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Polyphenols in *Coreopsis tinctoria* Nutt. fruits and the plant extracts antioxidant capacity evaluation

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Abstract: The aim of this research was to investigate the chemical composition of *Coreopsis tinctoria* Nutt. fruits extract, to highlight the potential of ultrasound assisted extraction in the fast preparation of extracts rich in polyphenols using different solvents (55%, 78% and 96% hydrous ethanol) and to evaluate the antioxidant potential of formulated extracts. LC-MS/MS was used to characterize the ethanolic extract from *Coreopsis tinctoria* Nutt. dried fruits. The extract contains different flavonoids (marein, flavanomarein, quercetagetin-7-O-glucoside, okanin aurone, leptosidin, luteolin, apigenin) and phenolic acids (chlorogenic acid, caffeic acid). Several parameters that could affect extraction efficiency were evaluated. Finally, this study focused on determination of plant extracts total phenolic content and their antioxidant capacity. The experimental results allowed the selection of the optimum operating parameters leading to the highest total polyphenolic content, expressed as gallic acid equivalents, and avoiding the degradation of phenolic compounds (ethanol 55%; extraction temperature 323.15 K, extraction time 30 min, liquid/solid ratio 20/1). A good relationship between total phenolic content and antioxidant capacity was obtained.

Keywords: *Coreopsis tinctoria Nutt. fruits • Polyphenols • Ultrasound assisted extraction • Antioxidant capacity*

1. Introduction

A wide range of nutritional supplements composed from the constituents obtained by solid-liquid extraction from medicinal plants is available on the herbal medicinal products market in Romania. However, extracts from plants cultivated for ornamental purposes are much less studied, and particularly for the extracts obtained from species of the genus *Coreopsis* (Asteraceae).

Coreopsis tinctoria Nutt. is a native from North America. In Romania it is commonly cultivated for ornament in gardens [1]. From blossoms or the aerial part of the plant during flowering soluble pigments are extracted and used for coloring textiles [2]. North American Indians have used the plant to treat several disorders including diarrhea, internal pains, bleeding, to strengthen blood and as an emetic. *Coreopsis tinctoria*'s use has been referred in a traditional Chinese formula for diabetes since Yuan Dynasty. The infusion of *Coreopsis*

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tinctoria flowering tops has been used in Portugal to control diabetes [3-4].

In 2010 Dias *et al.* concluded that the flavonoid-rich fraction of *Coreopsis tinctoria* Nutt. promoted glucose tolerance regain through pancreatic function recovery in streptozotocin-induced glucose-intolerant rats, and the results suggested that glucose tolerance regain is due to all phenolic compounds present in *Coreopsis tinctoria* extracts and not only to the main flavonoid, marein [4].

There are several phytochemical studies on the genus *Coreopsis* [4-14], but information on the chemical composition of the species, is scarce. Flavonoids such as chalcones, aurones, flavones, flavanones were described in the flowers and leaves of *Coreopsis tinctoria* Nutt. but no information is available on the chemical composition of the extracts from the plant fruits, therefore, the present study is mainly focused on the analysis of the fruits of *Coreopsis tinctoria* Nutt. species.

In our previous paper [15], regarding techniques for extracting polyphenols from *Coreopsis tinctoria* Nutt. fruits, the experimental results proved that the use of ultrasound-assisted extraction is much simpler and more effective than maceration technique, refluxing procedure and Soxhlet extraction for separating polyphenolic compounds from *Coreopsis tinctoria* Nutt. fruits in ethanol. Comparison of these ethanol extraction techniques revealed that they produce extracts with different characteristics. We have 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, producing the highest concentration of polyphenols in dried extract, was ultrasound procedure.

In this paper, the total polyphenol content of samples obtained by ultrasound-assisted extraction from dried fruits of *Coreopsis tinctoria* Nutt. was determined. We have evaluated a number of parameters which may affect the efficiency of extraction, namely: temperature and extraction time and we have also studied the influence of the solid-liquid ratio using different quantities of plant (g) and the same volume (20 mL) of solvent. Finally, we have compared the obtained results using three different solvents for the ultrasound-assisted extraction.

Flavonoids from fruit extracts were identified by LC-MS/MS and their antioxidant capacity was evaluated using the ABTS and DPPH assays, both based on the SET mechanism (Single Electron Transfer). The relationship between total polyphenolic content of fruit extracts and their antioxidant activities was established. Determination of antioxidant capacity was achieved, using two long-lived free radicals, namely the $ABTS^{\bullet+}$ radical cation and the $DPPH^{\bullet}$ radical.

2. Experimental procedure

2.1. Chemicals and reagents

Plants of *Coreopsis tinctoria* Nutt. were cultivated in Bucharest and their fruits were collected at maturity. The plant material was air-dried in a thin layer at room temperature till constant mass and further stored in dark paper hermetic tight bags to protect them from humidity and light. *Coreopsis tinctoria* Nutt. plant was authenticated by botanists from Bucharest Botanical Garden based on available literature [16].

The solvents used for experiments developed to investigate solid–liquid extraction of phenolic compounds were 55%, 78% and 96% ethanol. 96% ethanol (obtained from ethanol 96% p.a. from a Romanian company: Chemical Company (with main impurities: CH_3COOH max. 0.005%, NH_3 max. 0.0003%, methanol max. 0.1%, aldehydes and ketones max. 0.002%, isopropyl alcohol max. 0.002%, Pb max. 0.0001%) and distilled water).

On the day of the experiment the other two solvents were prepared as follows:

- for 78% ethanol, 16.3 mL of 96% ethanol and 3.7 mL of distilled water
- for 55% ethanol, 11.5 mL of 96% ethanol and 8.5 mL of distilled water.

The commercial standards of flavonoids were purchased from Sigma.

Gallic acid (3,4,5-trihydroxybenzoic acid, provided from Sigma-Aldrich, >99%), 50 μg mL^{-1} solution was used as standard chemical for spectrophotometric analysis.

Folin-Ciocalteu's phenol reagent (lithium sulphate – 12.2%, disodium wolframate dihydrate - 2%, hydrocloric acid – 9.5%, phosphoric acid – 6.9%, bromine – 0.1%, water - 67%, disodium molybdate dihydrate - 2%, sodium thiosulphate – 0.3%, provided from Sigma-Aldrich), sodium carbonate 7.5% solution (Merck), were used as reagents in spectrophotometric analysis.

ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6 sulfonic acid) diammonium salt, provided from Fluka, >99%), DPPH (2,2-diphenyl-1-picryl-hydrazyl, provided from Sigma), Trolox ((±)-6-hydroxy-2,5,7,8-tetramethylchloramane-2-carboxylic acid, provided from Sigma-Aldrich) and ammonium persulfate (provided from Sigma, ≥98%) were used for the determination of the antioxidant capacity of the plant extracts.

2.2. Apparatus

Chromatographic separation was performed with a liquid chromatography system model Agilent 1260 (Waldbronn, Germany) coupled with a triple quadrupole mass spectrometer model API 3200 (AB Sciex, Toronto,

Ontario, Canada). The LC-MS/MS was controlled with Analyst 1.5.1. software.

All spectrophotometric measurements were performed on a Jasco V-650 (Tokyo, Japan) spectrophotometer.

2.3. Extraction procedure – ultrasound-assisted extraction

1.0 g of dried fruits of *Coreopsis tinctoria* Nutt. were extracted with 20 mL of solvent ethanol/water (55/45, 78/22 or 96/4, v/v) in an ultrasonic apparatus (Elmasonic S 80 H, frequency 60 Hz, power 750 W). The bath temperature was varied in different experiments, *i.e.*, 303.15 K, 323.15 K and 343.15 K. Extraction times of 15 min, 30 min and 60 min were tested. The solid-liquid ratio was also varied, namely 0.5 g, 1 g and 2 g of dried fruit of *Coreopsis tinctoria* Nutt. were extracted in 20 mL of solvent. All 21 extracts were filtered and concentrated until dryness.

2.4. LC-MS/MS analysis

Analyses were performed with an electrospray ionization source (ESI) operated in positive-ion mode. ESI conditions were the following: temperature 500°C, curtain gas at 25 psi, nebulizer gas at 50 psi, heated gas at 50 psi, positive ionization mode source voltage 5kV. Nitrogen was used as curtain and collision activated dissociation (CAD) gas, while nebulizer and heated gas were supplied with zero grade air. Full scan mass spectra were obtained by scanning the first quadrupole from m/z 80 to 700 Da. MS/MS spectra were collected from the selected precursor ions after fragmentation with a collision energy of 30 eV and CAD 5.

Compounds were separated on a Phenomenex Synergi Fusion RP column (50×2 mm, 4 µm). Water + 0.1% formic acid as eluent A and acetonitrile:methanol (1:1) + 0.1% formic acid as eluent B were used. The mobile phase was delivered at 0.5 mL min^{-1} in gradient mode: 0–0.5 min 10% B, 2.5–4 min 50% B, 4.5–6 min 95% B, 6.1–8 min 10% B. Some of of the compounds (chlorogenic acid, luteolin, caffeic acid and apigenin) were identified by comparing retention time (t_R), and Q1 m/z values and MS/MS spectra with the results of standards tested under the same conditions, other compounds were tentatively identified based on literature data and analyzing their fragmentation pathways.

2.5. Determination of the total polyphenol content using spectrophotometry

The total polyphenol content (TPC) was determined by spectrophotometry, according to the method described by the International Organization for Standardization (ISO) 14502-1 [17].

0.1-0.5 mL of gallic acid 50 µg/mL solution was introduced into test tubes followed by 2.5 mL of Folin-Ciocalteu's phenol reagent (1/10 dilution in water). The tubes have been allowed to stand for 5 min. Then, 2 mL of Na_2CO_3 solution (7.5%) and distilled water up to 5 mL have been added and a period of 30 min was allowed for stabilization of the blue color formed. The absorbance against a reagent blank has been measured at 760 nm. Five-point calibration curve using 1-5 µg mL^{-1} gallic acid as standard was linear (y = 0.0974x + 0.0923, where y = absorbance and x = concentration in ppm, R^2 = 0.9994) in the reaction mixture and the absorbance range up to 1.000 AU.

5 mL of ethanol (96%) were added over each dried extract. Aliquots of fruits extracts (0.2 mL) were used instead of gallic acid and the procedure described above was applied. The concentration of polyphenols in samples was estimated on a standard curve of gallic acid. Data were expressed as mg gallic acid equivalent (GAE) per g dried extract.

2.6. Free-radical-scavenging activity by DPPH assay

The reaction mixture contained ethanol (96%), 670 µL DPPH and 70 µL of test samples at a final volume of 2000 µL [18]. The control solution contained only 1330 µL ethanol (96%) and 670 µL DPPH. The stock solution contained 1260 µL ethanol (96%), 670 µL DPPH and 70 µL Trolox working solution. After 3 min of incubation [19] at room temperature, the absorbance was recorded at 516 nm. A Trolox standard was used (0.25 mmol) and the results were expressed as TEAC (Trolox Equivalent Antioxidant Capacity). The DPPH radical-scavenging activity was calculated according to the following equation:

$$TEAC_{sample} = C_{Trolox} \times f \times \frac{A_{sample} - A_{blank}}{A_{Trolox} - A_{blank}} \times \frac{1}{m} \qquad (1)$$

where A_{blank} represents the maximum absorbance of the blank solution, A_{Trolox} represents the maximum absorbance of Trolox stock solution, A_{sample} represents the absorbance of sample, f is the dilution factor of the sample, C_{Trolox} is the concentration of Trolox in µmol L^{-1}, and m is the amount of sample into the measuring cell in g.

Each analysis was performed in triplicate on samples coming from the same extract.

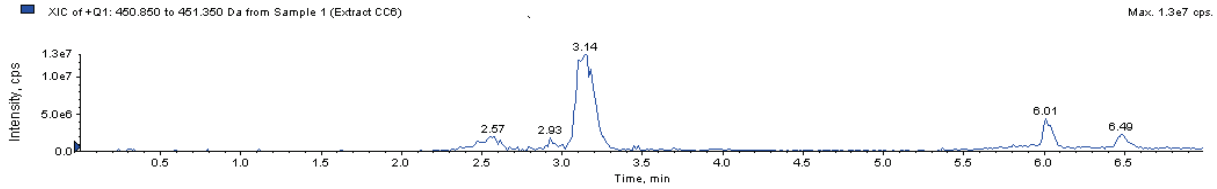


Figure 1. Extracted ion chromatogram for $[M+H]^+=451$, corresponding to marein/flavanomarein (full scan acquisition, 80-700 Da, ESI, positive).

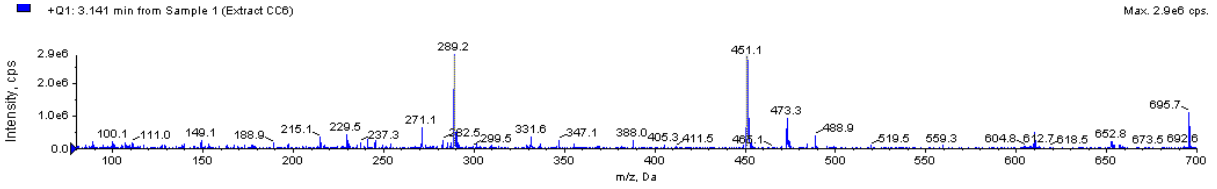


Figure 2. Full scan acquisition window showing the molecular ion $[M+H]^+=451$ at $t_R=3.14$ min.

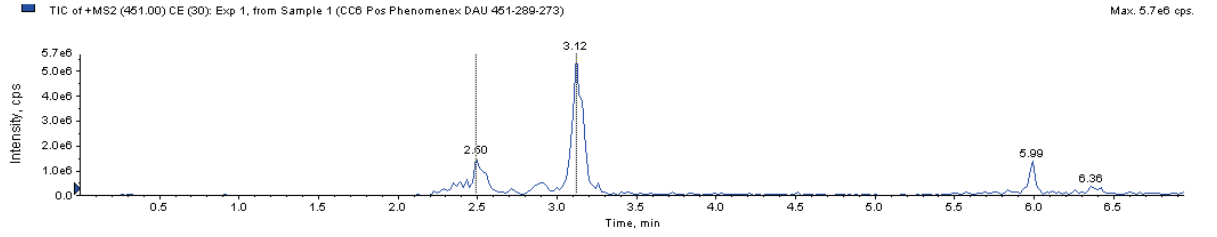


Figure 3. Chromatogram recorded for the fragmentation of ion $[M+H]^+=451$, at 30 eV collision energy (ESI, positive).

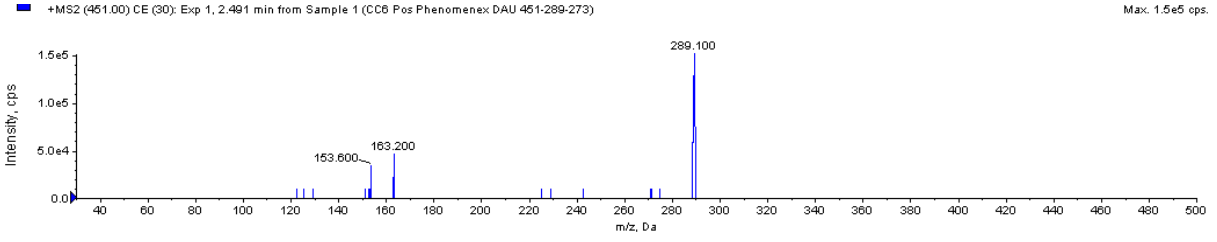


Figure 4. Mass spectrum of the compound with $t_R=2.49$, potentially flavanomarein (ESI, positive).

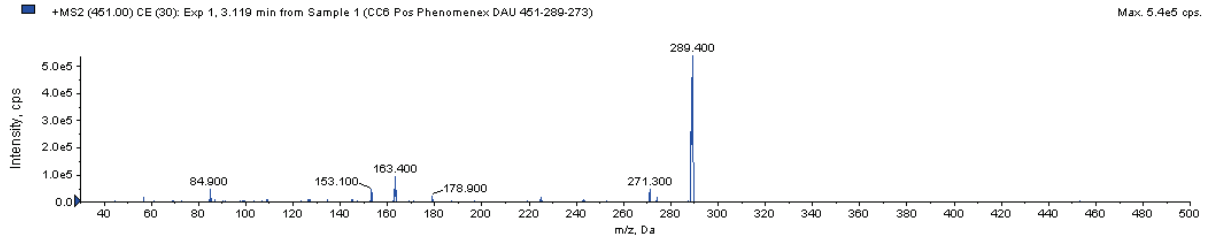
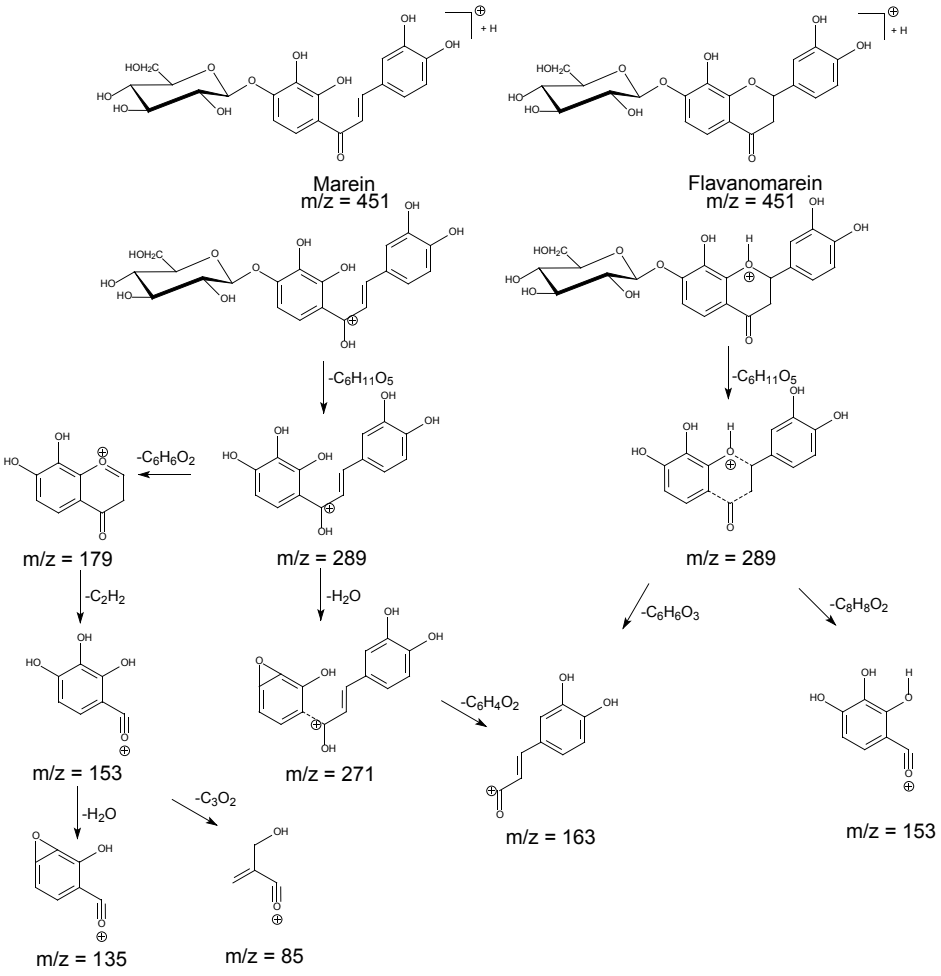


Figure 5. Mass spectrum of the compound with $t_R=3.12$, potentially marein (ESI, positive ion).

2.7. Scavenging Activity of ABTS Radical Cation

The ABTS radical cation ($ABTS^{\bullet+}$) scavenging activity was measured according to the methods described by Van den Berg *et al.* [20] and Re *et al*. [21] with some modifications. ABTS cations were produced by reacting ABTS stock solution (7 mmol) and ammonium persulfate (2.5 mmol). The mixture was incubated at room temperature in the dark for 12-16 h to allow completion of radical generation. 70 µL of test samples, 265 µL of distilled water were added to the ABTS working solution



Scheme 1. Proposed fragmentation pathway of flavanomarein (**2**) and marein (**3**).

to give a final volume of 2000 µL. The control solution contained only 2000 µL ABTS working solution. The stock solution contained 70 µL Trolox working solution, 265 µL of distilled water and 1665 µL ABTS working solution. After 3 min of incubation at room temperature, the absorbance was recorded at 731 nm and was expressed as TEAC (Trolox Equivalent Antioxidant Capacity) using Trolox as a standard [22,23]. The ABTS radical-scavenging activity was calculated according to Eq. 1.

Each analysis was performed in triplicate on samples coming from the same extract.

3. Results and discussion

3.1. LC-MS/MS analysis results

Compound identification was realized according to their molecular mass and existing literature data. Some of the compounds (chlorogenic acid, luteolin, caffeic acid and apigenin) were identified by comparing retention time (t_R), and Q1 m/z values and MS/MS spectra with the results of standards tested under the same conditions. Other compounds were tentatively identified based on literature data [4-14] and analyzing their fragmentation pathways.

The chromatograms and mass spectra of flavanomarein (2) and marein (3) are presented in Figs. 1-5; and their proposed fragmentation pathway in Scheme 1. A similar procedure was applied for each selected compound.

Qualitative data LC-MS/MS analyses are summarized in Table 1.

In the study by Dias *et al*. [4] on the identification of phenolic compounds in the ethyl acetate extract of *Coreopsis tinctoria* flowering tops by HPLC-DAD-ESI-MS/MS (ESI, negative), the flavonoids elution sequence was: chlorogenic acid < flavanomarein < flavanokanin < quercetagetin-7-*O*-glucoside < marein < okanin. The authors confirmed this order with commercial

Table 1. The results of qualitative LC-MS/MS analysis (ESI, positive ion) of the extract from dried fruits of *Coreopsis tinctoria* Nutt. by sonication with ethanol (30°C, 60 min, liquid-solid ratio 10/1).

No.	Proposed compound	Elemental Composition	Molecular weight	m/z +	Retention time, t_R (min)	Fragment
1	Quercetagetin-7-O-glucoside	$C_{21}H_{20}O_{13}$	480	481	2.92	319 $[MH-C_6H_{11}O_5]^+$
2	Flavanomarein	$C_{21}H_{22}O_{11}$	450	451	2.49	289.1 $[MH-C_6H_{11}O_5]^+$; 163.2 $[MH-C_6H_{11}O_5-C_6H_6O_3]^+$; 153.6 $[MH-C_6H_{11}O_5-C_8H_8O_2]^+$
3	Marein	$C_{21}H_{22}O_{11}$	450	451	3.12	289.4 $[MH-C_6H_{11}O_5]^+$; 271.3 $[MH-C_6H_{11}O_5-H_2O]^+$; 178.9 $[MH-C_6H_{11}O_5-C_6H_6O_2]^+$; 163.4 $[MH-C_6H_{11}O_5-H_2O-C_6H_4O_2]^+$; 153.1 $[MH-C_6H_{11}O_5-C_6H_6O_2-C_2H_2]^+$; 135 $[MH-C_6H_{11}O_5-C_6H_6O_2-C_2H_2-H_2O]^+$; 84.9 $[MH-C_6H_{11}O_5-C_6H_6O_2-C_2H_2-C_3O_2]^+$
4	Chlorogenic Acid *	$C_{16}H_{18}O_9$	354	355	2.12	163 $[MH-C_7H_{12}O_6]^+$; 145 $[MH-C_7H_{12}O_6-H_2O]^+$; 135.4 $[MH-C_7H_{12}O_6-CO]^+$
5	Leptosidin	$C_{16}H_{12}O_6$	300	301	5.76	165 $[MH-C_8H_7O_2]^+$
6	Okanin aurone	$C_{15}H_{10}O_6$	286	287	3.13	259 $[MH-CO]^+$; 231.2 $[MH-2CO]^+$; 213 $[MH-3CO]^+$; 185 $[MH-3CO-H_2O]^+$; 161.4 $[MH-3CO-H_2O-C_2H_2]^+$; 153.1 $[MH-C_8H_6O_2]^+$; 135 $[MH-C_8H_6O_2-H_2O]^+$
7	Luteolin*	$C_{15}H_{10}O_6$	286	287	3.75	269.5 $[MH-H_2O]^+$; 241.2 $[MH-H_2O-CO]^+$; 179 $[MH-H_2O-CO-C_5H_2]^+$; 161 $[MH-H_2O-CO-C_5H_2-H_2O]^+$; 153.3 $[MH-C_8H_6O_2]^+$
8	Apigenin*	$C_{15}H_{10}O_5$	270	271	4.02	253 $[MH-H_2O]^+$; 225 $[MH-H_2O-CO]^+$; 119 $[MH-H_2O-CO-C_6H_2O_2]^+$
9	Caffeic Acid*	$C_9H_8O_4$	180	181	3.96	163 $[MH-OH]^+$; 145 $[MH-OH-H_2O]^+$; 135 $[MH-OH-CO]^+$

** Standard was used to elucidate the compound*

standards or substances purified from plant extracts and characterized by NMR [4].

Taking into account the aforementioned study and the similar chromatographic conditions used in our research, we can consider that flavonoids elution sequence in the ethanolic extract obtained by sonication from dried fruits of *Coreopsis tinctoria* Nutt. was as follows: chlorogenic acid (t_R = 2.12 min) < flavanomarein (t_R = 2.49 min) < quercetagetin-7-*O*-glucoside (t_R = 2.92 min) < marein (t_R = 3.12 min) < okanin aurone (t_R = 3.13 min) < luteolin (t_R = 3.75 min) < caffeic acid (t_R = 3.96 min) < apigenin (t_R = 4.02 min) < leptosidin (t_R = 5.76 min).

The compounds with t_R = 6.01 min and t_R = 6.49 min were not identified.

3.2. Total polyphenols content (TPC) and free-radical scavenging activity of Coreopsis extracts against ABTS radical cation and DPPH radical

Figs. 6, 7 and 8 present the influence of different parameters (time, temperature and quantity of plant material) on the extraction process and antioxidant capacity using three ethanol-water solvent concentrations (55%, 78% and 96%). The best extraction yield was obtained using a 55% ethanol concentration whereas at 96% it provided the lowest extraction values. Thus, extraction of polyphenolic compounds from fruits of *Coreopsis tinctoria* Nutt. is strongly influenced by the nature of the solvent and the solute. In the almost all extraction experiments, the most efficient extractant seems to be 55% ethanol (except the samples extracted at 30°C).

The scavenging activity testing of *Coreopsis tinctoria* Nutt. fruits 96% ethanolic extracts against $ABTS^{\bullet+}$ cation radical did not afford any coloration. This behavior could be explained by the fact that the mentioned extract samples may have too low antioxidant content and consequently their concentrations are below the limit of quantification of the spectrophotometric method. This assumption is based on Re *et al*. report [21], showing that the high extinction coefficient of $ABTS^{\bullet+}$ (1.50×10^4 L mol^{-1} cm^{-1}) limits the antioxidant concentration range that can be accurately analyzed to about 1.5–70 μM final concentrations [24]. Antioxidant concentrations outside this range require too much or too little $ABTS^{\bullet+}$ for accurate optical measurements. For the reasons outlined above, the results of the ABTS assay in the case of extracts in 96% ethanol were not presented in Figs. 6-8.

The data presented in Fig. 6 showed that extracted polyphenolic compounds were thermolabile. In this study

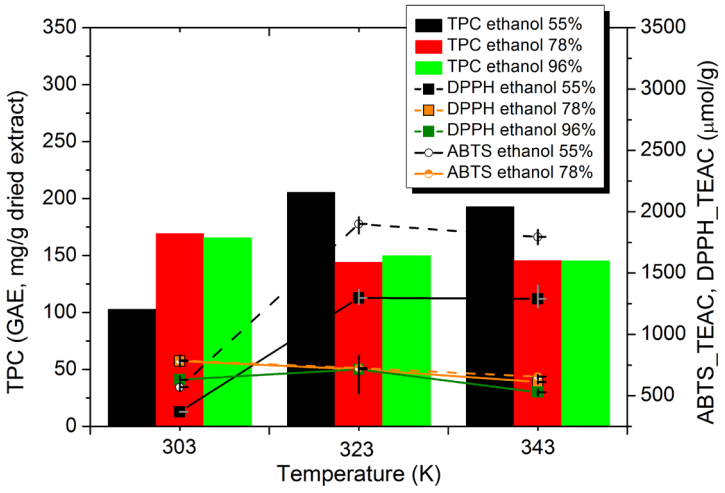


Figure 6. The influence of temperature on: a) the total polyphenolic content expressed as gallic acid equivalents (GAE, mg g^{-1} dried extract) vs. ethanol 55%, 78% and 96%; b) antioxidant capacity of the 55% and 78% ethanol extracts expressed as Trolox equivalents (TEAC, μmol g^{-1}) vs. ABTS and DPPH assays; Time: 60 min, Quantity of plant material: 1 g.

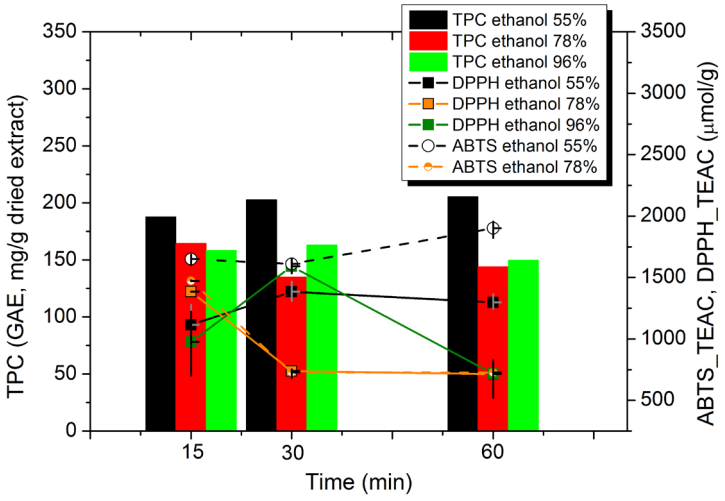


Figure 7. The influence of time on: a) the total polyphenolic content expressed as gallic acid equivalents (GAE, mg g^{-1} dried extract) vs. ethanol 55%, 78% and 96%; b) antioxidant capacity of the 55% and 78% ethanol extracts expressed as Trolox equivalents (TEAC, μmol g^{-1}) vs. ABTS and DPPH assays; Temperature: 323.15 K, Quantity of plant material: 1 g.

we have determined the total polyphenolic content by a spectrophotometric method, so there is no information on the decomposition of individual flavonoids identified by LC-MS/MS presented in Table 1.

By ultrasound-assisted extraction in ethanol 96% and 78% a decline of total polyphenolic content slowly occurs starting at 303.15 K, while in ethanol 55%, this phenomenon occurs after the temperature of 323.15 K. Furthermore, it can be seen that the use of 96% ethanol led to obtaining the smallest extracts (0.014 g – 0.046 g dried extract) and 78% ethanol use led to obtaining the greatest extracts (0.046 g – 0.104 g dried extract), which show that at the same time with the extraction of polyphenols, the extraction of unwanted compounds could be favored.

The 55% ethanolic extracts of *Coreopsis tinctoria* Nutt. fruits proved a good scavenging activity against the two radicals when the extracts were obtained in the temperature range 323.15 K – 343.15 K, the highest antioxidant capacity being measured for the 55% ethanolic extract at 323.15 K. The 78% ethanolic extracts of *Coreopsis tinctoria* Nutt. fruits demonstrated a lower

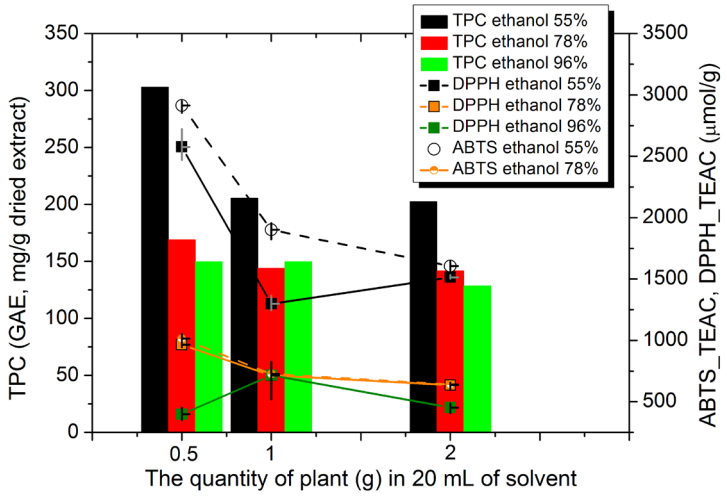


Figure 8. The influence of the quantity of plant (g) in 20 mL of solvent on: a) the total polyphenolic content expressed as gallic acid equivalents (GAE, mg g^{-1} dried extract) vs. ethanol 55%, 78% and 96%; b) antioxidant capacity of the 55% and 78% ethanol extracts expressed as Trolox equivalents (TEAC, μmol g^{-1}) vs. ABTS and DPPH assays; Time: 60 min, Temperature: 323.15 K.

Table 2. The results of correlation between TPC *vs.* DPPH, TPC *vs.* ABTS and ABTS vs. DPPH.

Correlation	Parameter influence	R^2
TPC *vs.* DPPH	Temperature*, 55% ethanol	0.9773
TPC *vs.* ABTS	Temperature*, 55% ethanol	0.9965
TPC *vs.* DPPH	The quantity of plant***, 55% ethanol	0.9317
TPC *vs.* DPPH	The quantity of plant***, 78% ethanol	0.9556
TPC *vs.* ABTS	The quantity of plant***, 55% ethanol	0.9280
TPC *vs.* ABTS	The quantity of plant***, 78% ethanol	0.9594
ABTS *vs.* DPPH	Temperature*, 55% ethanol	0.9916
ABTS *vs.* DPPH	Temperature*, 78% ethanol	0.9878
ABTS *vs.* DPPH	Time**, 78% ethanol	0.9986
ABTS *vs.* DPPH	The quantity of plant***, 78% ethanol	0.9999

** Temperature varied in the range 303.15-343.15 K*
*** Time varied in the range 15-60 min*
**** Quantity of plant material (g) in 20 mL of solvent: 0.5 g, 1 g, 2 g*
R^2 - correlation coefficient

anti-radical activity against the two tested radicals, and, in the same time, the anti-radical capacity decreases with increasing extraction temperature, possibly due to the thermal degradation of some active principles. The highest antioxidant capacity was measured to the extract obtained in 78% ethanol at 303.15 K. In the case of 96% ethanolic extracts the radical DPPH$^{\bullet}$ was scavenged by extracts obtained in the temperature range 303.15 K – 323.15 K, the highest antioxidant capacity was obtained at 323.15 K, but a further increase of the extraction temperature was not favorable.

Analyzing the data from Fig. 7, it can be considered that the optimum extraction time is 30 min when using ethanol 55% and ethanol 96%, while the optimum extraction time with ethanol 78% is 15 min. These data show that, at a temperature of 323.15 K thermal stability of extracted polyphenolic compounds depends on the type of solvent. The most suitable solvent is ethanol 55%, because using it the total polyphenolic content does not decrease with increasing extraction time.

The data presented in Fig. 8 showed a decrease in total polyphenolic content when doubling the plant amount (from 0.5 to 1 g) extracted in 20 mL of solvent, in turn further increase of the plant amount (g) in 20 mL of solvent, even a doubling of it from 1g to 2g does not have significant effects. Thus, increasing the liquid-solid ratio significantly increased the total polyphenolic content extracted in ethanol 55% and lead to a much slower increase for ethanol 78% and 96% solvents.

The highest antioxidant capacity against the two radicals was obtained when 55% ethanol was used to extract 0.5 g of plant material in 20 mL of solvent. The antioxidant capacity decreased significantly for 78% and 96% ethanol extracts. In the case of 96% ethanolic extracts, the highest antioxidant capacity against the radical DPPH$^{\bullet}$ was obtained from 1 g of plant material extracted in 20 mL of solvent.

From the data presented in Figs. 6-8 it can be seen that higher values were obtained in the case of ABTS assay compared to the DPPH assay (a possible explanation could be that DPPH does not react with

flavonoids without substituted OH in the B-ring or with aromatic acids with a single OH group, and ABTS does not discriminate between phenolic OH, providing a response related to total groups able to quench a radical reaction [25]), so that the extracts have a more prominent antioxidant capacity against $ABTS^{\bullet+}$ radical cation compared to that given against the radical $DPPH^{\bullet}$.

3.3. Correlation between TPC *vs.* DPPH, TPC *vs.* ABTS and ABTS vs. DPPH

We noted a good relationship between TPC and DPPH, TPC and ABTS, ABTS and DPPH values obtained for extract samples resulted in different working conditions (see high values of correlation coefficient in Table 2).

4. Conclusions

The experimental results we have obtained in the extraction of polyphenolic compounds from dried fruits of *Coreopsis tinctoria* Nutt. allowed the selection of the most appropriate extraction solvent (ethanol 55%), and to establish the operating parameters, leading to a high total polyphenolic content expressed as gallic acid equivalents (GAE, mg g^{-1} dried extract). The degradation of phenolic compounds was avoided (extraction temperature 323.15 K, optimum extraction time 30 min, liquid-solid ratio of 20/1).

The antioxidant capacity testing of the extracts using ABTS and DPPH assays revealed a more prominent antioxidant capacity against radical cation $ABTS^{\bullet+}$ than on the radical $DPPH^{\bullet}$. We noted a good relationship between TPC *vs*. DPPH, TPC *vs*. ABTS and ABTS *vs*. DPPH values obtained for extract samples resulted in different experimental conditions.

Taking into account that *Coreopsis tinctoria* Nutt. is very little studied for its phytotherapeuthical properties and in Romania is commonly cultivated for ornament in gardens, the results reported in this paper indicate that *Coreopsis tinctoria* Nutt. dried fruits are important sources of antioxidant compounds and can be considered as a potential raw material in pharmaceutical or nutraceutical manufacturing.

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