Scientific Bulletin. Series F. Biotechnologies, Vol. XXIII, 2019
ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364

# CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF *Hyssopus officinalis* L. SELECTIVE FRACTIONS OBTAINED BY DIFFERENT METHODS

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***Abstract***

*The aim of this study was to obtain selective fractions of Hyssopus officinalis L. by different methods and to investigate a possible correlation between their chemical content and antioxidant activity in order to establish a potential effect of this species on counteracting diseases associated with aging processes. HPLC analysis and quantitative determination of active principles from nine selective fractions show that the values were well correlated with the ones resulted by spectrophotometrically methods. The selective fractions have a total flavonoid content expressed as rutin from 1.236 to 19.060 % and respectively 0.126 to 16.783% polyphenolcarboxylic acids expressed as rosmarinic acid. It has been observed that the fractions with high content in polyphenolcarboxylic acids and flavonosides exhibit maximum antioxidant activity. There are selective fractions containing only one of the classes of compounds (flavonosides or polyphenolcarboxylic acids) in a higher amount that show great antioxidant activity. A strictly correlation between the flavones content of the selective fractions and antioxidant activity can not be made by this method.*

***Key words****: antioxidant activit , flavonosides, Hyssopus officinalis L., polyphenocarboxylic acids.*

## INTRODUCTION

In two previous studies we presented the results regarding the antioxidant action of some selective fractions obtained from the plant species cultivated in Romania with potential effect on the counteracting diseases associated with aging processes (Ashok & Rashid, 1999; Babovic et al., 2010).

In this paper are presented the results of researches regarding the obtaining of selective fractions of *Hyssopus officinalis* L. and their antioxidant action.

The species *Hyssopus officinalis* L., the *Lamiaceae* family, is known in the Romania in the traditional medicine for the therapeutic effect. Modern medicine has confirmed that, due to existing classes of active compounds in aerial parts, this species is beneficial in the treatment of certain diseases.

Classes of compounds with demonstrated therapeutic effects are flavonosides (apigenin, quercetin, diosmin, luteolin and glucosides thereof), polyphenolcarboxylic acids (chlorogenic, ferulic, caffeic and rosmarinic acids), volatile oil, calchones, triterpenes (ursolic and oleanolic acid), β-sitosterol and bitter principles (marubin). These compounds are responsible for the stomachic, carminative, antispasmodic, antiasthmatic, anticatarrhal, antiseptic, healing, antimicrobial and antioxidant effects (Colceru-Mihul et al., 2016; 2017).

Among chemical compounds derived from plant species, rosmarinic acid, caffeic acid or other compounds belonging to polyphenolcarboxylic acids class; diosmin, diosmetin or other flavonoidic compounds, are very well known for their antioxidant properties (Fathiazad & Hamedeyazdan, 2011; Istudor, 2001).

Compounds with antioxidant activity are regarded as basic elements of the anti-aging strategy because free radicals are considered the main responsible agents of premature aging and also of diseases associated to aging status (Marin et al., 1998).

## MATERIALS AND METHODS

The plant material consisting of aerial parts of *Hyssopus officinalis* L. (Hyssopi herba) was

obtained from culture, dried and ground as a fine powder (sieve VII).

Selective fractions obtainment:

Method I consisted of repeated extraction - two times of the active substances from 200 g Hyssopi herba, with 50% ethylic alcohol v/v (vegetal material/solvent ratio = 1/10 m/v for the first extraction and 1/5 m/v for the second extraction) at boiling temperature of the solvent for 1 hour per extraction with continuous mechanical stirring, followed by cooling and filtration of the extracts. The reunited hydroalcoholic solutions were rota-evaporated for alcohol removal. The resulting aqueous solutions were spray-dried and selective fractions HI were obtained.

Method II consisted of active principles extraction from 300 g Hyssopi herba, with 50% alcohol (plant/solvent = 1/10 m/v ratio) at boiling temperature for 1 hour with continuous mechanical stirring, followed by cooling and filtration of the extracts. Hydroalcoholic extract solution was evaporated to a volume of 1/1 m/v plant/solvent mixture and centrifuged. A precipitate (which was labeled as HII0 after drying) and an aqueous solution were obtained. In order to obtain selective fractions, aqueous solution was further processed by:

- 3 successive liquid-liquid extractions with ethylic ether, followed by solvent removal from the reunited etheric by rotaevaporation, resulting HIIA.
- 3 successive liquid-liquid extractions with chloroform, followed by solvent removal from the reunited chloroformic extracts by rotaevaporation, resulting HIIB.
- 3 successive liquid-liquid extractions with ethyl acetate, followed by solvent removal from the reunited ethyl acetate extracts by rotaevaporation, resulting HIIC.
- 3 successive liquid-liquid extractions with n-buthylic alcohol, followed by solvent removal from the reunited buthanolic extracts by rotaevaporation resulting HIID.
- adding acetone in a 2/1 v/v acetone/aqueous extract ratio resting at 4-6$^0$C for 24 hours, filtration and drying the precipitate resulting HIIE. This is shown in Figure 1.

Method III consisted of repeated extractions - two times of the active substances from 200 g Hyssopi herba, with methylic alcohol (plant/solvent ratio = 1/10 m/v for the first extraction and 1/5 m/v for the second extraction) at boiling temperature of the solvent for one hour per extraction with continuous mechanical stirring, followed by cooling and filtration of the extracts. Methanolic solutions were reunited, the solvent removed by rotaevaporation resulting HIII selective fractions.

Method IV consisted of macerating 200 g Hyssopi herba in acetone (plant/solvent ratio = 1/7 m/v), removing the solvent from acetone solution and re-extracting the residue in methanol. The active substances were extracted from moist plant material with 20% ethanol (plant/solvent ratio = 1/10 m/v) at boiling temperature of the mixture for 2 hours, followed by hydroalcoholic solution evaporation to an aqueous extract. Methanolic and aqueous extract were reunited and filtered. The resulting precipitate was dried and selective fractions HIV were obtained.

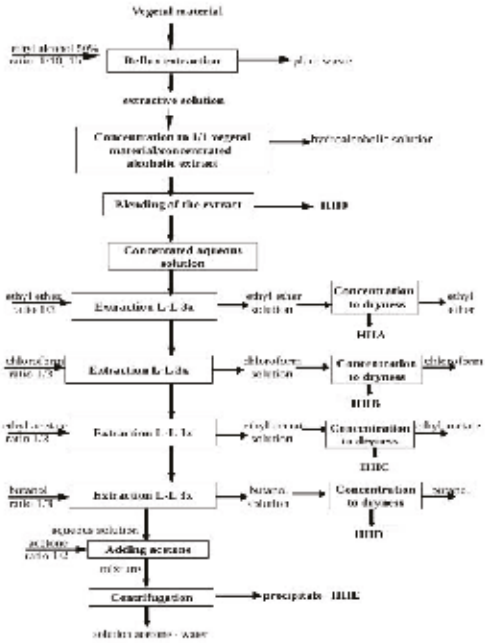


Figure 1. Selective fractions obtainment - method II

HPTLC analysis of selective fractions was performed using Silica Gel 60$F_{254}$ as stationary phase and a mixture of ethyl acetate - acetic acid - formic acid - water (100: 11: 11: 27 v/v/v/v) for chromatographic elution. The plates were scanned under 360 nm after the

derivatization with NP/PEG. The reference compounds for HPTLC analysis were from Sigma-Aldrich: caffeic acid, rosmarinic acid, chlorogenic acid, rutin, hyperoside and diosmin (Reich & Schibli, 2008; Romanian Pharmacopoeia, 1993).
HPLC analysis of selective fraction consisted in chromatographic separation on a Purosher ODS column (250 x 4.6 mm, 5 μ) at $40^0$C, using a gradient elution (both mobile phase and flow). The mobile phase was a binary gradient: water with orthophosphoric acid (pH = 2.5) and methanol. The eluent absorbance was monitored at 330 nm. The reference substances were from Sigma-Aldrich: caffeic acid, rosmarinic acid, rutin, diosmin and luteolin.
Quantitative determination of active principles from selective fractions consisted of determination of flavones by a colorimetric method based on their property to form intensely yellow complex with $Al_3^+$ and of determination on polyphenolcarboxylic acids by a colorimetric method based on the property of phenols to form nitrocompouds or nitro oxime with nitrous acid which give red stain when dissolve in alkaline solutions due to their weak acid character. For the quantification of flavones, rutin was used as reference substance and for polyphenolcarboxylic acids quantification rosmarinic acid was used as reference substance (Sanchez Moreno et al., 1998).

**Analysis of antioxidant action**

**DPPH assay:** In each reaction tube 100 μl vegetal extract of different concentrations was mixed with 3900 μl of 0.0025 g/l DPPH at room temperature for 30 min. 50% methanol solution was used as control. The reduction of the DPPH free radical was measured by reading the absorbance at 515 nm. Rosmarinic acid (from Sigma-Aldrich) was used as positive control. Inhibition ratio (percent) was calculated from the following equation (Wagner & Bladt, 1996):

% inhibition = [(absorbance of control – absorbance of sample)/absorbance of control] x 100

DPPH radicals react with suitable reducing agents losing color stoichometrically with the number of electrons consumed which is measured spectrophotometrically at 515 nm (Wagner & Bladt, 1996).
For determination of antioxidant activity, the selective fractions were chosen according to the yield obtained from 100 g plant and depending on the flavones and polyphenolcarboxylic acids content.

## RESULTS AND DISCUSSIONS

Nine Hyssopi herba selective fractions were obtained by experimental methods mentioned above. The quantities of product obtained from 100 g plant are shown in Table1.
Flavonoids (rutin, hyperoside, diosmin) and polyphenolcarboxylic acids (rosmarinic acid, caffeic acid, chlorogenic acid) were identified by HPTLC in most of selective fractions.
The content of caffeic acid, rosmarinic acid, diosmine, rutin and luteolin in each fraction was determined by HPLC method. The values obtained from individual assessment by HPLC were well correlated with the values obtained by the spectrophotometrically methods mentioned above. For example, rosmarinic acid content from the selective fractions, determined by HPLC, correlates with the polyphenolic acids content expressed in rosmarinic acid, determined by the headline method.

Table 1. The content of active principles of Hyssopi herba selective fractions

| Bioactive product | Product yieldt from 100 g plant | Flavonoids expressed as rutin % g/g | Polyphenol-carboxylic acids expressed as rosmarinic acid % g/g |
|---|---|---|---|
| HI | 19.21 g | 4.556 | 3.182 |
| HII0 | 2.90 g | 2.513 | 3.888 |
| HIIA | 0.41 g | 10.347 | 1.138 |
| HIIB | 0.65 g | 2.326 | 0.074 |
| HIIC | 1.15 g | 19.060 | 14.655 |
| HIID | 4.10 g | 2.853 | 1.030 |
| HIIE | 6.29 g | 2.178 | 2.726 |
| HIII | 15.10 g | 2.734 | 16.783 |
| HIV | 6.21 g | 1.236 | 3.962 |

The flavonoid content expressed as rutin and polyphenolcarboxylic acids expressed as rosmarinic acid of Hyssopy herba selective fraction are shown in Table 1.

The most affluent fractions in polyphenolcarboxylic acids expressed as rosmarinic acid are HIII (16.783%) and HIIC (14.655%) followed by HIV (3.962%), HII0 (3.888%) and HI (3.182%). HIIE contains 2.726% and the fraction with most low content in polyphenolcarboxylic acids are HIIE (2.726%), HIID (1.030%) and HIIB (0.074%).
The most affluent fractions in flavonosides expressed in rutin are HIIC (19.060%) and HIIA (10.347%) folowed by HI (4.556%). HIID, HIII, HII0, HIIB and HIIE containing 2.853%, 2.734%, 2.513%, 2.326% and 2.178%. The fraction with most low content in flavonosides expressed in rutin are HIV (1.236%).
Antioxidant activity of selective fractions is shown in Figure 2.

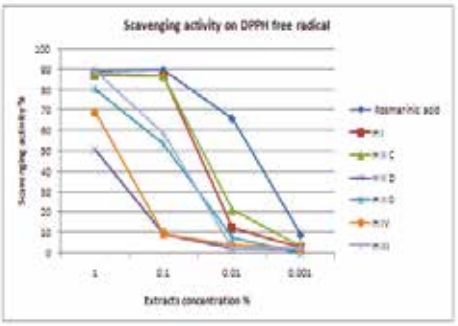


Figure 2. The antioxidant activity of the selective fractions from *Hyssopus officinalis*

Using the method for the analysis of the antioxidant activity described, it can be noted that rosmarinic acid in a percentage of 1, 0.1, 0.01 and 0.001% exhibits an antioxidant activity of 88.99%, 89.84%, 66.15% and 9.14%.
The selective fraction in a percentage of 1, 0.1, 0.01 and 0.001% exhibits an antioxidant activity.
The selective fraction HIII containing 16.783% polyphenolcarboxylic acids has a similar antioxidant activity as rosmarinic acid in a dilution of 1% and a much weaker activity in the dilution of 0.1% while the selective fraction HIIC containing 14.655% polyphenol-carboxylic acids has a slightly lower antioxidant activity compared to rosmarinic acid activity in both dilutions.
Some selective fractions with lower polyphenolcarboxylic acids content such as HI (3.182%), exhibit over 80% antioxidant activity in dilutions of 1% and 0.1%. Other selective fractions, such as HII0 (3.888%) show over 80% antioxidant activity only at 1% dilution.
Even the polyphenolcarboxylic acids content of the selective fraction HIV (3.962%) is similar to the content of HI, HII0 (which exhibits a good inhibitory potential), these fractions show a weaker antioxidant activity.
Comparing the antioxidant activity of selective fractions and the polyphenolcarboxylic acids expressed as rosmarinic acid and flavones expressed as rutin content it can be concluded that when the concentration of polyphenolcarboxylic acids increases the antioxidant activity also increases, though not an exact correlation can be made.
A correlation between the flavones content of the selective fractions and antioxidant activity can not be made by this method.

## CONCLUSIONS

From *Hyssopus officinalis* L. aerial parts (Hyssopi herba) nine selective fractions enriched in flavones and polyphenolcarboxylic acids were obtained by different methods.
Out of six selective fractions tested for antioxidant activity, three of them exhibited a scavenging activity comparable with the rosmarinic acid.
It can be concluded that a high content of polyphenolcarboxylic acids expressed as rosmarinic acid lead to a higher antioxidant activity but a precise correlation can not be made.
The antioxidant activity of the flavones was not highlighted by the method used in this study for the antioxidant activity evaluation.

## ACKNOWLEDGEMENTS

This research work has been financed by the Romanian National Authority for Scientific Research ANCS, Competitiveness Operational Programme COP-A1-A1.2.3-G-2015, Project title “Innovative technologies for new, natural health products”, ID P_40_406, SMIS 105542/ subsidiary “D” contract no. 28/2018.

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