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## New flavonoids from bioactive extract of Algerian medicinal plant *Launaea arborescens*

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## PEER REVIEW

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## Comments

The research presented by the authors complements previous work on the phytochemical study of this plant. In fact, after studying the methanol fraction of the extraction of this plant, the authors are interested in the butanol fraction by isolating and identifying new flavonoids using efficient chromatographic and spectroscopic methods. The description of these novel compounds of quite complex structures is a very interesting scientific result to use to search for active substances of this medicinal plant used by the people of these regions.

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## ABSTRACT

**Objective:** To investigate the butanol fraction of the water/acetone extract and isolate of the new flavonoids from *Launaea arborescens*.

**Methods:** The compounds were isolated by liquid chromatographic methods and their structures were identified by using spectroscopic analysis.

**Results:** The isolated compounds were identified as: 7-O-[ $\alpha$ -rhamnopyranosyl 4',5,6-Trihydroxy flavone 1,4',5'-Di-Methoxy 7-(5''-Me Hexan)1-oyl flavanone 2, 3''-isopropyl pyrano [1'':7,4'':6] 3',4',5',5-Tetrahydroxy flavanone 3,5,4',5'-Tri-Hydroxy 7-(3''-Me butan) -yl flavanone 4, 5,7-Dihydroxy-2',4',5' -trimethoxy-isoflavanone 5,5,6,7,4'-tetrahydroxy flavonol 6,7-O-[ $\alpha$ -rhamnopyranosyl-(1->6)- $\beta$ -glucopyranosyl]- 4',5,7-tri-hydroxy-flavanone 7,7-O-[ $\alpha$ -rhamnopyranosyl-(1->6)- $\beta$ -glucopyranosyl] 3',5-Dihydroxy 4'-Methoxy flavanone 8.

**Conclusions:** The presence of different types of bioactive flavonoids in *Launaea arborescens* extract can explain the large ethnopharmacological uses and the potential activity of this medicinal plant.

## KEYWORDS

*Launaea arborescens*, Asteraceae, Flavanone, Isoflavanone, Glycosid flavanone

## 1. Introduction

*Launaea arborescens* (*L. arborescens*) is a medicinal plant having capacities of important propagation. Following its biotope, associate to different species, it is frequently notably in the whole region of Algerian southwest of Wadi-Namous until the region of Karzaz. According to

our ethnopharmacological survey<sup>[1,2]</sup>, *L. arborescens* is used for treatment of the illnesses gastric. Following our phytochemical works achieved on the polyphenols of the methanolic extract of aerial part of *L. arborescens*, we are also interested to investigate the butanol fraction of the water/acetone extract and isolate of the new flavonoids from this plant<sup>[3]</sup>.

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## 2. Materials and methods

### 2.1. General experimental procedure

UV spectra were obtained in MeOH solvent with Unicam UV 300 spectrophotometer. IR spectra were obtained with a Avatar 320 FT-IR spectrophotometer. The NMR spectra were taken on a Bruker GP 250 ( $^1\text{H}$ , 300 MHz;  $^{13}\text{C}$ , 125 MHz) Spectrometer. EIMS spectra were obtained on a VG Trio-2 spectrometer. TLC was carried out on silica gel 60 F<sub>254</sub> plates (Merck, Germany). Column chromatography was performed over silica gel 60 (Merck, particle size 230–400 mesh).

### 2.2. Plant materials

The aerial part of *L. arborescens* were collected in March 2000 from Bechar (hammada, Oued saoura, Bechar, Algeria). The botanical identification and a voucher specimen was conserved at the phytochemical herbarium of Phytochemistry and Organic Synthesis Laboratory of University Center of Bechar under to accession number CA99/25[2,4].

### 2.3. Extraction and isolation

The dried aerial part of plants (200 g) of *L. arborescens* were extracted with acetone–water (70:30) using soxhlet apparatus; reflux for 6 h was performed. The residue was evaporated in vacuum apparatus until two third, then the third of aqueous residue was partitioned sequentially with *n*-hexan, ethyl ether, EtOAc and *n*-BuOH[5]. To purify and to identify the constituents of butanol fraction (2.36 g), one achieved some separations by liquid chromatography on column, one using a column in glass of type 20 mm/300 mm (29/39) filled with a stationary phase of silica gel (0,20 mm) and the mobile phase chosen for this separation is acetone/toluene/formic acid (60:80:10)[6].

## 3. Results

The separation has been done previously on a mass of 533 mg of butanol extract in the same conditions. We regrouped the final results after several separations and analysis chromatographic (Table 1).

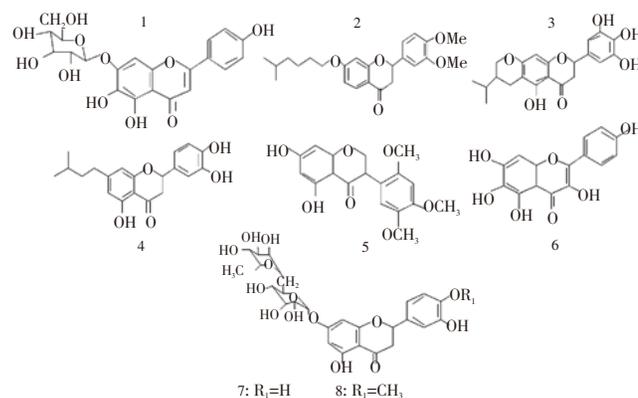
**Table 1**

Results of liquid column chromatography (C1,C2,C3: first, second and third column).

Compounds	$R_f$	Mass (mg)	Yield (%)	Column	Fractions
1	0.16	32	6.00	C2	99–101
2	0.20	40	7.50	C2	68–71
3	0.30	53	9.94	C3	62–67
4	0.40	35	6.57	C3	58–61
5	0.74	77	14.44	C1	40–47
6	0.80	30	5.63	C1	35–37
7	0.90	79	14.82	C1	23–30
8	0.96	167	31.33	C3	6–22

TLC analysis of the samples separated by liquid chromatography on column revealed the existence of eight products to different  $R_f$  of which one recovered them after spraying of the solvents for determination of the structures using spectroscopic methods (Table 1).

We have successfully separated three products in the first column (5, 6, 7), two other in the second column (C1, C2) and three in third column (3, 4, 8) by this protocol (Figure 1).



**Figure 1.** Structures of flavonoids isolated from aerial part of *L. arborescens*.

7-*O*-[ $\alpha$ -rhamnopyranosyl 4',5,6-Trihydroxy flavone. (1):  $T_f=169^\circ\text{C}$ .  $\text{UV}_{\text{max}}$  (MeOH): 237, 275, 331 nm. [35] IR(KBr): 3415, 2924, 1613, 1503, 1388, 1257, 1071, 815, 755  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR: 6.59 (H-3, br s), 6.98 (H-8, s), 7.84 (H-2', d,  $J=6.8$  Hz), 6.91 (H-3', d,  $J=7.3$  Hz), 6.91 (H-5', d,  $J=7.3$  Hz), 7.84 (H-6', d,  $J=6.8$  Hz), 5.09 (H-1'', d,  $J=5.9$  Hz), 3.59 (H-2'', m), 3.56 (H-3'', m), 3.43 (H-4'', t,  $J=8.3$  Hz), 3.59 (H-5'', m), 3.74 (H-6''a, m), 3.98 (H-6''b, d,  $J=11.7$  Hz). RMN  $^{13}\text{C}$ : 166.77 (C-2), 103.49 (C-3), 184.39 (C-4), 147.92 (C-5), 131.86 (C-6), 152.75 (C-7) 95.82 (C-8), 151.75 (C-9), 107.47 (C-10), 123.29 (C-1'), 129.56 (C-2'), 117.00 (C-3'), 162.78 (C-4'), 117.00 (C-5'), 129.56 (C-6'), 102.66 (C-1''), 74.73 (C-2''), 77.52 (C-3''), 71.42 (C-4''), 78.58 (C-5''), 62.55 (C-6'').

4',5'-Di-Methoxy 7-(5''-Me Hexan)1-oyl flavanone (2) molecular formula  $\text{C}_{24}\text{H}_{30}\text{O}_5$ , 72.34 (C), 7.59 (H), 20.07(O), MW=398.50, UV spectra: 254, 276, 336, IR (KBr): 3405, 2924, 2853, 1771, 1738, 1509, 1684, 1613, 1252, 1127, 1061, 1383, 755, 815  $\text{cm}^{-1}$ ,  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz): 5.36 (t, H-2, 3 Hz, 1.8 Hz), 2.95 (d, H-3a, 1.8), 2.88 (d, H-3b, 3 Hz), 7.392 (d, H-5, 6.8 Hz), 7.325 (d, H-6, 6.8 Hz), 7.28 (s, H-8), 7.455 (d, H-6', 7.2 Hz), 7.303 (d, H-5', 7.2 Hz), 7.37 (s, H-2'), 3.738 (t, 7 Hz, H-1'', 2H), 3.668 (s,  $\text{OCH}_3$ , 6H), 1.67 (m, 1.6 Hz, 2H-2, 2H-3, 2H-4, H-5, 7H), 0.86 (d, 6.6 Hz, H-6, 6H),  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 300 MHz):

3''-isopropyl pyrano [1'':7,4'':6] 3',4',5',5'-Tetrahydroxy flavanone (3): molecular formula  $\text{C}_{21}\text{H}_{22}\text{O}_7$ : 65.28 (C), 5.74 (H), 28.98 (O), MW=386.41, UV spectra: 238, 271, 335 IR (KBr): 3404, 2923, 2847, 1738, 1459, 1607, 1170, 1121, 1377, 618,  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz): 5.32 (H-2), 2.37 (H-3), 7.21 (s, H-8), 7.28 (s, H-2'), 7.28 (s, H-6'), 3.68 (d, H-1''a, 6 Hz), 3.7 (d, H-1''b, 6Hz), 2.13 (H-3''a, 1.2 Hz), 2.12 (H-3''b, 1.2 Hz), 0.75 (H-4''), 0.90 (H-5''), 1.63 (m, H-2'', 1H),  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 300 MHz):

5,4',5'-Tri-Hydroxy 7-(3''-Me butan) -yl flavanone (4): molecular formula  $C_{20}H_{22}O_5$ , 70.16 (C), 6.48 (H), 23.36 (O), MW=342.40, UV spectra: 249, 270, 327, IR (KBr): 3475, 2923, 2847, 1650, 1536, 1612, 1383, 1252, 1033, 760, 618,  $^1H$  NMR ( $CDCl_3$ , 300 MHz): 5.32 (H-2, 1H), 2.8 (H-3, 2H), 7.058 (H-6, 1H), 7.019 (H-8, 1H), 7.70 (H-2', 1H), 7.487 (H-5', 1H), 7.675 (H-6', 1H), 2.20 (H-1'', 2H), 1.85 (H-2'', 2H), 1.27 (H-3'', 1H), 0.87 (H-4'', 6H),  $^{13}C$  NMR ( $CDCl_3$ , 300 MHz): 76 (C2), 63 (C3), 184.9 (C4), 100.57 (C5), 116.44 (C6), 110.33 (C7), 111.366 (C8), 130.65 (C9), 87.00 (C10), 39 (C1''), 38 (C2''), 30.25 (C3''), 27.25 (C4''a, C4''b).

5,7-Dihydroxy-2',4',5'-trimethoxy-isoflavanone (5), MW=346.34, molecular formula,  $C_{18}H_{18}O_7$ , 62.42% (C), 5.24% (H), 32.34% (O), Tf=192 °C. UV max (MeOH): 253, 279, 325.[31] IR (KBr): 3470, 2951, 2847, 1733, 1613, 1536, 1383, 1258, 1132, 1000, 815, 755  $cm^{-1}$ .  $^1H$  NMR: 4.57 (H2a, dd, 10.8, 17.0), 4.44 (H2b, dd, 10.7, 5.5), 4.33 (H3a, dd, 11.5, 5.5), 5.99 (H6, d, 2.1), 5.97 (H8, d, 2.1), 6.77 (H3', s), 6.86 (H6', s), 12.32 (5'-OH, s), 3.8 (2'-OCH<sub>3</sub>, s), 3.86 (4'-OCH<sub>3</sub>, s), 3.74 (5'-OCH<sub>3</sub>, s).  $^{13}C$  NMR: 70.6 (C-2), 47.2 (C-3), 197.5 (C-4), 165.2 (C-5), 96.4 (C-6), 166.5 (C-7), 95.1 (C-8), 164.0 (C-9), 103.1 (C-10), 115.3 (C-1'), 152.8 (C-2'), 99.7 (C-3'), 150.7 (C-4'), 144.1 (C-5'), 116.5 (C-6'), 56.4 (2'-OCH<sub>3</sub>), 56.0 (4'-OCH<sub>3</sub>), 56.8 (5'-OCH<sub>3</sub>).

5,6,7,4'-tetrahydroxy flavonol (6): Tf=196 °C. MW=302.24,  $C_{15}H_{10}O_7$ , 59.61% (C), 3.33% (H), 37.05% (O), UV max (MeOH): 258, 277, 354. IR(KBr): 3399, 1635, 1514, 1376, 1143, 1405, 777  $cm^{-1}$ .  $^1H$  NMR: 8.05 (2H, d, 9, H-2', 6'), 6.91 (2H, d, 9, H-3', 5'), 6.60 (1H, s, H-8).  $^{13}C$  NMR: 146.8 (C-2), 135.6 (C-3), 175.9 (C-4), 160.7 (C-5), 127.3 (C-6), 162.5 (C-7), 94.5 (C-8), 156.2 (C-9), 103.1 (C-10), 121.7 (C-1'), 129.5 (C-2'), 115.4 (C-3'), 159.2 (C-4'), 115.4 (C-5'), 129.5 (C-6').

7-O-[ $\alpha$ -rhamnopyranosyl-(1->6)- $\beta$ -glucopyranosyl]-4', 5, 7-tri-hydroxy-flavanone (7), molecular formula:  $C_{27}H_{32}O_{14}$ , 55.86 (C), 5.56 (H), 38.58 (O), MW=580.55. UV max (MeOH): 248, 274, 333. IR(KBr): 3383, 2913, 1613, 1488, 1389, 1252, 1193, 1072  $cm^{-1}$ .  $^1H$  NMR: 5.42 (H-2, dd,  $J=12.2$ ; 2.9 Hz), 2.48 (H-3a dd,  $J=17.1$ ; 2.9 Hz), 3.62 (H-3b, dd,  $J=17.1$ ; 2.9 Hz), 6.05 (H-6, t,  $J=2.4$  Hz), 6.07 (H-8, m), 6.76 (H-2', m), 6.74 (H-3', H-5', d), 7.31 (H-2', H-6', d), 5.09 (H-1'', t,  $J=7.3$  Hz), 3.62 (H-2''), 3.22 (H-3''), m), 3.13 (H-4'', dd,  $J=9$ ; 5 Hz), 3.53 (H-5'', m), 3.42 (H-6''a), 3.80 (H-6'', d,  $J=10.3$  Hz), 4.71 (H-1''', s), 4.47 (H-2''', m), 4.45 (H-3'''), 3.64 (H-4''', d,  $J=10.2$  Hz), 4.45 (H-5'''), 1.09 (H-6''', d,  $J=6.3$  Hz).  $^{13}C$  NMR: 79.88 (C-2), 43.36 (C-3), 198.46 (C-4), 163.99 (C-5), 96.35 (C-6), 166.10 (C-7), 97.50 (C-8), 164.17 (C-9), 104.55 (C-10), 129.80 (C-1'), 116.45 (C-2', C-6'), 129.70 (C-3', C-5'), 159.07 (C-4'), 98.52 (C-1''), 77.44 (C-2''), 73.06 (C-3''), 70.82 (C-4''), 77.32 (C-5''), 61.69 (C-6''), 101.63 (C-1'''), 71.63 (C-2'''), 71.71 (C-3'''), 72.63 (C-4'''), 69.53 (C-5'''), 19.30 (C-6''').

7-O-[ $\alpha$ -rhamnopyranosyl-(1->6)- $\beta$ -glucopyranosyl]3',5-Dihydroxy 4'-Methoxy flavanone (8). [26,33] UV max (MeOH): 245, 286, 330 nm. IR (KBr): 3384, 2929, 1613, 1503, 1263, 1176, 1383, 810, 761  $cm^{-1}$ ,  $^1H$  NMR: 5.50

(H-2, dd,  $J=12.2$ ; 2.9 Hz), 2.77 (H-3a, dd,  $J=17.1$ ; 2.9 Hz), 3.28 (H-3b, m), 6.12 (H-6, d,  $J=2.0$  Hz), 6.12 (H-8, d,  $J=2.0$  Hz), 6.93 (H-2', m), 6.95 (H-5', d,  $J=8.3$  Hz), 6.90 (H-6', t,  $J=8.3$  Hz), 4.97 (H-1'', d,  $J=7.3$  Hz), 3.27 (H-2'', m), 3.22 (H-3'', m), 3.12 (H-4'', t,  $J=9.3$  Hz), 3.53 (H-5'', m), 3.42 (H-6''a, m), 3.80 (H-6''b, m), 4.52 (H-1''', s), 3.64 (H-2''', m), 3.43 (H-3''', m), 3.14 (H-4''', t,  $J=9.3$  Hz), 3.40 (H-5''', m), 1.08 (H-6''', d,  $J=6.3$  Hz).  $^{13}C$  NMR: 78.5 (C-2), 2.21 (C-3), 198.4 (C-4), 162.64 (C-5), 95.58 (C-6), 165.28 (C-7), 96.52 (C-8), 163.50 (C-9), 103.40 (C-10), 131.06 (C-1'), 114.23 (C-2'), 146.51 (C-3'), 148.11 (C-4'), 112.23 (C-5'), 118.05 (C-6'), 55.85 (MeOH), 99.61 (C-1''), 76.42 (C-2''), 73.13 (C-3''), 69.73 (C-4''), 75.58 (C-5''), 66.18 (C-6''), 100.75 (C-1'''), 70.40 (C-2'''), 70.84 (C-3'''), 72.21 (C-4'''), 68.45 (C-5'''), 17.96 (C-6''').

#### 4. Discussion

For all compounds isolated, the absorption bands in UV characterized by the lengths of maximal waves of basis situated between 203–254 nm (band I) correspond to double link C=C link. The band II situated between 260–284 nm corresponding to the carbonyl groups (ketone function) have associated to the transition of strong energy ( $\pi \rightarrow \pi^*$ ) (bande II)[7,8]. Who do the bands of absorption have maximal languor waves more elevated located toward 306–345 nm (band III) associated to the transition of weak energy ( $\pi \rightarrow \pi^*$ )[6].

The UV specter of the compound (8) present a maximum has 286 nm (band II) and another has 330 nm (band III), that indicates the presence of a flavanone. The use of the displacement reagents permits to determine the positions of the substituting. One displacement of + 22 nm of the band II is observed after the addition of  $AlCl_3$ , indicating the presence of hydroxyl group in the C-5 position. The addition of a basis as: (NaOAc or NaOMe) doesn't have any effect, showing the absence of hydroxyl group in C-7 position[9].

In infra red spectra, the bands matched toward (3383–3415  $cm^{-1}$ ) correspond to the elongation vibration of  $\dot{O}-H$  (valence vibration), the aliphatic links C-H is presented in the IR specter by fine and intense bands toward 2934  $cm^{-1}$  (asymmetric valence vibration of the  $CH_3$ ). The frequencies of vibrations situated between (2913–2929  $cm^{-1}$ ) correspond to the asymmetric valence vibrations of  $CH_2$ , the absorption bands to 2853  $cm^{-1}$  associate has vibrations of symmetrical valence of the  $CH_2$ [6]. The frequencies of vibration 990  $cm^{-1}$  corresponds to the vibrations of distortion out plan of the unsaturated hydrocarbons. The vibrations of valence of the ketone cyclic to six linkages or more, or aliphatic ketone (C=O) to be located toward 1717  $cm^{-1}$ , in the same way 1738  $cm^{-1}$  corresponds to the carbonyl functions for a saturated ester. The vibrations have 1766  $cm^{-1}$  and 1771  $cm^{-1}$  for the

vinyl esters of alcohol that possess the fragment of structure following:  $-\text{CO}-\hat{\text{O}}-\text{C}=\text{C}$ , this group entails an increasing importance of the frequency of vibration carbonyl (acetate of vinyl absorbs  $1776\text{ cm}^{-1}$  and the phenyl acetate absorbs to  $1770\text{ cm}^{-1}$ ). The vibration of distortion outside of the plan of C–H aromatic depends mainly of the position of the various substituting fixed on the benzene core and not of their nature<sup>[9]</sup>.

We note the existence of the bands toward  $1150\text{--}1200\text{ cm}^{-1}$  in all separated products, what confirms the presence of an aromatic alcohol corresponds to the vibration (OH aroma), and also the strips toward  $1033$  to  $1170\text{ cm}^{-1}$  made us think about a primary, secondary alcohol or even tertiary. One noticed that all these separated compounds represent an intense band toward  $1600\text{--}1700\text{ cm}^{-1}$ , this band indicates the existence of ketone function this grouping (C=O) is conjugated probably<sup>[10]</sup>.

The specters of these products indicate the presence of an aromatic cycle substituted confirmed by the vibrations of elongation (C=C) situated toward  $1500\text{ cm}^{-1}$  and  $1600\text{ cm}^{-1}$ , although the vibrations (=C–H aromatic) don't appear well on the IR specter due to the masking by the large bands of the function–OH in all compounds safe for: compound 2. This last link is identified well by the vibration of distortions (=C–H aromatic) that absorbs toward  $700\text{ cm}^{-1}$ . The vibrations of elongation of  $-\text{CH}_3$ ,  $-\text{CH}_2-$  appears between  $2847\text{ cm}^{-1}$  to  $2951\text{ cm}^{-1}$ . The vibrations that are in the domain of absorption between  $1104\text{ cm}^{-1}$  to  $1275\text{ cm}^{-1}$  extra in all IR specters of the products, representing the ether oxide group. The band of absorption toward  $1250\text{ cm}^{-1}$  characterizes an ether–oxide aliphatic aryle–aliphatic. A band of absorption in IR situates to  $924$ ,  $984\text{ cm}^{-1}$  characterize a link ethylic trans, and the strip to  $1601\text{ cm}^{-1}$  can be probably assigns to the vibration of valence of the C=C group conjugated. From these primary results of UV spectra and IR spectra, the al compounds can possess the structure general of a flavanone, flavonol and flavone<sup>[9]</sup>.

The compound 1 is a yellow strong product isolated in second column with 6% yield identified as 7–O–[ $\alpha$ -rhamnopyranosyl 4',5,6–Trihydroxy flavone. In  $^1\text{H}$  NMR spectra, we observe 14 signals, two singular signals appears toward 6.59 and 6.98 corresponds to benzenic protons respectively 3 and 4 positions, four other signals appears in weak fields as the unarmored doublets toward 6.91 and 7.84 ppm, the rest of signals represent the protons of sugar moiety or one has two diastereoisotopic protons of the methylene group. These results are confirmed by  $^{13}\text{C}$  NMR spectra, that presents 21 signals, six signals appears between 60.2–103 ppm corresponds to rhamnopyranosyl carbons, substituted by an oxygen in 7 position, proved by the unarmored carbons 7 and 1'', the more unarmored carbon appears to 184 ppm correspond to carbonyl group

(C=O) in position 4<sup>[6]</sup>. The compound 2 isolated in second column with 7.5% yield, identified as 4',5'–Di–Methoxy 7–(5''–Me Hexan)1–oyl flavanone, the characteristic signals of diastereoisotopic protons appears as two doublets toward 2.95 and 2.88 ppm, so the carbonyle group appears in unarmored zone to 190 ppm, these results proved that this compound is a flavanone substituted with an alkoyl of seven carbon in the unarmored position. The compound 3 and 4 isolated in third column in yield respectively to 9.94% and 6.57%, compound 3 possessed two asymmetric carbons, therefore it presents six diastereoisotopic protons assigned to three methylene groups in 3, 2'', 4'' positions. This proton is chemically non equivalent and appears in different chemical shifts. Compound 4 identified as 5,4',5'–Tri–Hydroxy 7–(3''–Me butan) –yl flavanone, NMR spectra proved the centesimal analysis, the alkyl substituted in 7 position,  $^1\text{H}$  NMR spectra present a signal to 0.87 correspond a two methyl equivalent integrated to 6H, these remarks show that the substituted alkyl correspond to the (3''–Me butanyl). Compound 5 isolated in first column identified as isoflavanone, it is a white powder. In  $^1\text{H}$  NMR spectra, we notice two unarmored diastereoisotopic protons of methylene group in position 2, it appears under shape two doublet respectively toward to 4.57 and 4.44 ppm<sup>[11,12]</sup>. In the first column, we arrived to isolate a flavonol (compound 6) in 5.56%, this compound is identified as 5,6,7,4'–tetrahydroxy flavonol,  $^1\text{H}$  NMR spectra present three unarmored signals for five protons, one corespond to a protons in position 8, the other signals correspond respectively to tow protons chemicaly equivalent. Glycoside 7 and 8 isolated respectively in first and third column. The compounds have already been isolated from *Citrus* spp. (Rutaceae), *Mentha piperita* (Lamiaceae) and *Myoporium tenuifolium* (Myoporaceae)<sup>[13]</sup>, it was identified by analysis of its spectroscopic data  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR experiments and comparison with those previously reported for the hesperidin (hesperetin–7–rutinoside). The hydrolysis and comparison with authentic samples using the same procedure described in the literature were used to identify the carbohydrates Lrhamnose and D–glucose of the rutinoside unit. The same spectrum shows a cross peak of H–2' [ $\delta\text{H}$  7.12 (s)] with  $\delta\text{C}$  121.0 (C–2',  $^1\text{J}_{\text{CH}}$ ) and 161.2 (C–3',  $^2\text{J}_{\text{CH}}$ ) which were used to confirm the location of the hydroxyl group at C–3'.

The presence of different types of bioactive flavonoids in *L. arborescens* extract can explain the large ethnopharmacological uses and the potential activity of this medicinal plant.

#### Conflict of interest statement

We declare that we have no conflict of interest.

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## Comments

### Background

Phytochemical study of medicinal plants is an important area of research for a good understanding of the biological properties of these plant species. Ethnopharmacological studies on *L. arborescens* in Algeria have shown medicinal properties from which the interest to know the different bioactives components of this plant.

### Research frontiers

In this work, the research aims to isolate and determine the structures of flavonoids present in the butanol fraction of the extract of the plant. The separation and identification of the target compounds were performed respectively by liquid chromatography and by spectroscopic methods.

### Related reports

According to ethnopharmacological survey authors, *L. arborescens* is used for the gastric illnesses. These works have been developed to study the phytochemical constituents that are behind the pharmacological properties of this plant.

### Innovations and breakthroughs

Polyphenols of the methanolic extract of aerial part of *L. arborescens* were isolated precedently.

Following these initial phytochemical studies, the authors' work in this part relates to the study of the butanol fraction of the water/acetone extract and isolating new flavonoids from this plant.

### Applications

The new flavonoids extracted and isolated from this plant *L. arborescens* could be tested to determine which of these flavonoids are the most biologically actives in the pharmacological action of this plant.

### Peer review

The research presented by the authors complements previous work on the phytochemical study of this plant. In fact, after studying the methanol fraction of the extraction of this plant, the authors are interested in the butanol fraction by isolating and identifying new flavonoids using efficient chromatographic and spectroscopic methods. The

description of these novel compounds of quite complex structures is a very interesting scientific result to use to search for active substances of this medicinal plant used by the people of these regions.

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