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DETERMINATION OF THE FLAVONOIDS OF BROCCHIA CINEREA FROM ALGERIA

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Brocchia cinerea is a plant from North Africa, member of the Asteraceae family; the plant is highly used in Algeria to relieve some ailments. Various classes of compounds are naturally encountered in the plant as tanins, polyphenols and flavonoids, these compounds could play a primordial pharmacological role giving to the plant its therapeutic proprieties. In the current study, the plant's flowers were collected from two sites in Algeria, in order to extract the flavonoids; which were subsequently identified using HPLC-DAD-MS. The results of the analysis identified 10 flavonoids: 2,2':3',2'':3'',2'''-Quaternaphthalene-1,1',1'',1''',4,4',4'',4'''-octone, Quercetin 3-O-beta-D-galactopyranoside, Hesperidin, Sakuranin, Quercetin-4'-glucoside, Flavone, 2',5,5',6-tetrahydroxy-3,4',7-trimethoxy-, tetraacetate, 6,11,12,17-Tetraacetoxy-5,18-trinaphthylenedione, Isoorientin, Luteolin, 4H-1-Benzopyran-4-one, 5,6-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-3,7-dimethoxy-. Multivariate statistics have been conducted in order to elucidate the variabilities. Close chemical patterns were noted between the samples, with small inter-region and inter-samples variabilities, except for one sample that standed as an outlier presenting different flavonoid levels pattern. This is crucial to understand the variabilities in the pharmacological proprieties of Brocchia

Keywords: *Flavonoids, Brocchia cinerea, Asteraceae*

INTRODUCTION

Brocchia cinerea (Delile) Vis. is a woolly herbaceous annual plant of 5-15 cm, tomentose, featured by whitish-green leaves and stems that are covered in thick, tiny hairs[1]. The stems are erect or diffuse, and the flowers tubular, whereas the leaves are thickly divided at an upper part with two or three segments, in the stem of the upper branch there are yellow inflorescences. The plant thrives in desert conditions (xerophilic plant) and favors sandy loam soils[2,3]. Usually found on non-saline wadi beds, growing on gravelly sandy soils[4] **(Fig.1).** Geographically, it is widely distributed in North Africa, particularly in the Saharan regions of Algeria and Morocco, the Red Sea region, Sinai, the Qattara depression and Mali[5]. The classification of the plant according to Cronquist and APG is presented in **Table1**. Traditionally, *Brocchia cinerea* (Delile) Vis is widely used to treat several diseases like colic, cough, diarrhea and digestive disorders. The herb is typically applied as a decoction, maceration, infusion, and inhalation[6,7,8]. Besides, it is used as an antiseptic, antipyretic, analgesic, and anti-inflammatory, as well as to treat rheumatism[8,9]. Furthermore, it is also used in the treatment of fever and cough, and as poultices for headaches and migraine[10]. In addition to its medicinal uses, nomads use the plant as a tea additive to improve the taste of tea. Thanks to its high preservative properties, it is commonly added to goat butter[11].

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Fig.1: Photo of Brocchia cinerea.

Table1: Cronquist and APG classification of Brocchia cinerea.

Classification Cronquist 1981	Classification APG
- Super Kingdom: Eukaryote - Kingdom: Plantae - Infra reign: Cormophyta Viridiplantae - Under reign: Tracheophyta - Phylum: Spermatophyto - Subphylum: Magnoliophyta (Angiospermae) - Class: Magnoliopsida - Subclass: Asteridae (upper asteridae) Euasterids II - Order: Asterales - Family: Asteraceae - Subfamily: Asteroideae - Tribe: Anthemideae - Subtribe: Cotulinae - Gender: Cotula - Species: Cotula cinerea Del.	- Kingdom: Plantae - (Clade) Angiosperms - (Clade) Eudicotyledons - (Clade) Basal Tricolpate - (Clade) Asterids - (Clade) Euasteridi II - Order: Asterales - Family Asteraceae - Subfamily: Asteroideae - Tribe: Anthemideae - Subtribe: Cotulinae - Gender: Cotula - Species: Cotula cinerea Del.

The phytochemical screening of the flowers of *Brocchia cinerea*, showed the presence of saponins, essential oils, tannins, flavonoids, steroids and terpenoids[4,5,7,9,12,13]. In fact, the chemical composition of the hydro-ethanolic extract of the flowers of *Brocchia cinerea* harvested at the flowering stage in Tougourt (south-eastern Algeria) was studied by Dendougui et al[14], new germacranolide-like sesquiterpene lactone and seventeen new flavonoids were isolated from the genus *Cotula* for the first time. (I) chrysospenol-D, (II) chrysosplenetin, (III) oxyayanin-B, (IV) axillarin, (V) 3 -methylquercetin, (VI) pedaletin, (VII) isokaempferide, (VIII) apigenin, (IX) luteolin, (X) 6-hydroxyluteolin, (XI) 3-glucosylisorhamnetin, (XII) 3-methyl-7-glucosylquercetin, (XIII) 7-O-β-D-glucosylapigenin, (XIV) 7-0-β-glucosylluteolin, (XV) 7-O-β-D-glucosyl-quercetin, (XVI) 7-O-ß-D-glucosylaxillarin and (XVII) 7-O-ß-D-diglucosylluteolin. Besides, six sesquiterpene lactones (three germacranolides, two guaianolides and one eudesmanolide) were isolated from the chloroform extract of the aerial parts of *Brocchia cinerea*[11].

Studies on methanolic extracts of the flowers of *Brocchia cinerea* harvested in Errachidia (Morocco) at the flowering stage revealed that the main phenolic compounds were luteolin-4'-O-glucoside; 4,5-dicafeoylquinic acid; 3,5-dicafeoylquinic acid; 3,4-dicafeoylquinic acid; cryptochlorogenic acid; chlorogenic acid and neochlorogenic acid[11]. The structures of this compound were elucidated by HPLC-DAD-MS. In spite of the above-mentioned experiments, the phytochemistry of the plant has not been sufficiently studied, due mainly to its restricted localization. Aiming to enrich the literature, the objective of the present study was to determine the flavonoids extracted from two sites in Algeria, understand and explain the variabilities exciting with regard to their levels amounts.

MATERIALS AND METHOD

Plant material

A voucher sample was deposited at the CRSTRA (center for scientific and technical research on arid regions), University of Mohamed Khider Biskra (specimen No.003CRSTRA0039).

The plant's flowers have been sampled in April 2016 from Biskra and El Oued, two towns in the southern east of Algeria. The samples 1 to 5 have been collected from Biskra, while that the samples 6 to 10 have been harvested from El Oued.

Reagents

- Distilled water used for extraction and cleaning provided by the laboratory of toxicology, university hospital of Batna.
- Methanol used for extraction of flavonoids, Sigma Aldrich SZBC2860V.
- Acetonitrile, mobile phase, Sigma Aldrich STBH0134.
- Formic acid, mobile phase, Chel Lab 22 2691711 600.
- Quercetin primary reference standard sigma Aldrich, 6151-25-3

Laboratory equipment

- Analytical balance, Sartorius
- Liquid chromatography for the determination of the flavonoids: Agilent type P4000 equipped with DAD detector G4212B and autosampler G1329B, monitored by Agilent Masshunter Qualitative Analysis B 07.00 software. The stationary phase was a Zorbax C18 column from Chromapack (Netherlands), :50 mm x 2.1 mm x 5 um . A pre-column (10 mm x 2.0 mm x 5 um) made of the same phase was included, the oven temperature was set to 40 ° C, and the mobile phase consisted of Solvent A, which contained 0.1 % formic acid in water, and Solvent B, which contained just acetonitrile. The determination was recorded in the region from 200 to 450 nm, the flow rate was 0.4 mL/min, and the volume injected 5 uL. The compounds were tested in both positive and negative ion mode for the study of flavonoids, utilizing a triple quadrupole mass spectrometer QQQ 6420. The ensuing conditions were set: Nebulizer pressure 25 Psi, gas flow 7 L/min, the capillary voltage and temperature were set at 350 °C and 4.0 kV, respectively. Scan mode was used to conduct the analysis from 100 to 1000 m/z.

Statistical test

The data analysis and statistics were carried out using the R programming language through R studio software.

Sample pretreatment

80 mL of methanol heated to reflux for 1 hour with 100 mg of the flowers; the extract was filtered and spiked to 100 mL. After undergoing filtration through a 0.45 mm diameter Acro-discs filters, the obtained methanolic extracts (average weight 0.15g/5mL ±0.02: 0.16±0.02 for samples of Biskra, and 0.14±0.006 for samples of El oued) have been then subjected to HPLC-DAD-MS analysis[15]. The internal standardization approach was employed to calculate the flavonoids' % concentration at 335 nm[16].

RESULTS and DISCUSSION

Results

Total flavonoid concentrations obtained with the internal standardization method for the assay of flavonoids using Quercetin (spectrophotometry)[16] are summarized in **Table 2**. The concentrations of total flavonoids are expressed in mg/g of dry plant matter. The table reveals that *Brocchia cinerea* is richer in total flavonoids with an average concentration of 44.58 ± 13.3 mg/g compared to *Matricaria pubescens*, another plant from the Asteraceae family[16], which contains only 36.57 ±11.52 mg/g. it is noteworthy to mention the high variability in total flavonoid concentrations, ranging from 25mg/g to 68mg/g, with higher concentration shown in the samples of Biskra (average concentration 48.76mg/g).

The screening of the samples by HPLC-DAD-MS has identified 10 flavonoids; which contents in each sample are grouped in the **Table 3**. Sakurunin showed to be the prevalent major compound with concentrations ranging from 19.69 till 49.6% depending on the sample, followed by Quercetin-4'-glucoside with concentrations from 0 to 21.56%, then 4H-1-Benzopyran-4-one, 5,6-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-3,7-dimethoxy- and Luteolin, with close levels stretching respectively from 0 to 27.3 and 2.78 to 19.99%, Flavone, 2',5,5',6-tetrahydroxy-3,4',7-trimethoxy-, tetraacetate comes behind showing contents from 7.72 to 17.73%, the flavonoids: Isoorientin, 6,11,12,17-Tetraacetoxy-5,18-trinaphthylenedione, Hesperidin, Quercetin 3-O-β-D-galactopyranoside and 2,2':3',2":3",2"'-Quaternaphthalene-1,1',1",1"',4,4',4",4"'-octone showed minor contents ranging from 0 to 9.64%.

Table 2: Results of total flavonoids concentrations in samples of Brocchia cinerea (Delile) Vis.

Samples of Biskra	Concentration in mg/g	Samples of El Oued	Concentration in mg/g
1	42,68	**6**	25,07
2	40,58	**7**	58,14
3	68,52	**8**	50,22
4	50,94	**9**	42,59
5	41,10	**10**	25,97
Average	48,76 ± 11.8		40,4 ± 14,65
General average	44,58 ± 13.3		

Table 3: Results of qualitative and quantitative analysis by liquid chromatography of methanolic extracts of Brocchia cinerea aerial parts.

N°	RT (min)	Compounds	Mw	1	2	3	4	5	6	7	8	9	10
1	1,76	2,2':3',2":3",2"'-Quaternaphthalene-1,1',1",1"',4,4',4",4"'-octone	626	6,69	5,24	5,55	8,43	3,52	5,06	3	9,64	3,68	3,98
2	3,04	Quercetin 3-O-β-D-galactopyranoside	464	3,62	2,85	4,55	3,39	5	4,08	0,9	0	0	2,42
3	3.08	7-O-β-D-diglucosyl luteoline	610	1,6	4,16	2,52	5,53	4,45	1,67	0	4,57	2	3,96
4	5.397	Luteolin 7 O-glucoside	448	21,6	20,6	25,7	19,69	24,5	26,8	23	49,6	24,2	31,6
5	5.72	Quercetin-7'-glucoside	464	21,56	21,3	16,7	12,91	14,1	13,9	22	0	12,8	12,6
6	6.302	Flavone, 2',5,5',6-tetrahydroxy-3,4',7-trimethoxy-, tetraacetate	544	13,21	14,3	10,5	13,08	7,72	9,04	16	17,7	8,55	8,05
7	6,47	6,11,12,17-Tetraacetoxy-5,18-trinaphthylenedione	640	0	1,75	1,5	1,29	0,88	2,63	3,7	7,02	2,16	0,55
8	6,59	Isoorientine	448	5,68	2,91	4,43	2,25	2,89	1,98	3,1	8,63	2,56	1,49
9	8,04	Luteoline	286	13,1	12,4	13,8	19,99	9,59	16,9	18	2,78	19,5	18,5
10	10	oxyayanin-B	360	12,93	14,5	14,8	13,41	27,3	17,9	10	0	24,6	16,9
Total				100	100	100	100	100	100	100	100	100	100

Mw: molecular weight, RT: Retention time.

Discussion

The multivariate principal components analysis of the data showed 3 components explaining 83% of the variance, with PC1 accounting for 53.13%, PC2 17.45 % and PC3 12.77%, the flavonoids: 2,2':3',2":3",2"'-Quaternaphthalene-1,1',1",1"',4,4',4",4"'-octone and Sakuranin are shown to have a contrasting evolution alike Hesperidin and Luteolin, in fact higher contents of the formers are coincided with lower levels of the latters, same pattern is noted between: Flavone, 2',5,5',6-tetrahydroxy-3,4',7-trimethoxy-, tetraacetate, 6,11,12,17-Tetraacetoxy-5,18-trinaphthylenedione and Isoorientin from one side and Quercetin 3-O-β-D-galactopyranoside and 4H-1-Benzopyran-4-one, 5,6-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-3,7-dimethoxy- from the other. Samely higher contents of Hesperidin occurs with low contents of Quercetin-4'-glucoside.

Besides the samples 1,2,3,4,5,6,7,9,10 are noted to have high amounts of Sakuranin and Quercetin-4'-glucoside, and on the other hand featured by lower contents of 2,2':3',2":3",2"'-Quaternaphthalene-1,1',1",1"',4,4',4",4"'-octone, Quercetin 3-O-β-D-galactopyranoside, and Isoorientin, opposing the sample 8 characterized by the highest amount of Sakuranin, topping 49%, high levels of Flavone, 2',5,5',6-tetrahydroxy-3,4',7-trimethoxy-, tetraacetate(17%) Isoorientin(8.63%), and 6,11,12,17-Tetraacetoxy-5,18-trinaphthylenedione(7%) **(Fig.2)**, indeed, the cluster analysis showed a big cluster regrouping the samples 1,2,3,4,5,6,9,10, with the sample 7 close (distant on the PC 2 axis), while that the sample 8 stands far on the axis of PC1; the principal component with highest variability, demonstrating the difference in the flavonoids chemical pattern **(Fig.3)**.

It is noteworthy to mention, that the samples from each site form sub-cluster when referring to PC3 axis (which accounts for the smallest variance) with the subcluster of samples from El Oued on the top **(Fig.4)**. The variability in the flavonoid levels between the plants could be due to the existence of several varieties with various genetic materials resulting in different chemical contents, the other explanations may be attributed to the age

of the plant, since some older plants could accumulate higher flavonoid contents namely quercetin-3-O-galactoside, quercetin-3-O-glucoside, kaempferol-3-O-glucoside and kaempferol-3-O-glucuronide for the case of *Vitis vinifera* L[17], furthermore, in stress conditions as extreme temperatures and high salt are noted to increase the levels of some flavonoids in some plans as *Capsicum* cultivars and Rice[18,19], on the other hand, some plant diseases as Rust may induce higher flavonoid compounds, especially anthocyanin and catechin regarding Malus plants[20], furthermore, the accumulation of some flavonoids as Catechin and Proanthocyanidin in the leaves of some plants have been noted to result from injury caused by herbivores or pathogens[21], while that in other species belonging to the Brassicaceae the flavonoid levels drop considerably[22], it is also shown that light exposure plays a crucial role, with flavonoid concentration increases following higher photosynthetic active radiation[23,24], on the other hand, the role played by the use and the nature of the fertilizer remains controversial[24, 25]. The difference in the flavonoids chemical pattern between the plants may result in a difference in the pharmacological activities and the therapeutic efficiency of the used plant, and also may explain the differences in the folk applications of the plant and its broad utilizations.

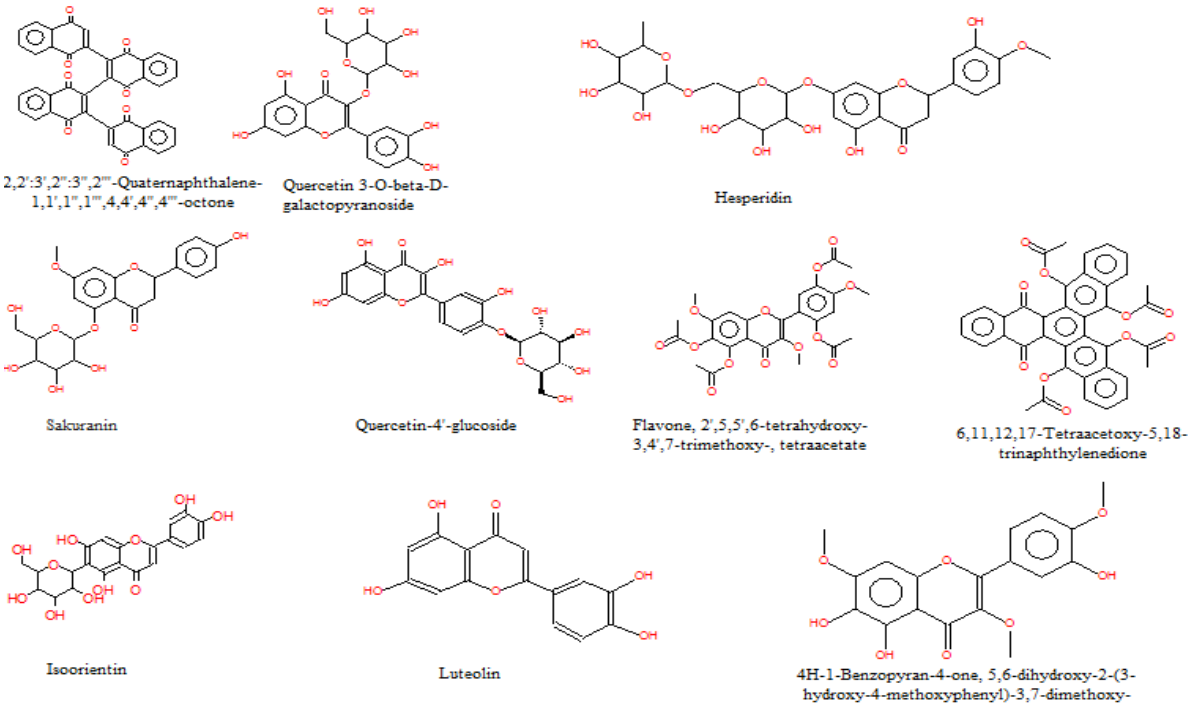

Fig. 2: Structures of the identified flavonoids.

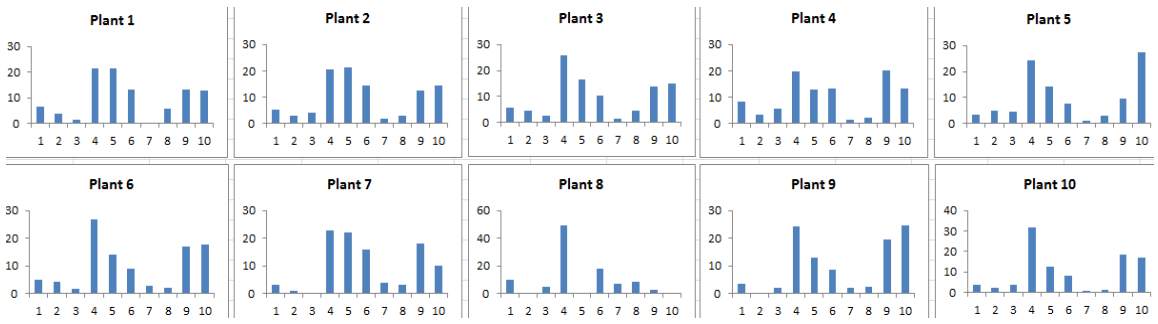

Fig.3: The flavonoids pattern of the samples.

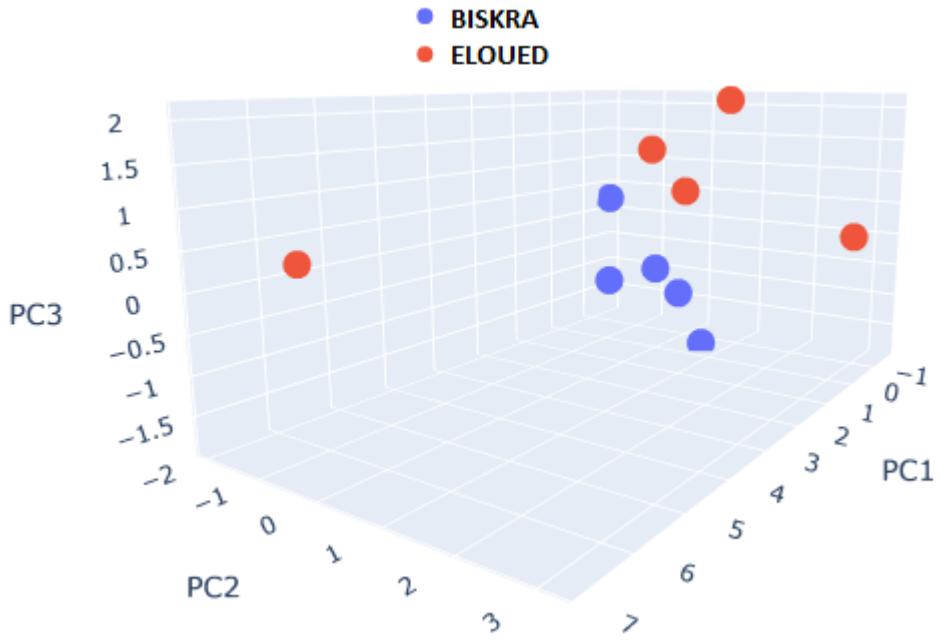

Fig. 4; The distribution of the flavonoid's contents of the samples according to the multivariate analysis.

Conclusion

Brocchia cinerea is a wild plant exclusive to North Africa, featured by its specific aspect, and the plethora of therapeutic uses rooted in the culture of the local population from immemorial history, due certainly to its phytochemical compounds, from which stand the flavonoids as an important family of secondary metabolites, interesting in their pharmacological activities, the study focused on their determination from two sites, following extraction and chromatographic analysis, 10 flavonoids have been identified, with Sakuranin, Quercetin-4'-glucoside,

Luteolin and 4H-1-Benzopyran-4-one, 5,6-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-3,7-dimethoxy- as the major ones, variabilities according to the contents have been noted resulting in different chemical patterns between the plants.

A more extensive study with higher samples is needed to confirm the results that may be important to understand the variabilities in the pharmacological proprieties of *Brocchia,* so to distinguish the good chemical pattern, which may be exploited in the pharmaceutical industry.

Author Contributions

Study conception and design (Mohammed tahar Benmoussa, Abdelhakim Bounab, Youcef Hadef); Acquisition of data (Mohammed tahar Benmoussa, Said Nadji); Analysis and interpretation of data (Said Nadji, Mohammed tahar Benmoussa); Provision of reagents/resources (Mohammed tahar Benmoussa, Abdelhakim Bounab); Drafting of manuscript (Said Nadji, Mohammed tahar Benmoussa).

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تقدير مركبات الفلافونويد في نبات Brocchia cinerea من الجزائر

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[4]مختبر الكيمياء التحليلية ، قسم الصيدلة ، كلية الطب ، جامعة عنابة

الخلاصة Brocchia cinerea : نبات ينتمي إلى العائلة النجمية ينمو بشكل طبيعي في شمال أفريقيا ويستعمل النبات بكثرة في الجزائر للتخفيف من بعض الأمراض. توجد فئات مختلفة من المركبات بشكل طبيعي في النبات مثل التانينات والبوليفينول والفلافونويد، والتي يمكن أن تلعب دورًا أساسيًا في المزايا العلاجية للنبات الموجودة في .Brocchia في الدراسة الحالية، تم استخدام زهور النبات لاستخلاص الفلافونويدات من عينات تم الحصول عليها من موقعين في الجزائر، والتي تم تحديدها لاحقا باستخدام HPLC-DAD-MS. حددت نتائج التحليل ١٠ فلافونويدات: ٢,٢':٣',٣'':٢'',٢'''-Quaternaphthalene-1,1',1'',1''',4,4',4'',4'''-octone, Quercetin 3-O-beta-D-galactopyranoside, Hesperidin, Sakuranin, Quercetin-4'-glucoside, Flavone, 2',5,5',6-tetrahydroxy-3,4',7-trimethoxy-, tetraacetate, 6,11,12,17-Tetraacetoxy-5,18-trinaphthylenedione, Isoorientin, Luteolin, 4H-1-Benzopyran-4-one, 5,6-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-3,7-dimethoxy-

تم إجراء إحصائيات متعددة المتغيرات من أجل توضيح المتغيرات. وقد لوحظت أنماط كيميائية متقاربة بين العينات، مع وجود تباينات صغيرة بين المناطق وبين العينات، باستثناء عينة واحدة كانت بمثابة حالة متطرفة تقدم نمطًا مختلفًا لمستويات الفلافونويد.