Evaluation of Phenolic Compounds Content and Antioxidant Capacity of Some Medicinal Plants, Potentially Used for Their Preventive Role Against Various Oral Diseases

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The oral cavity diseases represent a public health issue. Antioxidants have an important role in the prevention of these diseases. In this study we tested different types of plants in order to evaluate the compounds with antioxidant capacity and in order to create a topical product which prevents oral cavity diseases. Phenolic compounds and flavonoids were analyzed in four different species of medicinal plants from spontaneous flora of Bihor County. The antioxidant capacity of the extracts was evaluated by: CUPRAC assay, ABTS method and FRAP method. Our results show that the studied medicinal plants represent rich sources of compounds with antioxidant capacity, especially Polygonum aviculare and Fumaria officinalis.

Keywords: phenolic compounds, flavonoids, antioxidant capacity, oral cavity diseases

One of the public health issues are the oral cavity diseases. A good balance of oxidants and antioxidants is important for oral health and for systemic health too [1].

Different chemical and pharmaceutical waste pollutants [2] (e.g. alcohol, nicotine, superoxide, hydrogen peroxide, antibiotics, hydroxyl radicals, etc.) are factors that can disturb the balance of oxidants in oral tissues, causing oxidative stress [3-9]. Recent studies on antioxidants have demonstrated their important role in offset this imbalance [10,11]. Many antioxidant compounds possess antiinflammatory, antiatherosclerotic [12], antiproliferative, antitumor [13], antimutagenic, anticarcinogenic, antibacterial or antiviral activities [13-18]. An important group of antioxidants are phenolic compounds [10,12]. Several thousands of natural phenolic compounds have been identified in plants, some of them being obtained in aromatic plants distillation [19]. Thereare classified in two groups: flavonoids and non- flavonoids [20-23].

People are familiar with antioxidants taken systemically, such as foods and vitamin supplements, but topical antioxidants may have action on oral mucosa cells. Research studies are currently under way to examine the effectiveness of combinations of antioxidants applied topically to oral cells. Results from clinical studies, though incomplete, are positive. In addition, published studies confirmed that antioxidants that work on skin cells also have an effect on oral, gingival, and periodontal cells [1,3-9,11].

In this study we tested four different species of medicinal plants from unpolluted spontaneous flora of Bihor County in order to evaluate the compounds with antioxidant capacity and in order to create a topical pharmaceutical formulation which could be used to prevent the oral cavity diseases.

Experimental part

Plant material

We performed a comprehensive study in which we followed, during 2017, in eight different areas, from Bihor county of Romania, the spontaneous medicinal plants populations. The aerial parts of creeping jenny, *Glechoma hederacea (Lamiaceae Family);* common knotgrass, *Polygonum aviculare (Polygonaceae Family);* common fumitory, *Fumaria officinalis (Fumariaceae Family);* red clover, *Trifoliumpratense (Fabaceae Family)* were studied. They were collected from three different unpolluted regions of Oradea area (O1, O2, O3), Beiu' area (B1, B2,B3), and two different unpolluted regions of Cefa area (C1, C2).The identified areas O1, O2, O3, B1, B2, B3 are placed in wooded areas, with clayey soil. The area C1 and C2, are in areas with sandy, wet soil.

In case of *Glechoma hederacea* and *Polygonum aviculare*, we harvested the flowering stems and leaves, in each area, between April and June 2017. Between May and July 2017 in case of *Fumaria officinalis* and *Trifoliumpratense*we harvested the flowering stems and leaves, in each area, between May and July 2017. We harvested the studied plants in different times of the year due to the differences in their periods of maximum development and flowering.

A specimen from each of the studied species was deposited in the Pharmaceutical Botany Herbarium of Faculty of Medicine and Pharmacy Oradea. Plant materials were dried at room temperature and ground before extraction. We used plant extracts to measure the total

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quantity of phenolic acids and flavonoids. After that we evaluated the antioxidant capacity of the extracts.

Study of the bioactive compounds of plant extracts

The determination of total phenolic contents using Folin-Ciocalteu method

The alcoholic extract solutions(tinctures) were prepared by maceration in 70⁰alcohool, at room temperature (20 °C) for 10 days. The residue was removed by decantation. Total phenolic contents were determined by using the Folin-Ciocalteu reagen [14,24]by using Gallic acid as standard, with some modifications. The extract solution (0.1 mL) containing 1000 µg of extract was mixed with 46 mL distilled water in a volumetric flask and 1 mL Folin-Ciocalteu (Merck) reagent was added; the flask was thoroughly shaken. The mixture was allowed to react for 3 minutes and 3 mL aqueous solution of 2% Na_2CO_3 were added. At the end of the 2 h incubation at the room temperature, the absorbance of each mixture was measured at 765 nm, in Shimadzu UV-1700 Pharmaspec UV-Vis Spectrophotometer. The same procedure was also applied to the standard solutions of Gallic acid, and a standard curve was obtained.

With the Folin-Ciocalteu method we measured the OH groups of the samples taken into study in alkaline conditions (adjusted with sodium carbonate). The absorbance at the 765 nm wavelength increases proportional with the number of OH groups of the anthocyanins. The calibration curve (fig.1) was obtained with a solution of known concentration of gallic acid (20-100 ppm), and the concentration of polyphenol extracts was calculated from the regression equation and expressed as mg Gallic acid equivalents (GAE) / 100 g dry sample [14,24,25].

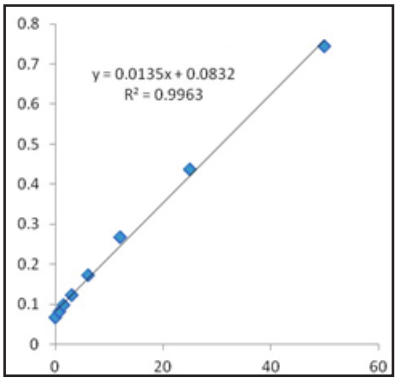

Fig. 1. The calibration curve made with Gallic acid for Folin-Ciocalteu method in alcoholic medium

*T*he determination of total flavonoid contents

The content of total flavonoids was determined by using a colorimetric method which has been described previously [25-28]. 1 mL of the sample (containing 0.1 mg / mL dry weight) is mixed with 4 mL water and placed in a 10 mL volumetric flask.

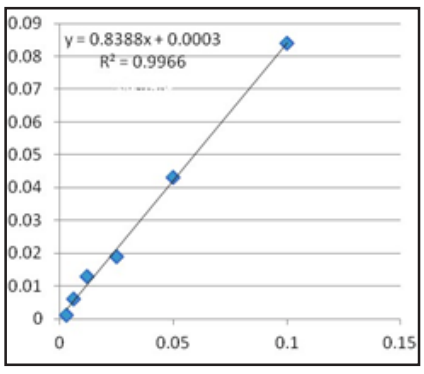

Fig. 2. Calibration curve made with quercetin in alcoholic medium (surroundings, environment)

First there were added 0.3 mL ground $NaNO_2$ 5%, after five min 0.3 mL $AlCl_3$ 10% and after 6 min, 2 mL of NaOH 1M. The volumetric flask is filled to the mark with distilled water. The solution is mixed in the volumetric flask and it is read the absorbance at 510 nm in the Shimadzu UV-1700 Pharmaspec UV-Vis spectrophotometer. The calibration curve (fig. 2) shall create its standard using quercetin (QE) [29-31].

Study of the antioxidant capacity. FRAP method (Ferric Reducing Antioxidant Power)

FRAP method is a simple spectrophotometric method that assesses the antioxidant power of the studied samples, being based on the reduction of ferric tripyridyltriazine complex [Fe(III)-TPTZ] by a reductant, at an acid *p*H. The stock solutions included: 300 mM acetate buffer; 270 mg $FeCl_3 \cdot 6\ H_2O$ dissolved in 50 mL distillated water; 150 mg TPTZ and 150 µl HCl, dissolved in 50 mL distillated water. The working FRAP solution was freshly prepared by mixing 50 mL acetate buffer, 5 mL $FeCl_3 \cdot 6\ H_2O$ solution and 5 mL TPTZ solution. Trolox was used as a standard solution, the calibration curve was made for concentrations between 0-300 µM, having the correlation coefficient $R^2 = 0.9956$ and the regression equation ($y = 0.0017x + 0.0848$), where *y* represents the absorbance detected at 595 nm. The results are expressed as µmol Trolox equivalents (TE)/100 µL extract [32,33].

CUPRAC assay (Cupric Ions (Cu^{2+}) Reducing Power)

In order to determine the cupric ions (Cu^{2+}) reducing antioxidant capacity the method proposed by Karaman et al. (2010) was used with slight modifications [34]. To this end, 0.25 mL $CuCl_2$ solution (0.01 M), 0.25 mL ethanolic neocuproine solution (7.5×10^{-3} M) and 0.25 mL CH_3COONH_4 buffer solution (1 M) were added to a test tube, followed by mixing with the plants extracts. The total volume was adjusted to 2 mL with distilled water and thoroughly mixed. The tubes were stoppered and kept at room temperature. Absorbance was measured at 450 nm against a reagent blank, 30 min later. Increased absorbance of the reaction mixture indicates increased reduction capability [35-37].

ABTS Method (Determination of Antioxidant Capacity using the ABTSÿ⁺ Radical Cation)

The test method of antiradical capacity, with the application of ABTS (2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) is known and widely used to determine the antioxidant activity of substances of whatever nature. Based on the given method, it was determined the antioxidant activity of both pure substances, and antioxidant complexes. The ABTSÿ⁺ radical is generated by the oxidation of ABTS with potassium persulfate and is reduced by the addition of hydrogens atom.

ABTS method or TEAC (Trolox Equivalents Antioxidant Capacity) is based on the ability of antioxidants to reduce the life of the cation radical ($ABTS^+$), a green – blue chromophore that absorbs at 734 nm, compared to Trolox. $ABTS^+$ is produced by reacting stock solution ABTS – 2,2 Azinobis-(3 ethyl 6 sulfonic acid) (7mM) with potassium persulfate (2.45 mM) for 12-16 h. In order to study the antioxidant activity, $ABTS^+$ solution is diluted with ethanol until an absorbance of 0.70±0.02 to 734 nm is obtained. After the addition of 100 µL of sample to 2900 µL $ABTS^+$ solution, the mixture was monitored spectrophotometrically at 734 nm. The calibration curve was made with the Trolox standard [15,16,26,38-40].The calculation formula is the same as for the DPPH test.The reduction of

the extinction values (% Inhibition) of the DPPH solution is calculated according to the equation:

$$\%\ Inhibition = (Abs_{DPPH} - Abs_{SAMPLE})/Abs_{DPPH} x100$$

Results and discussions

Bioactive compounds of plant extracts

The total amount of polyphenols, determined by the Folin-Ciocalteu method, is highest in *Polygonum aviculare*, followed by *Fumaria officinalis, Glechoma hederacea,*and *Trifoliumpratense*, but we also obtained high values in the cases of other species introduced in the study, as it can be seen in table 1.

In the same time, total flavonoid content is the highest in *Polygonum aviculare* followed by *Fumaria officinalis, Glechoma hederacea* and *Trifoliumpratense*, as it can be seen in table 2.

Antioxidant capacity

After analyzing the total polyphenolic content and the total flavonoid content, we determined the areas with the highest values, in case of *Glechoma hederacea* areaO1, in case of *Polygonumaviculare*area O3, in case of *Fumaria officinalis* area B1 and in case of *Trifolium pratense* area B1 (tables 1 and 2). From extracts obtained from plants harvested in the respective areas we evaluate the antioxidant capacity by: CUPRAC assay, ABTS method and FRAP methodas it can be seen in figures 3, 4, and 5.

The obtained results by using those three methods of determining the antioxidant capacity, showed that the ethanol extracts obtained from the *Polygonumaviculare* from O3 area and *Fumaria officinalis* from B1 area, have shown a high capacity of reducing ABTS, FRAP and CUPRAC radicals, at the beginning of the vegetation period in April and May.

Dentists around the world are of particular interest in the treatment of orofacial system diseases with new, herbal-available pharmaceutical formulations [41-43].

Month	O1	O2	O3	B1	B2	B3	C1	C2
Glechoma hederacea								
April	368.17	324.65	327.15	311.97	301.45	320.55	225.16	240.64
May	362.65	361.37	352.66	309.46	272.73	241.237	248.20	272.17
June	350.31	351.07	340.71	321.24	330.57	303.411	247.05	223.35
Polygonum aviculare								
April	401.45	430.78	454.60	321.78	342.52	314.51	360.27	313.48
May	378.72	412.34	432.05	332.27	345.15	327.80	280.36	320.54
June	390.55	387.35	397.06	332.46	298.23	324.56	365.32	292.00
Fumaria officinalis								
May	392.65	361.37	352.66	309.46	272.73	241.23	266.32	172.06
June	350.31	351.07	340.71	321.24	330.57	303.41	260.77	213.48
July	328.17	324.65	327.15	311.97	301.45	320.55	250.36	220.24
Trifoliumpratense								
May	232.75	281.30	331.16	342.60	303.24	341.37	266.32	172.06
June	320.15	328.00	304.41	329.34	280.71	362.41	260.77	213.48
July	218.27	227.25	271.22	270.19	247.14	276.52	250.36	220.24

Table 1
TOTAL POLYPHENOLIC CONTENT IN MEDICINAL PLANTS TESTED (mgGAE/ 100 DW)

Months	O1	O2	O3	B1	B2	B3	C1	C2
Glechoma hederacea								
April	28	23	25	24	25	27	22	23
May	28	28	26	24	23	28	20	21
June	25	26	23	25	21	24	17	19
Polygonum aviculare								
April	33	36	38	34	35	34	27	26
May	30	32	35	34	30	30	25	25
June	29	28	32	30	27	30	25	25
Fumaria officinalis								
May	26	29	29	36	33	32	25	26
June	28	27	29	32	29	29	21	25
July	25	27	28	31	28	28	20	22
Trifoliumpratense								
May	30	26	29	29	28	28	27	25
June	27	24	25	24	27	25	22	25
July	25	24	25	23	25	24	19	22

Table 2
TOTAL FLAVONOID CONTENT IN MEDICINAL PLANTS TESTED (mgQE/ 100 DW)

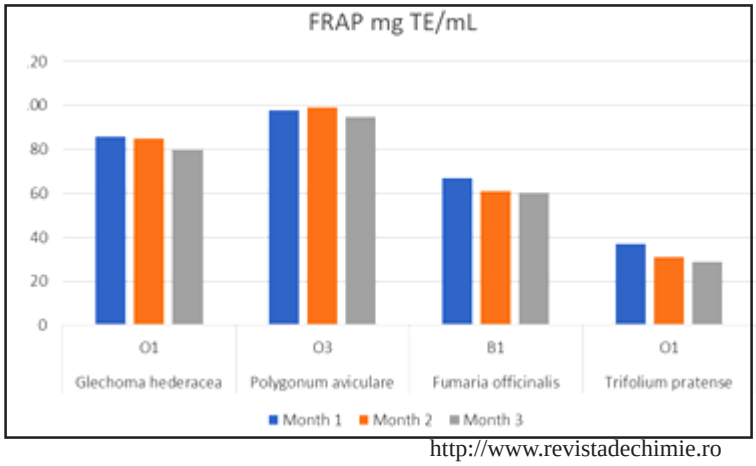

Fig. 3. Capacity of reducing FRAP radicals (FRAP mg TE/mL)

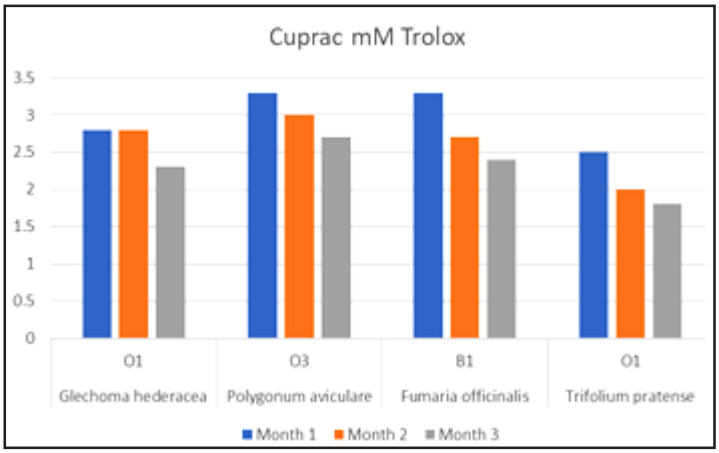

Fig. 4. Capacity of reducing CUPRAC radicals (Cuprac mM Trolox)

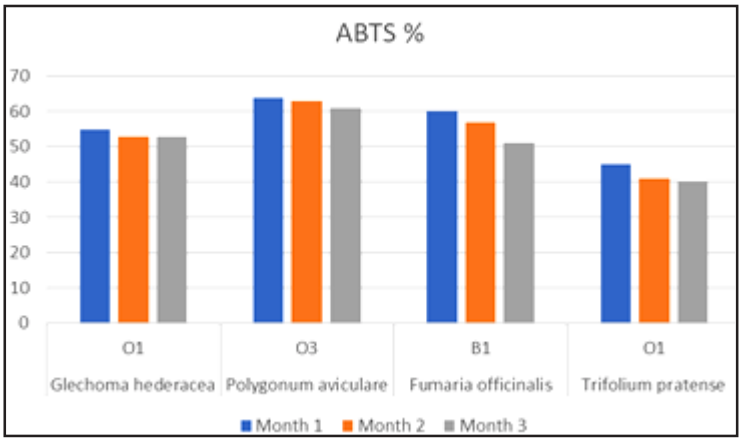

Fig. 5. Capacity of reducing ABTS (%)

Conclusions

By analyzing the obtained results, clear correlation between pedoclimatic conditions and the polyphenols and flavonoids content of studied species can be observed, especially in the beginning of the vegetation period. *Polygonum aviculare* and *Fumaria officinalis* have the highest antioxidant capacity out of all the studied species. We will continue our studies concerning these species in order to be able to include them in a topical pharmaceutical formulation, which could be used to prevent the oral cavity diseases.

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Manuscript received: 14.12.2018