EFFECT OF SOLVENT ON SOLID-LIQUID EXTRACTION OF PHENOLIC COMPOUNDS FROM WALNUT (JUGLANS REGIA L.) MEMBRANE SEPTUM

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Abstract: *The extractive efficiency of phenolic compounds from plant material is greatly depended on the solvent. In conventional processes polyphenols are extracted from vegetable material using different solvents in a temperature range from 40 to 90 ^{0}C. In this study, was proposed to extract polyphenolic compounds from walnut membrane septum (woody septum) using the Soxhlet extraction, and water ethanol as a solvent mixtures with different concentrations as a solvent. The obtained extracts were evaluated for total polyphenol content by Folin-Ciocalteu method, and UV spectra of the investigated extracts* were *also analyzed. In the course of these studies it was found that optimum ratio of water and ethanol for the extraction of polyphenols from walnut membranes is 30% of the mixture.*

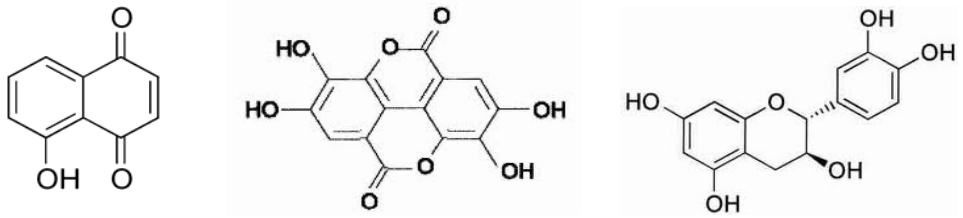
Cuvinte cheie: *walnut membrane septum/woody septum, phenolics, Folin Ciocalteu assay, UV spectra.*

I. Introduction

Walnut (Juglans regia L.) is a valuable crop being the nut very popular and largely consumed. Not only dry fruits (nuts) but also green walnuts, shells (inner and outer), kernels, barks, green walnut husks (epicarp) and leaves have been used in food, cosmetic and pharmaceutical industries [1, 14].

It is well known that walnuts membrane septum contains high amounts of antioxidants, namely polyphenols (juglone, rutin, ellagic acide, tannins). In recent years, polyphenols production has become a very important issue because of their increasing commercial interest in the field of pharmaceutical, food and nutraceutical industries [2].Their antioxidant activity can be attributed to the polyphenolic constituents, including the ellagitannins, present primarily in the pellicle and septum [3].

Stampar et al. (2006) identified thirteen phenolic compounds in walnut membrane septum: chlorogenic acid, caffeic acid, ferulic acid, sinapic acid, gallic acid, ellagic acid, protocatechuic acid, syringic acid, vanillic acid, catechin, epicatechin, myricetin, and juglone (Fig. 1) [4]. For this reason walnut septum/membrane has important value for health care. In Iran traditional medicine, the septum of fruit of Juglans regia L. has been traditionally used to treat diabetic patients and other diseases [5, 15].



Juglone Ellagic acide Catechin

Fig. 1. Chemical structures of polyhenolic compounds of walnut membrane septum

The choice of water/ethanol mixtures as solvent was made on the basis of literature data: they have revealed to be more efficient in extracting phenolic constituents than the corresponding mono-component solvent system [7].

II. Materials and methods

2.1. Samples

Experimental runs on polyphenols extraction were performed on samples of walnut membrane septum derived from walnut residues kindly collected from local producers in September 2011. The walnut

membrane septum were dried by natural method approximately 7 days, in a dark place, in order to remove the moisture initially present.

2.2. Chemicals

Folin-Ciocalteu's phenolic reagent, sodium carbonate and gallic acid were supplied by Sigma-Aldrich. Ethanol (99,9%) and was provided by Eco-Chimie (Chisinau, Republic of Moldova). All reagents were of analytical grade. Distilled water was used throughout.

2.3. Extraction

Dried walnut membrane septum were ground until the powder condition before extraction. The dried powder of septum was extracted with 0, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100% ethanol for 2 h at 60^0C and liquid-to-solid ratio 10 ml per gram. The extracts of tasted septum were filtered with paper filter and after were used in the experiments. Obtained extracts were analyzed for the total polyphenol content assays and UV-vis spectra.

2.4. Total polyphenol compound

For quantification of total polyphenol content, the Folin-Ciocalteu's method was used [8]. A volume of 0.5 ml of Folin-Ciocalteu's reagent was added to a dark flask, containing 0.5 ml of the each extract sample and 10 ml of distilled water. After 5 min, 8 ml of a 7.5% aqueous sodium carbonate solution was added to the mixture and the content was mixed thoroughly. The samples were kept in dark for 2h and then the absorbance was measured at 765 nm with HACH LANGE DR-5000 UV/vis spectrophotometer. Three parallel samples were analyzed. Gallic acid was used for constructing the standard curve, obtained in advance and shown in Figure 2.

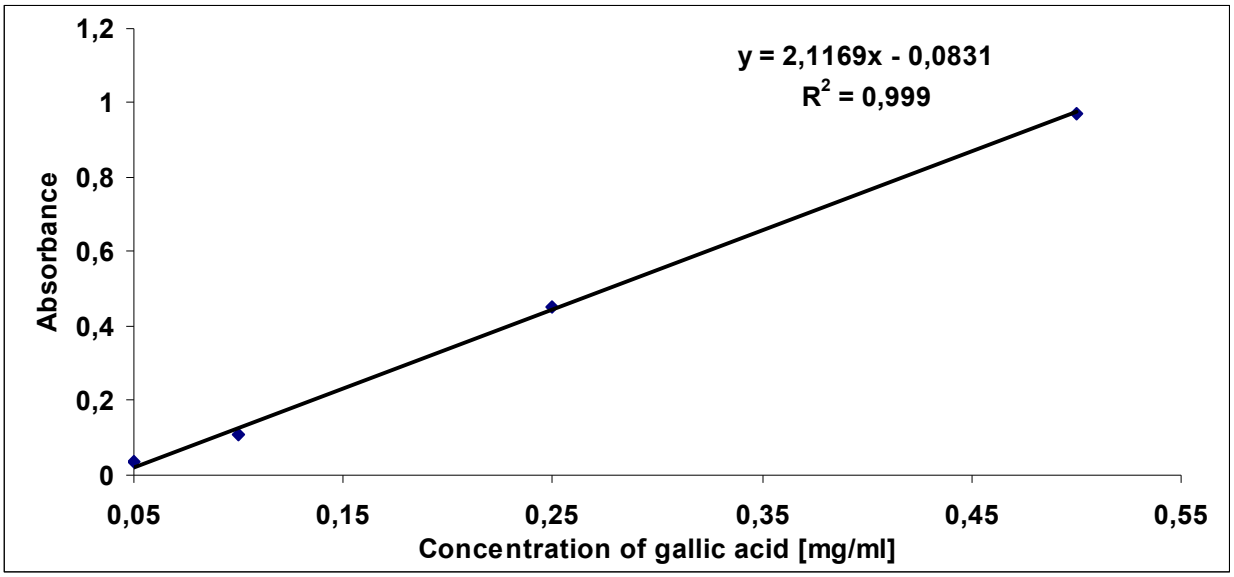


Fig. 2. Standard curve of gallic acid.

Concentration range of gallic acid was of 0.05-0.5 mg/ml. The results of total polyphenol content were expressed as mg of gallic acid equivalents per ml of extract (mg GAE/ml).

2.5. Statistical analysis

Variance analysis of the results was carried out by least square method with application of coefficient Student and Microsoft Office Excel program version 2007. Differences were considered statistically significant if probability was greater than 95% (p-value <0.05). All assays were performed by triplicate at room temperature 20 ± 1 ^{0}C. Experimental results are expressed as average ± SD (standard deviation) [9].

III. Results and discussion

Several procedures have been used : extraction using fats and oils,organic solvents, aqueous alkaline solutions and supercritical carbon dioxide [10]. Several studies showed that polyphenol content differed with

solvents polarities. For instance, absolute methanol was used for the extraction of tea polyphenols [11], and 50% acetone for extraction of wheat total phenolics [12], which were found to bemore effective than water. In addition, Hayouni reported that water and organic solvents used individually or in mixture such as acetone/water/acetic acid (90/9.5/0.5) and ethyl acetate/methanol/water (60/30/10) affected significantly total polyphenol contents of Quercus coccifera L. and Juniperus phoenicea L. fruit extracts [13]. Alcoholic solvents have been commonly employed to extract phenolics from natural sources: they give quite high yield of total extract even though they are not highly selective for phenols.

Walnut membrane septum are valuable plants which contain an impressive amount of biologically active substances that have a wide range of uses. During this study it was investigated the influence of water/ethanol solvent mixtures at different concentrations on the level of total polyphenol content of extracts from walnut membrane septum. Obtained experimental data are demonstrated in figure 3.

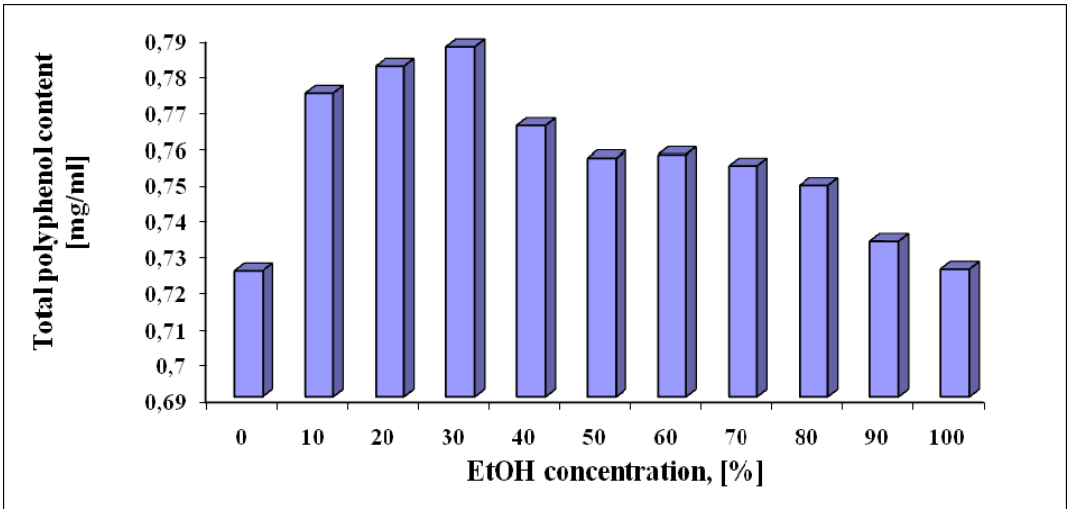


Fig. 3. *Dependence of solvent type on the level of polyphenol compounds extraction from walnut membrane septum.*

Figure 4 illustrates the UV/Vis spectra of the extracts from walnut septum in the wavelength range 190 - 1100 nm. The spectrum of the extracts display strong peaks, typical for phenolic compounds at 400 and 450 nm.

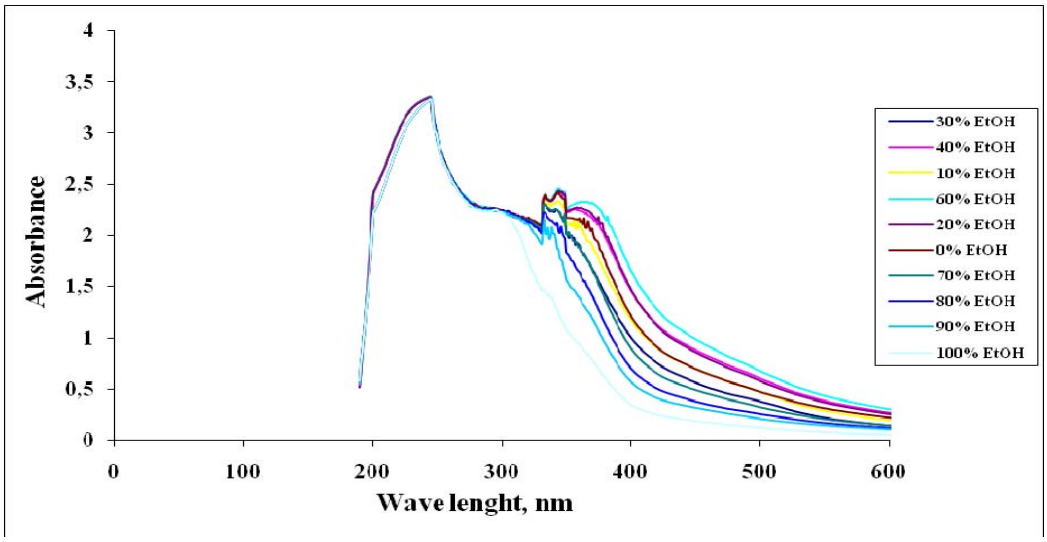


Fig. 4. UV/Vis spectra of the extracts from walnut membrane septum.

Conclusions

In this study water and ethanol, two environmentally and food safe solvents were used to optimize solid-liquid extraction of phenolic compounds from walnut membrane septum. Total polyphenol content of walnut septum extracts was evaluated using Folin-Ciocalteu reagent. It was established that optimal solvent for antioxidant extraction from walnut membrane septum is 30% mixture of water and ethanol.

Acknowledgements

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