OPTIMIZATION OF THE EXTRACTION PROCESS IN ORDER TO ISOLATE ANTIOXIDANT COMPOUNDS FROM WALNUT LEAVES

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ABSTRACT

In the popular perception, nuts were considered to have high content of fats and therefore were seen as unhealthy foods, which are indicted in different cardio-vascular diseases or diabetes. This perception has changed after the lately researches, which proved their healthy fatty acid profile of the walnut and its products. The walnut (*Juglans regia L.)* consumption is now associated with a reduced risk of coronary heart diseases, cancer and all other oxidative stress mediated diseases. Different studies had shown that the walnut leaves contain several phenolic compounds which contribute to their biological properties. Researchers are focused on walnut because it contains an important phenolic compound called juglone and it is used in the treatment of inflammatory and infectious diseases. They also inhibit the Gram positive and Gram negative bacteria and also fungi.
The present research has the priority to establish the proper method of extraction using walnut leaves and a mixture of solvents in different volumetric proportions. Primary the walnut leaves were extracted with a mixture of ethanol and water in proportions 50:50; 60:40; 70:30; 80:20. After the extracts were obtained, they were concentrated and the final extract was used to analysis.

Keywords: walnut leaves, antioxidant properties, heart diseases.

1. INTRODUCTION

A healthy diet must include several foods that contain essential fatty acids and also an important amount of antioxidant compounds. Walnuts (*Juglans regia* L.) possess one of the highest antioxidant capacity, therefore it is recommended to consume these nuts daily. The benefits of walnuts consumption consist in the protection for cardio-vascular diseases by decreasing LDL-cholesterol and increasing HDL-cholesterol (Bernal et al., 2011), normalizes the level of blood glucose and prevention of the harmful effects of the free radicals.
The increased interest in foods and supplements obtained from plants which contain high amounts of antioxidant compounds is the basis for further researches that proved the chemical composition and bioactive properties. Therefore the process of extraction must be improved to the highest capacity using modern techniques and materials.
The walnut leaves are believed to have an important role in maintaining a normal level of blood glucose, even decreasing it (Hasan et al., 2011). A recent study showed that the methanolic extract of Juglans regia's leaves decreases the level of blood glucose on diabetic rats and also human due to the capacity of walnut compounds to regenerate β-cells which are insulin-producers (Teimori, 2009).
Several researchers analysed the influence of different solvents on the antioxidant capacity in the extraction process. They proved that it is a strong connection between the solvent used in the extraction and the antioxidant properties and extraction yield, based on the different polarity of the compounds obtained (Fernandez-Agullo et al., 2012).
An important compound contained in walnut leaves is *juglone* (5-hydroxy-1,4-naphthoquinone), a quinone with a powerful cytotoxic activity and it has been proved that its action mechanism is DNA intercalation or it acts like an alkylating agent.
An important property of the walnut leaves is the antimicrobial activity which is dependent with the concentration of the extract, therefore for a low concentrated extract no inhibition of the bacteria is found (Pereira et al., 2007). In the extraction process the concentration and solvents occupies a central role, and the optimization of this process must take into consideration these two factors.

2. MATERIALS AND METHODS

Materials. The plant parts used for the present study were fresh walnut leaves harvested from Dobrogea county. They were naturally dried and after that procedure, were mixed with a food processor until a powder was obtained.

Methods. The extract was obtained from the powdered walnut leaves with a mix of solvents (ethanol:water) in four different proportions: 50:50; 60:40; 70:30; 80:20 by volume. For the extraction process it was used an ultrasounds water bath and after that the extract was concentrated with rotavapor and filtrated. The analysis carried on thus the obtained extract were:

1. Determination of the antioxidant capacity using the DPPH method.
2. Determination of flavonoids using the spectrophotometric method, reference substances were quercitin and rutin.
3. Determination of polyphenols using the Folin-Ciocalteu method, reference substances were gallic acid and tannic acid.
4. Determination of water content.
5. Determination of lipids using Soxhlet method.

3. RESULTS AND DISCUSSION

The effects of polyphenolic compounds are of great interest due to the antioxidant and antiproliferative activities. In the present study, the polyphenols determination assay proved that the highest concentration of compounds was extracted with the 50:50 solvent ethanol:water, as shown in Fig. 1. The 70:30 and 80:20 solvent ethanol:water extracts showed also a high level of polyphenols due to the higher ratio of alcohol. The variation of phenolic compounds is due to their capacity to dissolve in different solvents in different proportions.

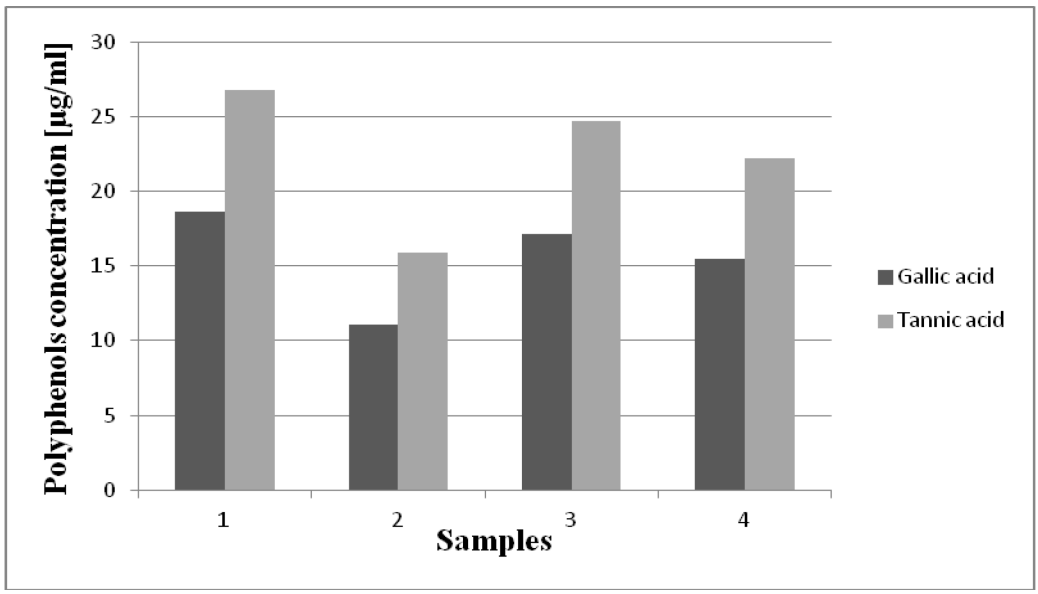

Figure 1. Variation of polyphenols in extracts with different solvents

1 - extract with ethanol:water = 50:50; 2- extract with ethanol:water = 60:40; 3 – extract with ethanol:water = 70:30; 4 – extract with ethanol:water = 80:20

Flavonoids are compounds that protect cells from free radicals effects which decrease the defense capacity of the human body. A recent research showed that flavonoids are able to replace vitamin E and their antioxidant capacity can depend on their chemical structure (Fernandez et al., 2010).
Researchers proved recently that flavonoids inhibit the LDL oxidation, therefore the antioxidant activity is situated in the top of the antioxidant substances.
The present study showed that flavonoids are influenced by the type and concentration of solvents in the extracts. In Fig. 2 it is revealed that the most powerful solvent is the one with the lowest concentration (50% ethanol). The quantities of quercitin and rutin is the highest in 50:50 ethanol:water extract of walnut leaves.

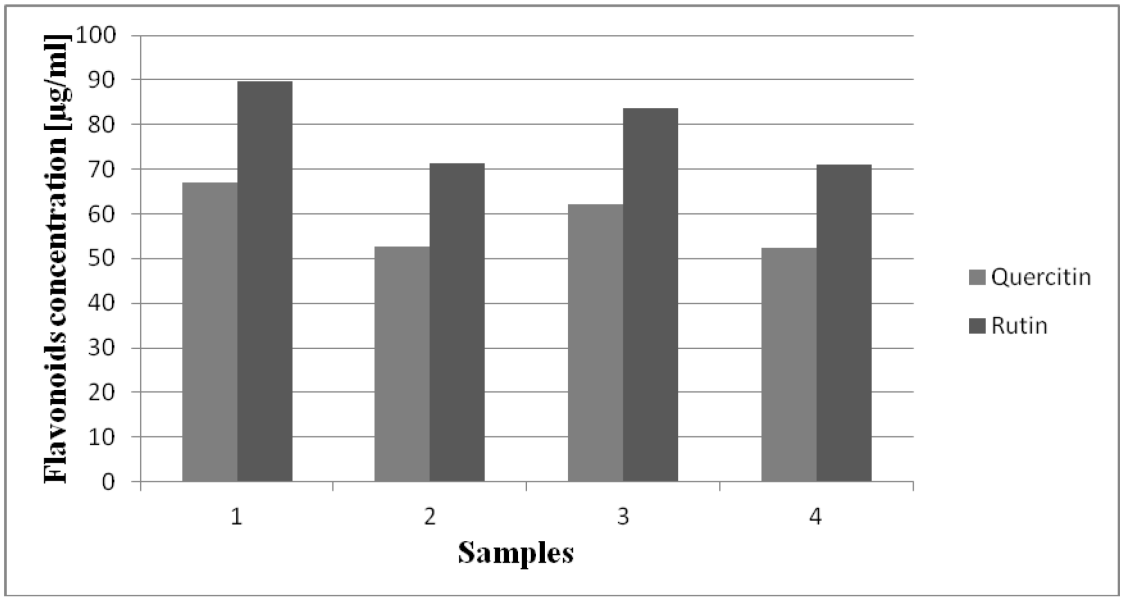

Figure 2. Variation of flavonoids in extracts with different solvents

1 - extract with ethanol:water = 50:50; 2- extract with ethanol:water = 60:40; 3 – extract with ethanol:water = 70:30; 4 – extract with ethanol:water = 80:20

The DPPH analysis for antioxidant capacity proved that the ability of walnut leaves extract to inhibit the free radicals is best highlighted with the ethanol:water solvent in proportion of 50:50. A high antioxidant activity is also showed by the 80:20 ethanol:water solvent extract, as it is shown in Fig. 3.

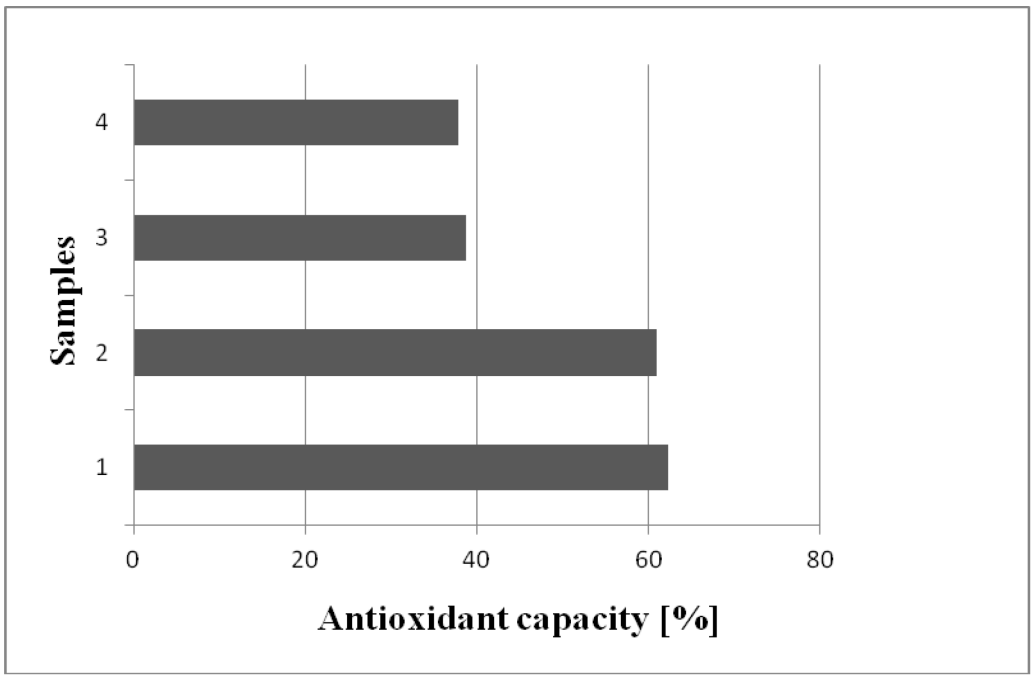

Figure 3. Variation of antioxidant capacity in extracts with different solvents

1 - extract with ethanol:water = 50:50; 2 - extract with ethanol:water = 60:40; 3 - extract with ethanol:water = 70:30; 4 - extract with ethanol:water = 80:20

4. CONCLUSIONS

The walnut leaves can be extracted with different mixes of solvents and the most appropriate were proved to be mixes of ethanol and water. The best ratio of these two solvents is 50:50 by volume, because as it was shown, it has the highest levels of polyphenols, flavonoids and antioxidant capacity. The 80:20 ethanol:water solvent appears to be also a good option for the walnut leaves extraction, showing that it almost reaches the values of the 50:50 solvents.

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