

## Flavonol 3-*O*-Glycosides from Three Algerian *Bupleurum* Species

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**Abstract:** Flavonoids distribution in three algerian *Bupleurum* (Apiaceae) species has been investigated. Quercetin (**1**), quercetin 3-rutinoside (**2**) and isorhamnetin 3-rutinoside (**3**) were found in the endemic species *B. plantagineum* Desf. Three kaempferol glycosides, kaempferol 3-glucoside (**4**), kaempferol 3-galactoside (**5**), kaempferol 3-rutinoside (**6**) and three quercetin glycosides, quercetin 3-rutinoside (**2**), quercetin 3-glucoside (**7**) and quercetin 3-galactoside (**8**), have been isolated from *B. fruticosum* L. while isorhamnetin (**9**), isorhamnetin 3-galactoside (**10**) and isorhamnetin 3-galactorhamnoside (**11**) were found in *B. spinosum* L. Seven flavonols are reported here for the first time from the genus.

**Keywords:** *Bupleurum*; *B. plantagineum*; *B. fruticosum*; *B. spinosum*; flavonoids.

### 1. Plant Source

*Bupleurum* (Apiaceae) species are used in traditional medicine to treat various diseases [1,2]. *B. plantagineum* Desf and *B. fruticosum* L were collected from Bejaia and *B. spinosum* L was collected from Batna, in June 2006. Each plant was authenticated by Prof. Gérard De Bélair (Annaba, Algeria). Voucher specimens were deposited in the Herbarium of the Laboratory of Therapeutic Substances (LOST) at Mentouri University–Constantine, (LOST/Bp/06/06), (LOST/Bf/06/06), and (LOST/Bs/06/06), respectively.

### 2. Previous Studies

*Bupleurum* species have been reported to possess anti-inflammatory [3,4], antioxidant and hepatoprotective effects [5,6]. Saikosaponins [7-9] and flavonoids [10-12] have been reported from *Bupleurum* species. There are two recent studies on *B. spinosum*, in one of them [11], isolation of

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three flavonoids reported besides polyacetylenes and terpenoids from species including *B. spinosum* [7]. A very recent review article, published in 2011 presents chemistry and pharmacology of the genus *Bupleurum* [13]. The genus *Bupleurum* is represented by fourteen species in Algeria [14]. However, there is almost no study on Algerian *Bupleurum* species including subject species of this study *B. fruticosum* L. and *B. spinosum*, except for a study on the essential oil of *B. plantagineum* Desf. [15] which is the endemic species to Algeria. In addition, one study is found on Algerian *B. montanum* Coss. which reports on the isolation of some flavonoids [16].

### 3. Present Study

Air-dried and powdered aerial parts (1 kg) of each *Bupleurum* species were extracted with 70% MeOH. The residue was dissolved in water and extracted with petroleum ether, dichloromethane, ethyl acetate and n-BuOH, successively.

The n-BuOH extract of *B. plantagineum* Desf. (15 g) was subjected to a MN-SC6 polyamid column chromatography being eluted with a gradient system of toluene/MeOH with increasing polarity. Two main fractions (A-B) were collected. Fraction A (108 mg) was further purified by Whatman 3MM paper chromatography using 15% AcOH and BAW (n-BuOH-AcOH-H<sub>2</sub>O, 4:1:5 (upper phase) leading to compound **1** (35 mg) which was purified by column flash chromatography over sephadex LH-20 in MeOH. Fraction B (150 mg) was subjected to TLC on polyamid MN-DC6 eluted with H<sub>2</sub>O-MeOH-metylethylketone-acetylacetone (13:3:3:1) affording compounds **2** (25 mg) and **3** (13 mg).

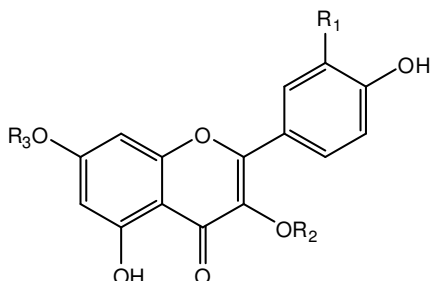
The n-BuOH extract of *B. fruticosum* L. (20 g) was subjected to a MN-SC6 polyamid column chromatography being eluted with a gradient system of toluene/MeOH with increasing polarity. Four main fractions (A-D) were collected. Fraction A (203 mg) was further purified by Whatman 3MM paper chromatography using 15% AcOH and BAW (n-BuOH-AcOH-H<sub>2</sub>O, 4:1:5 (upper phase) leading to compounds **4** (12 mg), **5** (18 mg) and **6** (22 mg) which were purified by column flash chromatography over sephadex LH-20 in MeOH. Fraction B (110 mg) was subjected to TLC on polyamid MN-DC6 eluted with H<sub>2</sub>O-MeOH-metylethylketone-acetylacetone (13:3:3:1) leading to compounds **7** (12 mg) and **8** (15 mg).

The n-BuOH extract of *B. spinosum* L. (15 g) was subjected to a MN-SC6 polyamid column chromatography being eluted with a gradient system of toluene/MeOH with increasing polarity. Two main fractions (A-B) were collected. Fraction A (90 mg) was further purified by Whatman 3MM paper chromatography using 30% AcOH and BAW (n-BuOH-AcOH-H<sub>2</sub>O, 4:1:5 (upper phase) leading to compounds **9** (10 mg) and **10** (13 mg) which were purified by column flash chromatography over sephadex LH-20 in MeOH. Fraction B (85 mg) was subjected to TLC on polyamid MN-DC6 eluted with H<sub>2</sub>O-MeOH-metylethylketone-acetylacetone (13:3:3:1) leading to compounds **11** (14 mg). Compounds **1-11** were identified by using UV, <sup>1</sup>H NMR, <sup>13</sup>C NMR and MS analyses and literature data [17-21].

The pure compounds were treated with 2M HCl at 100°C for 1 h for the acid hydrolysis. The hydrolysates were extracted with EtOAc and the aglycones were identified by their UV spectra in methanol and by comparison of their R<sub>f</sub> with authentic samples. Sugars were identified in the aqueous residue by comparison with authentic samples on silica gel TLC impregnated with 0.2 M NaH<sub>2</sub>PO<sub>4</sub>, solvent Me<sub>2</sub>CO-H<sub>2</sub>O (9:1), revealed with aniline malonate.

Compounds (**1-3**), isolated from *B. plantagineum* Desf., were identified as quercetin (**1**), quercetin 3-rutinoside (**2**) and isorhamnetin 3-rutinoside (**3**). Kaempferol glycosides, kaempferol 3-glucoside (**4**), kaempferol 3-galactoside (**5**), kaempferol 3-rutinoside (**6**) and quercetin glycosides, quercetin 3-rutinoside (**2**), quercetin 3-glucoside (**7**) and quercetin 3-galactoside (**8**), have been isolated from *B. fruticosum* L. while isorhamnetin (**9**), isorhamnetin 3-galactoside (**10**) and isorhamnetin 3-galactorhamnoside (**11**) were found in *B. spinosum* L. These flavonols/flavonol glycosates are different from those reported from the Italian species which consist of tamarixetin, tamarixetin 3-robinobioside and tamarixetin 3-galactoside [11]. All these flavonols are reported for

the first time from the studied species, but seven flavonols (**4-8**, **10**, **11**) are reported for the first time from the genus.



	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	<b>7</b>	<b>8</b>	<b>9</b>	<b>10</b>	<b>11</b>
<b>R<sub>1</sub></b>	OH	OH	Me	H	H	H	OH	OH	Me	Me	Me
<b>R<sub>2</sub></b>	H	Rut	Rut	Glc	Gal	Rut	Glc	Gal	H	Gal	Gal-Rham
<b>R<sub>3</sub></b>	H	H	H	H	H	H	H	H	H	H	H

Quercetin (**1**) [17-19]; Quercetin-3-*O*-rutinoside (**2**) [17-19]; Isorhamnetin-3-*O*-rutinoside (**3**) [17-19]; Kaempferol 3-glucoside (**4**) [18,19]; Kaempferol 3-*O*- $\beta$ -D-galactoside (**5**) [20]; kaempferol 3-*O*-rutinoside (**6**) [18,19]; quercetin 3-*O*- $\beta$ -D-glucoside (**7**), [17-20]; Quercetin 3-*O*- $\beta$ -D-galactoside (**8**) [20,21]; Isorhamnetin (**9**) [17-19]; Isorhamnetin 3-galactoside (**10**) [20]; Isorhamnetin 3-*O*- $\alpha$ -L-rhamnosyl(1 $\rightarrow$ 2)- $\beta$ -D-galactoside (**11**) [21].

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