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STUDY OF ANTIOXIDANT CAPACITY OF FLAVONOIDIC COMPOUNDS FROM *TILIA PLATYPHYLLOS* EXTRACT

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ABSTRACT. Identification of compounds from medicinal plants is one of the oldest fields for the application of thin-layer chromatography (TLC). The past few years have seen tremendous growth in the use of herbal medicines worldwide.

The flavonoids are one of the most numerous and important group of natural compounds and they have many biological properties.

This paper is concerning to test the antioxidant capacity of flavonoids from Tilia Platyphyllos by treated the methanolic extract of plant with nitroxidic radical and analyzed of these samples by TLC in situ coupled with UV spectroscopy.

INTRODUCTION

Natural compounds offer without doubt the richest resources of chemical diversity. They can be found not even in medicinal plants. The term medicinal and aromatic plants (MAP) include not only the plants used for medicinal purposes, but also aromatic plants and spices [1]. Interest in research concerning the constituents and biological activities of MAP has significantly increased in recent year [2].

The principal aim of MAP researched development is to produce new or more efficient phytopfarmacons. This can be basically divided into four categories: pulverized MAP in which all the compounds occurring in the plant are presented; raw extracts of the MAP which contain only substances soluble in the extraction solvent used (e.g. aqueous extracts of different plants, tea, are the common raw extracts); purified extracts which occurring as a result of selective purification. They contain only certain types of biologically active compounds. The fourth category – used very often in conventional therapy, but rarely in phytotherapy - is that of isolated active substances, with purity greater than 99%.

Most of these categories are biological complex matrices; therefore planar chromatography is the first choice and, generally, the most useful analytical method in the research and development of MAP, because the widely used silica can be applied as the only stationary phase. Because of the usefulness of thin layer chromatography (TLC) when cost-effectiveness is essential, TLC or HPTLC is widely used as a standard technique for rapid and accurate identification of plant materials [3] or finished product, and for purity testing of raw materials and formulations in different pharmacopeial prescriptions. A disadvantage of planar chromatography is the skill and experience required because of relatively large number of factors influencing the result [4].

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One of the most important and most widespread groups of natural compounds present in the composition of MAP with a lot of therapeutic properties is the flavonoid class [5]. Flavonoids occur in a variety of structural forms and they are phenolic compounds with a basic C_6-C_3-C_6 skeleton. First of all, they are remarkable because of their antioxidant capacity. Conveniently, the 5000 different flavonoids are divided into 12 classes according to the oxidation level of the central C_3 unit [6]. Paper chromatography, TLC, HPTLC, high performance liquid chromatography (HPLC), column chromatography (ion-exchange resins, polyamide powder, gel material), and countercurrent chromatography have been the most commonly used methods for the separation and purification of flavonoids [7]. TLC and HPTLC is a technique that is applicable to all classes of flavonoids and is especially useful for rapid analysis of partly purified mixtures derived from paper or column chromatography and various TLC or HPTLC systems have been described in the literature [8-12].

The aim of this study is to confirm the presence of flavonoids in *Tilia Platyphyllos* methanolic extract and to illustrate the antioxidant capacity of these compounds. *Tilia Platyphyllos* can be included in MAP category. *Tilia Platyphyllos* has been used traditionally as a calming agent in treatment of anxiety, indigestion, and common cold and as a remedy for ear infection and fever, but medical studies demonstrated its antispasmodic and diuretic actions.

EXPERIMENTAL

All the solvents were of analytical grade. The solution of tempol (Fluka, purity > 97%) was prepared by dissolving 0.0195g tempol in methanol.

The spectrophotometric measurements were performed using Spekol 20 (Carl - Zeiss Jena) spectrophotometer.

The chromatographic experiments was realized on silicagel plates Sil G F_{254} (Merck).The samples (50μL) were applied on plates as belts. The mixtures ethyl acetate – methyl-ethyl-ketone – formic acid – water (50 : 30 : 10 : 10, v/v) were used as mobile phase. The plates were developed on a 12cm distance, in saturated N-chambers at room temperature and then they were dried and the detection was performed in UV light either at 254nm or at 365nm after spraying the plates with diphenyl-borate-amino-buthylic alcohol (NTS).

The densitogram and the *in situ* UV spectra were recorded at 254nm using a Desaga CD60 densitometer.

RESULTS AND DISCUSSION

This research followed the next steps:

1) Establishing the total quantity of flavonoids contained in extract;
2) Testing the assumption that flavonoides react with the nitroxydic radical;
3) Qualitative analysis of simple extract in comparison with the extract treated with the radical for confirming the antioxidant capacity of flavonoides.

The extract of *Tilia Platyphyllos* is made by extraction of dry plant (flowers) in methanol and then the solution was concentrated by evaporation [13].

The total quantity of flavonoids from extract was determined spectrophotometrically at 430nm, using a calibration curve. Different concentration solutions, prepared from rutozid standard according to Pharmacopoeia [14] were used for

plotting the curve. The calibration curve is presented in Figure 1. The total quantity of flavonoidic compounds was expressed in rutozid units. In the *Tilia Platyphyllos* extract the total quantity of flavonoidic compounds, determined from calibration curve, was 17.74 mg/100mL (A = 0.480).

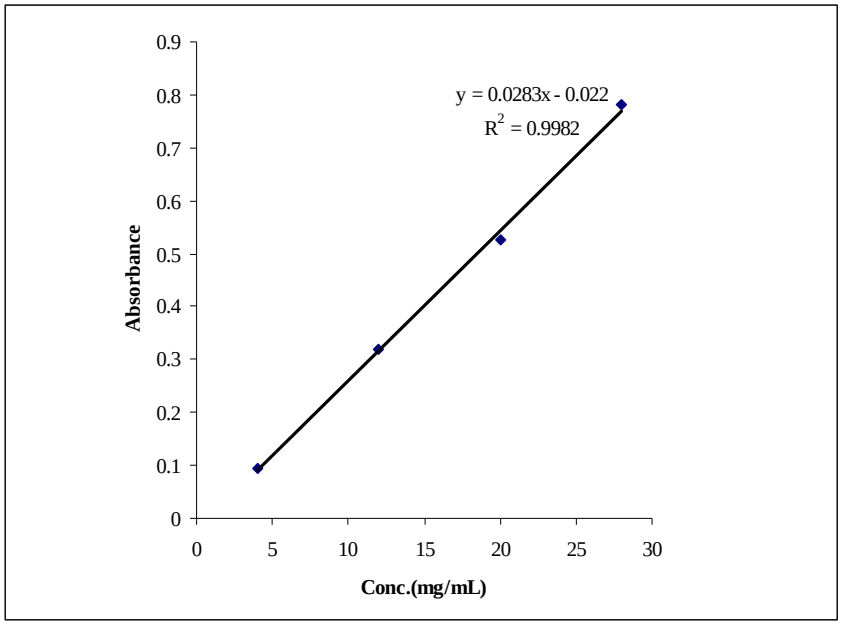

Fig. 1. *The calibration curve for spectrophotometric determinations of flavonoids.*

For testing the assumption that flavonoids act as antioxidant, the *Tilia Platyphyllos* extract was treated with methanolic solution of tempol. Tempol, 4-hidroxy-2,2,6,6-tetramethyl-piperidin-N-oxyl, is a free stable nitroxidic radical. Using different molar ratio extract: radical (1 : 1, 1 : 5) and the same spectrophotometric measurements it came out that absorbance, measured at 430nm decreased and this is the first proof that flavonoidic compounds react with nitroxidic radical and they have antioxidant capacity.

Qualitative analysis of samples – simple *Tilia Platyphyllos* extract and extract treated with tempol – was made by TLC coupled with UV spectroscopy.

The densitogram (Figure 2) shows that *Tilia Platyphyllos* extract contains four flavonoides. Comparing the densitograms of samples (Figure 2) it can be observed that the suitable spots of each flavonoidic compound decreased in intensity and this is another proof of antioxidant capacity.

There are also compared the *in situ* UV spectra of each flavonoidic compounds from *Tilia Platyphyllos* and from treated extract (Figure 3 – 6). From these figures it came out that the absorbance of flavonoidic compounds decreased in intensity or the place of the absorbency maximum is changed.

In conclusion, these researches confirm the fact that flavonoidic compounds act like antioxidant agents. As a remark we can say that these researches prove the advantages of TLC *in situ* coupled with UV spectroscopy.

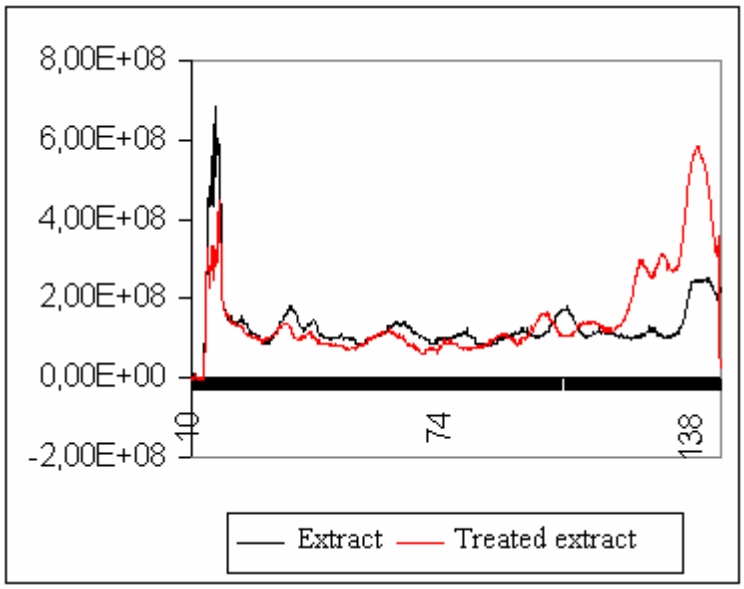

Fig. 2. *The densitograms of extract and treated extract with tempol*.

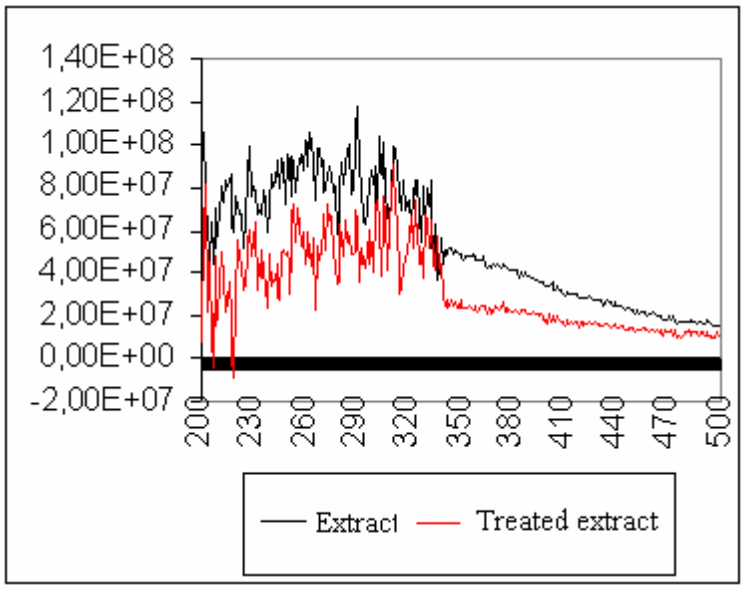

Fig. 3. *The UV spectra of flavonoidic compound I*.

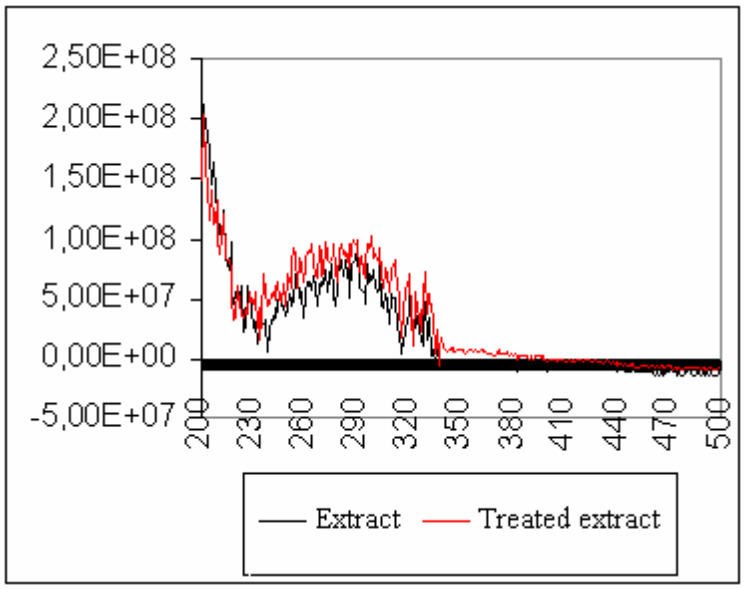

Fig. 4. *The UV spectra of flavonoidic compound II.*

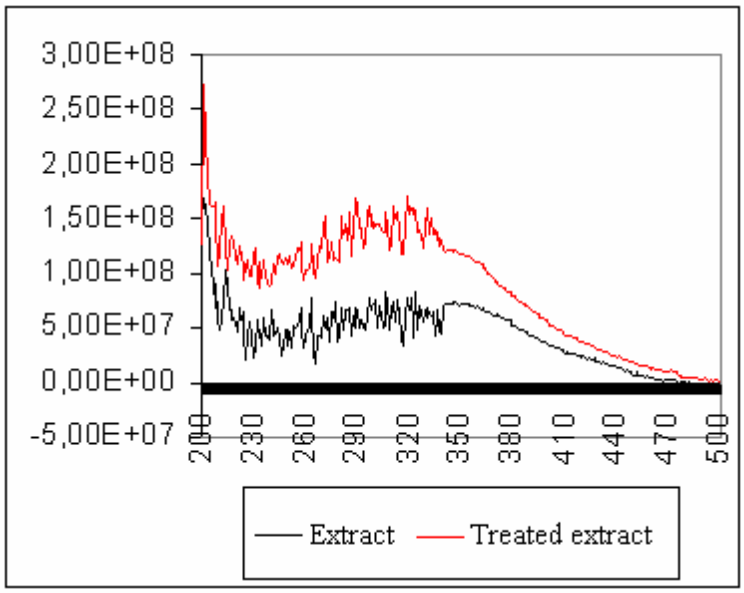

Fig. 5. *The UV spectra of flavonoidic compound III.*

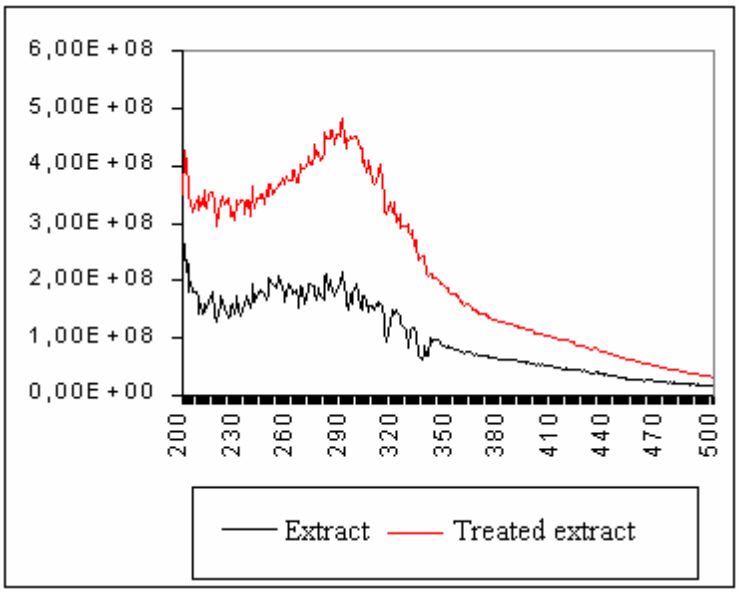

Fig. 6. *The UV spectra of flavonoidic compound IV.*

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