ORIGINAL PAPER

FITOCHEMICAL AND ANTIOXIDANT CHARACTERIZATION OF AUTUMN CROCUS (*COLCHICUM AUTUMNALE*) FLOWERS AND ROOTS PLANT EXTRACTS

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Abstract. *The present paper describes the important components of the Autumn crocus (Colchicum autumnale) plant studied. The aim of our study was to characterize antioxidant activity, protein, carbohydrate and phytochemical compounds determinations (polyphenols, tannins, flavonoids, terpenoids) of the hidroalcoolic extract obtained using autumn crocus (Colchicum autumnale) plant. The samples (flowers and roots) were analyzed by UV–VIS, FTIR and RAMAN spectroscopy. Antioxidant activity of the extracts was evaluated using DPPH method.*

Keywords: *Colchicum autumnale, phytocompounds, antioxidant activity.*

1. INTRODUCTION

From ancient times, plants have been used intuitive for medicinal purposes. Many of them show high antioxidant activity and are used to treat various diseases [1]. Despite the major and important discovers applied in modern medicine in recent period, plants still make an important contribution to health care. Therefore, a large number of plants have been investigated and various spices have been reported to exhibit antioxidant activity [2].

Autumn crocus (*Colchicum autumnale*), belong to the Liliaceae family, is a plant which contain a natural alkaloid, colchicine ($C_{22}H_{25}NO_6$). It was used in the past for the treatment of diverse medical causes, like gout or rheumatism. It is a very toxic plant when it is used for homemade preparations [3, 4]. The purple flowers, develop from underground bulbs, in autumn, and are usually the most visible feature of this plant. The young leaves appear in the spring but die back before flowering [3]. Now, it is used in all world for breeding studies and as drug to treat diseases like gout, familial Mediterranean fever and primary biliary cirrhosis [5]. Also, colchicine is was detected to be a potent anticancer agent but its medical application in cancer chemotherapy is limited because of its relatively high toxicity [6].

The overall alkaloid concentration of the autumn crocus it is found between 0.1%- 2%. The highest colchicine level has been detected in flowers and seeds, but all parts of the plant

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are toxic. The alkaloid content fluctuates from 0.5% to 1.2% in seeds, 1.2% to 2% in fresh flowers, 0.15% to 0.4% in fresh leaves and 0.1% to 0.6% in the underground bulbs [3].

Colchicine, (fig. 1), is a highly poisonous water-soluble alkaloid, extracted from plants of the genus Colchicum. Despite her poison capacity, colchicine has approved by the Food and Drug Administration (FDA) for the treatment of dangerous diseases, lige gout, Familial Mediterranean fever (FMF), secondary amyloidosis (AA) or Scleroderma [7].

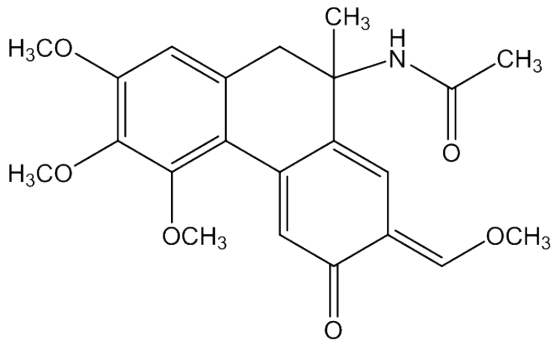

Figure 1. Structure of colchicine.

2. MATERIALS AND METHODS

2.1. MATERIALS

Autumn crocus (*Colchicum autumnale*) was collected from a Romanian forest, near Targoviste city. All solvents used for extraction were of analytical grade. Methanol was purchased from Merck. Distilled water was internal laboratory obtained, using Liston equipment. Standard substances utilised for curve calibration of methods were linalool (Merck), vanillin (Scharlau), gallic acid (Merck), catechin (Sigma-Aldrich). For determinations of phytochemical methods it was used also aluminium chloride (AlCl3 from Sigma-Aldrich), $NaNO_2$, NaOH, H_2SO_4, Na_2CO_3, HCl and Folin–Ciocalteu reagent (Merck). Benedict and Millon reagents, utilized for carbohydrate and protein determination, were bought from Sigma-Aldrich. Also, 2,2-diphenyl-1- picryl-hydrazyl-hydrate stable free radical, DPPH, used for antioxidant determination was purchased from Merck.

Figure 2. Autumn crocus *(*flowers and roots*)* plant.

2.2. METHODS

The Autumn crocus (Fig. 2) flower and root (bulb) were exhaustively extracted separately in a mixed solution (methanol: distilled water) for 24 hours, were filtered and the macerates were kept in a brown volumetric dark flask in order to avoid degradation.

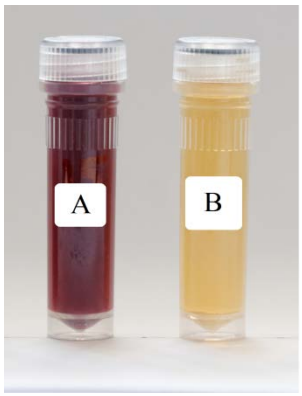

Figure 3. Colour of Autumn crocus (*Colchicum autumnale*) extracts: A) flowers and B) roots

2.2.2. Characterization Methods

UV-VIS Spectroscopy

The absorption spectra of the samples were recorded on a double beam M400 Carl Zeiss Jena UV-VIS spectrophotometer from 250 to 550 nm, at the resolution of 1 nm, with 1 nm slit width and 0.3 nm/s scan rate.

FTIR Spectroscopy

For Fourier transformed IR spectroscopy, the spectra were collected using a Perkin Elmer Spectrum GX instrument. Scans in the range of 400–4000 cm^{-1} were accumulated for each spectrum at a spectral resolution of 4 cm^{-1}.

RAMAN spectroscopy it was used a portable Raman spectrometer IR in two lengths Xantus-2 (utilizes integrated software combining open architecture with customizable, user defined settings for optimized sampling parameters that result in comprehensive and actionable data analysis) – Rigaku, with options of 785 and 1064 nm stabilized laser, providing high sensitivity. This device does not require special preparation of samples; liquid and solid samples can be analyzed.

Antioxidant activity (AA%)

The principle of AA % method consists in reducing in the presence of an antioxidant molecule, giving rise to colored methanol solutions. The utilization of DPPH method gives an easy and rapid result to antioxidant activity against free radicals [8-10].

Phytochemical Analyses. The phytochemical quantification procedures were used for the determination of total tannins, total flavonoids, total pholyphenols and total terpenoids existent in the autumn crocus plant. The assays are presented in Table 1.

Table 1. Phytochemical assays effectuated for Autumn crocus (Colchicum autumnale) plant

Assay	Reagents	Conditions	Monitoring system and standard curve	Reference
Total tannins	0.5 mL extract + 3 mL 4% vanillin:MeOH + 1,5 mL HCl	15 minutes incubation at room temperature	Absorbance = 500 nm; Catechin curve calibration standard	[8]
Total flavonoids	1 mL extract + 4 mL distilled water + 0,3 mL $NaNO_2$ (5%); After 5 min: 0,3 mL$AlCl_3$(10%) After 5 min: 2 mL 1M NaOH + 2,4 mL distilled water	30 minutes incubation at room temperature	Absorbance = 510 nm; Catechin curve calibration standard	
Total polyphenols	1 mL diluted extract + 5 mL Folin Ciocalteau reagent; After 8 min: 4 mL Na_2CO_3	60 min incubation at room temperature	Absorbance = 765 nm; Gallic acid curve calibration standard	
Total terpenoids	2 mL extract + 1 mL 2% vanillin:H_2SO_4	heated at 60ºC/20 min; cooled at 25ºC/5 min	Absorbance = 608 nm; Linalool curve calibration standard	

Carbohydrate and protein analyses:

The methods of carbohydrate and protein determination are presented in Table 2.

Table 2. Carbohydrate and protein methods performed on the Autumn crocus *(Colchicum autumnale)* flower and root extracts

Phytocompound name	Reagent	Test method	Precipitate colour	Reference
Carbohydrate	Benedict	1 ml extract + 5 ml Benedict reagent; 5 min boiling	Green-blue colour	[11]
Protein	Millon	1 ml extract + 5-6 pic. Reactiv Millon; heating	White precipitate which changes its color in red by heating	

3. RESULTS AND DISCUSSION

The components and phytosynthesis of extracts were confirmed by modern analytical techniques (UV-VIS, FTIR, RAMAN).

UV-VIS results

UV-visible spectroscopy was the first analyze of the Autumn crocus plant. The UV-Vis analysis (fig. 4) presented the UV-VIS spectra obtained for the flower and root hidroalcoolic extracts. The wavelength spectrum was made between 250-550 nm. It was identified the maxima wavelengths specific colchicines at 350-355 nm, most evident at root extract.

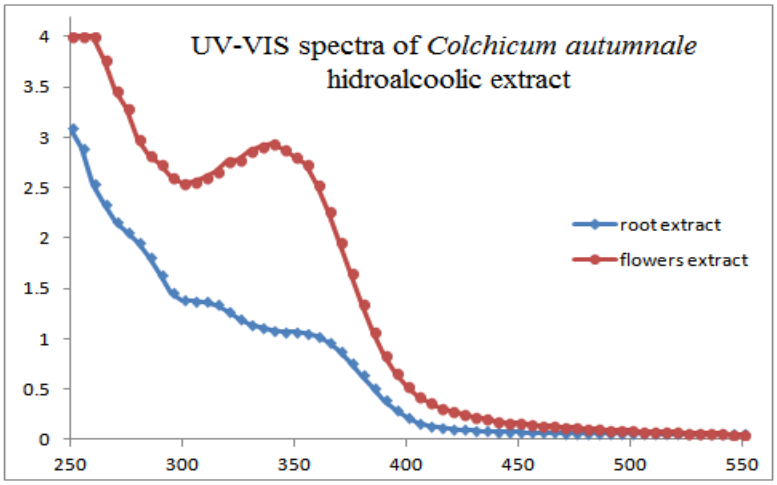

Figure 4. The UV–VIS absorption spectra of Autumn crocus (*Colchicum autumnale)* flower and root extracts.

At flower extract, are observed in the 300-350 nm are, the peaks which correspond to phenolic acids, more than in root extract. On root extract spectrum, at 280 nm it is identified the specific wavelength for phenolics acids and between 300-320 nm are found flavonoids and colchicine [4, 12].

For preparation of the calibration curve, it was used colchicine freshly extract obtained in our lab, from *Colchicum autumnale* plant. It was prepared standard solutions of different concentrations: 1.35; 0.945; 0.725 and 0.495 ml 10^{-4} M (Table 3). The regression equation for the calibration curve was y = 1.8223x +0.1132 and a good result for R^2 = 0.9943 (Fig. 5).

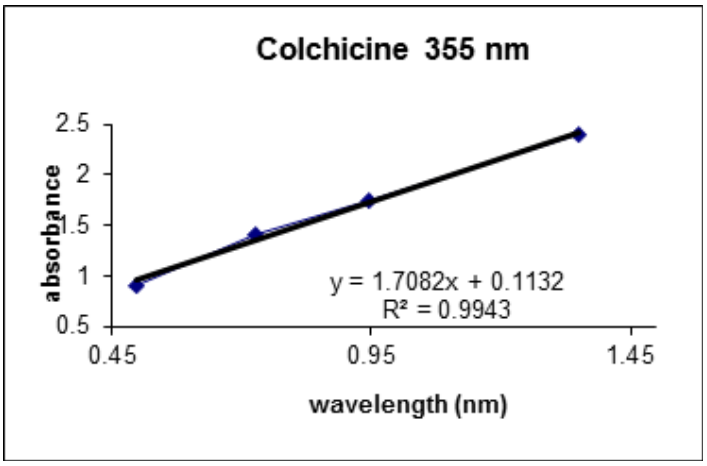

Figure 5. Calibration curve of colchicine.

Table 3. Calibration data of colchicine

Cx10^{-4} (M)	A=355 nm
1.35	2.395
0.945	1.744
0.725	1.4093
0.495	0.9087

FTIR results

In order to obtain FTIR spectrum of Autumn crocus extract samples was recorded in the region 300 - 5000 cm^{-1} (Fig. 6). FTIR spectra have been presented OH, H_2O picks at 3350 cm^{-1}; at 2935 cm^{-1} are found CH groups; 1598 cm^{-1} it is associated to amides I, carboxyl and C=C bounds from alchenes and aromatics groups, at 1397 cm^{-1} methyl groups, C-O bonds appear at 1238 cm^{-1}. At 1078 cm^{-1} are found bands of aromatic rings or CH_2, OH and C-O aliphatic groups [1, 13].

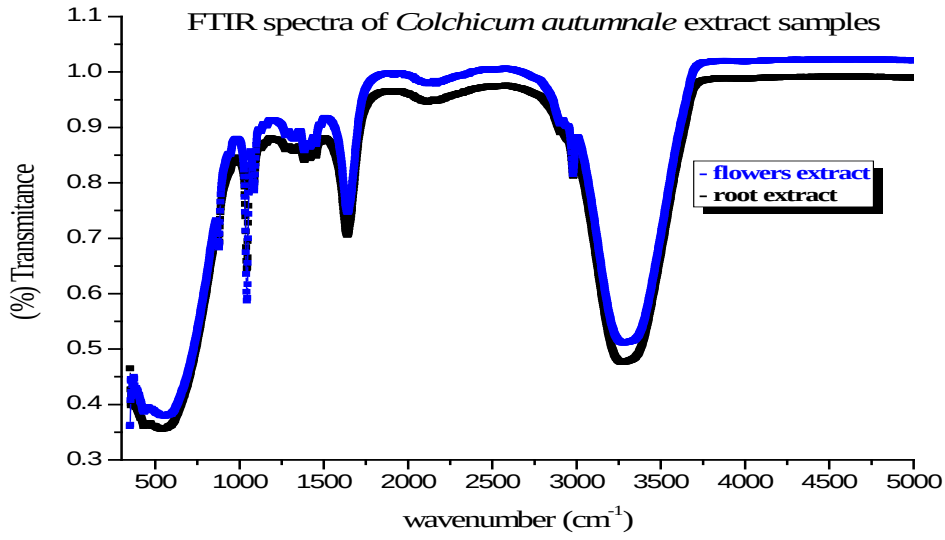

Figure 6. FTIR spectra of flowers and roots Autumn crocus *(Colchicum autumnale)*.

Characteristic for colchicine: The C=O stretching vibrations are observed at 1660 and 1610 cm^{-1}. The N–H stretching vibrations occur in the region 3500-3000 cm^{-1}. The hetero aromatic structure shows the presence of C–H stretching vibrations in the region 3000-3100 cm^{-1}, which is the characteristic region for the ready identification of the C–H stretching vibration. The asymmetric CH_2 stretching vibrations are generally observed in the region 3100-3000 cm^{-1}, while the symmetric stretching vibrations are generally observed between 3000 and 2900 cm^{-1}. The C–C aromatic stretching bands known as semi-circle stretching were found at 1500-1700 cm^{-1}. The asymmetric CH_3 stretching vibrations are at 2900-3000 cm^{-1}. C–N stretching vibrations: 1100-1230 cm^{-1}. C–O stretching vibrations are at 915-1300 [1, 11, 13].

RAMAN results

RAMAN spectra of Autumn crocus flower and root extracts samples were recorded in the region 200-2000 cm^{-1} (fig. 7). At 1060-1127 cm^{-1} are found C-C groups. Aromatic rings are situated at the 860-100 cm^{-1} region. The Raman spectra were characterized by a very strong CH_2 stretching vibration signal around 2840-2950 cm^{-1}. Samples contain peak at 1710 cm^{-1} that has been assigned to the C=O. CH_3 group is found in the 1375-1410 cm^{-1} zone [11].

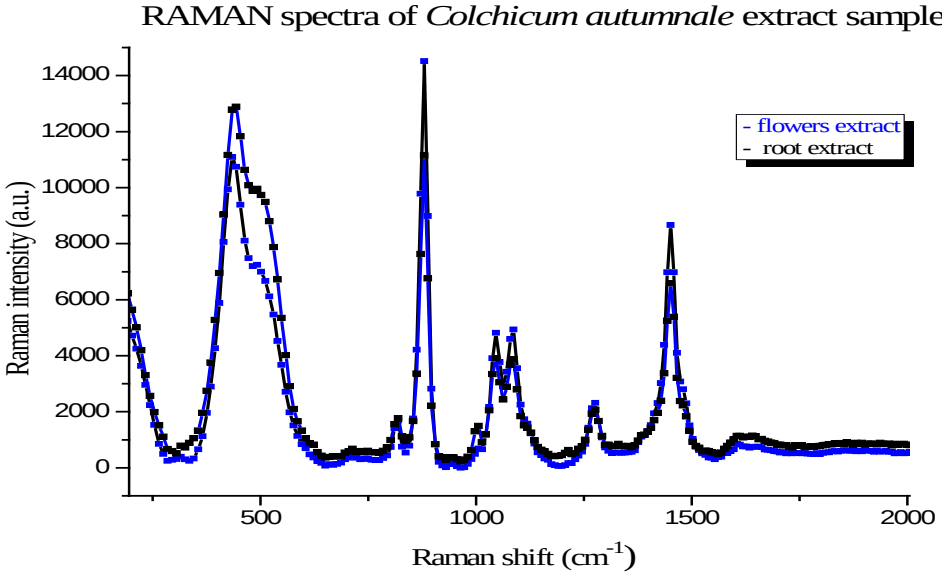

Figure 7. RAMAN spectra of Autumn crocus (*Colchicum autumnale*) flowers and roots extracts.

Antioxidant activity results

In order to establish the antioxidant activity of the studied samples, their inhibitory effect against free radicals was evaluated using the DPPH method [8]. DPPH solution, a violet solution, was prepared in that day in the laboratory (0.02 mg DPPH/mL MeOH). The final solutions consisted of mixing 0,5 ml extract sample + 1 mL of DPPH freshly prepared solution, which were agitated a few minutes and then left in the dark for an hour. The mixtures were tested by reading the absorbance λ=517 nm at UV-VIS Specord M 400 Carl Zeiss Jena spectrophotometer. As a blank, the same steps as in the preparation of samples were followed, a mixed solution prepared between 0.5 mL MeOH + 1 mL of 0.02 mg/mL DPPH solution it was measured at the same wavelength, then agitated a few minutes and left in the dark for an hour.

The antioxidant activity (AA%) percentage was calculated using the formula [8]:

$$AA\% = [(A_{Control} - A_{Sample} / A_{Control}] \times 100$$

where: $A_{Control}$ is the absorbance of a DPPH solution without sample, A_{Sample} is the absorbance of the sample mixed with 0.02 mg/mL DPPH freshly prepared solution.

Table 3. Antioxidant activity results for Autumn crocus flowers and roots extracts

Sample	AA%
Autumn crocus flowers extract	52,81 %
Autumn crocus roots (bulb) extract	34,60 %

Carbohydrate and protein results are presented in table 4. It is observed that carbohydrate and protein are not present in the hidroalcoolic extracts samples studied.

Table 4. Carbohydrate and protein results for Autumn crocus flowers and roots extracts

Sample name	Carbohydrate			Protein		
	Colour at cold temperature	Colour at hot temperature	Result	Colour at cold temperature	Colour at hot temperature	Result
Autumn crocus flower extract	Emerald green solution	Red brick prepipitate;	-	Bei suspension	Bei suspension	-
Autumn crocus root extract	Emerald green solution	Mustard suspension	-	White-yellow suspension	Bei suspension	-

Phytochemicals results are detailed in the table 5. It was observed the flower extract contain a major phytocompounds than root sample.

Table 5. Phytochemicals results for Autumn crocus flowers and roots extracts

Phytocompound determination	Autumn crocus flower extract (mg/L)	Autumn crocus root (bulb) extract (mg/)L
Flavonoides	100.1	82
Tannins	86.4	66.3
Terpenoides	245.76	227.057
Polyphenols	177.635	138.728

4. CONCLUSIONS

Colchicum autumnale extract was used to. The phytosynthesis of Autumn crocus extract was confirmed by spectral studies (UV-VIS, FTIR and RAMAN) that revealing the presence of colchicine, polyphenols and flavonoids in the plant extract studied. The second step, was to determinate total flavonoids, polyphenols, terpenoids, tannins, carbohydrate, protein and antioxidant activity from Autumn crocus (*Colchicum autumnale*) flower and root extracts, an indigene plant picked from a wood near Targoviste area. The existence of phenolic compounds in the snake fruit was confirmed by the Folin-Ciocalteu method. ATR-FTIR and RAMAN results demonstrated the major amount of colchicines, alkaloids but it was found also polyphenols and flavonoids. The antioxidant capacity was measured by the free radical scavenging methods DPPH and the autumn crocus solution samples showed good antioxidant capacity at flowers = 52,81% than roots = 34,60 %. All results of phytochemical analyses were made in triplicate and calculated using results of calibration curves, with very good regression indices.

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