zone [a]	MIC (µg/ml)
Escherichia coli ATCC 25922	-	80
Escherichia coli	-	80
Staphylococcus aureus ATCC 43300	12	80
Staphylococcus aureus	11	80
Pseudomonas aeruginosa ATCC 27853	11	80
Pseudomonas aeruginosa	11	80
Klebsiella pneumoniae	-	80
Enterobacter sp	-	80
Proteus mirabilis	13	80

[a]: *(128 µg/ml)*

CONCLUSION

Six flavonoids have been isolated for the first time from the butanolic extract of the aerial parts of *R. phyteuma* L.: Apigenin (**1**), Luteolin (**2**), Apigenin 7-O-*β*-glucoside (**3**), Luteolin 7-O-*β*-glucoside **4**), Luteolin 7-O-*β*-galactoside (**5**) and Luteolin 3'-O-*β*-glucoside (**6**). A significant phenolic content in agreement with good radical scavenging activity were found for this extract. The chloroformic extract was tested against a panel of Gram + and Gram – bacteria but the growth of the used strains was midly inhibited.

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