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Determination of Flavonoid and Polyphenol Compounds in *Viscum Album* and *Allium Sativum* Extracts

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ABSTRACT
Ethnopharmacology is a new interdisciplinary science that appeared in Europe of the '90, in France, as a necessity claimed by the return to the traditional remedies of each nation. The aim of this study is to identify and quantify the active ingredients of the species *Viscum album* and *Allium sativum*, in order to provide a complex chemical characterisation, which is necessary for the use of these plants' extracts as natural ingredients in the pharmaceutical industry. The following methods were used: (1) the plant material was harvested from the west-side of Romania (Europe) in July 2014; (2) it was dried quickly and the main active principles were extracted using ethylic alcohol solution (50%); (3) the quantitative analyses of the flavonoids and polyphenols were performed according to a procedure described in the Romanian Pharmacopoeia. FT-IR results showed that the *Viscum album* extract had the highest content of polyphenolic compounds, for both flavonoids and polyphenols. This is the reason why it can be concluded that alcoholic extracts of mistletoe must be used as supplements for diabetics who require diets with flavonoids or for patients with cancers, degenerative diseases, and particularly cardiovascular diseases, who need an increased amount of polyphenols.

***Key Words*:** garlic, mistletoe, rutin, catechol, cinarin, FTIR.

INTRODUCTION

The mistletoe (*Viscum album*), belonging to the *Loranthaceaeste* family, is a semiparazit herbaceous plant, which grows on the stems of the host plant, having the appearance of branchy bushes, with stems reaching a length that varies between 30 and 100 cm, thickened at nodes, where it breaks easily. The leaves are arranged in opposite pairs, the flowers have yellow-greenish coloration, while the fruits are hyaline, with many seeds, green in the incipient stage of development and whitish when reaching maturity. The fruits usually appear from October to December. This plant parasites the birch, the fir, the ash, the rose, the apple and the wild pear trees. The young branches with leaves are used for pharmaceutical purposes. Harvesting is possible all year round, however specialists recommend the cold season for this activity. Its most important chemical compounds are: triterpenic saponins, flavonoids, acetylcholine, viscin, viscitoxin, mineral substances, amino acids, C, E vitamins and mucilage (Bujor, 2003; Vicas *et al.*, 2009; Zuber, 2004).

The garlic (*Allium sativum*), belonging to the *Liliaceae* family, is a plant which has been grown for more than 5000 years for both culinary and therapeutic purposes. The part used for its medicinal properties is the bulb, which is formed of several cloves, wrapped in individual membrane. Its best known chemical compound is allicin, which has an extensive pharmacological action. The allicin has hypotensive, antimicrobial, antitumoral and immunomodulatory properties (Thomas and Ali, 2003; Bayan *et al.*, 2014).

Having as starting point the present knowledge, the aim of the article hereby is to provide a qualitative and quantitative characterization of the flavonoid content of the two plants.

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MATERIALS AND METHODS

Plant material

The plant material used for extraction consisted of leaves of *Viscum album*, which parasite the pear tree and home-grown bulbs of *Alium sativum*. The plants were harvested from Groseni village (Arad County, Romania) in July 2014. The harvested material was dried quickly at a temperature of 90°C, for 48 hours. It was deposited under the shade, at a temperature of 20°C (Markham, 1982; Meriga *et al.*, 2012).

Reagents

Rutin and catechol, used as standard samples, were purchased from Sigma-Aldrich. All the other reagents used for this research were at analytical grade purity.

Plant extraction

In order to obtain the alcohol extract, 1g plant material (*Viscum album* and *Allium sativum*), was pulverized and subjected to extraction with 100 mL ethylic alcohol (50%), under reflux on a water bath for 30 minutes. The boiling solution was filtered through cotton in a graded flask, and after cooling down it was completed to 100 mL, by washing the residue with the same solvent (solution A – EV1, EU1).

Determination of flavonoids content

The quantitative analysis of the flavonoids was performed according to the method presented in the Romanian Pharmacopoeia (F.R. X, 1993), using ethanol rutin as the standard substance.

10 mL solution A was diluted with methanol (R) to 25 mL in a graded flask. After stirring, the solution was rested for 10 minutes. Afterwards, the solution was filtered, removing the first fractions of the filtrate. 0.5 mL filtrate was mixed with 5 mL of sodium acetate 100g/L (R) and 3 mL of aluminium chloride 25 g/L; after a preliminary stirring it was enriched with methanol (R) to 25 mL, in a graded flask (sample solution). After 15 minutes, it was enriched once more with methanol (R) to 25 mL and

the absorption of the solution was determined at 430 nm, by using a solution which had been obtained under similar circumstances as the sample solution as compensation liquid. The new solution was obtained from 5 mL filtrate, 8 mL water and methanol (R) to 25 mL, in a graded flask.

The flavonoids content of the extracts was determined from the standard calibration curve for rutin, and it was expressed in rutin equivalents or mg rutin/mL extract. The standard calibration curve for rutin was obtained by using a rutin solution, having a concentration that varied between 0.01 and 0.07 mg rutin/mL.

Determination of polyphenols content

The quantitative analysis of the polyphenols was made using as standard substance, the piro-catechol, cinarin respectively (F.R. X, 1993) and the standard reactive (R) as they are presented in Pharmacopoeia.

The research was carried out with the same plant material, which was presented in the previous paragraphs.

In order to obtain the alcoholic extract, 1 g plant material was subjected to extraction with 100 mL ethylic alcohol (50%), under reflux on water bath for 30 minutes. The boiling solution was filtered through cotton in a graded flask, and after cooling down it was completed to 100 mL by washing the residue with the same solvent (solution A – EV1, EV2).

Determination of total polyphenols content

5.0 mL solution A was added to 5 mL of phosphotungstic acid, solution (R). After stirring, it was filtered, thus removing the first fractions of the filtrate. Then, 5.0 mL filtrate was diluted with sodium carbonate (R) 200 g/l for 10 mL, in a graded flask (sample solution). After 1 minute, the absorption of the solution was determined at 660 nm, by using a solution, which was made of 0.5 mL of filtrate and brought with water to 10 mL, in a graded flask, as compensation liquid.

The content of polyphenol compounds of the extracts was determined by using the standard calibration curve for catechol, and it was expressed in mg catechol/mL extract. The standard calibration curve was obtained by using a solution with a concentration varying between 0.001 and 0.005 mg/mL catechol.

Identification of flavonoid debris by FTIR spectroscopy

The FTIR spectra for the extracts were determined with a Spectrum Two FTIR Spectrometer (Perkin Elmer). The FTIR spectroscopy highlighted valance vibrations ν C=O, through strips having between 1630 and 1665cm^{-1}, which are typical for the flavonoids structure; valance vibrations ν C-O through strips having between 1000 and 1300cm^{-1}, and in-plane deformation vibrations, δ C-H through strips having between 600 and 980cm^{-1}.

Compliance with ethics requirements

Authors declare that they have no conflict of interest and there were not any procedures involving animal or human subjects in this research.

RESULTS AND DISCUSSION

Determination of flavonoids content

The flavonoids content of the *Viscum album* and *Allium sativum* extracts was expressed as rutin equivalent (standard equation of the curve: y=0.626x+0.024; R^2= 0.933), as it can be seen in table 1.

The calibration curve for rutin is shown in figure 1, and the flavonoids content for each plant material analysed in this study is shown in table 1.

The flavonoid content for *Viscum album* extract is equal to 0.4245 mg rutin/mL extract, and for *Allium sativum* extract is equal to 0.1823 mg rutin/mL. The highest flavonoids content was identified in the *Viscum album* extract.

Table 1: Total flavonoid content expressed in rutin.

Extract	Absorption, 430 nm	Concentration mg rutin/mL extract	Flavonoids g% (rutin, %)
EV1	0.389	0.4245	1.6980
EU1	0.167	0.1823	0.7292

Table 2: Polyphenols content of the pirochatechina type of the analysed extracts.

Extract	Absorption, 660 nm	Total polyphenols content (mg catechol)	%Polyphenol compounds
EV1	0.989	0.0475	23.75
EU1	0.665	0.0336	16.80

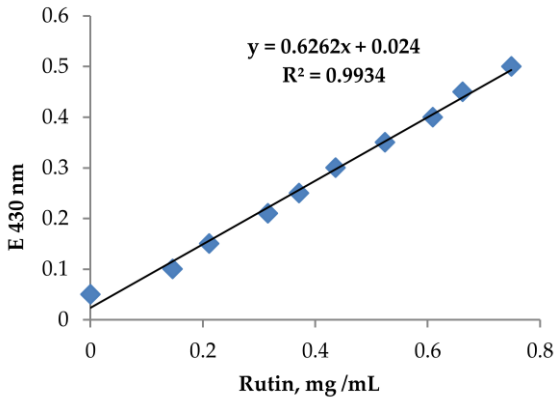

Figure 1: Standard calibration curve for rutin.

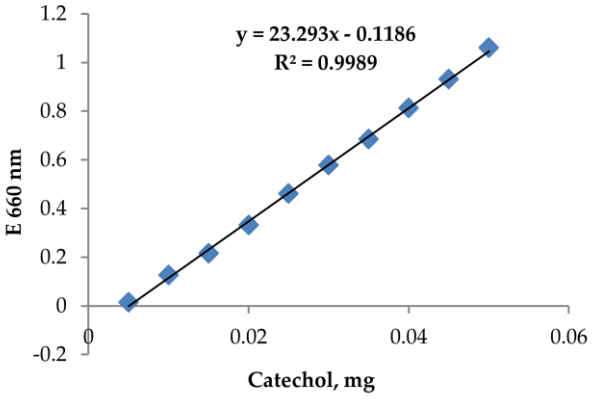

Figure 2: Calibration curve for catechol.

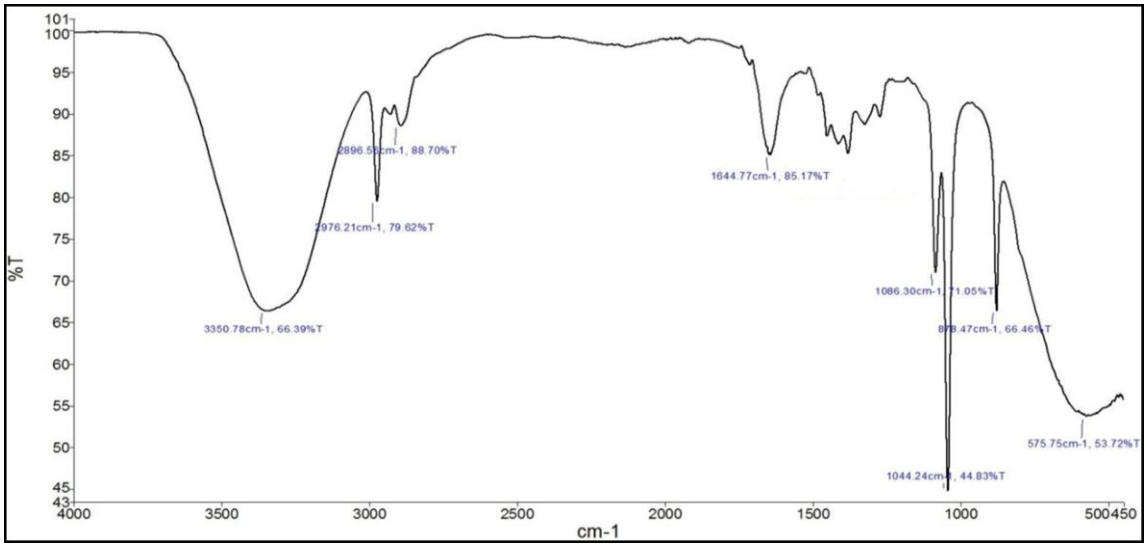

Figure 3: FTIR Spectrum for the Viscum album extract (EV1).

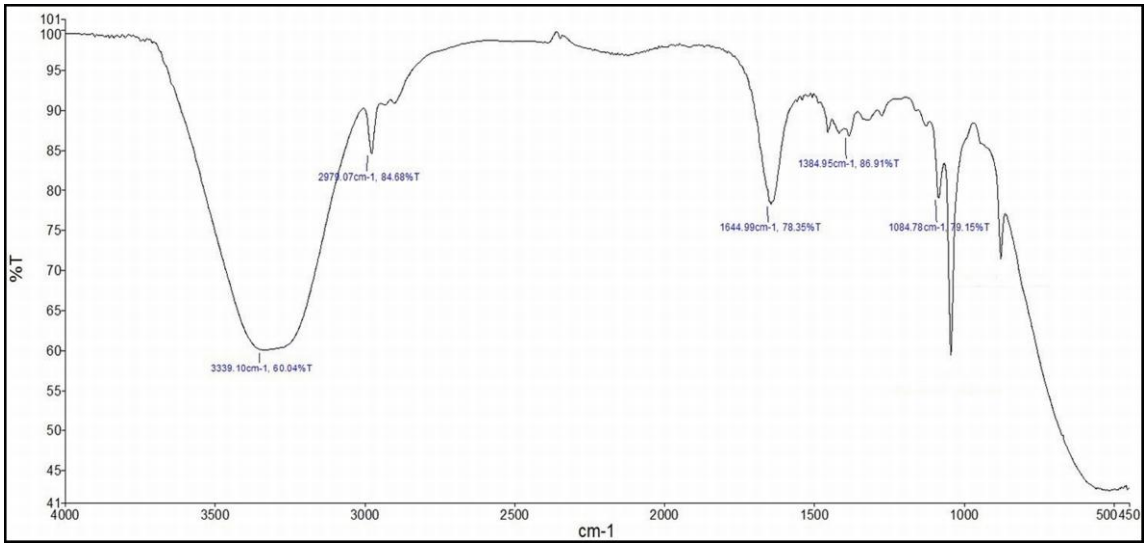

Figure 4: The FTIR Spectrum for the Allium sativum extract (EU1).

Determination of polyphenols content

The polyphenols content was expressed as catechol equivalent (standard equation of a line: y = 23.29x-0.018; R^2 = 0.998). The calibration curve obtained for catechol is shown in figure 2, and polyphenols content obtained for each extract is shown in table 2.

The polyphenols content for *Viscum album* extract is equal to 0.0475 mg catechol/mL extract, and for the *Allium sativum* extract is equal to 0.0336 mg catechol/mL. The highest content of polyphenol compounds was identified in the *Viscum album* extract.

Identification of flavonoid debris with the FTIR spectroscopy

The FTIR spectrum for the *Viscum album* extract (EV1) (figure 3) shows the following characteristic peaks: 3200cm^{-1}; 2986cm^{-1}; 2896cm^{-1}; 1644cm^{-1}; 1384cm^{-1}; 1086cm^{-1}; 1044cm^{-1}; 877cm^{-1}; 575cm^{-1} respectively. The 3300cm^{-1}, 2970cm^{-1} and 2856cm^{-1} peaks are specific to the OH group and to the CH_2 symmetric frequency (CH_2 stretching frequency). The peak having the value of 1644cm^{-1} represents the C=C relation; 1384cm^{-1} peak corresponds to the OH phenolic group; the 1086cm^{-1} peak corresponds to the C-H relation, which is specific for the aromatic nucleus; the 1044cm^{-1} peak corresponds to the ketone group, while 878 and 575cm^{-1} peaks correspond to the in-plan deformation vibrations, δ C-H.

The FTIR Spectrum for *Alium sativum* (EU1) (figure 4) shows the following characteristic peaks: 3339cm^{-1}; 2979cm^{-1}; 1644cm^{-1}; 1384cm^{-1}; 1084cm^{-1}; 1044cm^{-1} and 877cm^{-1} respectively. The 3339cm^{-1} and 2979cm^{-1} peaks are specific for OH group and for the CH_2 symmetric frequency (CH_2 stretching frequency). The peaks having the value of 1644cm^{-1} represents the C=C relation; the 1384cm^{-1} peak corresponds to the OH phenolic group; the 1084cm^{-1} peak to the C-H relation, which is characteristic for the aromatic nucleus; the 1044cm^{-1} peak corresponds to the ketone group, while the 878cm^{-1} peak corresponds to the in-plan deformation vibrations, δ C-H.

CONCLUSION

The presence of flavonoids and polyphenols in the two species was studied both qualitatively through IR spectroscopy, and quantitatively (quantitative dosage) by using the spectroscopy method. The IR spectroscopy highlighted valence vibrations C=O, through strips between 1630 and 1665cm^{-1}, typical for the flavonoids structure; valence vibrations C-O, through strips between 1000 and 1350cm^{-1} and in-plan deformation vibrations, C-H, through strips between 600 and 980cm^{-1}. The flavonoids content was determined quantitatively, for the plant material subjected to study and it had values between 0.73 and 1.70 (% rutin) for the *Viscum album* extracts. Cathecol type polyphenols (catechol) of the plant material were dosed quantitatively. The research pointed out a content of cathecol type polyphenols of approximately 16.4-23.75%. The *Viscum album* extract had the highest content of polyphenolic compounds, for both flavonoids and polyphenols.

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