АНТИОКСИДАНТНА АКТИВНОСТ НА ЕКСТРАКТИ ОТ МАГАРЕШКИ БОДИЛ (*ONOPORDUM ACANTHIUM L.*)

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ANTIOXIDANT ACTIVITY OF EXTRACTS FROM COTTON THISTLE (*ONOPORDUM ACANTHIUM L.*)

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ABSTRACT

Cotton thistle is a popular herb widely used in the folk medicine. Except the other ingredients, cotton thistle contains substances with antioxidant properties: tocopherols, flavonoids, phenolic acids. The aim of this work is to study the polyphenolic content of extracts from cotton thistle in order to determine and quantify their antioxidant capacity. In order to determine the most powerful solvent composition that extracts maximum solid matter from the plant, extractions are carried out with mixtures containing ethanol and water in different ratio. The study shows that in terms of total yield of extract best results are obtained with 20% ethanol and hydromodule (solid:liquid) 1:10. Extracts produced at the conditions of maximum yield contain also the highest amount of polyphenols. The antioxidant capacity of the extracts is proportional to their polyphenolic content.

***Key words**: Cotton thisle, extraction, polyphenols, antioxidant capacity.*

INTRODUCTION

Onopordum acanthium L. (common name Cotton thistle) is a noxious weed with a worldwide distribution. It is biannual herbaceous prickly plant 100-300 cm in height, highly branched with broad leaves with longitudinal spines and red-purple flowers. It occurs most commonly in wastelands, pastures, riverbanks and well drained sandy or gravelly soils. It has been found also in agricultural fields (1, 2). Usable area is ground section with basket and leaves without the lower part of stem. The leaves contain anthocyanidins, saponins, bitter sesquiterpen lactone arktsiopikrin. The flower basket contains inulin. The known physiological action is diuretic, stimulation of the secretion of the glands in the digestive track. Bulgarian folk medicine recommends use of extract of Cotton thistle as a restorative and invigorating the body means. Small doses of extract from Cotton thistle increase excitability of nervous system, whereas larger doses push it down. The plant has also antimicrobial and antibiotic action (3).

Among the other ingredients, cotton thistle contains substances with antioxidant properties: tocopherols, flavonoids, phenolic acids (4). Antioxidant compounds are very important and beneficial for human health. They neutralize the free radicals thus preventing negative actions on body's tissues, development of abnormal cells (cancer), premature ageing, etc. Phenolic compounds are recognized as main representatives of the natural antioxidants presently identified. However, cotton thistle is not studied for antioxidant activity. The aim of this work is to study the polyphenolic content of extracts from cotton thistle in order to determine and quantify their antioxidant capacity.

MATERIALS AND METHODS

Plant material

The plant was purchased as dry herb containing leaves and flowers. Before extraction they were disintegrated to small particles at about 1 mm size.

Chemicals used

Folin-Ciocalteu (FC) reagent (Sigma 2N solution), gallic acid (Sigma), dehydrated Na_2CO_3 (Valerus), ethanol (96%, Valerus), $DPPH^+$ (Sigma), and methanol (99.9% LabScan) were used for determination of total content of polyphenols (TPC) and antioxidant capacity (AOC). The other supplementary chemicals and reagents were acquired from Sigma–Aldrich.

Extract preparation

All extracts are processed in the same manner: A number of identical samples are prepared by mixing in glass flasks a given amount of dry and ground plant with a given amount of solvent. Then the flasks are vortexed in a thermostatic shaking device. The temperature is chosen to be not too high (40^oC) in order to prevent eventual thermal destruction of active components. For determination of process development in the course of time, the liquid phase of each flask is sampled only once at different time in order to avoid big process disturbance (which occurs when taking many samples from the same flask). The samples are analyzed for determination of total extract quantity necessary for drawing of extraction isotherm. After the process is finished, analyses are also made for determination of total polyphenolic content (TPC) and antioxidant capacity (AOC) of the extracts.

In order to determine the most powerful solvent composition that extracts maximum solid matter from the plant, extractions are carried out with mixtures containing ethanol and water in different ratio.

Analytical methods

Total extract determination

Samples of the extracts are evaporated at mild temperature conditions (105^0C) until reaching steady weight in order to determine the quantity of dry solid matter extracted from the plant.

Determination of TPC

Total polyphenols are determined by the well known method of Folin–Ciocalteu (5) using Gallic acid as a standard for deriving of calibration line. The method is based on color reaction of polyphenols with Folin-Ciocalteu reagent. The color intensity of the resulting solution is proportional to substance concentration. Double-beam UV-VIS-spectrophotometer (UNICAM®-Helios β) is used to analyze the samples. Light absorption was measured at 765 nm (6). In order to avoid accidental errors, each analysis is repeated at least three times. The results are expressed in mg Gallic acid equivalent to 1 g dry extract.

Antioxidant capacity (AOC)

DPPH method is largely used because of its simplicity and stable results (7). This assay evaluates the capacity of an extract to neutralize free radicals of DPPH solution. It is also based on color reaction. The light absorbance is measured with the same spectrophotometer (UNICAM®-Helios β) at 517 nm (8). The free radical inhibition capacity IC is calculated by the expression

$$IC\ [\%] = (1 - A_s/A_o)\ x100 \qquad (1)$$

where A_o is absorption of blank sample, A_s is absorption of extract containing sample.

RESULTS AND DISCUSSION

Fig. 1 (cumulative extracted mass **m** plotted against the time **t**) illustrates the process development in the course of time when using solvent of different composition. It is seen that regardless of solvent composition, the extraction isotherms become horizontal after about 40minutes, i.e. no material is further extracted, quasi-equilibrium state is attained, and the process can be considered as completed. The plateaus, which correspond to the total extracted amount, are located at different levels indicating different quantity of total extract.

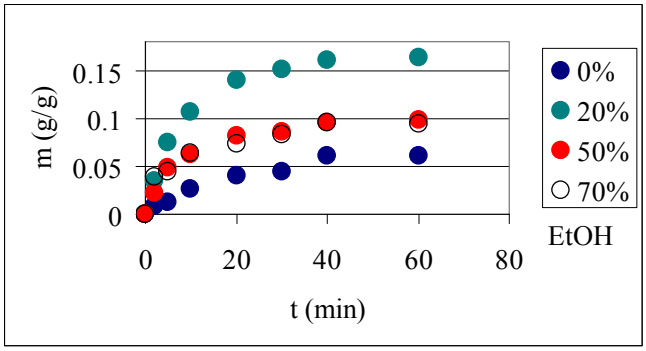

Fig.1. Kinetic curves of the extracts with different solvents

Fig. 2 depicts explicitly the total mass (M) extracted with different solvents at equilibrium state.

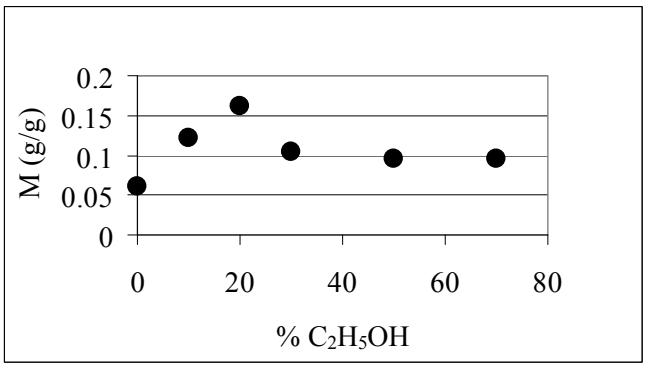

Fig. 2. Total dry extract mass obtained with different solvent composition

Lowest results are obtained using ethanol-free water. Highest amount is extracted with 20 % ethanol. Extraction with more concentrated solvent does not improve the extraction efficiency being also economically unattractive. The conclusion is that 20% ethanol-in-water mixture can be regarded as optimal for this process.

The influence of solid/liquid ratio on the extracted quantity is shown on Fig. 3.

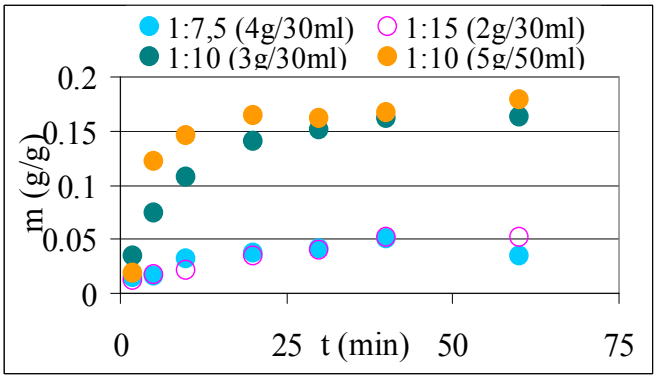

Fig. 3. Kinetic curves for the extraction of different amounts of material at different hydromodule

Fig. 3 depicts results for the extraction of solids with different solid – liquid ratio. The results show that the yield increases initially with increasing the amount of extracted material, i.e. when reducing the hydromodule. It means that the amount of solvent is sufficient and the solubility does not limit the process under these conditions. A likely explanation for this result is that an increased solid phase quantity corresponds to larger contact surface between the phases and results in a larger yield. However, later reduction of hydromodule leads to insufficient solvent quantity, not enough

for dissolving the existing soluble matter (process controlled by solubility). This graph shows also that the hydromodule 0.1 (1:10) chosen for caring out the majority of our experiments, provides appropriate extraction conditions not limited by the solubility.

The extraction carried out at the same solid-liquid ratio (1:10), but with 5 times bigger quantity of raw material 5g/50ml, has shown identical process path, and identical yield has been obtained. This result is an initial step towards scaling-up the process, verifying the applicability at conditions when the amount of processed material is increased.

Fig. 4 represents the polyphenol content of the solid matter (dry extract) obtained with different solvents. As it is seen, extracts with 20% ethanol contain the greatest amount of polyphenols. We recall that the same solvent produces the biggest amount of total extract, too (see Fig. 2).

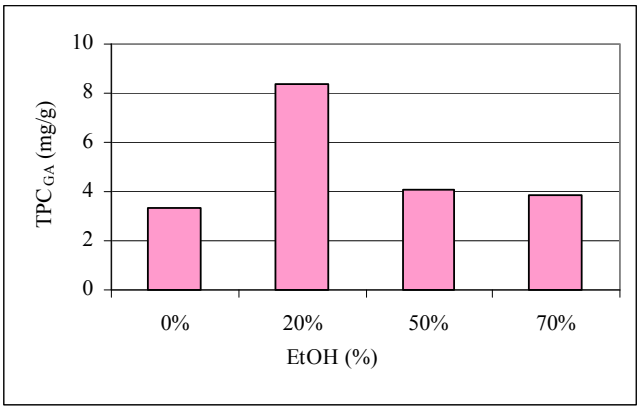

Fig. 4. Polyphenol content of dry extracts obtained with different solvents

Fig.5 is a comparative graph showing the inhibition capacity IC50 (ml/l) of original liquid extracts, expressed as ml of extract necessary to reduce the half of the free radicals in 1 l of reference solution (0.004 % DPPH). It is seen that 50% inhibition is achieved with the least amount of extract obtained by using 20% ethanol. It means that this extract contains the highest amount of antioxidant compounds.

In order to express AOC in more understandable terms, IC50% approach is applied also to solutions of a popular antioxidant ascorbic acid (AA) - vitamin C (fig. 6). The value of AOC_{AA} means that AOC of this quantity of vitamin C is equivalent to AOC of 1 g of plant. 20 % extract shows the highest antioxidant capacity.

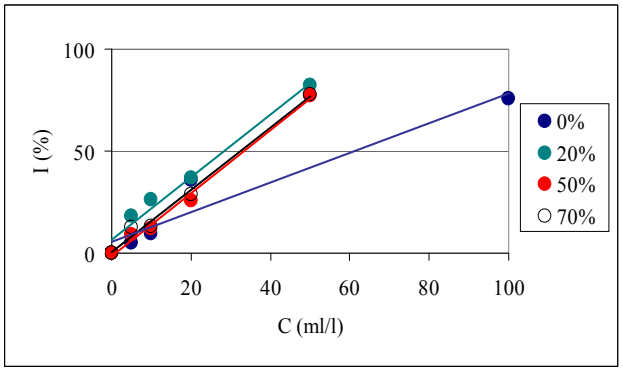

Fig.5. Inhibition capacity of different extracts EtOH

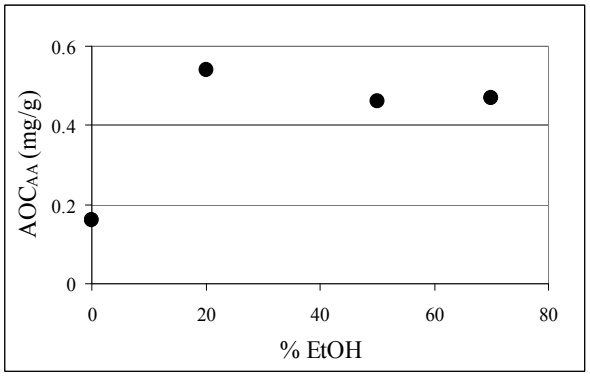

Fig.6. AOC of extracts with different solvents

Fig. 7 shows that Cotton thistle has comparable and even higher AOC than that of other popular plants.

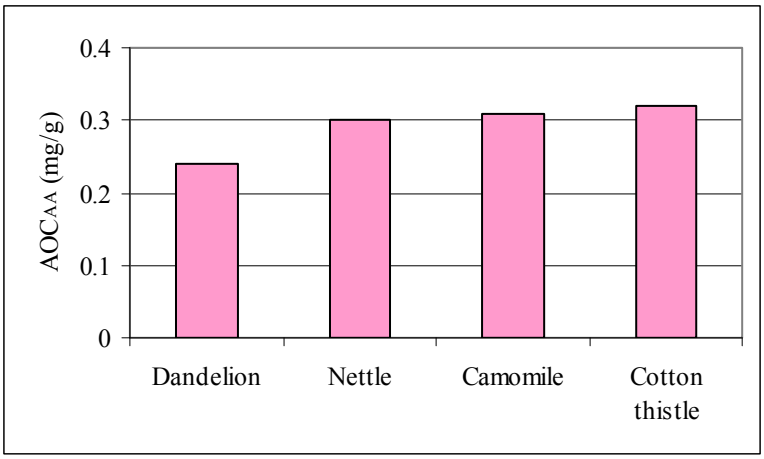

Fig. 7. Comparison of AOC of different plants

CONCLUSIONS

The study on the extraction of Cotton thistle (Onopordum acanthium) with water-ethanol solutions at different operational conditions has shown that:

- In terms of total yield of extract best results are obtained with 20% ethanol and hydromodule (solid:liquid) 1:10;
- The process duration is determined by the time necessary for attaining equilibrium state – about 40 minutes.
- Extracts produced at the conditions of maximum yield contain also the highest amount of polyphenols;
- The antioxidant capacity of the extracts is proportional to their polyphenolic content. It is confirmed by the fact that the highest antioxidant capacity is shown by the extracts with the biggest concentration of polyphenols;
- In comparison to other popular medicinal plants Cotton thistle is relatively rich source of antioxidants.

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