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Original Research

Bioactive flavones isolated from Tunisian *Artemisia campestris* L. Leaves

Rafika Metoui[1,2], Jalloul Bouajila[2], Mansour Znati[2,3], Sylvie Cazaux[2], Mohamed Neffati[1], Ahmed Akrout[1*]

[1] Range Ecology Laboratory, University of Gabès, Institute of Arid Lands, 4119 Medenine, Tunisia
[2] Laboratory of IMRCP UMR CNRS 5623, Faculty of Pharmacy of Toulouse, University of Toulouse, University of Paul-Sabatier, F-31062 Toulouse, France
[3] Laboratory Heterocyclic Chemistry, Natural Products and Reactivity (LR11ES39), Team: Medicinal Chemistry and Natural Products and Reactivity, Faculty of Science of Monastir, University of Monastir, 5019 Monastir, Tunisia

Correspondence to: akirite@yahoo.fr

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Abstract: Four flavones were isolated from dried leaves of *Artemisia campestris* L. 2',4',5,7-tetrahydroxy-5',6-dimethoxyflavone, eupatilin and dimethoxy-centaureidin were reported for the first time in this species whereas cirsiliol was previously identified but it was isolated for the first time. Their structures were elucidated by spectroscopic methods, including 1D and 2D NMR experiments and mass spectrometry analysis. In addition, all isolated flavones were evaluated for their antioxidant, anti-inflammatory, anti-superoxide dismutase, anti-xanthine oxidase and cytotoxic activities. The results showed that all isolated compounds exhibited potent anti-xanthine oxidase activity with IC_{50} ranging from 3.3 to 6.8 µM, which was higher than that of the control compound allopurinol (8.2 ± 0.6 µM). In addition, cirsiliol was found to be the most cytotoxic against OVCAR-3, IGROV-1and HCT-116 cell lines at 15µM, with inhibition percentage values of 53.7, 48.8 and 40.9%, respectively. All compounds also showed weak to moderate anti-inflammatory and anti-superoxide dismutase activities.

Key words: *Artemisia campestris*; Anti-inflammatory; Anti-superoxide dismutase; Anti-xanthine oxidase; Cytotoxic activity.

Introduction

Plants are of significant interest for their flavors and for their medicinal properties. Plants are recognized for their ability to produce several secondary metabolites, which are used to treat a variety of diseases (1).

Artemisia is a genus of small herbs and shrubs which includes about 400 species distributed in the Mediterranean region, Western Asia, South Western Europe, and the Arabian Peninsula (2). It belongs to the important family Compositae (Asteraceae), one of the most numerous plant groupings, which consists of about 2250 species in Tunisia, of which more than 172 were inventoried in the center and the south (3). The flora of Tunisia include six *Artemisia* species: *A. arborescens*, *A. atlantica*, *A. herba-alba*, *A. saharae*, *A. vulgaris and A. campestris* (4). *Artemisia* species are used in traditional medicine all over the world for analgesic, anti-inflammatory and antispasmodic properties and to treat fever, malaria, ulcers, diabetes, gastrointestinal disorders and cancer (5).

A.campestris, the subject of this study, locally named as "T'gouft", is an approximately 1 meter height shrub widespread in the south-eastern regions. This species is widely used in Tunisian folk medicine as a decoction or infusion for their antivenin, for its anti-inflammatory, anti-rheumatic and antimicrobial activities and to treat gastric disturbances, diarrhea, abdominal cramps, hypertension and rheumatism (6, 7). Previous studies have revealed that the phenolic profile of *A. campestris* is quite complex, consisting of flavones, flavonols, flavanones, dihydroflavonols and their methyl ethers. The isolation of coumarins and acetophenones have been also reported (8, 9).Several compounds have been isolated and identified from different extracts of this species, such as flavonoids (10).The shoots of *A.campestris* were the subject of a previous phytochemical investigation leading to the identification by LC/MS of 39 molecules including coumarins, flavones, flavonols, phenolic acids and sesquiterpenes. The major phenolic compounds are principally luteolin-7-*O*-rutinoside, rhamnetin, isorhamnetin, hydroxycoumarin, kaempferolrutinoside and threedi-*O*-caffeoylquinic acid isomers (11).

The chemical composition and bioactivities of the dried leaf extracts of A. campestris have not been previously investigated. The current study describes the isolation and the structure elucidation of four flavones 2',4',5,7-tetrahydroxy-5',6-dimethoxyflavone[1], eupatilin[2], dimethoxycentaureidin [3] and cirsiliol [4] from ethyl acetate and dichloromethane extracts of A. campestris dried leaves. Their structures were established principally by NMR spectroscopy and mass spectrometry analysis. Furthermore, we report the antioxidant, anti-inflammatory, anti-xanthine oxidase, anti-superoxide dismutase and cytotoxic activities of the isolated compounds.

Materials and Methods

Collection of plant material

The aerial parts of *A. campestris* were collected from Beni-Khedache (a mountainous region in the south-east

of Tunisia) in August 2013 and kindly identified by Professor Neffati Mohamed (Range Ecology Laboratory, Institute of Arid lands, Medenine, Tunisia). Avoucher specimens (AC0813) have been deposited in this laboratory in the same institute. The plant's raw materials were cleaned of dust andopen air-dried in shadowed place (20-26 °C) for two weeks. Then, the leaves were separated from the other parts and used for the analyses.

Extraction and isolation

Samples (200 g of the dried leaves of *A. campestris*) were macerated in 1000 ml hexane and kept in contact in the dark in the closed bottles for 72 hours at room temperature (20-26°C). All filtrates were collected together and the solvent was totally evaporated under reduced pressure to yield 0.5% of hexane extract, which was weighed and stored in an amber vial at 4°C for further analysis. Then, the same sample was successively treated with dichloromethane, ethyl acetate, n-butanol and water under the same conditions as with hexane to yield 4.9, 3.7, 3.9 and 4.1% dry weight, respectively.

The ethyl acetate dry extract (5 g) was fractionated by silica gel normal phase flash column chromatography using successive gradients of cyclohexane/Ethyl acetate (170ml × 100:0; 500ml × 80:20; 400 ml × 60:40; 500 ml × 50:50; 800 ml × 30:70; 600 ml× 0:100), and 500 ml MeOH to afford ten fractions (F_1- F_{10}) based on the TLC analyses. The fraction F_5 (1.68 g) was again subjected to silica gel flash column chromatography eluting with gradient of EtOAc/cyclohexane (200 ml × 100:0; 170 ml × 90:10; 170 ml × 80:20; 200 ml × 70:30; 200 ml × 60:40; 800 ml × 50:50; 200 ml× 40:60; 330ml× 35:65;1000 ml × 30:70) and finally 200ml methanol to afford ten sub-fractions ($F_{5\text{-}1}$- $F_{5\text{-}10}$) according to the TLC analysis. In the fifth sub-fraction ($F_{5\text{-}5}$) was formed a yellow precipitate which was recovered after filtration and washing successively with cyclohexane and ethyl acetate to give compound (1) (20 mg).

The dichloromethane dry extract (8 g) was further fractionated by silica gel normal phase flash column chromatography using successive gradients of dichloromethane/Ethyl acetate (110 ml × 100:0; 110ml × 90:10; 100 ml × 80:20; 100 ml× 70:30; 100 ml × 60:40; 100 ml ×50:50; 20 ml × 40:60; 150ml × 30:70; 80 ml× 20:80; 170 ml× 10:90; 600 ml × 0:100) and finally with 200 ml methanol to afford seven fractions (F_1-F_7) according to the TLC analysis. The precipitation of the fraction F_3 (4g) and F_4 (1.5 g) in ethyl acetate gives two compounds: compound 2 (40 mg) and compound 3 (15 mg), respectively. The liquid layer obtained after precipitation of the fraction F_3(4 g)was fractioned by silica gel normal flash column chromatography eluting successively with gradients of cyclohexane/Ethyl acetate (400ml × 100:0; 110ml × 90:10; 600ml × 80:20; 600ml × 70:30; 400ml ×50:50; 200 ml×0:100) and finally with 300 ml methanol to afford five main sub-fractions ($F_{3\text{-}1}$-$F_{3\text{-}5}$) based on the TLC analysis. The sub-fraction $F_{3\text{-}3}$ showed a yellow precipitate in ethyl acetate which was recovered after filtration and washing with dichloromethane to give compound 4 (20 mg).

Structural identification of the compounds

^{1}H- NMR (300 MHz),^{13}C- NMR (75 MHz) and 2D NMR spectra were recorded on a Bruker AM-300 spectrometer using DMSO-d_6 as solvent and non-deuterated residual solvent as the internal standard. Chemicals shifts (δ) are given in parts per million (ppm) and coupling constants (J) in Hertz (Hz). DCI-HRMS of compound 1 was run in a GCT 1er Waters. DCI-MS of compounds 2-4 were obtained with a DSQ Thermo Fisher Scientific (DCI/ NH3).

Biological activities

Anti-oxidant activity (DPPH assay), anti-inflammatory activity (anti-5-lipoxygenase assay) and cytotoxic activities were investigated using methods adopted by Bekir *et al.* (12) with slight modifications. The anti-xanthine oxidase and anti-superoxide dismutase activities were evaluated according to protocols described by El Euch *et al.* (13).

Statistical analysis

All data were expressed as means and ± standards deviations of triplicate measurements. Statistical significance was determined through analysis of variance (ANOVA). p values of less than 0.05 were considered to be statistically significant.

Results

Structure determination

The following compounds were identified through comparison of their ^{1}H NMR, ^{13C} NMR and 2D NMR data with literature and their agreement with the proposed structures. The structures of compounds 1–4 are presented in Figure 1.

Compounds 1-4 were identified as 2',4',5,7-tetradroxy-5',6-dimethoxyflavone [1] (14), eupatilin [2] (15,16), dimethoxycentaureidin [3] (17) and cirsiliol [4] (18,19). All these compounds were isolated for the first time from the dried leaves of A. campestrisL. 2',4',5,7-tetrahydroxy-5',6-dimethoxyflavone, eupatilin and dimethoxycentaureidin had not been previously reported in A. campestris, whereas cirsiliol was previously identified in this species.

Compound 1*: *2',4',5,7-tetrahydroxy-5',6-dimethoxyflavone yellow powder

DCI-HRMS m/z: 347.0807 $[M + H]^+$(calcd for $C_{17}H_{14}O_8$,

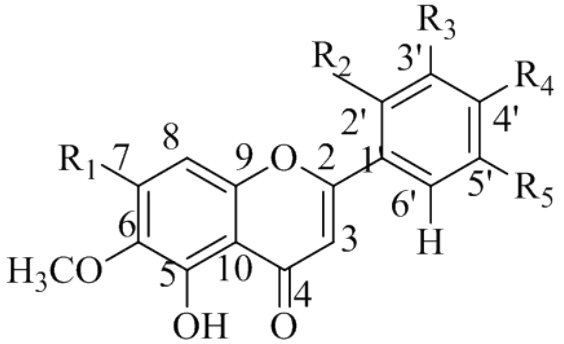

Figure 1. Structures of compounds 1-4 isolated from A. campestris dried leaves. Compound 1 (2',4',5,7-tetradroxy-5',6-dimethoxyflavone): R_1: OH; R_2: OH; R_3: H; R_4: OH; R5: OCH_3 . Compound 2 (Eupatilin): R_1: OH; R_2: H; R3: OCH_3; R_4:OCH_3; R_5: H . Compound 3 (Dimethoxycentaureidin): R_1: OH; R_2: H; R_3: OH; R_4: OCH_3; R_5: H . Compound 4 (Cirsiliol): R_1: OCH_3; R_2: H; R_3: OH; R_4: OH;; R_5: H

347.0689), 331 [M - CH_3]$^+$, 328 [M - H_2O]$^+$, 279 [M + H - C_3O_2]$^+$. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 3.74 (3H, s, 5'-OCH_3), 3.79 (3H, s, 6-OCH_3), 6.55 (1H, s, H-3'), 6.61 (1H, s, H-8), 7.02 (1H, s , H-3), 7.37 (1H, s, H-6′), 9.99 (1H, s, 4'-OH), 10.33 (1H, s, 2'-OH), 10.38 (1H, s, 7-OH), 13.00 (1H, s, 5-OH). ^{13}C NMR (75 MHz, DMSO-d_6,) δ:182.6 (C-4), 162.2 (C-2), 157.4 (C-7), 153.4 (C-2′), 153.1 (C-4′), 152.8 (C-5), 152.8 (C-9), 148.1 (C-3′), 131.5 (C-6), 112.1 (C-6′), 106.9 (C-3), 94.7 (C-8), 107.4 (C-1′), 104.8 (C-5′), 104.3 (C-10), 60.4 (3'-O-CH_3), 56.9 (6-O-CH_3).

Compound 2: Eupatilin: yellow amorphous powder

DCI-MS m/z: 345.0 [M + H]$^+$, 331 [M - CH_3]$^+$, 315 [M - 2 CH_3]$^+$. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 3.76 (3H, s, 6-O-CH_3), 3.85 (3H, s, 3'-OCH_3), 3.87 (3H, s, 4'-OCH_3), 6.63 (1H, s, H-8), 6.95 (1H, s, H-3), 7.10 (1H, d, J=3.0 Hz, H-2'), 7.55 (1H, d, J = 9.0 Hz, H-5'), 7.66 (1H, dd, J=9.0,3.0 Hz, H-6'), 7.66 (1H, s, H-3), 13,00 (1H, s, 5-OH), 10.69 (1H, s, 7-OH).^{13}C-NMR (75 MHz, DMSO-d_6) δ: 182.6 (C-4), 163.8 (C-2), 157,8 (C-5)**,** 153.2 (C-7), 152.9 (C-9), 152.6 (C-4'), 149.4(C-3'), 131.8 (C-6), 123,4 (C-1'), 120.5 (C-6')**,** 112.1 (C-2'), 109.8 (C-5'), 104.6 (C-10), 103.8 (C-3), 94.8 (C-8), 60.4 (6- OCH_3), 56,3 (3′-OCH_3) 56,2 (4′-OCH_3).

***Compound 3: Dimethoxycentaureidin*: yellow powder**

DCI-MS m/z: 331.0 [M + H]$^+$, 315 [M - CH_3]$^+$, 303 [M - CO]$^+$, 134 [^{1,3}B$^+$ - CH_3]. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.79 (3H, s, 6-OCH_3), 3.92 (3H, s, 4'-OCH_3), 6.65 (1H, s, H-8), 6.93 (1H, s, H-3), 6.95 (1H, d, J=9.0 Hz, H-5′), 7.60 (1H, d, J=2.0 Hz, H-2′), 7.58 (1H, dd, J=9.0,2.0 Hz, H-6′), 13.12 (1H, s, 5-OH), 9.99 (1H, s, 3'-OH), 10.71 (1H, s, 7-OH). ^{13}C-NMR (75MHz, DMSO-d_6) δ: 182.6 (C-4), 164.2 (C-2), 157.7 (C-7) , 153.2 (C-5), 152.8 (C-9), 151.1 (C-3′), 148.1 (C-4′),131.8 (C-6), 122.0 (C-1′), 120.8 (C-6′), 116.2 (C-5′), 110.6 (C-2′), 104.5 (C-10), 103.2 (C-3), 94.8 (C-8), 60.5 (4'-O-CH_3), 56.4 (6- O-CH_3).

***Compound 4*: *Cirsiliol*: yellow amorphous powder**

DCI-MS m/z: 331.0 [M + H]$^+$, 315 [M - CH_3]$^+$, 171 [^{1,4}A]$^+$, 129[^{1,3}A$^+$ - C_2H_2O]$^+$. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 3.75 (3H, s, 6-OCH_3), 3.95 (3H, s, 7-OCH_3), 6.76 (1H, s, H-3), 6.90 (1H, d, J=9.0 Hz, H-5′), 6.93 (1H, s, H-8), 7.46 (1H, s, H-2'), 7.49 (1H, d, J=9.0 Hz, H-6′), 13.09 (1H, s, 5-OH), 8.32 (1H, s, 3'-OH), 8.27 (1H, s, 4'-OH). ^{13}C-NMR (75MHz, DMSO-d_6) δ: 182.6 (C-4), 164.7 (C-2), 159.0 (C-7), 153.0 (C-5), 152.8 (C-9), 150.4 (C-4′), 146.3 (C-3′), 132.3 (C-6), 121.8 (C-1′), 119.5 (C-6′), 116.4 (C-5′), 113.9 (C-2′), 105.5 (C-10), 103.1 (C-3), 91.9 (C-8), 60.5 (7-O-CH_3), 56.4 (6-O-CH_3).

Biological activities

The isolated compounds were investigated for their antioxidant, anti-xanthine oxidase, cytotoxic, anti-inflammatory and anti-superoxide dismutase activities. The results of these assays are presented in Table 1.

Antioxidant activity: **DPPH radical scavenging assay**

It is important to mention that 2',4',5,7-tetrahydroxy-5',6-dimethoxyflavone and dimethoxycentaureidin had not been previously tested for this activity. DPPH radical scavenging activity was measured to evaluate the antioxidant activity of compounds 1-4 isolated from A. campestris dried leaves. The results of the antioxidant activity are presented in Table 2. Our findings reveal that both compounds 1 and 4 have very notable antioxidant activity with IC_{50}values of 9.13 ± 0.2; 7.96 ± 0.1 µM, respectively, which were higher than the positive control ascorbic acid (IC_{50} = 26.68 ± 0.1 µM). Compounds 2 and 3 showed lower antioxidant activity with an IC_{50} higher than 29.1 ± 0.2 and 30.3 ± 1.1 µM, respectively.

Anti-xanthine oxidase activity

It is important to mention that 2',4',5,7-tetrahydroxy-5',6-dimethoxyflavone and dimethoxycentaureidin had not been previously tested for this activity.

Xanthine oxidase (XOD) serves as an important biological source of oxygen-derived free radicals that contribute to the oxidative damage of living tissues (20). The results of this investigation are presented in Table 2. All compounds exhibited an activity higher than that of

Table 1. Antioxidant (DPPH assay), anti-inflammatory, anti-xanthine oxidase (XOD), anti-superoxide dismutase (SOD) and cytotoxic (HCT-116, IGROV-1,MCF-7 and OVCAR-3 cells lines) activities of four compounds isolated from *A. campestris* dried leaves.

Compound	IC_{50} (µM) Antioxidant activity	IC_{50} (µM) Anti-XO oxidase activity	Anti-inflammatory activity (IP % at 30 µM)	Anti-SOD activity (IP % at 30 µM)	Cytotoxic activity (IP % at 15 µM)			
					HCT-116	IGROV-1	OVCAR-3	MCF-7
1	9.1±0.2 [b]	5.5± 0.1 [b]	46.85±2.02 [a]	28.27 ±0.23[c]	42.4±2.0[b]	3.7±0.1[c]	na	4.3±0.2
2	29.1± 0.2 [c]	3.3± 0.0 [a]	25.55±0.36 [b]	40.80±0.81[b]	57.8±5.2[a]	24.7±2.9[b]	na	17.6±3
3	30.3± 1.1 [c]	6.84± 0.0 [c]	13. 93±0.93 [c]	43.81±0.92[a]	na	na	8.3±0.1	na
4	7.9±0.1 [a]	5.5± 0.1 [b]	48.51±1.37 [a]	29.01±0.59[c]	40. 9±3.9[b]	48.8±3.5[a]	53.9±3.9	na
allopurinol	-	8.2 ± 0.6	-	-	-	-	-	-
Ascorbic acid	26.7 ± 0.1	-	-	-	-	-	-	-
NDGA (20 µM)	-	-	54.0±4.2	-	-	-	-	-
Tamoxifen (5 µM)	-	-	-	-	100.7±4.7	96.3±5.3	98.8±4.9	98.5±5.6

Results are expressed as Means ± Standard deviation (SD) of three replicates. na: not active. Within each column, values with different upper case letters means a significant difference at level $p < 0.05$.

the positive control compound allopurinol (IC_{50}= 8.22 ± 0.6µM). The highest activity was displayed by eupatilin (IC_{50} = 3.34 ± 0.01 µM), followed by 2',4',5,7-tetrahydroxy-5',6-dimethoxyflavone (IC_{50} = 5.49 ± 0.09µM), cirsiliol (IC_{50} = 5.51 ± 0.06µM) and finally dimethoxycentaureidin (IC_{50}= 6.84 ± 0.01µM).

Anti-inflammatory activity: 5-Lipoxygenase inhibitory activity

It is important to mention that 2',4',5,7-tetrahydroxy-5',6-dimethoxyflavone and dimethoxycentaureidin were not previously tested for this activity. The results for 5-lipoxygenase inhibitory activity are presented in Table 1. All compounds showed activity lower than that of the positive control compound NDGA. Among the four compounds tested, 2',4',5,7-tetrahydroxy-5',6-dimethoxyflavone and cirsiliol displayed the highest anti-inflammatory activity with percentages of inhibition of 46.85 and 48.51%, respectively, which may be considered to be moderate activity compared to NDGE (54.0% at 20 µM), whereas the two other compounds exhibited weak activity.

Anti-superoxide dismutase activity: anti-SOD activity

The results for anti-SOD activity are presented in Table 1. To our knowledge, no studies on anti-superoxide dismutase activity of any of our compounds, except the compound cirsiliol**,** have been previously reported to date. Our results showed that, at a concentration of 30 µM, all the compounds displayed moderate to weak anti-SOD activity with an inhibition percentage ranging from 28.3% for 2',4',5,7-tetrahydroxy-5',6-dimethoxyflavone to 48.5% for dimethoxycentaureidin. Eupatilin and dimethoxycentaureidin displayed the highest anti-SOD activity with inhibition percentages of 40.80 and 43.81%, respectively, whereas compounds 2',4',5,7-tetrahydroxy-5',6-dimethoxyflavone and cirsiliol exhibited moderate activity (29.01 and 28.27%, respectively).

Cytotoxicity assay

The cytotoxic activity of the four compounds isolated from *A. campestris* dried leaves against the human colon cell line HCT-116, breast cancer line MCF-7 and ovary cell lines IGROV-1 and OVCAR-3 were assessed using MTT assay, which reliably detects the proliferation of cells. It is important to mention that 2',4',5,7-tetrahydroxy-5',6-dimethoxyflavone and dimethoxycentaureidin had not been previously tested for their cytotoxic activity against these cell lines. The results for the cytotoxicity assay are presented in Table 1. The HCT-116 cell line was shown to be the most sensitive cell against the four compounds tested, whereas the MCF-7 cell line was the most resistant cell line. The cytotoxic activity of all compounds was lower than that of the positive control compound tamoxifen. The most active compound was cirsiliol, with inhibition percentage values of 53.7% and 48.8% against the OVCAR-3 and IGROV-1 cell lines at the concentration of 15 µM, respectively, but it showed no activity against MCF-7. Eupatilin displayed the highest cytotoxic activity against the HCT-116 cell line with an inhibition percentage of 57.8%, but it exhibited lower activity against IGROV and MCF-7 cell lines (24.7 and 17.6% respectively). 2',4',5,7-tetrahydroxy-5',6-dimethoxyflavone showed important activity only on the HCT-116 cell line (42.4%), whereas dimethoxycentaureidin was not active against any of the cell lines except the OVCAR-3 cell line where it presented low activity (8.3%). This difference is due to the selectivity of the product towards the cancer cell lines.

Discussion

Our findings for compound 2 are comparable to those obtained by Mettion *et al*. (21) who showed that compound 2 presented an IC_{50} value of 21.5±1.8 µM, but our results for compound 4 are different from those indicated by Lin *et al*. (22) (IC_{50} = 21.8 ± 1.2 µM). This difference could be attributed to the protocols or purity of products.

The potent antioxidant activity of compound 4 could be attributed to the presence of ortho hydroxyl groups at C-3'and C-4' of ring B (23).On the other hand, the strong activity of compound 1 could be explained by the presence of four free phenolic hydroxyl groups at positions C-5, C-7, C-4' and C-2'in the structure of compound 1. These are explained by the mesomeric effects, which stabilize the molecule after the removal of hydrogen from this structure.

Many structure-activity relationship investigations have been performed on the antioxidant activity of flavonoids. However, only a few studies were, in fact, quantitative and they showed that the antioxidant activity of flavonoids depends strongly on the number and position of hydroxyl groups in the molecule (24, 25). Therefore, the spatial arrangement of substituents is perhaps a greater determinant of antioxidant activity. Consistent with most polyphenolic antioxidants, both the configuration and total number of hydroxyl groups substantially influence several mechanisms of antioxidant activity (26). Also, the presence of 5-OH groups in combination with a 4-carbonyl function and the C2-C3 double bond in this structure increases the radical scavenging activity. Our findings on the relationship between the radical scavenging activity and the chemical structure of phenolic compounds showed great similarities with the hierarchy of antioxidant effectiveness described by Rice-Evans *et al*. (23). Moreover, it was noticed that all the compounds 1 to 4 have a free hydroxyl group at the C-5 position and 2–3 double bond in conjugation with a 4-carbonyl group, although they displayed great differences in their inhibitory activities against free radicals. Therefore, it is likely that the 5-hydroxyl group has little influence on activity. Interestingly, the presence of a double bond between C-2 and C-3 in the C ring does not seem to be a prerequisite for antiradical activities (20).

Our findings for eupatilin are comparable to those reported by Hajdú *et al*. (27) who reported strong antixanthine activity for this compound, but with an IC_{50} lower than that of our value (1.33 µM). In contrast, for cirsiliol, our value was lower than that announced by Lin *et al*. (28) (10.7 µM). These differences between the results may be attributed to differences in the methods of analyses used or to the purity of the isolated compounds. Biochemically, this enzyme inhibitor is associated with the hydrogen binding of phenolic hydroxyls or carbonyls of the substrate with the amide carbonyls

or amino group in the peptide chain of the enzyme (30). The potent XOD inhibitory activity of the compound eupatilin may be attributed to the 3',4'-dimetyhoxyl groups besides the $C_{2,3}$ double bond, the carbonyl group at C-4 and the hydroxyl groups at C-5 and C-7 (31).

Compound 2, isolated from Artemisia eargyi folium, was previously tested for anti-inflammatory activity and results showed that this compound may reduce H_2O_2-induced cytotoxicity and 5-lipoxygenase expression and LTB_4 production by controlling the MAPK and JNK signaling pathways through antioxidative action in feline esophageal epithelial cells *in vivo* with an IC_{50} value of 0.10 μM (32).

It seems that the presence of a methoxyl group at C-4' enhances the activity of flavones, whereas the substitution of a hydroxyl group with a methoxyl group at C-3' decreases this activity. Cirsiliol, isolated from *Sideritis javalambrens*, was previously tested against anti-SOD activity in a nonenzymic system and was found to be inactive (33).

Eupatilin, isolated from *Artemisia asiatica Nakai,* was previously tested against another type of breast cancer (MCF-10A-ras cells) where it inhibited the growth of MCF10A-ras cells in a concentration-dependent and time-related manner (34). In addition, cirsiliol showed moderate cytotoxic activity against another type of colon cancer (Caco-2) with an IC_{50} of 96.0 l μM (35).

The difference in the level of cytotoxic activity between the four compounds tested may be attributed to the structure of these flavones. The influence of the B-ring substituents on the cytotoxic activity can be examined by comparison of the activity of compounds 2',4',5,7-tetrahydroxy-5',6-dimethoxyflavone, eupatilin and dimethoxycentaureidin, which have the same A-ring structure. Among these three compounds, dimethoxycentaureidin exhibited the lowest cytotoxic activity against the four cell lines tested, which may be attributed to the presence of a hydroxyl group at C-3' and a methoxyl group at C-4'. On the other hand, the presence of two methoxyl groups en ortho (C-3' and C-4'), such as in eupatilin, or a methxoyl group at C-5' and two hydroxyl groups at C-4' and C-2', such as in 2',4',5,7-tetrahydroxy-5',6-dimethoxyflavone, increases the cytotoxic activity against HCT-116 cell lines. IGROV-1 and OVCAR-3 cell lines seem to be more sensible against flavones with the 3',4'-dihydroxy substituent pattern on the B-ring and a methoxyl group at C-7, as in cirsiliol. After investigating the influences of substituents at the A and B-rings of flavones on cell growth inhibition in a limited number of flavones, we concluded that it is difficult to suggest rules that are commonly applicable to the four tumor cell lines (36). Our results provide further support to previous studies, which have underlined the importance of this structural feature for interaction with the different cellular mechanisms involved in cancer growth.

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