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-glucosidase inhibitory activities of
Centaurea papposa extracts**

LC-MS analysis, antioxidant and alpha-glucosidase inhibitory activities of *Centaurea papposa* extracts

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Abstract

This work aimed to ascertain the phenolic compounds and assess the antioxidant capacity and alpha-glucosidase inhibitory activity of *Centaurea papposa* extracts. Phenolic compounds were appraised using LC-MS technique. Moreover, antioxidant activity was investigated using DPPH, ABTS, CUPRAC and FRAP assays. *In vitro* alpha-glucosidase inhibitory effect was carried out. LC-MS analysis revealed the presence of 21 compounds among which 13 were phenolic acids, 6 flavonoids, 1 phenolic aldehyde and 1 benzopyrone. The ethyl acetate extract exhibited the highest activity in ferric reducing antioxidant power (FRAP) assay (IC_{50} : $22.9 \pm 2.8 \mu\text{g/mL}$). Nevertheless the *n*-butanol extract was the most active in cupric reducing antioxidant capacity assay (IC_{50} : $3.1 \pm 0.1 \mu\text{g/mL}$). A significant alpha-glucosidase inhibitory activity was displayed by dichloromethane extract (IC_{50} : $227.6 \pm 4.4 \mu\text{g/mL}$).

Introduction

Diabetes mellitus is a chronic disease of metabolic disorder (Pallavi et al., 2015) which is treated by either insulin or oral anti-diabetic drugs. Among the oral anti-diabetic drugs, alpha-glucosidase inhibitor retards the liberation of d-glucose from dietary complex carbohydrates and delays glucose absorption, resulting in reduced postprandial plasma glucose level and suppression of postprandial hyperglycemia. Plants such as *Camellia sinensis* (Hara and Honda, 1992), *Ipomoea batatas* (Matsui et al., 1996), berry (McDougall and Stewart, 2005), *Syzygium zeylanicum*, *Cleistocalyx operculatus*, *Horsfieldia amygdalina* and *Careya arborea* (Truong et al., 2007) show alpha-glucosidase inhibitor activity.

The genus *Centaurea* L. (Asteraceae, Carduaceae) is characterized by biosynthesis of sesquiterpene lactones (Medjroubi et al., 1998) and flavonoids (Akkal et al., 2003). They have long been used in traditional medicine to treat fever, diabetes and hemorrhoid and peptic ulcer (Honda et al., 1996; Kargioğlu et al., 2008). *Centa-*

urea papposa is a species belonging to the *Centaurea* section, subsection *Acrolophus* (Cass.) DC (Hilpold et al., 2011), increasing wild in Algeria and Tunisia.

The chemical profile of the endemic species *C. papposa* of Algeria, has not been reported before. For this reason, this plant was chosen to explore its chemical compounds and quantities of the phenolic compounds by LC-MS/MS and to evaluate the α -glucosidase inhibitory activities and antioxidants *in vitro* by different methods.

Materials and Methods

Chemicals used

For LC-MS/MS analysis: The analytical standards of quinic acid, malic acid, fumaric acid, gallic acid, protocatechic acid, pyrocatechol, chlorogenic acid, 4-OH-benzoic acid, vanillic acid, caffeic acid, syringic acid, vanillin, salicylic acid, *p*-coumaric acid, rutin, ferulic



acid, sinapic acid, hesperidin, isoquercitrin, rosmarinic acid, nicotiflorin, α -coumaric acid, rhoifolin, quercitrin, apigetrin, coumarin, myricetin, fisetin, cinnamic acid, liquiritigenin, quercetin, luteolin, naringenin, apigenin, hesperetin, kaempferol and chrysin were purchased from Sigma-Aldrich, Italy. HPLC-grade acetonitrile, ammonium formate and formic acid were purchased from Sigma-Aldrich, Italy.

For extracts and biological activities: Quercetin, potassium persulfate, ferrous chloride, dichloromethane, ethyl acetate, *n*-butanol, α -tocopherol, acarbose and ethylenediamine tetraacetic acid (EDTA) were obtained from E. Merck, Germany. Folin-Ciocalteu's reagent (FCR), neocuproine, butylated hydroxytoluene (BHT), DPPH dye, sodium carbonate, aluminum chloride, phosphate buffer, ammonium acetate buffer, potassium ferricyanide, butyl hydroxyanisole (BHA), methanol and ethanol were obtained from Sigma Chemical Co., Germany. 2,20-Azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), *p*-nitrophenyl- α -D-glucopyranoside solution, α -glucosidase, ascorbic acid, tannic acid were obtained from Fluka Chemie, Germany.

Plant material

The aerial parts of the plant were collected in full bloom in Annaba (North East of Algeria) in September 2016. They were identified by Dr. Hamel Tarek, Department of Plant Biology and Environment, Badji Mokhtar University, Annaba, Algeria. A reference specimen was deposited in the herbarium of the laboratory under the reference code No.: ChifaDZUMCAPBC000039. The samples were dried in the shade at room temperature in a ventilated place and cut into small pieces.

Preparation of extracts

The air-dried powdered aerial parts of *C. papposa* (600 g) were successively macerated with dichloromethane (6 L \times 3) and methanol (4L \times 3) at room temperature for 24 hours. After concentration under reduced pressure, dichloromethane (10 g) and methanol (56 g) extracts were obtained. The methanol extract was dissolved in hot distilled water. The resulting solution was successively extracted by solvents with increasing polarity ethyl acetate and *n*-butanol evaporated under reduced pressure to obtain 2 g of ethyl acetate and 16 g of *n*-butanol extracts.

LC-MS method development and validation

The LC-MS analyses of phenolic compounds were performed using a Nexera model Shimadzu UHPLC coupled to a tandem MS instrument. The liquid chromatography was equipped with LC30AD binary pumps, CTO-10ASvp column oven, DGU-20A3R degasser and SIL-30AC autosampler. The chromatographic separation was performed on an RP-C18 Inertsil ODS-4 (100 mm \times 2.1 mm, 2 μ m) analytical column. Reversed-

phase ultrahigh performance liquid chromatography was optimized to achieve optimum separation for 37 phytochemical compounds and to overcome the suppression effects. The column temperature was fixed at 35°C. The elution gradient consisted of eluent A (water, 10 mM ammonium formate and 0.1% formic acid) and eluent B (acetonitrile). The following gradient elution program was applied: 5-20% B (0-10 min), 20% B (10-22 min), 20-50% B (22-36 min), 95% B (36-40 min), 5% B (40-50 min). The solvent flow rate was maintained at 0.25 mL/min and the injection volume was settled as 4 μ L.

MS detection was performed using a Shimadzu brand. LCMS 8040 model tandem mass spectrometer equipped with an ESI source operating in negative ion mode. LC-ESI-MS/MS data were collected and shipped by LabSolutions Software (Shimadzu) software. Multiple reaction monitoring (MRM) was used to quantify it. The working conditions of the mass spectrometer were passed as interface temperature, 350°C; DL temperature, 250°C; temperature of the thermal block, 400°C; nebulization gas flow (nitrogen), 3L/min; and drying gas stream (nitrogen), 15 L/min.

A complete LC-MS/MS method was optimized and validated for the quantification of 37 phytochemical fingerprint compounds (17 flavonoids, 15 phenolic acids, 3 non-phenolic organic acids, 1 benzopyrene and 1 phenolic aldehyde) on the species studied. The performance characteristics of the method were determined using standard solutions, enriched and non-enriched samples. In this context, the developed method has been fully validated in terms of linearity, accuracy (recovery), interday and intraday precision (repeatability), detection and quantification limits (LOD/LOQ) and uncertainty relative standards (U% at 95% confidence level [$k = 2$]) (Table I).

Determination of antioxidant activity

DPPH radical scavenging assay

DPPH assays based on measurement of the scavenging capacity of antioxidants towards a stable free radical α , α -diphenyl- β -picrylhydrazyl (DPPH; C18H12N5O6, M=394.33). The odd electron of the nitrogen atom in DPPH was reduced by receiving a hydrogen atom from antioxidants to the corresponding hydrazine (Kedare and Singh, 2011).

The antiradical activity of crude extracts obtained from the species *C. papposa* was evaluated by the free radical DPPH assay (Blois, 1958). A solution of 40 μ L of the sample (extract or standard) at different dilutions was completed with 160 μ L of the DPPH methanol solution (0.1 mM). DPPH reagent in methanol was used as a blank. After 31 min, the absorbance of each solution was detected at 517 nm using a microplate reader. Butyl hydroxyl toluene (BHT) and butyl hydroxyanisole (BHA) were used as positive controls. The percentage of

radical scavenging activity was calculated as follows:

$$\% \text{ Inhibition} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

Where, A_{control} is the absorbance of control reaction (containing all reagents except the test extract or standard), and A_{sample} is the absorbance of the test extract or standard

The result was expressed as IC_{50} value ($\mu\text{g/mL}$) corresponding of sample concentration that inhibits 50% DPPH of free radical.

ABTS radical scavenging assay

The ABTS antioxidant assay measures $ABTS^+ \bullet$ radical production induced by potassium persulfate.

The ABTS scavenging activity was determined according to the method described earlier (Re et al., 1999). $ABTS^+$ was produced by the reaction between 7 mM of ABTS in water and 2.45 mM potassium persulfate, stored in the dark at room temperature for 12 hours before use. $ABTS^+$ solution was then diluted with methanol to obtain an absorbance of 0.70 ± 0.02 at 734 nm. After the addition of 160 μL of diluted $ABTS^+$ to 40 μL of different concentrations of crude extracts and standards (BHT, BHA), the absorbance was measured at 734 nm after 10 min of the initial mixing using a 96-well microplate reader. The scavenging activity of $ABTS^+$ radical was expressed as the inhibition percentage using the following equation:

$$\% \text{ Inhibition} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

Where, A_{control} is control absorbance reaction (containing all reagents except the test extract or standard), and A_{sample} is the absorbance of extract or standard

Reducing power assay (FRAP)

FRAP assay measures the change in absorbance at 593 nm which indicated with the appearance blue colored Fe^{2+} -tripyriddytriazine compound from colorless oxidized Fe^{3+} form by the action of electron donating antioxi-

dants (Popova et al., 2014).

The FRAP of the crude extract was determined according to the method described earlier (Marco, 1968). In which, 10 μL of different concentrations of extracts were mixed with 40 μL of phosphate buffer (0.2 M, pH 6.6) and 50 μL of potassium ferricyanide (10 mg/mL). Then, the reaction mixture was incubated at 50°C for 20 min, after that the reaction mixture was acidified with 50 μL of trichloroacetic acid solution (10%), and 10 μL of ferric chloride solution (0.1%) was added to this solution. The absorbance was measured at 700 nm.

Alpha-glucosidase inhibitor activity

The α -glucosidase inhibitor activity was investigated using the method described elsewhere (Lordan et al., 2013). A volume of 50 μL of extract solution and 50 μL of p-nitrophenyl- α -D-glucopyranoside solution 5 mM (in phosphate buffer 100 mM, pH 6.9) were mixed and incubated at 37°C for 10 min. Then, 100 μL of alpha-glucosidase (0.1 U/mL) was added and the absorbance was recorded at 405 nm after 0 and 30 min respectively. The pharmacological inhibitor, acarbose, was included as a positive control.

The activity of α -glucosidase was calculated as follows:

$$\% \text{ Activity} = [(A_{\text{Absorbance of control}} - A_{\text{Absorbance of extract}}) / A_{\text{Absorbance of control}}] \times 100$$

Statistical analysis

In this study, analyses were carried out in triplicate of each sample and each experiment was realized out in triplicate ($n=3$). The mean value and standard deviation were calculated from the data obtained. Data of bioassays were the subject of one-way analysis of variance (ANOVA) using the SPSS 18.0 software (SPSS Inc.) followed by Tukey's test. The level of significance was fixed at $p < 0.05$.

Box 1: Cupric reducing antioxidant capacity

Principle

The cupric ion reducing antioxidant capacity (CUPRAC assay) evaluates the capacity of antioxidants to reduce the Cu^{2+} to Cu^+ in the presence of a chelating agent. It is efficient for glutathione and thiol-type antioxidants, for which the FRAP test is nonresponsive.

Requirements

Ammonium acetate buffer; Cu (II) solution; Extract; Neocuproine; UV-Vis spectrophotometer

Procedure

Step 1: Ammonium acetate buffer (60 μL ; 1 M, pH 7.0) solution, neocuproine solution (50 μL ; 7.5 mM) and Cu (II) solution (50 μL , 10 mM) were added to a test tube.

Step 2: Mixed well using vortex mixer.

Step 3: 40 μL of the sample solutions at different concentrations were added.

Step 4: Mixed well using vortex mixer.

Step 5: After 30 min, the absorbance was read at 450 nm using spectrophotometer.

Analysis of results

The results were given as 0.5 ($\mu\text{g/mL}$) which corresponding to concentration indicating 50% of absorbance intensity and results were compared with those of standards BHT and BHA.

References

Apak et al., 2004; Rubio et al., 2016

Table I

Phytochemicals in *C. papposa* extracts

No.	Analyte	Retention time	Amount ($\mu\text{g/g}$ extract)		
			Dichloromethane extract	Ethyl acetate extract	<i>n</i> -Butanol extract
1	Quinic acid	1.1	NI	200579.5	162905.0
2	Malic acid	1.2	NI	1 436.7	1 201.4
3	Fumaric acid	1.5	NI	NI	NI
4	Gallic acid	3.0	NI	2 807.1	2 599.3
5	Protocatechuic acid	4.9	NI	3 221.9	2 640.1
6	Pyrocatechol	6.5	NI	NI	NI
7	Chlorogenic acid	7.1	7.6	4539.3	4188.0
8	4-OH-Benzoic acid	7.4	NI	827.8	704.1
9	Vanillic acid	8.6	183.4	1834.9	1539
10	Caffeic acid	8.8	1.6	650.9	594.7
11	Syringic acid	9.0	NI	145.4	120.8
12	Vanillin	10.9	54.8	NI	NI
13	Salicylic acid	11.2	17.4	197.4	194.0
14	<i>p</i> -Coumaric acid	11.5	9.9	471.01	416.2
15	Rutin	12.6	NI	NI	NI
16	Ferulic acid	12.6	214.8	654.2	546.3
17	Sinapinic acid	12.7	NI	NI	NI
18	Hesperidin	12.7	NI	61.8	67.7
19	Isoquercitrin	13.4	NI	19.0	33.0
20	Rosmarinic acid	14.5	NI	31.4	29.1
21	Nicotiflorin	14.7	NI	NI	NI
22	<i>o</i> -Coumaric acid	15.5	NI	NI	NI
23	Rhoifolin	16.1	NI	283.6	268.0
24	Quercitrin	16.4	NI	783.5	661.4
25	Apigenin	16.6	NI	1658.7	1567.7
26	Coumarin	17.4	1075.4	2521.8	2430.5
27	Myricetin	18.7	NI	NI	NI
28	Fisetin	19.3	NI	NI	NI
29	Cinnamic acid	25.6	NI	NI	NI
30	Liquiritigenin	25.6	NI	NI	NI
31	Quercetin	28.2	NI	NI	NI
32	Luteolin	28.3	NI	NI	NI
33	Naringenin	30.7	NI	NI	NI
34	Apigenin	31.4	146.1	1608.0	1506.3
35	Hesperetin	31.8	NI	NI	NI
36	Kaempferol	31.9	NI	NI	NI
37	Chrysin	36.7	NI	NI	NI
Total detected products			9	20	20
NI : Non-identifier					

Results

Identification and quantification of contents

Following the LC-MS results, phenolic acid contents in

C. papposa extracts were higher than their of flavonoid contents (Table I). The LC-MS analysis of ethyl acetate extract revealed the presence of quinic acid, malic acid, gallic acid, protocatechuic acid, chlorogenic acid, vani-

Table II

Antioxidant activities of *C. papposa* extracts

Extract	DPPH IC ₅₀ (µg/mL)	ABTS IC ₅₀ (µg/mL)	CUPRAC A0.50 (µg/mL)	Reducing power A0.50 (µg/mL)
Dichloromethane	NA	>100	>100	> 200
Ethyl acetate	18.1 ± 0.6	11.7 ± 0.2	4.9 ± 0.0	22.9 ± 2.8
<i>n</i> -Butanol	17.0 ± 0.9	22.6 ± 1.2	3.1 ± 0.1	42.1 ± 2.9
Butyl hydroxyl toluene	6.8 ± 0.5	1.6 ± 0.0	9.6 ± 0.9	NT
Butyl hydroxyl anisole	6.8 ± 0.5	1.0 ± 0.0	3.6 ± 0.2	NT
Ascorbic acid (4 µg/µL)	NT	NT	NT	6.8 ± 1.2
Tannic acid (4 µg/µL)	NT	NT	NT	5.4 ± 0.9
α-Tocopherol (4 µg/µL)	NT	NT	NT	34.9 ± 2.4

Values were expressed with means ± SD of three parallel measurements, (p<0.05). SD: Standard deviation, *C. azarolus*: *Crataegus azarolus*, ABTS: 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid, IC₅₀: Half maximal inhibitory concentration; nt: not tested, na: not absorbance

Table III

Alpha-glucosidase inhibitory assay of *C. papposa* extracts

Extracts (4 µg/µL)	IC ₅₀ (µg/mL)
Dichloromethane	227.6 ± 4.4
Ethyl acetate	791.9 ± 1.8
<i>n</i> -Butanol	NA
Acarbose	275.4 ± 1.6

llic acid, syringic acid, apigetrin, apigenin and coumarin showed the highest concentrations (200579.8, 1436.7, 2807.1, 3221.9, 4 539.34, 1834.9, 1658.7, 2521.8, 1608.0 µg/g extract) respectively. While 4-OH-benzoic acid, caffeic acid, salicylic acid, *p*-coumaric acid, ferulic acid, rosmarinic acid, hesperidin, isoquercitrin, rhoifolin and quercitrin were found with the lowest values.

On the other hand, the same previously cited phenolic compounds were detected in the *n*-butanol extract. Finally, coumarin showed the highest values (1075.4 µg/g extract) followed by ferulic acid, vanillic acid, apigenin and vanillin in dichloromethane extract.

Antioxidant activity

For DPPH test, maximum scavenging activity was found in *n*-butanol extract (IC₅₀ value: 17.0 ± 0.9 µg/mL), followed by ethyl acetate extract (IC₅₀ value: 18.1 ± 0.6 µg/mL). Dichloromethane extract was inactive. In the ABTS method, the ethyl acetate extract exhibited the highest activity (IC₅₀ value: 11.7 ± 0.2 µg/mL) among all extracts, followed by *n*-butanol (IC₅₀ value: 22.6 ± 1.2 µg/mL). Results of CUPRAC of extracts were compared with those of BHA and BHT (Table II). Activity (absorbance) increased linearly with the increasing of extract amount. *n*-Butanol extract exhibited the highest activity, it was better than those of standards (A_{0.50} value: 3.1 ± 0.1 µg/mL). Ethyl acetate extract indicated the higher activity, it was better than that of BHT, but

lower than the BHA (4.9 ± 0.0 µg/mL). Ethyl acetate extract exhibited higher ferric reducing power ability than the α-tocopherol (22.9 ± 2.8 µg/mL), which is adjoining to *n*-butanol extract (42.1 ± 2.9 µg/mL) but this activity was relatively lower than that of ascorbic acid and tannic acid (Table II).

Alpha-glucosidase inhibitor activity

Dichloromethane extract showed a greater inhibition activity compared to acarbose (IC₅₀ value: 227.6 ± 4.4 µg/mL). Ethyl acetate extract (IC₅₀ value: 791.9 ± 1.8 µg/mL) exhibited weak inhibitory activity against alpha-glucosidase. The *n*-butanol extract, however, was inactive (Table III).

Discussion

Biological potential and total phenolic contents of *C. papposa* are reported for the first time in this work. The LC-MS/MS analysis of *C. papposa* extracts revealed the presence of 21 phenolic compounds. The protocatechuic acid, chlorogenic acid, caffeic acid, syringic acid, *p*-coumaric acid, ferulic acid, coumarin, salicylic acid, vanillic acid and apigenin presented the highest values. Previous experiments on *Centaurea* have shown the presence of major phenolic compounds, quercetin, quercetin-3-β-D-glucoside and protocatechuic from *C. amaena* and *C. aksoyi* (Albayrak et al., 2017), chlorogenic, caffeic, ferulic, and *p*-coumaric acids, isoquercitrin, and coumarin from *C. cyanus* (Escher et al., 2018), β-sitosterol 3-glucoside, protocatechuic acid, scopoletin, chlorogenic acid, cen-taurein, kaempferol-3-glucoside, jacein, arctiin, querce-tin-3-glucoside and janerin from *C. isaurica* (Flamini et al., 2004). Sixteen compounds including protocatechuic acid, hexoside and ferulic acid were determined in the methanol extract of *C. baseri* using LC/MS (Kösen et al., 2016).

The ethyl acetate and *n*-butanol extract of *C. papposa*

have the best antioxidant effect, it was more potential than other *Centaurea* species investigated in earlier studies from *C. pulchella* (Zengin et al., 2010), *C. calcitrapa* subsp. *calcitrapa*, *C. ptosimopappa*, *C. spicata* (Erol-Dayi et al., 2011), *C. kurdika*, *C. rigida*, *C. amanicola*, *C. cheirolopha* and *C. ptosimopappoides* (Aktumsek et al., 2013).

A good correlation between total phenolic contents and antioxidant activity was demonstrated. Indeed, ethyl acetate and butanol which are richer with these compounds were generally significantly more actives. From literature, it has been well noted that medicinal plants with high amounts of phenols and flavonoids have potent antioxidant actions (da Silva et al., 2006; Ksouri et al., 2009; Falleh et al., 2011; Dehshiri et al., 2013).

On the other hand, the dichloromethane extract exhibits strong alpha-glucosidase inhibitory activity, the TLC profiling revealed with sulfuric vanillin that indicated the presence of terpenes (visible spots: blue, green, violet pink) might contribute to this activity (Ouattara et al., 2016).

Conclusion

A total of 21 compounds were identified of which the main constituents were flavonoids and phenolic acids. The ethyl acetate and *n*-butanol extracts of *C. papposa* have strong antioxidant properties *in vitro*. The dichloromethane extract exhibits strong alpha-glucosidase inhibitory activity, this result therefore clearly indicates the potential of this extract to manage hyperglycemia.

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Conflict of Interest

Authors declare no conflict of interest

References

Akkal S, Benayache F, Medjroubi K, Jay M, Tillequin F. *Seguin E.* flavonoids from *Centaurea furfuracea*. *Biochem Systematics Ecol.* 2003; 31: 641-43.

Aktumsek A, Zengin G, Guler GO, Cakmak YS, Duran A. Anti-oxidant potentials and anticholinesterase activities of methanolic and aqueous extracts of three endemic *Centaurea L.* species. *Food Chem Toxicol.* 2013; 55: 290-96.

Albayrak S, Atasagun B, Aksoy A. Comparison of phenolic components and biological activities of two *Centaurea* sp. obtained by three extraction techniques. *Asian Pac J Trop*

Med. 2017; 10: 599-606.

Apak R, Güçlü K, Özyürek M, Karademir SE. Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. *J Agric Food Chem.* 2004; 52: 7970-81.

Blois MS. Anti-oxidant determinations by the use of a stable free radical. *Nature* 1958; 181: 1199-200.

da Silva JFM, de Souza MC, Matta SR, de Andrade MR, Vidal FVN. Correlation analysis between phenolic levels of Brazilian propolis extracts and their antimicrobial and antioxidant activities. *Food Chem.* 2006; 99: 431-35.

Dehshiri MM, Aghamollaei H, Zarini M, Nabavi SM, Mirzaei M, Loizzo MR, Nabavi SF. Antioxidant activity of different parts of *Tetrataenium lasiopetalum*. *Pharm Biol.* 2013; 51: 1081-85.

Erol-Dayi Ö, Pekmez M, Bona M, Aras-Perk A, Arda N. Total phenolic contents, antioxidant activities cytotoxicity of three *Centaurea* Species: *C. calcitrapa* subsp. *calcitrapa*, *C. ptosimopappa* *C. spicata*. *Free Radic Antioxid.* 2011; 1: 31-36.

Escher GB, Santos JS, Rosso ND, Marques MB, Azevedo L, do Carmo MAV, Dagher H, Molognoni L, do Prado-Silva L, Sant'Ana AS, da Silva MC, Granato D. Chemical study, antioxidant, antihypertensive, and cytotoxic/cytoprotective activities of *Centaurea cyanus L.* petals aqueous extract. *Food Chem Toxicol.* 2018; 118: 439-53.

Flamini G, Stoppelli G, Morelli I, Ertugurl K, Dural H, Tugay O. Secondary metabolites from *Centaurea isaurica* from Turkey and their chemotaxonomical significance. *Biochem Syst Ecol.* 2004; 32: 553-57.

Falleh H, Ksouri R, Medini F, Guyot S, Abdelly C, Magné C. Anti-oxidant activity and phenolic composition of the medicinal and edible halophyte *Mesembryanthemum edule L.* *Ind Crops Prod.* 2011; 34: 1066-71.

Hara Y, Honda M. Inhibition of rat small intestinal sucrose and alpha-glucosidase activities by tea polyphenol. *Biosci Biotechnol Biochem.* 1992; 57: 123-24.

Hilpold A, Schönswetter P, Susanna A, Garcia-Jacas N, Vilatersana R. Evolution of the central Mediterranean *Centaurea cineraria* group (Asteraceae): Evidence for relatively recent, allopatric diversification following transoceanic seed dispersal. *Taxon* 2011; 60: 528-38.

Honda G, Yeşilada E, Tabata M, Sezik E, Fujita T, Takeda Y, Takaishi Y, Tanaka T. Traditional medicine in Turkey VI. Folk medicine in West Anatolia: Afyon, Kütahya, Denizli, Muğla, Aydın Provinces. *J Ethnopharmacol.* 1996; 53: 75-87.

Kargioğlu M, Cenkeci S, Serteser A, Evliyaoğlu N, Konuk M, Kök MŞ, Bağcı Y. An ethnobotanical survey of Inner-West Anatolia, Turkey. *Hum Ecol.* 2008; 36: 763-77.

Kedare SB, Singh RP. Genesis and development of DPPH method of antioxidant assay. *J Food Sci Technol.* 2011; 48: 412-22.

Kösen YB, Işcan G, Göger F, Akalm G, Demirci B, Başer KHC. Chemical composition and biological activity of *Centaurea baseri*: New species from Turkey. *Chem Biodivers.* 2016; 13: 1369-79.

- Ksouri R, Falleh H, Megdiche W, Trabelsi N, Mhamdi B, Chaieb K, Bakrouf A, Magné C, Abdely C. Antioxidant and antimicrobial activities of the edible medicinal halophyte *Tamarix gallica* L. and related polyphenolic constituents. *Food Chem Toxicol.* 2009; 47: 2083-91.
- Lordan S, Smyth TJ, Soler-Vila A, Stanton C, Ross RP. The α -amylase and α -glucosidase inhibitory effects of Irish seaweed extracts. *Food Chem.* 2013; 141: 2170-76.
- Marco GJ. A rapid method for evaluation of antioxidants. *J Am Oil Chem Soc.* 1968; 45: 594-98.
- Matsui TC, Yoshimoto C, Osajima K, Oki T, Osajima Y. *In vitro* survey of alpha-glucosidase inhibitory food components. *Biosci Biotech Biochem.* 1996; 60: 2019-22.
- Medjroubi K, Benayache F, Benayache S, Akkal S, Kaabeche M, Tillequin F, Seguin E. Eudesmanolide from *Centaurea granata*. *Phytochemistry* 1998; 49: 2425-27.
- McDougall GJ, Stewart D. The inhibitory effects of berry polyphenols on digestive enzymes. *Biofactors* 2005; 23: 189-95.
- Ouattara LH, Kabran GRM, Kadja AB, Tano MB, Akhanovna J, Bekro YA. Phytochemical survey and antioxidant activity of plant extracts from Côte D'ivoire used in traditional treatment of hemorrhoids. *Int J Innov Appl Stud.* 2016; 15: 881-93.
- Pallavi B, Chandresh V, Kanika K, Tammama T. *In vitro* evaluation of antidiabetic and antioxidant activity of Seabuckthorn (*Hippophae rhamnoides* L.). *J Med Plants Res.* 2015; 9: 929-32.
- Popova A, Mihaylova D, Alexieva I. Study on the antioxidant activity of dried *Allium bulgaricum* leaves. *Proc Biotech Food Technol.* 2014; 53: 27-31.
- Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med.* 1999; 26: 1231-37.
- Rubio CP, Hernández-Ruiz J, Martínez-Subiela S, Tvarijonavičiute A, Ceron JJ. Spectrophotometric assays for total antioxidant capacity (TAC) in dog serum: An update. *BMC Vet Res.* 2016; 12: 166.
- Truong TM, Nghiem NT, Pham GT, Nguyen VC. Alpha-glucosidase inhibitory and antioxidant activities of Vietnamese edible plants and their relationships with polyphenol contents. *J Nut Sci Vitaminol.* 2007; 53: 267-76.
- Zengin G, Cakmak YS, Guler GO, Aktumsek A. *In vitro* antioxidant capacities and fatty acid compositions of three *Centaurea* species collected from Central Anatolia region of Turkey. *Food Chem Toxicol.* 2010; 48: 2638-41.
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