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# DETERMINATION OF ANTIOXIDANT CAPACITY OF *HYPERICUM PERFORATUM L.* FLOWERS IN MARAMURES COUNTY BY VOLTAMETRIC METHOD WITH DIFFERENTIAL PULS

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***Abstract***

*Hypericum perforatum is a medicinal plant with a high antioxidant capacity due to its high content in flavonoids and polyphenols, as demonstrated by us in this work.*

*Plant was harvested from a pollution-free area in Maramures County in June when the active principles: flavonoids and polyphenols are in high concentrations in the plant. After harvesting from the plant, an extract was prepared to determine the antioxidant effect using the polarograph with differential pulse voltammetry method. The results obtained were calculated using the Trace Master 5 Software calculation algorithm and from the data analysis it was concluded that the St. John's wort flowers have a high concentration of polyphenols.*

**Keywords:** plant, antioxidant, polyphenols, St. John's wort

## INTRODUCTION

*Hypericum perforatum L.* (St. John's wort, measles, etc.) is a widespread medicinal species as a geographical area and unpretentious to climatic conditions (Pallag, 2015). Although drought-resistant it prefers moist, nitrogen-rich and light soils to concentrate volatile oil (Bojor, 2018).

Many compounds have been identified in the chemical composition of the plant product: hypericin, hyperforin, flavonoids, hisperidine, isoquercetin, rutin, biflavones, tannins, polyphenolic compounds, phytosterols with many beneficial effects on the body (Pallag, 2015, Bojor, 2018).

The main effect of St. John's wort extract is the antidepressant effect, and in present there are many studies that have demonstrated this (Vollmer, Rosenson, 2004, Bojor, 2018, Sarris et al., 2012, Behnke et al., 2002, Gastpar et al., 2006). It also has other effects: antioxidant, antibacterial, antiviral (studied *in vitro* on HIV), anticarcinogen (hyperforin could offer new perspectives in the fight against cancer), oily extract St. John's wort if used externally has a healing, anti-inflammatory and skin repairing effect (Suntar et al., 2010, Barnes et al, 2001, Schempp et al., 2000, Sun et al., 2011, Henderson et al., 2002).

Different chemical methods can be used to determine the antioxidant capacity and in this paper the method of voltammetry plus differential was used. This method showed that the analyzed St. John's wort flowers has antioxidant capacity, total content of polyphenols was highlighted with ascorbic acid and gallic acid by two methods: algebraical and graphical (Ikawn et al., 2003, Ciobanu et al., 2018, Li et al., 2018, Brand-Williams et al., 1995, Sun et al., 2018).

The working electrode is used to measure and characterize chemical reactions of interest. Usually the working electrode is used in combination with auxiliary electrodes and reference electrodes, the system being tri-electrode. The working electrode can be made of gold, platinum, silver or inert carbon (glass carbon and pyrolytic carbon). Most often chemically modified working electrodes are used to research organic molecules in solutions (Jurca et al., 2016, Dae-Ok et al., 2003, Seruga et al., 2011, Romani et al., 2000).

## MATERIAL AND METHOD

### MATERIALS

Polarograph TraceLab 150 stand, working electrode made of graphene modified with graphene Metrohm, Germany, reference electrode from Ag/AgCl, KCl with double junction Metrohm, Germany, platinum wire counter electrode Metrohm, Germany, potassium chloride Silver Chemicals Romania, distilled water, rated flask, ascorbic acid Silver Chemicals Romania, gallic acid Silver Chemicals Romania, St. John's wort alcoholic extract.

### METHOD

After the solutions of 0.1 M KCl, 0.04 M ascorbic acid, 0.04 M gallic acid were prepared and the St. John's wort flower extract was obtained, this extract was introduced into the 15 mL electrochemical cell in which the three electrodes are found: the electrode working, reference electrode and counter electrode.

### DRAWING VOLTAGRAMS FOR ASCORBIC ACID AND GALLIC ACID SOLUTIONS

In the voltametric cell over 10 mL 0.1 M KCl, the ascorbic acid solution was first added in 0.1 mL steps, recording a differential pulse voltammogram after each addition and then also the 0.04 M gallic acid solution.

**DRAWING VOLTAGRAMS FOR ALCOHOLIC STRAWBERRY EXTRACT WITH THE REAGENTS: ASCORBIC ACID AND GALICIC ACID**

In the voltametric cell of the device we introduced 10 mL of 0.1 M KCl solution. After the first voltammogram for KCl was recorded, I added 0.5 mL alcoholic extract of St. John's wort flowers, recorded the voltammogram and then in 0.5 mL steps to the final volume of 2.5 mL, I added the solution of ascorbic acid, using the standard addition method. The indicator electrode was used to record the voltammograms. I did exactly the same with the gallic acid solution.

## RESULTS AND DISCUSSION

In order to be able to determine the antioxidant capacity of the alcoholic extract of St. John's wort flowers, the potential values of ascorbic acid and gallic acid must first be checked. Depending on the volume of ascorbic acid and gallic acid solution that were added, the current intensities that occurred at 400 mV are shown in Table 1 and Table 2.

*Table 1.*

The value of the current intensity, depending on the volume of ascorbic acid solution added

| Added AA solution volume (mL) | Current intensity (V) |
|---|---|
| 0.1 | $3.351 \cdot 10^{-7}$ |
| 0.2 | $4.349 \cdot 10^{-7}$ |
| 0.3 | $4.969 \cdot 10^{-7}$ |
| 0.4 | $5.370 \cdot 10^{-7}$ |
| 0.5 | $6.117 \cdot 10^{-7}$ |
| 0.6 | $6.520 \cdot 10^{-7}$ |
| 0.7 | $8.113 \cdot 10^{-7}$ |
| 0.8 | $9.375 \cdot 10^{-7}$ |
| 0.9 | $9.665 \cdot 10^{-7}$ |
| 1.0 | $1.004 \cdot 10^{-6}$ |

*Table 2.*

The value of the current intensity, depending on the volume of gallic acid solution added

| Volume of added gallic acid solution (mL) | Current intensity (V) to 400 mV | Current intensity (V) to 800 mV |
|---|---|---|
| 0.1 | $2.185 \cdot 10^{-6}$ | $1.457 \cdot 10^{-7}$ |
| 0.2 | $2.890 \cdot 10^{-6}$ | $1.627 \cdot 10^{-7}$ |
| 0.3 | $3.938 \cdot 10^{-6}$ | $2.747 \cdot 10^{-7}$ |
| 0.4 | $5.007 \cdot 10^{-6}$ | $4.410 \cdot 10^{-7}$ |
| 0.5 | $5.273 \cdot 10^{-6}$ | $4.614 \cdot 10^{-7}$ |
| 0.6 | $6.294 \cdot 10^{-6}$ | $4.081 \cdot 10^{-7}$ |
| 0.7 | $7.366 \cdot 10^{-6}$ | $6.214 \cdot 10^{-7}$ |
| 0.8 | $8.401 \cdot 10^{-6}$ | $7.551 \cdot 10^{-7}$ |
| 0.9 | $8.933 \cdot 10^{-6}$ | $8.800 \cdot 10^{-7}$ |
| 1.0 | $9.736 \cdot 10^{-6}$ | $1.045 \cdot 10^{-6}$ |

Figure 1 and Figure 2 illustrate the voltammograms obtained by adding ascorbic acid and gallic acid solutions to the voltametric cell over the 0.1 M KCl solution.

Figure 3 and Figure 4 show the calibration curves of the potential values at 400 mV and 800 mV for ascorbic acid solution and gallic acid solution.

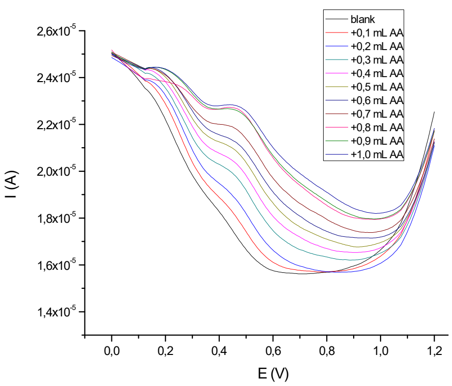


Fig. 1. Differential pulse voltammograms for ascorbic acid solution

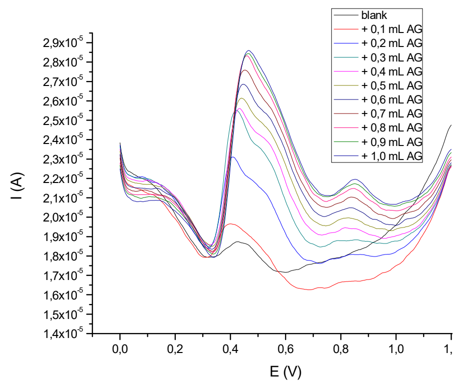


Fig. 2. Differential pulse voltammograms for gallic acid solution

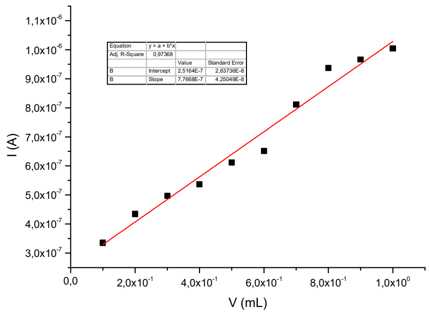


Fig. 3. Linearity of ascorbic acid solution

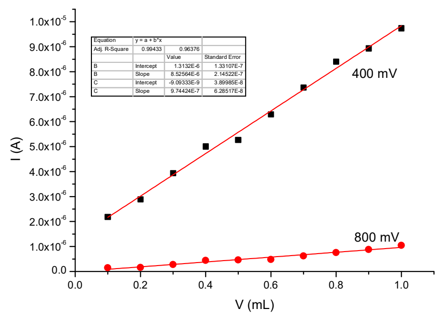


Fig. 4. Linearity of the gallic acid solution

The analysis of the calibration line demonstrates the linearity of the points for the two potentials 400 mV and 800 mV, which leads to the conclusion that this method is useful for dosing ascorbic acid and gallic acid in different products.

The values of the current intensities (baseline) corresponding to the voltammograms for the alcoholic extract of St. John's wort using ascorbic acid are presented in table 3 and the voltammogram in figure 5 and for gallic acid the values of the voltammograms are presented in table 4 and the voltammogram in figure 6.

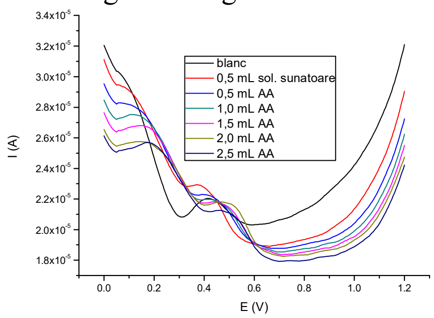


Fig. 5. Differential pulse voltammograms for alcoholic extract from flowers of sounds with ascorbic acid

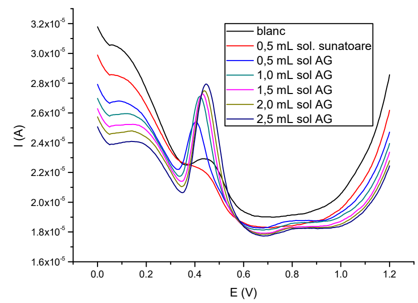


Fig. 6. Differential pulse voltammograms for alcoholic extract from flowers of gallic acid ringers

*Table 3.*

Intensity values as a function of concentration

| Concentration (mol/L) | Intensity (A) |
|---|---|
| 0 | $25.76 \cdot 10^{-6}$ |
| $1.90476 \cdot 10^{-3}$ | $26.54 \cdot 10^{-6}$ |
| $3.63636 \cdot 10^{-3}$ | $27.64 \cdot 10^{-6}$ |
| $5.21739 \cdot 10^{-3}$ | $28.47 \cdot 10^{-6}$ |
| $6.66666 \cdot 10^{-3}$ | $29.53 \cdot 10^{-6}$ |
| $8.00000 \cdot 10^{-3}$ | $31.11 \cdot 10^{-6}$ |

*Table 4.*

Intensity values as a function of concentration

| Concentration (mol/L) | Intensity (A) |
|---|---|
| 0 | $3.842 \cdot 10^{-6}$ |
| $1.90476 \cdot 10^{-3}$ | $4.262 \cdot 10^{-6}$ |
| $3.63636 \cdot 10^{-3}$ | $6.424 \cdot 10^{-6}$ |
| $5.21739 \cdot 10^{-3}$ | $6.912 \cdot 10^{-6}$ |
| $6.66666 \cdot 10^{-3}$ | $7.428 \cdot 10^{-6}$ |
| $8.00000 \cdot 10^{-3}$ | $8.479 \cdot 10^{-6}$ |

If it is represented graphically the intensities of the currents obtained depending on the concentration, we will obtain a line from which the concentration of the alcoholic extract solution of St. John's wort flowers can be determined in terms of antioxidant capacity relative to ascorbic acid figure 7 and for gallic acid figure 8.

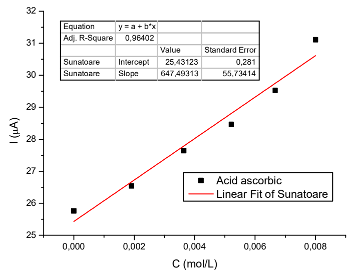


Fig. 7. Right calibration for alcoholic extract from flowers of sounds with ascorbic acid

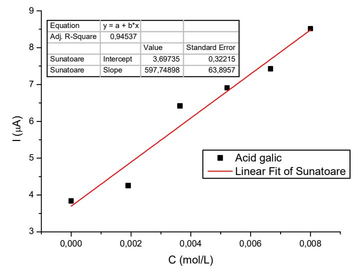


Fig. 8. Right calibration for alcoholic extract from flowers of gallic acid ringers

Calibration line for alcoholic extract of St. John's wort flowers:
- with ascorbic acid has the correlation coefficient R = 0.96402 and the equation is: I (A) = 25.43123 + 647.49313 · C (mol/L),
- with gallic acid has the correlation coefficient R = 0.96402 and the equation is: I (A) = 3.69735 + 597.74898 · C (mol/L).

The concentration of the alcoholic extract solution of St. John's wort flowers with ascorbic acid and gallic acid can be determined by two methods:

1. Algebraical: where from the equation of the line at the value of I = 0 is determined concentration as follows:
- with ascorbic acid: 0 = 25.43123 + 647.49313 · C (mol/L), C = $3.92764 \cdot 10^{-2}$ mol/L depending on ascorbic acid or C = 6.91737 g ascorbic acid/L.
- with gallic acid: 0 = 3.69735 + 597.74898 · C (mol/L), C = $6.17902 \cdot 10^{-3}$ moles gallic acid/L or C = 1.05043 g gallic acid/L.

2. Graphycal: where the concentration is obtained following the intersection of the extension line with the Ox axis, the concentration being taken in absolute value.

Following the analysis of the two graphs, figure 9 and 10 shows that the concentration value of the alcoholic extract of St. John's wort flowers determined with:
- ascorbic acid is: C = $6.5 \cdot 10^{-4}$ moles ascorbic acid/L or C = $11.4478 \cdot 10^{-2}$ g ascorbic acid/L
- gallic acid is: C = $1.15574 \cdot 10^{-3}$ moles of gallic acid/L or C = $19.64758 \cdot 10^{-2}$ g gallic acid/L.

The antioxidant capacity of the alcoholic extract of St. John's wort flowers as a function of ascorbic acid and gallic acid can be calculated using the data (coefficients of the right equation) in Table 5.

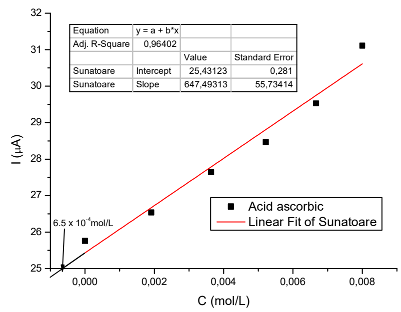


Fig. 9. Determination of the concentration for alcoholic extract from flowers of sound rings with ascorbic acid

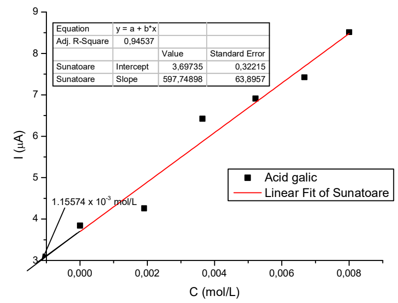


Fig. 10. Determination of the concentration for alcoholic extract from flowers of gallic acid ringers

*Table 5.*

Antioxidant capacity of alcoholic extract of St. John's wort flowers depending on ascorbic acid or gallic acid

| **Reactive** | **Intercept** | **Pant** | **R** | **Antioxidant capacity expressed in ascorbic or gallic acid equivalent/L** | |
|---|---|---|---|---|---|
| | | | | **Calculated** | **Graphic** |
| Ascorbic acid | 25.43123 | 647.49313 | 0.96402 | $3.92764 \cdot 10^{-2}$ | $0.65 \cdot 10^{-3}$ |
| Gallic acid | 3.69735 | 597.74898 | 0.94537 | $6.17902 \cdot 10^{-3}$ | $1.15574 \cdot 10^{-3}$ |

From data analysis obtained for the extract of *Hypericum perforatum* we can observe that it has a concentration of $3.92764 \cdot 10^{-2}$ moles ascorbic acid/L and $6.17902 \cdot 10^{-3}$ mol gallic acid/L.

## CONCLUSIONS

The differential pulse voltammetry method used showed that the analyzed St. John's wort flowers have antioxidant capacity, total content of polyphenols being highlighted using the reagents used (ascorbic acid and gallic acid) by the two methods: algebraic and graphical. The method used showed that ringing flowers have antioxidant capacity, therefore this plant can be used in the fight against free radicals to preserve the health of the population.

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