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ANTIOXIDANT CAPACITY OF *HYPERICUM PERFORATUM* FLOWERS IN MARAMURES COUNTY

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Abstract

Hypericum perforatum is a medicinal plant that has a wide spread throughout our country. The active principles it contains has made it beneficial in the treatment of depressive diseases because they have fewer adverse effects than synthetic drugs.

In this paper, the antioxidant activity of Hypericum perforatum flowers was studied, harvested during the period when it contained the highest amount of hypericin and hyperforin, responsible for the antidepressant action. First an alcoholic extract from flowers was obtained from which it was determined: polyphenolic compounds using the Folin-Ciocâlteu method, the total of flavonoids using colorimetric and spectrophotometric methods and then the antioxidant capacity using two tests: DPPH and FRAP.

Keywords: ringtones, antioxidant, antidepressant, hypericin, hyperforin, flavonoids

INTRODUCTION

St. John's wort, *Hypericum perforatum*, is part of the *Hypericum* family along with other plant species. Of the existing species it is the most used in therapy because it is the richest in active principles. St. John's wort can be used internally to treat depression, viral and bacterial diseases, inflammation or externally St. John's wort oil to heal wounds, burns, eczema, ulcers, cuts, hemorrhoids (Voller, Rosenson, 2004, Bojor, 2018, Sarris et al., 2012, Behnke et al., 2002, LaaKmann et al., 2002, Gastpar et al., 2006).

The Folin-Ciocâlteu method is widely used to identify the content of total polyphenols. By this method, total polyphenol content is calculated as galic acid equivalents/g vegetable product; the method takes place in the basic medium using sodium carbonate when the phenolic hydroxyl groups contained in the phenolic and polyphenolic compounds of the ethanolic extract of the plant product are identified. Folin – Ciocâlteu is a mixture of phosphomolybdate and phosphotungstat. Sodium carbonate first reacts with phenolic hydroxyl groups, forms sodium phenolates, which then react with the Folin-Ciocâlteu reagent, forming compounds that color the solution in blue, which is all the more intense as there are several phenolate groups (Everette et al., 2010, Ikawm et al., 2003, Dejeu et al., 2019).

Flavonoids have high antioxidant capacity, i.e. they can act against free radicals, have anti-inflammatory and immune properties, can help the human body to maintain it's health and fight against viruses, bacteria, etc. The total flavonoids was determined by using a colorimetric and spectrophotometric methods. Most of the researchers use two tests for the rapid determination of antioxidant capacity: DPPH the name comes from the reagent that uses 1,1-diphenyl-2-picrilhydrazil and FRAP, which comes from English, Ferring Reducing Antioxidant Power (Arnao, 2003, Li et al., 2018, Williams et al., 1995, Benzie, Strain, 1996, Mot et al., 2011).

The DPPH technique is performed to determine the antioxidant capacity in vitro or the ability to release hydrogen ions of compounds extracted from different plant products (Sacalîs, 2020, Guzel et al., 2019).

Also a method of determining the antioxidant capacity of compounds in plant products is FRAP method. This method is based on the reduction reaction of the ferric tripiridiltriazine complex (Fe(III)-TPTZ), which must be freshly prepared, the reduction being made to the ferrous tripiridiltriazine complex ((Fe(II)-TPTZ). This requires an acid reducer and pH (Jurcă et al., 2016).

MATERIALS AND METHODS

MATERIALS

Dried flowers of *Hypericum perforatum*, analytical balance KERN ABT 220-5DNM, methanol Silver Chemicals Romania, ethanol Silver Chemicals Romania, Soxhlet device, rotavapor Hei-VAP Advantages Heidolph, semi-automatic pipette pipet4u Performance 0.5-5mL, bidistilled water, Folin-Ciocâlteu reagent, IKA VORTEX 3, sodium carbonate Silver Chemicals Romania, spectrophotometer PG Instruments T70 +, sodium nitrite Silver Chemicals Romania, aluminum chloride Silver Chemicals Romania, sodium hydroxide Silver Chemicals Romania, solution DPPH and FRAP freshly prepared.

OBTAINING ALCOHOLIC EXTRACT FROM HYPERICUM PERFORATUM FLOWERS BY SOLID-LIQUID EXTRACTION METHOD

The extract was obtained by the solid-liquid extraction method using the Soxhlet apparatus. This type of extraction is used when we want to separate one or more components from a solid phase using a liquid phase.

I put a one gram of dried and crushed flowers in a paper cartridge, which I placed in the extraction space of the extractor. As extraction solvent I used the methanol that I put in a flask in which the boiling process takes place. After the extraction method was completed, I concentrated the obtained

extract using a rotary evaporator. It operated at a speed of 80 rpm, a pressure of 200 atmospheres, at a temperature of 40 °C. I concentrated it until there was a residue in the flask in the form of a film, which I then took up with 100 mL of ethanol. In this case, we used tap water as a coolant.

DETERMINATION OF POLYPHENOLIC COMPOUNDS FROM THE ALCOHOLIC EXTRACT OF HYPERICUM PERFORATUM BY THE FOLIN-CIOCÂLTEU METHOD

0.1 mL of freshly prepared extract, 1.7 mL of double-distilled water and 0.2 mL of Folin-Ciocâlteu reagent diluted 10 times are placed in a test tube. The contents of the test tube are shaken with a vortex and then left to stand for 5 minutes. 1 mL of 20% Na_2CO_3 solution is introduced into the test tube to obtain a basic medium (pH = 10) for the reaction to take place between the Folin-Ciocâlteu reagent and the phenolates present in the extract and the solution to turn blue. Reaching the highest intensity of the blue color is obtained after the test tube is kept in the dark for 90 minutes and then its absorbance is read on the UV-VIS spectrophotometer, at a wavelength of 765 nm compared to the standard ethanol. The tests are done in triplicate.

DETERMINATION OF TOTAL FLAVONOID CONTENT

Measure 1 mL of the sample extract and place in the test tube with 4 mL of double-distilled water and 0.3 mL of 5% $NaNO_2$ solution. The test tube mixture was stirred with a vortex and allowed to stand for 5 minutes, then 0.3 mL of $AlCl_3$ 10% solution was added. The contents are mixed, left to stand for 6 minutes and then treated with 2 mL of 1M NaOH solution, 2.4 mL of double-distilled water and again shaken vigorously. Read the absorbance of the samples on the UV-VIS spectrophotometer at a wavelength of 510 nm using quartz cuves. The test is done in triplicate.

Prepare another blank sample: from 4 mL of distilled water, 0.3 mL of 5% $NaNO_2$ solution is stirred, leave to stand for 5 minutes, then 0.3 mL of 10% $AlCl_3$ solution is added and again left to stand for 6 minutes, then add 2 mL of 1M NaOH solution, 2.4 mL of double-distilled water and shake vigorously.

DETERMINATION OF THE ANTIOXIDANT CAPACITY OF *HYPERICUM PERFORATUM* ALCOHOLIC EXTRACT BY THE DPPH METHOD

Place in the test tube 2.9 mL of freshly prepared $6x10^{-5}$ M DPPH solution, 0.1 mL alcoholic extract which is then shaken and kept in the dark at room temperature for 15 minutes. Then read the absorbance at a

wavelength of 515 nm. It is considered as blank, the solution of DPPH. The test is performed in triplicate.

DETERMINATION OF THE ANTIOXIDANT CAPACITY OF THE ALCOHOLIC EXTRACT OF *HYPERICUM PERFORATUM* BY THE FRAP METHOD

Place in the test tube 0.1 mL of alcoholic extract, 0.5 mL of FRAP solution, 2 mL of double distilled water, shake the contents, leave to stand at room temperature for 60 minutes, read the absorbance on the UV-VIS spectrophotometer at wavelength of 595 nm using a quartz tub. It is used as a standard Trolox and the samples are made in triplicate.

RESULTS AND DISCUSSION

After reading the absorbance of the ethanolic extract at the wavelength of 765 nm, using the equation of the calibration curve present in figure 1. The concentration of the total polyphenols, expressed in mg galic acid equivalents (GAE)/100 g dry sample, shall be calculated. The equation of the calibration right is:

$Y = 0.0135X + 0.0832$, $R^2 = 0.9963$

Where: Y – absorption of gallic acid solutions of different concentrations, read at 765 nm using a UV-VIS spectrophotometer; X – concentration of gallic acid solutions.

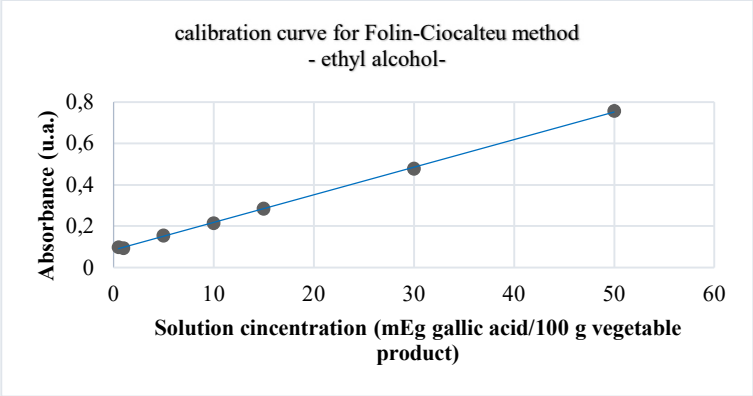

Fig. 1. Calibration curve for ethanolic solutions of different concentrations in gallic acid

The results obtained for the total polyphenols by the Folin-Ciocâlteu method for the extract from the ringing flowers are shown in table 1.

Table 1.

Calculation of the average total polyphenol concentration of St. John's wort flower extract expressed in mg GAE/g dry product

Sample	Sample absorption read at 765 nm	Sample concentration (mg GAE/1 g dry matter) (= 1000X)	Average sample concentration (mg GAE/g dry matter)
Alcoholic extract from *Hypericum perforatum* flowers	0.0973	65.911	66.281±0.889
	0.0990	67.170	
	0.0971	65.763	

From the analysis of the data obtained it can be concluded that the alcoholic extract of *Hypericum perforatum* has an appreciable content of total polyphenols (66.281±0.889). The result is consistent with other determinations by other researchers (62.9 mg GAE/g dry matter (Ciobanu et al., 2018), 75.44-121.19 mg GAE/g dry matter (Shabani et al., 2019), 107.38 mg GAE/G dry matter (Tusevski et al., 2019)).

Calibration curve, presented in figure 2, for the determination of flavonoids was drawn using standard quercetin solutions, of different concentrations.

The equation of the calibration line made with quercetin in aqueous medium is:

Y = 0.8259 X -0.0028

where: Y - absorbance of the sample of ethanolic extract read at 510 nm; X - flavonoid concentration of the sample of ethanolic extract expressed in mg equivalents gram of quercetin/mL.

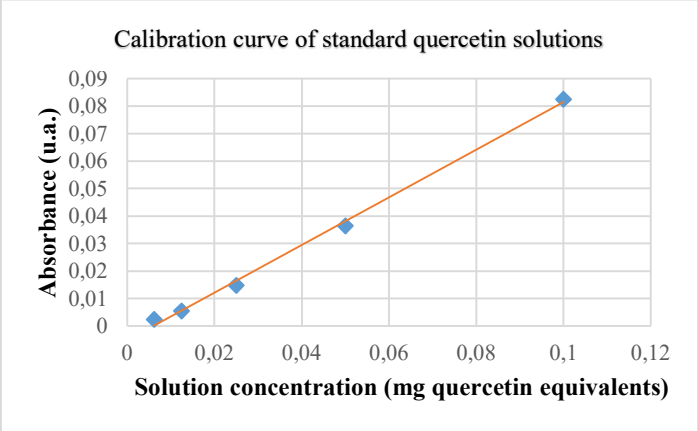

Fig. 2. Calibration curve performed with quercetin standard in aqueous medium

The results of the analyzes are shown in Table 2.

Table 2.

Calculation of the average flavonoid concentration of ethanolic extract of St. John's wort flowers expressed in mg EQ/g dry matter

Sample	Sample absorption read at 510 nm	Sample concentration in flavonoids (mg EQ/mL alcoholic extract)	Sample concentration in flavonoids (mg EQ/g dry matter) (=100X)	Average sample concentration in flavonoids (mg EQ/g dry matter)
Alcoholic extract from *Hypericum perforatum* flowers	0.851	1.034	103.378	108.019±4.641
	0.902	1.095	109.553	
	0.915	1.111	111.127	

From the analysis of the data in the table, we can conclude that the alcoholic extract of *Hypericum perforatum* flowers has a high concentration of flavonoids. The data obtained are consistent with those provided by current studies of some researchers: 108.65-125.35 mg GAE/g dry matter (Shabani et al., 2019) 68.59 mg GAE/g dry matter (Tusevski et al., 2019).

The percentage of DPPH inhibition is calculated using the equation:

$$\%\, inhibition = \frac{A_{blanc} - A_{sample}}{A_{blanc}} \cdot 100$$

where: A_{blanc} – blanc absorption read at 515 nm (t = 0 minutes); A_{sample} – sample absorption read at 515 nm (t = 15 minutes).

The results obtained after performing the DPPH method are presented in Table 3.

Table 3.

DPPH test results on alcoholic extract from ringing flowers

Sample	Blanc absorbance	Sample absorbance	Inhibition percentage, %	Average inhibition percentage, %
Alcoholic extract from *Hypericum perforatum* flowers	0.6959	0.193	72.27	72.86±0.59
		0.187	73.12	
		0.186	73.20	

The data provided by table 3. show that the alcoholic extract of flowers of *Hypericum perforatum L.* has a high percentage of inhibition, so a good ability to neutralize free radicals (72.86 ± 0.59), as evidenced by other existing studies (92.45% (Sun et al., 2018)).

The antioxidant capacity according to the FRAP method in alcoholic extracts can be calculated using the regression equation:

$$y = 0.0017\, x + 0.0872$$

where: y - the absorbance of the sample read on the UV-VIS spectrophotometer at 595 nm, expressed in u.a., x - concentration in µmoles equivalent Trolox/sample of 0.1 mL alcoholic extract.

Table 4 shows the results obtained for the antioxidant capacity of the alcoholic extract by the FRAP method.

Table 4.

Results of the FRAP method on alcoholic extract from *Hypericu perforatum* flowers

Sample	Sample absorption read at 595 nm	Sample concentration (µmoli TE/100 g dry matter)	Average sample concentration (µmoli TE/100 g dry matter)
Alcoholic extract from *Hypericu perforatum* flowers	0.206	69.882	72.431±2.745
	0.210	72.235	
	0.215	75.176	

The data provided in Table 4 show that the alcoholic extract of Hypericum perforatum flowers has a lower antioxidant capacity (72.431 ± 2.745 moles TE/100 g dry matter) than rosehips (94.685 ounces TE/100 g dry matter), but higher than sea buckthorn fruit (45.2437 µmoles TE/100 g dry matter) (Sacalîs, 2020). The data obtained are consistent with the data reported in other papers (78.117 µmoles TE/100 g dry matter (Guzel et al., 2019)).

CONCLUSIONS

From the analysis of the obtained data it can be concluded that the alcoholic extract of *Hypericum perforatum* has an appreciable content of total polyphenols (66.281 ± 0.889 mg GAE/g dry matter), has a high concentration of flavonoids (108.019 ± 4.641 mg EQ/g dry matter) and a high percentage of inhibition, so a good neutralizing capacity of free radicals (72.86 ± 0.59%) and an antioxidant capacity (72.431 ± 2.745µmoles TE/100 g dry matter) lower than rosehip fruit, but higher than sea buckthorn fruit.

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