TECHNIQUES FOR EXTRACTING POLYPHENOLS FROM *COREOPSIS TINCTORIA* NUTT. FRUITS

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The literature does not present any information on ultrasonic assisted extraction of phenolic compounds from Coreopsis tinctoria Nutt. *fruits using solvents with different polarities. The objective of this paper is to highlight the potential of ultrasound assisted extraction in the fast preparation of extracts rich in polyphenols of Coreopsis tinctoria* Nutt. *fruits in good yields. Several parameters that could affect extraction efficiency were evaluated. Finally, the results of the ultrasonic assisted extraction were compared with those obtained by using three extraction techniques, namely: maceration, refluxing and Soxhlet extraction.*

Keywords: maceration, refluxing, soxhlet, ultrasound-assisted extraction, polyphenols, *Coreopsis tinctoria* Nutt

1. Introduction

Coreopsis tinctoria Nutt. (Asteraceae) is native from North America, in Romania is commonly cultivated for ornament in gardens. The plant is used to treat several disorders including diarrhea, internal pains, bleeding, to strengthen blood, as an emetic and to control diabetes. [1-3]

In the present study we used the fruits of *Coreopsis tinctoria* Nutt., to highlight the total content of polyphenols, using several extraction techniques, namely: ultrasonic extraction (U), maceration (M), Soxhlet extraction (S) and refluxing (R) using n-hexane, chloroform and ethanol as solvents.

Plants are able to synthesize an extensive array of secondary metabolites with a significant portion consisting of phenolic compounds and flavonoid compounds. Phenolic compounds and flavonoids accumulate in significant amounts in plants and appear to have a myriad of supplemental and adjacent functions in a plant's life cycle. It may be structural or protective roles in different

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tissues, as allelopathic agents, as attractants for pollinators and seed-dispersing animals, involvement in defense strategies, ultra violet protectants and signal molecules in symbiosis between plants and their environment. [4]

More than 6000 different flavonoids have been described until now, and the number continues to increase. Flavonoids are polyphenolic compounds comprising of 15 carbons, with 2 aromatic rings connected by a 3-carbon bridge. According to the modifications of the central C-ring, they can be divided into different structural classes including flavonols, flavan-3-ols, flavones, flavanones, isoflavones, and anthocyanidins, as shown in Fig. 1. Other flavonoid groups, which are found in relatively small quantities, are dihydroflavones, flavan-3,4-diols, coumarins, chalcones and aurones. [4]

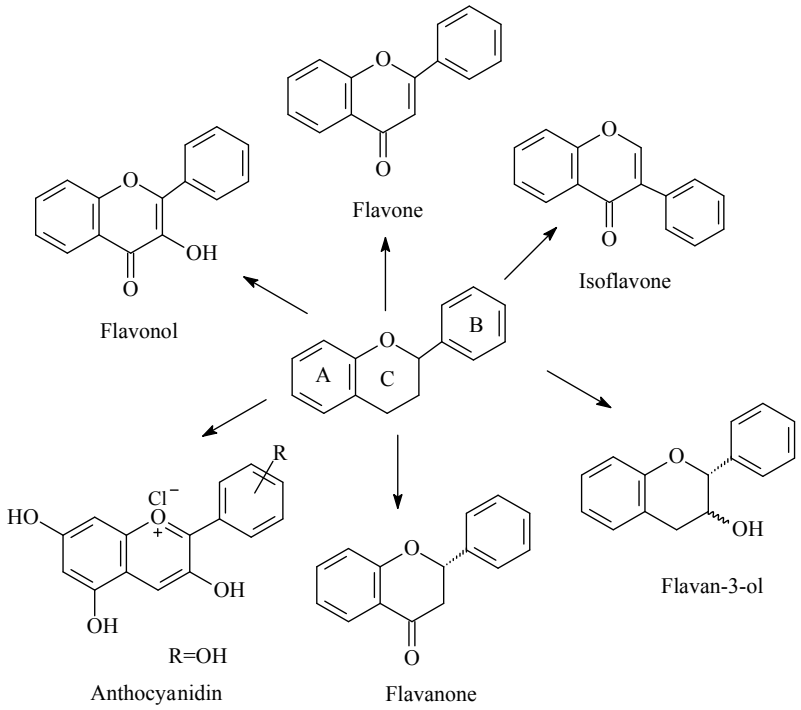

Fig. 1. Structures of the main flavonoid subgroups [4]

Beneficial actions of polyphenols on human health are most often attributed to their ability to act as antioxidants. Thus, flavonoids constitute one of the most common forms of phenolic compounds present in plants. Multiple health benefits have been proposed for flavonoids, although the biochemical and physiological mechanisms are not fully defined and quantified. Among others, the existence of a large number of flavonoids bearing different chemical substituent

and the limited knowledge of their tissue distribution and concentration, are the factors that complicate the understanding of their biological effects. [4]

The main methods for determining polyphenols are spectroscopy, chromatography and electrophoresis. Spectroscopic methods are useful only for the determination of large amounts of polyphenol compounds in a sample.

The amount of polyphenols is usually specific for each plant species and depends on the varietal features and the growing conditions of a plant. [5]

The extraction yield of antioxidant compounds from plant materials is influenced mainly by the conditions under which the process of solid-liquid extraction is carried out, using a solvent to separate a soluble fraction from a permeable solid. Choosing the appropriate solvent is one of the most relevant steps leading this operation. Chemical characteristics of the solvent and the diverse structure and composition of the natural product shows that each material–solvent system has a different behavior, which cannot be predicted. [6]

Extraction is a very important stage in isolation, identification and use of phenolic compounds and there is no single and standard extraction method. Solid–liquid extraction of phenolic compounds with different solvents and in some cases ultrasound assisted extraction are the most common techniques used to isolate these compounds.

Ideally, an extraction procedure should be exhaustive with respect to the compounds to be analyzed, rapid, simple, inexpensive and – for routine analysis – subject to automation. Usually, the traditional techniques require long extraction time and have low efficiency. Moreover, many natural products are thermally unstable and could be degraded during the extraction with increasing temperature. Classically, the extraction of phenolic compounds, fatty acids, terpenes, is performed by maceration, Soxhlet extraction. Such techniques are usually time-consuming and require large volumes of organic solvent. The use of large volumes of solvent implies in additional costs, due to the fee associated with the purchasing and disposal of toxic solvents, and environmental hazard. As followed in the last decade, alternative extraction techniques that reduce the volume of solvent and the time of extraction have been considered. For example, one of the new techniques is ultrasound-assisted extraction. [7]

The effect of ultrasound in liquid medium is given by acoustic cavitation. Factors that generate cavitation include acoustic power and frequency, but its effects depend on: solvents used, system vapor pressure, external temperature and pressure as well as the presence and nature of dissolved gases.

Passing high intensity ultrasound through a liquid medium leads to the generation of a field or cloud of bubbles. [8] These bubbles grow to a critical size and contract violently, generating extreme temperatures and pressures inside the bubbles. [8] Solvent and solute molecules present within the bubbles are

decomposed under these extreme conditions and generate more highly reactive radicals. [9]

In a solid–liquid system, the contraction of the cavitation bubbles may have different effects on the solid surface. Even though the mechanism is still disputed, it has been demonstrated that the vaporized molecules inside the bubble can form reactive free radicals upon the violent contraction of the bubble. [10] These radicals can initiate reactions, increase the reaction rate and even initiate new reaction pathways. [11]

The most important aspect for a successful ultrasound assisted extraction is to establish appropriate values for extraction parameters relating to the biological properties of the plant material to be extracted. The possible benefits of ultrasound in extraction are: mass transfer intensification [12] and an enhanced hydration process, which takes place simultaneously with plant material fragmentation. [13]

2. Materials and methods

2.1. Reagents and plant material

Gallic acid (Sigma-Aldrich, >99%), 50 μg/mL solution was used as standard chemical for spectrophotometric analysis.

Folin-Ciocalteu's phenol reagent (Sigma-Aldrich), sodium carbonate 7.5% solution (Merck), were used as reagents in spectrophotometric analysis.

The plant material consists of dried fruits of *Coreopsis tinctoria* Nutt. harvested in Romania. They were stored in dark hermetic tight bags to protect them from humidity and light.

2.2. Chemicals

The solvents used for experiments developed to investigate solid–liquid extraction of phenolic compounds were n-hexane, chloroform and ethanol. All the solvents (provided from Merck) were stored in dark flasks.

2.3. Ultrasonic extraction procedure (U)

1.0 g of dried fruits of Coreopsis tinctoria Nutt. were extracted with 20 mL of solvent (n-hexane, chloroform or ethanol) in an ultrasonic apparatus (Elmasonic S 80 H, frequency 60 Hz, power 750 W). The temperature of the bath was controlled at 30°C. The extraction time in this case was 1 h. The extracts were filtered and concentrated until dryness. The experiment was performed in triplicate.

2.4. Maceration procedure (M)

For 1.0 g of dried fruits of *Coreopsis tinctoria* Nutt. and 20 mL of solvent (n-hexane, chloroform or ethanol) a maceration procedure was employed, and the

extraction time in this case was 5 h. The extracts were filtered and concentrated until dryness. The experiment was performed in triplicate.

2.5. Soxhlet extraction procedure (S)

1.0 g of dried fruits of *Coreopsis tinctoria* Nutt. and 40 mL of n-hexane were refluxed by 5 h using Soxhlet apparatus. Chloroform (46 mL) and ethanol (50 mL) were used for comparison purposes. The extracts were filtered and concentrated until dryness. The experiment was performed in triplicate.

2.6. Refluxing procedure (R)

1.0 g of dried fruits of *Coreopsis tinctoria* Nutt. and 13 mL of solvent (n-hexane, chloroform or ethanol) were refluxed by 5 h using a refluxing apparatus. The extracts were filtered and concentrated until dryness. The experiment was performed in triplicate.

2.7. Determination of the total content of phenolic acids using spectrophotometer

The total polyphenol content (TPC) was determined by the spectrophotometric method, according to the method described by the International Organization for Standardization (ISO) 14502-1. [14] We used a spectrophotometer type: Jasco V 530.

2.7.1. Procedure for the calibration curve

0.1-0.5 mL of gallic acid 50 μg/mL solution were introduced into test tubes followed by 2.5 mL of Folin-Ciocalteu's phenol reagent of a 1/10 dilution in water. The tubes were allowed to stand for 5 min. Then, 2 mL of Na_2CO_3 solution (7.5%) and water up to 5 mL were added and a period of 30 min was allowed for stabilization of the blue color formed. The absorbance against a reagent blank was measured at 760 nm. Five-point calibration curve using 1-5 μg/mL gallic acid as the standard was linear (y = 0.20509x, where y = absorbance and x = concentration in ppm, R^2 = 0,9981) in the reaction mixture and the absorbance range up to 1.000 AU.

2.7.2. Procedure for fruits extracts

Aliquots of fruits extracts (0.2 mL) were used instead of gallic acid and the procedure described at section 2.7.1. was applied.

The concentration of polyphenols in samples was derived from a standard curve of gallic acid. Data were expressed as mg gallic acid equivalent (GAE) per 100 g plant material and mg gallic acid equivalent (GAE) per g dried extract, respectively.

3. Results and Comparative Discussions

Conditions under which the experiments were performed are shown in Table 1.

Table 1

Experimental conditions used for extraction of 1g fruits of *Coreopsis tinctoria* Nutt.

Process	Ultrasound treatment			Maceration			Soxhlet			Refluxing		
Solvent	C_6H_{14}	$CHCl_3$	C_2H_6O	C_6H_{14}	$CHCl_3$	C_2H_6O	C_6H_{14}	$CHCl_3$	C_2H_6O	C_6H_{14}	$CHCl_3$	C_2H_6O
Code	U-H	U-C	U-E	M-H	M-C	M-E	S-H	S-C	S-E	R-H	R-C	R-E
Time (hour)	1	1	1	5	5	5	5	5	5	5	5	5
Temperature (° C)	30	30	30	25	25	25	69	61	78	69	61	78
Initial volume (mL)	20	20	20	20	20	20	40	46	50	13	13	13
Final volume (mL)	10	12	15	10	11	10.9	37	38	42	6.1	6	7
Color	light yellow	light yellow	yellow	color-less	light yellow	dark yellow	opal	light yellow	yellow orange	opal	light yellow	dark yellow

The content of total polyphenols found (TPC) in the plant material and in the extracts was shown in Table 2.

Table 2

The test results of extracts from *Coreopsis tinctoria* Nutt. fruits

Code	A, 760 [nm]	GAE [ppm]	DF**	GAE [mg in 5 mL]	Total GAE [mg]	TPC [mg/ 100 g plant material]	Dried extract mass [g]	TPC, [mg/g dried extract]	Obser-vations
	a	b	c	d	e	f	g	h	
-	-	a/ 0.20509	-	$b*10^{-3}*$ 5 mL	c*d	(e*100 g plant material)/ 1 g plant material	-	(e*1 g dried extract)/ g	-
U-H	0.0919	-	-	-	-	-	0.020	-	slightly turbid, colorless
U-C	0.1164	-	-	-	-	-	0.002	-	slightly turbid, colorless
U-E	0.138	0.673	300	0.0034	1.0093	100.93	0.010	100.93	blue
M-H	0.1486	-	-	-	-	-	0.010	-	turbid, colorless

M-C*	0.0634	0.309	15	0.0015	0.0232	2.32	0.010	2.32	blue
M-E	0.1877	0.915	300	0.0046	1.3728	137.28	0.310	4.43	blue
S-H	0.1601	-	-	-	-	-	0.080	-	turbid, colorless
S-C	0.0458	-	-	-	-	-	0.020	-	colorless
S-E	0.5024	2.450	300	0.0122	3.6745	367.45	0.180	20.41	blue
R-H	0.052	-	-	-	-	-	0.020	-	slightly turbid, colorless
R-C*	0.09	0.439	15	0.0022	0.0329	3.29	0.005	6.58	blue
R-E	0.1494	0.728	300	0.0036	1.0927	109.27	0.390	2.80	blue

* Samples were read after filtering
** Dilution Factor

From the values shown in Table 2 it can be seen that polyphenolic compounds were extracted only by ethanol and chloroform. So, extraction of polyphenolic compounds from *Coreopsis tinctoria* Nutt. fruits is strongly influenced by the nature of the solvent and the dissolved substance. Of the three solvents used only ethanol is miscible in water and has a dielectric constant value above 5. Ethanol has dielectric constant of 24.6 and dipole moment of 1.69, while chloroform has dielectric constant of 4.81 and dipole moment of 1.04, and its solubility in water (g/100g) is 0.8. Among the solvents used hexane shows the lowest value of dielectric constant, dipole moment respectively, and its solubility in water (g/100g) is 0.0014.

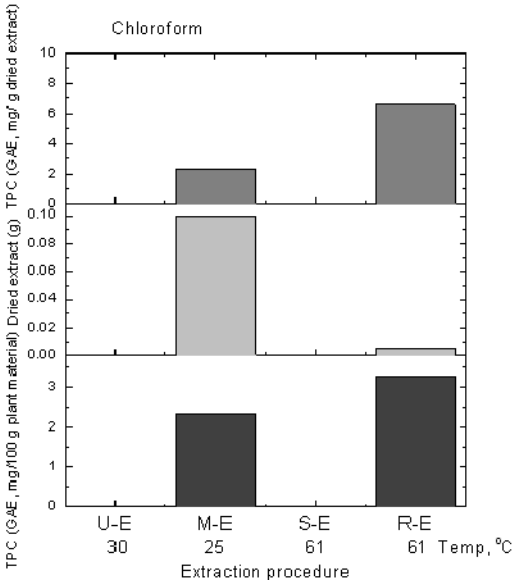

Fig. 2. Total polyphenols content expressed as gallic acid equivalents (GAE, mg/100g plant material) / Dried extract (g) / Total polyphenols content expressed as gallic acid equivalents (GAE, mg/g dried extract) vs. chloroform separation processes

Therefore, the extraction of polyphenolic compounds from fruits of *Coreopsis tinctoria* Nutt. is recommended to take place with solvents whose relative polarity to be as close to the water. Dissolved compounds by hexane and chloroform are non-polar compounds.

From the analysis of data presented in Table 2 it can be noticed that the use of chloroform resulted in obtaining the lowest TPC values, polyphenols were identified only in samples obtained by maceration and refluxing. The analysis of data presented in Fig. 2 shows that the most effective chloroform extraction technique of polyphenolic compounds is refluxing. If chloroform maceration was held at 25°C, refluxing was carried out at 61°C, so that the increase of extraction temperature provided an increased solubility of polyphenolic compounds that are extracted in chloroform.

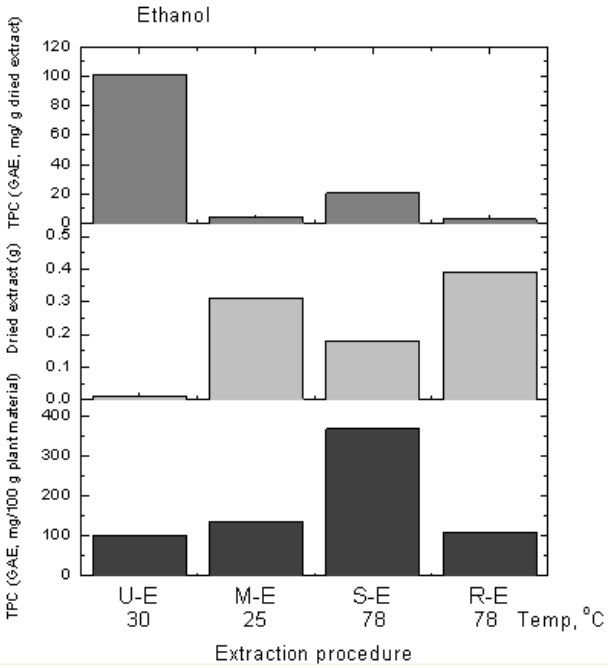

Fig. 3. Total polyphenols content expressed as gallic acid equivalents (GAE, mg/100g plant material) / Dried extract (g) / Total polyphenols content expressed as gallic acid equivalents (GAE, mg/g dried extract) vs. ethanol separation processes >>

From the analysis of data presented in Fig. 3 it can be noticed that all ethanol methods lead to the extraction of polyphenols, the highest amount of polyphenols is obtained in Soxhlet method, but the most selective extraction process, in which we have the highest concentration of polyphenols in dried extract, is ultrasound procedure.

Possible benefits of ultrasound in ethanol extraction are increased mass transfer, improved penetration, breaking cells, and capillary effects. In addition, it significantly reduces the extraction time and the amount of ethanol used.

4. Conclusions

Extraction of polyphenolic compounds from *Coreopsis tinctoria* Nutt. fruits is more effective the higher the relative polarity of the solvent used is. Following hexane extraction were not obtained polyphenolic compounds from *Coreopsis tinctoria* Nutt. fruits most likely dissolved compounds by hexane are non-polar.

When using chloroform extract quantities were reduced, polyphenols are put in evidence only for refluxing and maceration procedures.

Ethanol extraction of polyphenolic compounds from *Coreopsis tinctoria* Nutt. fruits proved to be the most effective.

The utilization of ethanol ultrasonic extraction has proven to be a much simpler and more effective ethanol extraction technique than the maceration, refluxing procedure and Soxhlet extraction for separating polyphenolic compounds from *Coreopsis tinctoria* Nutt fruits. The ethanol ultrasonic extraction can be carried out at lower temperature, lower solvent volume with economy in time.

Comparison of these ethanol extraction techniques revealed that they produce extracts with different characteristics, the highest concentration of polyphenols were obtained for ultrasound assisted extraction.

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