THE CHEMICAL PROFILE OF BASIL BIO-VARIETIES AND ITS IMPLICATION ON THE BIOLOGICAL ACTIVITY

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Abstract

Three species of basil were investigated in order to establish their antioxidant potential. The analyses were performed on the hidroalcoholic (50%) extracts from *Ocimum basilicum L.* (Ob), *Ocimum basilicum var. purpurascens* (Obr), and *Ocimum sanctum L.* (Os), included in bio-cultures from Romania. The phenolic content was quantified by the Folin-Ciocalteu method. A high performance liquid chromatography method (HPLC) was used to identify the main compounds of the hidroalcoholic extracts. In addition, the antioxidant activity was assessed by the diphenylpycrylhydrazyl (DPPH) radical method and metal chelating method. All extracts exhibited high ability for chelating metals, with Obr sample being the most efficient. The extracts were also effective as DPPH scavengers.

Rezumat

Scopul cercetării a constat în studiul chimic a trei specii de busuioc pentru a evidenția potențialul antioxidant al acestora. Extractele etanolice 50% utilizate pentru analiză au fost obținute din *Ocimum basilicum L.* (Ob), *Ocimum basilicum var. purpurascens* (Obr) și *Ocimum sanctum L.* (Os), specii aclimatizate în România sub formă de culturi bio. Conținutul în polifenoli a fost evaluat prin metoda Folin-Ciocâlteu, iar identificarea componentelor s-a realizat prin cromatografie de lichide de înaltă performanță (HPLC). Pentru stabilirea potențialului antioxidant s-au folosit capacitatea de scavenger de radicali liberi DPPH (difenilpicrilhidrazil) și capacitatea de chelatare a ionului feros. Toate cele trei extracte au manifestat o puternică capacitate de chelatare față de ionul metalic, proba Obr având acțiunea cea mai intensă. Extractele au fost de asemenea active față de radicalul difenilpicrilhidrazil.

Keywords: *Ocimum sp.* extracts, HPLC, antioxidant activity

Introduction

In the last two decades, many studies have illustrated that nutritional aport of antioxidant-rich plants, such as basil, coriander, fennel,

thyme, etc. can positively modulate the aging process, carcinogenesis, hyperglycemia, hyperlipidemia, atherosclerosis, hypertension, stroke and many neurological disorders [1,3,6,9,11-13,16,17,19-21]. Phenolic compounds are an important group of secondary metabolites. They are synthesized by the plant, in various stress conditions such as water stress, cold stress, intense sunlight, high winds, infections etc. [15]. Antioxidants protect the human body from reactive oxygen species (ROS) and free radicals, chemical species with unpaired electrons (e.g., superoxide, $O_2^{\cdot -}$, hydroxyl, $^{\cdot}OH$, perhydroxyl, $HO_2^{\cdot -}$, nitric oxide, $NO^{\cdot}$, peroxynitrite, $ONOO^-$) [2; 15; Halliwell, Chirico, 1993]. Therefore, the inner antioxidant mechanisms and the nutritional antioxidants are important for the protection of cells and tissues against oxidative damage, which can cause pathological conditions. Even though basil is most known for its volatile oil properties [4], we focused on the polyphenolic fraction, presuming that these compounds would be responsible for the vegetal material antioxidant potential. The aim of our study was to assess the antioxidant activity of the hidroalcoholic extracts from three species of *Ocimum* (Ob. Obr, Os) harvested from bio-cultures. This estimation may help in understanding the mechanisms of their antioxidant potential.

Materials and Methods

Plant material

The plants, harvested during August 2011, were included in bio-cultures from the Biological Research Center "Stejarul" Piatra Neamţ, Romania. The samples were deposited at the Department of Pharmacognosy, Faculty of Pharmacy, University of Medicine and Pharmacy "Gr.T.Popa" Iaşi, Romania, prior to the experiments.

Preparation of the hidroalcoholic extracts

2.5g of dried and ground plant material were mixed with 100mL ethanol 50% and then extracted at 85°C and then dry extracts were obtained by concentration in a hot oven at 30°C.

HPLC (LC/DAD) analysis

In order to identify the main compounds, an Agilent 1200 HPLC system (Agilent Technologies, PaloAlto, CA, USA) coupled with DAD (diode array detector) was used. The working conditions were: Agilent Zorbax Eclipse XDB-C18 column (4.6 x 150mm, 5µm); column temperature: 40°C; detection wavelengths: 241, 254, 282, 326 and 521 nm; flow rate: 0.6mL/min; gradient elution: acetic acid 1% (solvent A) and acetonitrile with acetic acid 1% (solvent B); the initial conditions were 100% A and 0% B; the gradient program was 6-38-100% solvent B at 5-70-

105 min, with two isocratic segments at 6% B and 38% B, after which we switched back to the initial conditions; sample injection (25 µL of 40mg/mL dry basil extracts in methanol). As standards we used caffeic, chlorogenic, ferulic, and rosmarinic acids, quercetin, rutin, hyperoside, luteolin-7-O-glucoside, apigenin-7-O-glucoside, luteolin, apigenin (LGStandards).

Polyphenolic assessment

The total phenol content in the extracts was determined using the Folin-Ciocalteu reagent and caffeic acid as standard. The sample (0.04mL) and 0.6mL of sodium carbonate (20%) were added to 0.2mL of Folin-Ciocâlteu reagent. After 2 hours of reaction, at room temperature (RT) and in the absence of light, the absorbance was measured at 765nm in a Jasco V-550 UV/VIS spectrophotometer. Tests were carried out in triplicate.

Flavonoids assessment

The flavonoids were determined using sodium nitrite and aluminium chloride as reagents and catechine as standard. The sample (0.25mL) was added to 0.75mL sodium nitrite 5% and left 6 minutes to react. Following, 150µL aluminum chloride 10% were added. After 5 minutes of reaction at RT, the absorbance was measured at 510nm in a Jasco V-550 UV/VIS spectrophotometer. Tests were carried out in triplicate.

Antioxidant capacity

Free radical scavenging activity (DPPH)

A dimethyl sulfoxide (DMSO) solution (50µL) of each sample, at three different concentrations, was placed in a cuvette and 2.950mL of DPPH methanolic solution 4% were added. After 5 minutes of reaction at RT, the absorbance was measured at 517nm using a Jasco V- 550 UV/VIS spectrophotometer. A sample containing 3mL methanol was used as a negative control. Quercetol was used as standard. Sample concentration providing 50% inhibition (IC_{50}) was obtained plotting the inhibition percentage against the sample concentration.

Fe^{2+} chelation activity

Freshly prepared iron chloride (2mM) was added to a reaction mixture containing 400µL DMSO extract solution, in four different concentrations, and 80 µL ferrozine, 5mM. A first measurement of the absorbance was performed at 562nm in a Jasco V- 550 UV/VIS spectrophotometer. After 10 minutes of reaction at RT, the absorbance was measured, for the second time, at the same wavelength. Caffeic acid was used as standard. Sample concentration providing 50% inhibition (IC_{50}) was obtained plotting the inhibition percentage against sample concentration.

Results and Discussion

Extraction yields and HPLC analysis

As indicated above, the hydro-alcoholic extracts (ethanol 50%) were obtained from 2.5 g of dried material, still the amount of dry extract that was obtained varied from one sample to the other. Thus, the drug extract ratio (DER) was as follows: Ob sample DER=2.5:1.21, Os sample DER=2.5:1.20 and Obr sample DER=2.5:0.89. Therefore, we might state that the lowest extractability corresponds to red basil variety sample, maybe due to some compounds that partially inhibit the extraction. It remains to be seen if this low rate of extraction will affect the polyphenol composition and the antioxidant activity.

The HPLC analysis of the three extracts allowed the identification of the following compounds: rosmarinic acid, chlorogenic acid, hyperoside, quercetin, rutoside, luteolin and apigenin. Out of all polyphenolic acids, rosmarinic acid was best represented in all three samples with values varing from 32.05 mg/1g dry extract in Os to 92.89 mg/1g dry extract for Obr, while Ob was in the middle with 45.33 mg/1g dry extract. Amongst flavones, apigenine was present in all samples, but in small quantities (0.74 - 0.85 mg/1g dry extract). Nevertheless, the levels of flavonoid compounds taken separately were lower, this being the main characteristic of all three samples.

Total phenolic content

As expected, the amount of the total phenols varied from one sample to the other, *Ocimum sanctum* extract being the richest with 28.99 mg polyphenols (expressed as mg of caffeic acid) for 1g dry extract, while Ob sample had the lowest quantity (17.17mg caffeic acid/1 g of dry extract) (Figure 1).

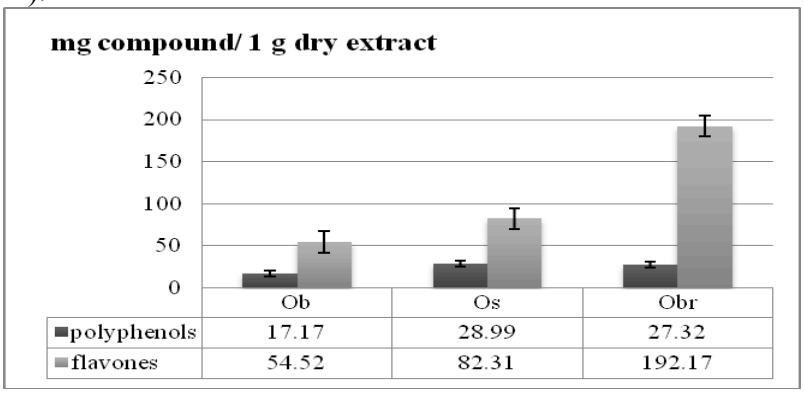

Figure 1

Total phenolic content (mg/g dry extract) of the hidroalcoholic extracts of the studied plants

The results revealed that the total phenol content of both Os and Obr samples was high, therefore, both can be considered a better source of polyphenols than Ob.

Flavonoids content

Unlike polyphenols, the findings revealed that Ob (54.52 mg catechine/1g dry extract) and Os (82.31 mg catechine/1g dry extract) samples had a lower content in flavonoids (expressed as mg catechine/1g dry extract) compared to that of Obr sample (192.17 mg catechine/1g dry extract), which was significantly higher.

All in all, the Obr sample had the highest polyphenol and flavone content of all species even though it had the lowest extractability rate. This may be due to the type of flavone and polyphenol derivatives which solubilize better in our chosen solvent (ethanol 50 %).

Antioxidant activity

Free radical scavenging activity (DPPH assay)

As expected, in terms of capacity for scavenging DPPH radicals, Obr extract showed the best activity, since it had the lowest IC_{50} values (0.8 mg/mL). In contrast, Os had the lowest ability for scavenging this radical, having the highest IC_{50} value (2.0 mg/mL). The analyses of the extracts revealed that an inverse correlation between the content of the total amount of polyphenols and IC_{50} values were observed in the case of Os extract.

It is notable that at concentrations of 15.67 mg/mL and 5 mg/mL, the values of the scavenging activity resemble for all samples, compared to the reference antioxidant, quercetin (Fig. 2). When lowering the concentration at 1mg/mL, the scavenging activity lowers as well, significantly. Still, even at this dilution, Obr sample has the highest value of all extracts.

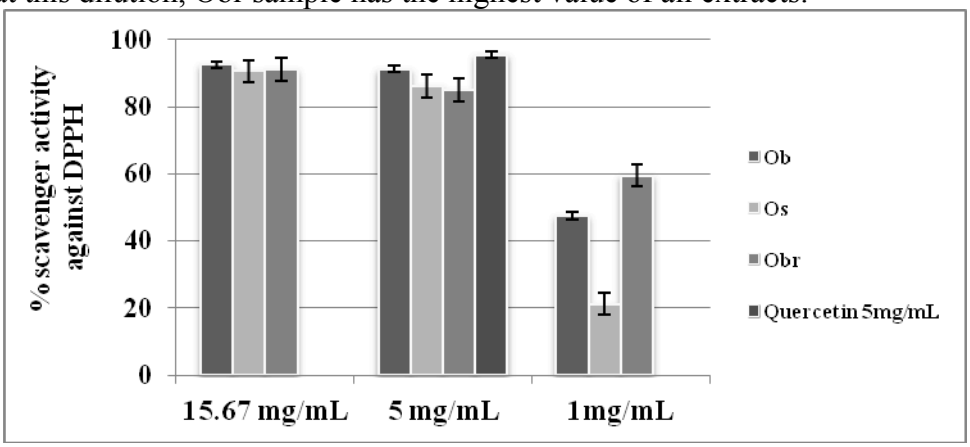

Figure 2

Free radical scavenging activity (%) for three dilutions.

Although some authors reported that the phenol compounds are responsible for the antioxidant activities of plants [18], more recent data [1,3,9] shows that this relationship may vary, depending especially on the method used for evaluating the antioxidant ability. In this study, an inverse correlation between the high polyphenols content was found for Os sample and its scavenging capacity, which was low. Such results suggest that in this extract there are some phenolic constituents contributing less effectively for the scavenging activity, by comparison to the other two extracts that are more active.

Fe^{2+} chelation assay

Experiments revealed that at 40mg/mL concentration the chelating activities were found to be 73.78% for Ob, 89.09% for Os and 81.16% for Obr. The results (fig.3) showed that the metal chelating activity lowers proportionally with the concentration. According to their IC_{50} values, extracts Ob (4.9 mg/mL) and Os (4.3 mg/mL) generally showed a weaker capacity for metal chelating. Obr (3.9 mg/mL) was, again, the most effective as Fe^{2+} chelating agent. As reported for DPPH radical-scavenging activity, in this assay there was also a correlation between flavonoids content and the ability for chelating metals. The analysis of the extracts of each plant revealed a positive correlation between flavonoids content and IC_{50}.

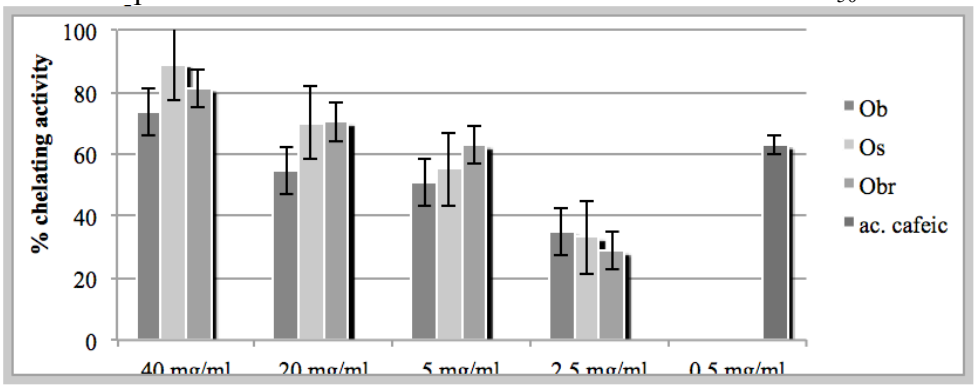

Figure 3

Metal chelating activity (%) for four dilutions for *Ocimum* samples.

Iron can catalyze reactions (e.g., Fenton reaction) that generate ROS. Also, iron decomposes lipid peroxides, favoring lipid peroxidation of biological membranes, thus generating cytotoxic aldehydes (e.g., malondialdehyde [MDA]). These can inhibit protein synthesis, inactivate enzymes, cross-link proteins, generate thrombin, and eventually leading to membrane rupture and to release cell and organelle contents [7,8,15].

Our data indicated that all samples could chelate efficiently and in a dose-dependent manner the iron they were in contact with. Os sample had the highest chelation activity due, maybe, to its extremely elevated flavonoids levels.

Conclusions

The hidroalcoholic extract of Ob had the lowest polyphenolic content in contrast to those of Os and Obr, respectively. On the other hand, the extract of Obr had significantly higher flavonoids content, comparing to the other. One must state that even with the lowest extractability rate (DER=2.5:0.89) red basil extract has to be considered the richest in antioxidant compounds, both *in vitro* tests indicating its scavenger and chelating potential. Nevertheless, further studies are needed for understanding/discovering the chemical structures of the components in the extracts responsible for the antioxidant activities.

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Manuscript received: December 21st 2012