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Antioxidant activity and chemical constituents of *Anthriscus vulgaris* Bernh. (Apiaceae) from Algeria

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Abstract: *The chloroform and ethyl acetate extracts obtained from the aerial parts of Anthriscus vulgaris Bernh. were analyzed by gas chromatography-mass spectrometry (GC-MS). 36 components have been identified in each extract. The major constituents were 1-monooleoylglycerol (20.72%), caffeic acid (15.20%), cinnamic acid (11.31%) and benzene acetic acid (10.95%). The phytochemical study led to the isolation and structural elucidation of three compounds, scopoletin, umckalin and 1-(3',4'-dihydroxycinnamoyl) cyclopentane-2,3-diol. Moreover the ethyl acetate extract was screened for its possible in vitro antioxidant activity by 2,2-diphenyl-1-picrylhydrazy l(DPPH) and lipid peroxidation inhibition assays in which it displayed a noticeable activity. This study provides the first biological and chemical investigation on Anthriscus vulgaris* Bernh. in Algeria.

Keywords: *Anthriscus vulgaris* Bernh., GC-MS, scopoletin, umckalin, DPPH

Introduction

Anthriscus vulgaris Bernh. [Syn. *Torilis japonica* (Houtt.) DC] is a wild biennial herb that belongs to the Apiaceae family, present in North Africa, in the Eurasian area and on central African mountains [1]. The fruits of this plant have been used as a folk medicine for the treatment of skin disease, impotence, and

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inflammation [2]. Some species from genus *Anthriscus* show interesting biological properties such as antioxidant [3], antiproliferative [4], antispasmodic, and anti-inflammatory activities [5]. Previous chemical investigations of the genus *Anthriscus* have led to the identification of a variety of secondary metabolites, including, terpenes [6], flavonoids [7], polyacetylenes, and lignans [8].

Our literature survey revealed no published reports on the chemical composition and antioxidant activities of Algerian *A. vulgaris*. Thus, in the present study, we examined for the first time the antioxidant activity and the chemical characterization of chloroform and ethyl acetate extracts obtained from the aerial parts of *A. vulgaris* by gas chromatography-mass spectrometry (GC-MS) supported by the isolation and identification of 3 compounds.

Material and Methods

Plant material

A. vulgaris was collected on June 2015 at Milia (North Eastern Algerian Province) and identified by Professor Mohamed Kaabache (Botany Department, Ferhat Abbas University, Setif, Algeria). A voucher specimen (AVu/06/2015) of the plant material has been deposited in the Herbarium of the research unit VARENBIOMOL, University Frères Mentouri, Constantine 1, Algeria.

Extraction and isolation

Air-dried aerial parts (2 kg) of *A. vulgaris* were macerated in an ethanolic solution (80%) for 24 h, three times. After filtration, the filtrates were combined and concentrated up to 38°C under reduced pressure. The residue obtained was diluted with distilled water (800 ml) under magnetic stirring and maintained at 4°C for one night to precipitate a maximum of chlorophylls. After filtration, the resulting solution was successively extracted with petroleum ether, chloroform, ethyl acetate and n-butanol. The organic phases were dried with Na2SO4, filtered using common filter paper and concentrated in vacuum up to 38°C to obtain the corresponding extracts: petroleum ether (3 g), CHCl3 (12 g), EtOAc (5 g) and n-BuOH (20 g).

The chloroform extract was subjected to silica gel column chromatography and eluted with 100% hexane; the polarity was increased by the progressive addition of ethyl acetate to obtain thirtyseven fractions (F1-F37). F33 and F34 were purified on a Sephadex LH 20 column eluted with methanol to obtain compounds 1 and 2. The ethyl acetate extract was chromatographed on a silica gel column using a gradient system of chloroform/methanol, to obtain forty fractions(F1-F40). F38 was chromatographed on a silica gel column eluted with an isocratic system (AcOEt/acid acetique/H2O 8:1:1) to afford compound 3.

Gas chromatography-Mass spectrometry

GC-MS was performed using a Bruker Scion SQ instrument, equipped with a Factor Four capillary column (VF-5 ms, 30 m; 0.25 mmi.d., film thickness 0.25 µm) coupled with a SQ (single quadrupole)

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detector. Helium was used as the carrier gas at a rate of 1 ml/min, injector temperature 280°C and ionization voltage 70 eV. Peaks were identified by comparing their retention times and MS fragmentation with those of the standards present in the commercial library NIST [17]

Total phenolic content (TPC)

The TPC was determined using the Folin-Ciocalteu reagent method [18]. 20 µl of ethyl acetate extract was mixed with 100 µl diluted Folin-Ciocalteu reagent (1:10) and 75 µl sodium carbonate solution (75 g/L). After placing them for 2h in darkness, the absorbance was measured at 765 nm in the microplate reader. The TPC is expressed as mg Gallic acid equivalents (GAE) per g of extract [19].

Antioxidant properties

DPPH free radical scavenging assay

The free radical DPPH scavenging activity was determined as described by Braca et al. [19]. Different volumes of ethyl acetate extract (2.5-300 µl) or vitamin C (antioxidant standard) was mixed with 3 ml of DPPH methanolic solution (0.004 %). The absorbance was measured at 517 nm after 30 min incubation in the dark. The free radical scavenging activity is expressed as the inhibition percentage of free radicals by the sample and calculated using the following equation:

DPPH scavenging effect (%) = [(AControl – ASample)/ AControl] × 100,

where A control is the absorbance of control and A sample is the absorbance of sample at 517 nm.

The IC50 values (the concentration of antioxidant which eliminates 50% of DPPH radicals) were defined for the extract and vitamin C.

Lipid peroxidation inhibition assay

Lipid peroxidation in egg vitellus is evaluated by the malondialdehyde assay (MDA) according to the methods described by Lizcano et al. [20] and Amrani et al. [21]. The fresh vitellus was homogenized in ice cold sodium phosphate buffer (20 mM, pH=7.4) to produce a 10% homogenate (v/v) and centrifuged at 4000 rpm for 20 min. 1ml aliquots of the supernatant were incubated with the extract or vitamin C at 37 ºC for 1 h in the presence of 0.07 M $FeSO_4$. After cooling, 1 ml of 20% trichloroacetic acid (TCA) and 1.5 ml of 1% thiobarbituric acid (TBA) was added, and the mixture was then heated at 100ºC for 15 min. The color of the complex was detected at 532 nm after centrifugation at 4000 rpm for 20 min to remove precipitated protein. The control group was run in parallel without sample under similar conditions, except that 1 ml TCA was added before incubation [22].

The lipid peroxidation (LPO) inhibitory activity (%) was calculated by the following equation:

LPO inhibition activity (%) = $[(A_{Control} - A_{Sample})/ A_{Control}] \times 100$.

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Statistical analysis

All data are expressed as the mean ± standard deviation (SD) of triplicate measurements. The statistically significant differences among mean values at the level of significance ($P<0.05$) were evaluated by one-way ANOVA (Graph Pad Prism 5 software).

Results and Discussion

Chemical composition

GC-MS analysis of the chloroform extract revealed the presence of several compounds, of which 36 were identified (Table 1). The main constituents were found to be, 1-monooleoylglycerol (20.72%), monopalmitin (13.11%), epimethendiol (11.45%), myrtenoic acid (10.96%), linolenic acid (8.04%), 13-*cis*-retinoic acid (6.09%) and aucubin (5.58%) (Table 1).

In the ethyl acetate extract, 36 components have been identified. The major constituents were found to be, caffeic acid (15.20%), cinnamic acid (11.31%), benzene acetic acid (10.95%), *p*-hydroxybenzoic acid (9.65%), benzoic acid 4-methoxy (8.09%) (Table 2).

Table 1. Chemical composition of chloroform extract obtained from *A. vulgaris* (*GC-MS* analysis)

No.	Chemical constituents	RT	%
1	Glycerol	13.004	0.17
2	Myrtenoic acid	13.153	10.96
3	13-cis-Retinoicacid	13.614	0.17
4	2-[(1*R*)-2,2,3-trimethylcyclopent-3-en-1-yl]acetaldehyde	16.906	0.16
5	2-Pinene	17.908	0.23
6	(-)-Myrtenol	18.139	0.30
7	5α-Androstan-17-one	18.39	0.13
8	4-terpineol	18.742	0.07
9	(-)-Myrtenol	19.775	0.24
10	Linolenic acid	20.038	8.04
11	4-Hydroxy-2-methoxy-benzeneacetic acid	21.019	0.27

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12	Farnesol	21.837	0.18
13	5,8,11-Eicosatrienoic acid	21.948	0.84
14	cis-2-Hexen-1-ol	23.954	0.21
15	Ritalinic acid	24.225	0.13
16	13-*cis*-Retinoic acid	26.863	6.09
17	L-(-)-Sorbofuranose	27.242	0.75
18	3-Vanilpropanol	27.629	0.95
19	Retinal	28.767	0.29
20	Neocurdione	28.919	0.18
21	D-Galactose	30.028	5.54
22	Digoxigenin	32.313	0.56
23	13-*cis*-Retinoic acid	33.249	1.92
24	19-norandrosterone	33.408	2.22
25	Epimethendiol	33.679	11.45
26	Linalool	33.825	0.98
27	Eudesmol	35.382	0.20
28	Octadecanoic acid	37.59	0.13
29	Etiocholan-3α-ol-17-one	40.207	0.19
30	Decanoic acid	43.697	0.41
31	Monopalmitin	44.309	13.11
32	17β-Hydroxy-5α-androstan-3-one	44.851	3.00
33	2-Monopalmitoylglycerol	46.721	1.82
34	1-Monooleoylglycerol	47.337	20.72
35	Aucubin	49.249	5.58
36	5β–Cholestane-3α,7α,12α,23ξ,25-pentol	49.335	0.35

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Table 2. Chemical composition of ethyl acetate extract obtained from *A. vulgaris* (GC-MS analysis)

No.	Chemical constituents	RT	%
1	Levulic acid	15.533	0.13
2	2-Propenoic acid, 3-methoxy, methyl ester	17.524	0.62
3	Phosphoric acid	17.838	0.46
4	Benzoic acid	20.334	0.27
5	Myrtenoic acid	21.746	0.54
6	Glycerol	22.377	1.68
7	Butanedioic acid	23.700	0.41
8	Salicylic acid	24.809	0.92
9	3-Nonanol	25.539	0.14
10	2-Pinene	27.059	0.67
11	Hydroquinone	27.321	0.58
12	(-)-Myrtenol	29.202	0.99
13	Linalool	29.54	0.48
14	2-Hydroxybenzoic acid	31.714	0.79
15	2-Hydroxymandelic acid, ethyl ester	31.814	1.68
16	Benzene acetic acid	32.516	10.95
17	1-Methylcyclohexanecarboxylic acid	32.68	0.50
18	1,2-Butanediol	33.483	0.67
19	2-Methyl-3-buten-2-ol	34.181	1.30
20	*p*-Hydroxybenzoic acid	35.963	9.65
21	4-(3-Hydroxypropyl)phenol	37.252	1.60
22	Gluconic acid	38.493	1.89

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23	D-Xylofuranose	38.702	0.76
24	4-Methoxybenzoic acid	38.800	8.09
25	L-(-)-Sorbofuranose	39.568	1.52
26	D-(-)-Tagatofuranose	39.804	2.49
27	D-(-)-Fructofuranose	39.925	3.69
28	Cinnamic acid	41.189	11.31
29	Mannonic acid	41.277	7.86
30	Altronic acid	41.357	1.07
31	Caffeic acid	43.376	15.20
32	Gulose	44.432	3.07
33	Alpha-D-Glucopyranose	46.985	2.00
34	D-(+)-Turanose	47.942	1.85
35	1-Tetralone	50.403	1.02
36	Arabitol	52.145	0.75

Compounds 1-3

Compound 1: $C_{10}H_8O_4$, ^{1}H-NMR (400 MHz, $CDCl_3$+MeOH-*d_4*, *δ*ppm, *J*/Hz): 7.69 (1H, *d,* J=12, H-4), 6.86 (1H, *s*, H-5), 6,61 (1H, *s*, H-8), 6,01 (1H, *d*, J=12,H-3), 3.81 (3H, *s*, OCH_3), ^{13}C-NMR (100MHz, $CDCl_3$+ MeOH-*d_4*, *δ*ppm): 163.61 (C-2), 147.20 (C-8a), 146.5 (C-6), 145.07 (C-4), 150.68 (C-7), 124.28 (C-4a), 108.7 (C-3), 107.60 (C-5), 103.21 (C-8), 55.96 (6-OCH_3).scopoletin [9].

Compound 2: $C_{11}H_{10}O_5$, ^{1}H-NMR (400 MHz, MeOH-*d_4*, *δ*ppm, *J*/Hz): 7.69 (1H, *d,* J=12, H-4), 6.66 (1H, *s*, H-8), 5,90 (1H, *d*, H-3), 3.80 (3H, *s*, OCH_3), 3.74 (3H, *s*, OCH_3).*umckalin* [10].

Compound 3: $C_{25}H_{24}O_{12}$, ^{1}H-NMR (400 MHz, DMSO-*d_6*, *δ*ppm, *J*/Hz): 7.43 (1H, *d*, J=16, H-8'), 6.99 (1H, *s*, H-2'), 6.93 (1H, *d*, J=8, H-6'), 6.66 (1H, *d*, J=8, H-5'), 6.17 (1H, *d*, J=16, H-7'), 5.15 (1H, *td*, J=9.2, 3.2, H-1), 3.86 (1H, *m*, H-3), 3.45 (1H, *m*, H-2), 1.92 (2H, *m*, H-4), 1.75 (2H, *m*, H-5), ^{13}C-NMR (100 MHz, DMSO-*d_6*, *δ*ppm): 147.0 (C-4'), 145.76 (C-8'), 144.1 (C-3'), 126.9 (C-1'), 126.9 (C-9'), 121.34 (C-6'), 115.98(C-5'), 114.68 (C-2'), 114.18 (C-7'), 74.23(C-2), 72.99 (C-1), 71.75 (C-3), 39.61 (C-4), 38.37 (C-5). 1-(3', 4' dihydroxycinnamoyl) cyclopentane-2,3-diol [11].

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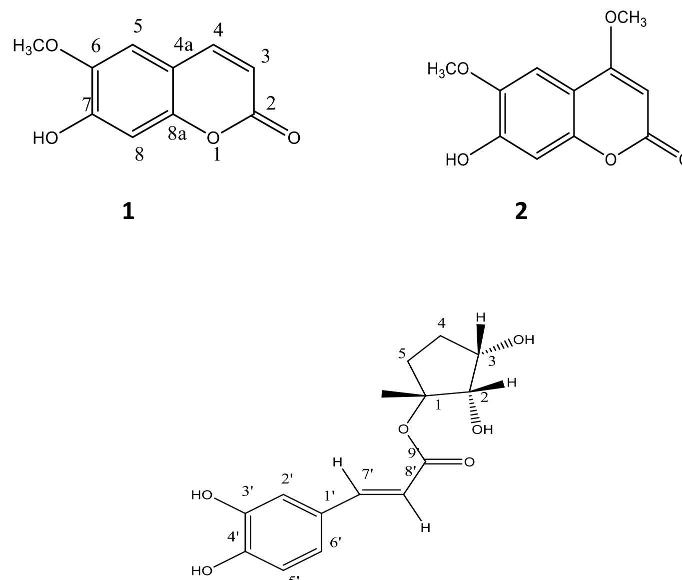

Figure 1. Chemical structure of compounds 1–3

Total phenolic content

The total amount of phenolic compounds of ethyl acetate extract was determined using gallic acid as a standard, the concentration of phenolic in the ethyl acetate extract was found to be (252.46±0.03 mg GAE/g extract). In comparison with other *Anthriscus* species, the result indicates that the amount of phenolic compounds obtained from *A. vulgaris* is higher than that obtained from *A. sylvestris* (85.45±1.16 mg GAE/g hydroethanolic 50% extract) [12].

DPPH radical scavenging activity

The free radical scavenging activity of the ethyl acetate extract of *A. vulgaris* is given in Figure 2. The antiradical capacity of ethyl acetate extract and vitamin C (positive control) is dose-dependent. The IC_{50} values are as follows: Vitamin C (IC_{50}= 5±0.15 µg/ml), ethyl acetate extract (IC_{50}= 46.96±0.25 µg/ml). These results suggest that the ethyl acetate extract of *A. vulgaris* has an interesting effect on scavenging DPPH radical. The compositions of the ethyl acetate extract that show good level of DPPH• radical scavenging activity is rich in caffeic acid and *p*-hydroxy benzoic acid. These compounds appear to be responsible for the antioxidant effect of *A. vulgaris* extract. Caffeic acid has been reported as an effective antioxidant *in vitro* assays such as lipid peroxidation analysis, reducing efficiency, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid ($ABTS^+$) and DPPH scavenging [13].

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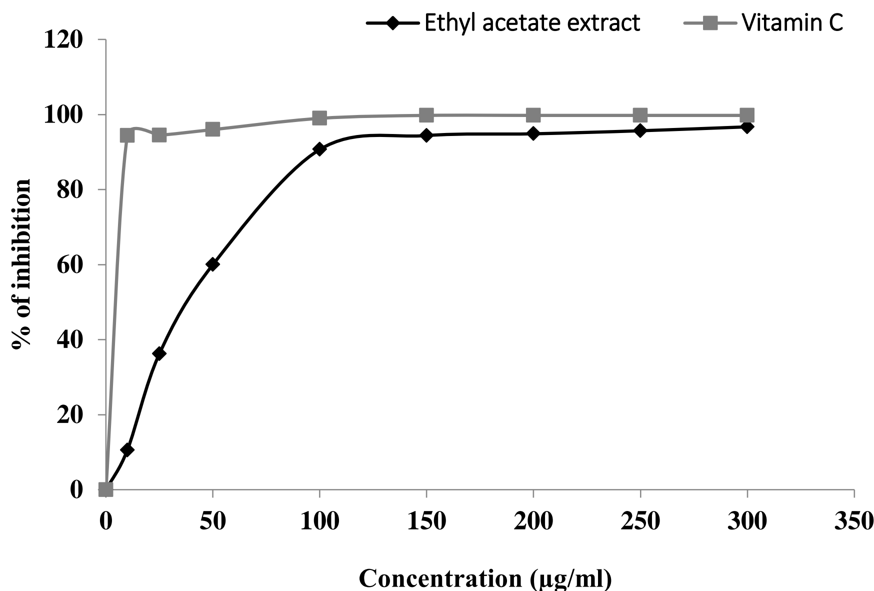

Figure 2. Scavenging effect of ethyl acetate extract of *A. vulgaris* and standard vitamin C on DPPH radical

Lipid peroxidation inhibition assay

Figure 3 showed the activity of ethyl acetate extract of *A.vulgaris* and vitamin C (standard) against non- enzymatic lipid peroxidation in egg *vitellus* homogenate, induced by $FeSO_4$ system. Ethyl acetate extract demonstrated the ability to inhibit the formation of MDA by iron chelating mechanism or by scavenging hydroxyl radicals that were generated by the iron dependent Fenton chemistry in a concentration-dependent manner. The inhibition of lipid peroxidation of ethyl acetate extract was increased with increasing concentration. The percentage of inhibition of lipid peroxidation by 500 µg/ml was found to be 67.23%. The percentage at this concentration for vitamin C was found to be 98.7%. The potent antioxidative activity of ethyl acetate extract of *A. vulgaris* might result from its high contents of polyphenolic compounds such as caffeic acid and p-hydroxybenzoic acid. The active components in the ethyl acetate extract can act synergically to exert their full antioxidant effect. Phenolic compounds such as caffeic acid has been shown to be a potent antioxidant in vitro in different oxidation systems [14], especially against non-enzymatic lipid peroxidation induced by $FeSO_4$ through an iron chelating mechanism, preventing the formation of free hydroxyl radicals and, therefore, inhibiting Fenton-induced oxidative damage [15]. Hydroxybenzoic acids itself and its derivatives showed antioxidant properties against different types of free radicals and can prevent or decrease overproduction of reactive species [16].

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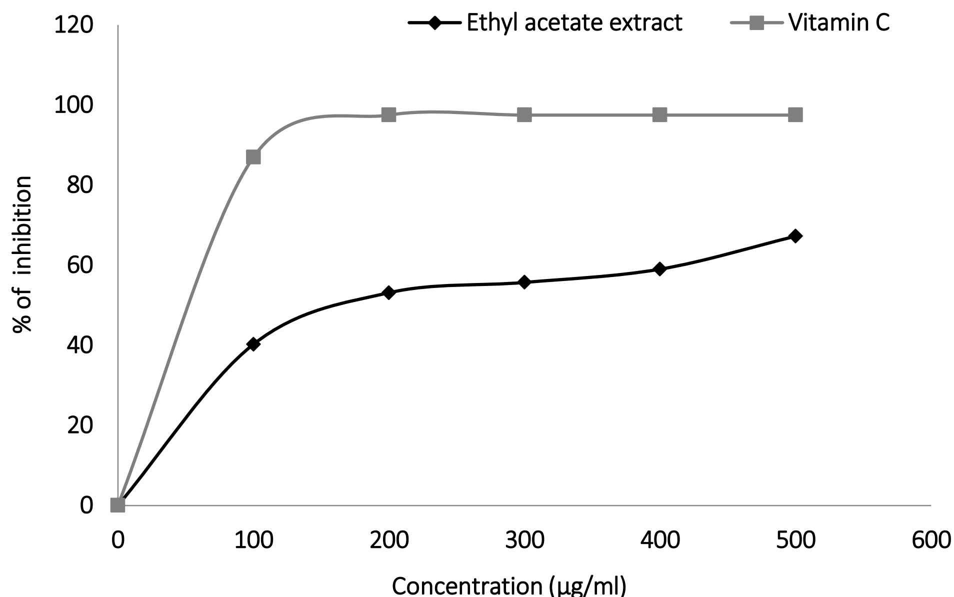

Figure 3. Effect of ethyl acetate extract of *A. vulgaris* and standard vitamin C on non-enzymatic lipid peroxidation

Conclusion

In this study, we report for the first time the antioxidant activities of *A. vulgaris* ethyl acetate extract, relating them to the chemical profile established by total phenolic GC-MS analysis of ethyl acetate and chloroform extracts, and isolation of pure metabolites. The antioxidant activities in the ethyl acetate extract are associated with a rich phenolic composition that often acts in a synergistic fashion to exert their antioxidant effect.

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References

[1] Reduron, J.; Spalik, K., Le genre *Anthriscus* (Apiaceae) dans la flore française, *Acta Botanica Gallica*. **1995**, *142*, 55-96.

[2] Lee, M.C.; Ryu, K.C., Studies on the constituents in the fruits, *Buli KH Pharma Sci*., **1978**, *6*, 61-67.

[3] Fejes, S.; Blázovics, A.; Lugasi, A.; Lemberkovics, E.; Petri, G.; Kéry, A., *In vitro* antioxidant activity of *Anthriscus cerefolium* L. (Hoffm.) extracts, *J Ethnopharmacol*. **2000**, *69*, 259-265.

Corresponding author: benaissaouahiba@yahoo.com
Full Paper
DOI: 10.2478/asn-2020-0007
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[4] Ikeda, R.; Nagao, T.; Okabe, H.; Nakano, Y.; Matsunaga, H.; Katano, M.; Mori, M., Antiproliferative constituents in Umbelliferae plants. III. Constituents in the root and the ground part of *Anthriscus sylvestris* Hoffm, *Chem. Pharm. Bull*. **1998**, *46*, 871-874.

[5] Lee, E.B.; Cho, S.I., Kang, S.S., Kim, K.R.; Kim, T.H., Isolation of torilin from *Torilis japonica* fruits and its analgesic and anti-inflammatory activities, *Saengyak Hakhoechi*, **1999**, *30*, 137-144.

[6] Hui, C.; He-Zhong, J.; Yong-Chao, L.; Guo-Qing, W.; Yun, G.; Chao- Ying, Ma., Antitumor Constituents from *Anthriscus sylvestris* (L.)Hoffm, *APJCP*, **2014**, *15 (6)*, 2803-2807.

[7] Olaru, O.T.; MihaiNiţulescu, G.; Orțan, A.; Dinu-Pîrvu, C.E., Ethnomedicinal, Phytochemical and Pharmacological Profile of *Anthriscus sylvestris* as an Alternative Source for Anticancer Lignans, *Molecules*, **2015**, *20*, 15003-15022.

[8] Hendrawati, O.; Woerdenbag, H. J.; Michiels, P.; Aantjes, H.G.; Van Dam, A.; Kayser, O., Identification of lignans and related compounds in *Anthriscus sylvestris* by LC–ESI-MS/MS and LC-SPE–NMR, *Phytochemistry*, **2015**, *72*, 2172–2179.

[9] Giesemann, A.; Bichland, B.; Lieberei, R.; Identification of scopoletin a phytoalexin of the rubber tree *Hevea brasiliensis*, *J Phytopath*, **1986**, *117*, 373-376.

[10] Kolodziej, H.; Kayser, O.; Tan, N., Novel Coumarin Sulfates from *Pelargonium sidoides*, In: Rauter A.P.; Palma F.B.; Justino J.; Araújo M.E.; dos Santos S.P. (Eds). Natural Products in the New Millennium: Prospects and Industrial Application. Proceedings of the Phytochemical Society of Europe, Springer, Dordrecht, **2002**, 59-64.

[11] Haibo, W.; Maruleedharan, G.N.; Gale, M. S.; Alden, M. B.; Ian, G.J., Novel antioxidant compounds from tart cherries (*Prunus cerasus*), *J. Nat. Prod*. **1999**, *62*, 86-88.

[12] Olaru, O. T.; Mniţulescu, G.; Orţan, A.; Băbeanu, N.; Popa, O.; Ionescu, D.; Dinu-pîrvu, C.E.; Polyphenolic content and toxicity assessment of *Anthriscus sylyestris* Hoffm., *Rom Biotechnol Lett*, **2016**, *22*, 12054-12061.

[13] Khan, F.A.; Maalik, A.; Murtaza, G., Inhibitory mechanism against oxidative stress of caffeic acid, *J Food Drug Anal*, **2016**, *24*, 695-702.

[14] Szwajgier, D.; Pielecki, J.; Targonski, Z., Antioxidant activities of cinnamic and benzoic acid derivatives, *Acta Sci. Pol*., *Technol. Aliment*., **2005**, 129-142.

[15] Genaro-Mattos, T. C.; Maurício, Â.Q.; Rettori, D.; Alonso, A.; Hermes-Lima, M., Antioxidant Activity of Caffeic Acid against Iron-Induced Free Radical Generation - A Chemical Approach, *PLoS One*, **2015**, *10* (*6*), e0129963.

[16] Veliká, B.; Kron, I, Antioxidant properties of benzoic acid derivatives against superoxide radical, *Free Rad. Antiox*, **2012**, *2*, 62–67.

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[17] Mikaia, A.; White, E. V.; Zaikin, V.; Zhu, D.; Sparkman, O. D.; Neta, P.; Zenkevich, I.; Linstrom, P.; Mirokhin, Y.; Tchekhovskoi, D., NIST Standard Reference Database 1A. **2014**.

[18] Singleton, V. L.; Rossi, J. A. J., Colorimetry of total phenolics with phosphomolydbic-phosphotungstic acid reagents, *Amer. J. Enol. Viticult*. **1965**, *16*, 144-158.

[19] Braca, A.; DE Tommasi, N.; Di Bari, L.; Pizza, C.; Politi, M.; Morelli, I., Antioxidant principales from *Bauhinia terapotensis*, *J Nat Prod*, **2001**, *64*, 892-895.

[20] Lizcano, L. J.; Viloria-Bernal, M.; Vicente, F.; Berrueta, L. A.; Gallo, B.; Martínez-Cañamero, M.; Ruiz-Larrea, M.B.; Ruiz-Sanz, J. I, Lipid oxidation inhibitory effects and phenolic composition of aqueous extracts from medicinal plants of Colombian Amazonia, *Int J Mol Sci*, **2012**, *13*, 5454-5467.

[21] Amrani, A.; Lahneche, A. Ma.; Benaissa, O.; Boubekri, N.; Demirtas, I.; Zama, D.; Benayache, F, Benayache, In vitro antiproliferative and inhibition of oxidative DNA damage activities of n-butanol extract of *Limonium bonduelli* from Algeria, *Braz. Arch. Biol. Technol*., **2019**, Vol. *62*, e19170779.

[22] Cao, U.; Ikeda, I., Antioxidant activity and antitumor activity (*in vitro*) of xyloglucan selinious ester and surfated xyloglucan, *Int. J. Biol. Macromol*., **2009**, *45*, 231-235.

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