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HPTLC FINGERPRINT USE, AN IMPORTANT STEP IN PLANT-DERIVED PRODUCTS QUALITY CONTROL

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Abstract

Romanian flora comprises a significant number of vegetal species, some of these already established in terms of chemical composition and pharmacological potential. Also, currently on the national profile market there are many plant-derived products for internal or external use that contain standardized extracts or herbal powders. So, it is very important for the plant-derived products production process to have quality control methods for raw materials and for finished products. In the context of complex chemical composition of vegetal raw material, depending on the cultivating region, the climate (temperature, humidity, light and wind), the harvest time and plants part used, the chromatographic fingerprint can certify the species, chromatographic fingerprint of vegetal products being one of the most simple and feasible method of quality control. Accordingly, High-performance thin-layer chromatography (HPTLC) has become one of the most important tool for quality control of plant-derived products on basis of its simplicity and accurately, as well. It can serve as a tool for instance, depending on the chromatographic conditions, HPTLC method can identify numerous classes (16) of vegetal compounds. Given these, this paper was aims at presenting HPTLC chromatographic phenolic fingerprint of some valuable Romanian vegetal species as follows: Juglans regia L. - walnut, Morus nigra L. - mulberry tree, Althaea officinalis L.- marshmallow, Carum carvi L.- caraway, Crataegus monogyna Jacq. - hawthorn, Tilia cordata Mill. - linden, Achillea millefolium L. - yarrow determined by HPTLC, species present in many plant-derived products.

Key words*: chromatographic phenolic fingerprint, HPTLC, Romania, vegetal species.*

INTRODUCTION

Romania has different bio-geographic regions and ecosystems, being considered a link between Europe and Central Asia. The flora and fauna of Romania represent a renewable resource of significant value if protected and exploited on a sustainable basis. Romania possesses about 50 % of Europe's flora and fauna with more than 3,500 plant species (Pârvu, 1997).

With this abundance of resources, the local producers that uses native flora as raw material, are in a continuous expansion. In the same time, the interest of the consumers for the plant-derived products is increasing, given the fact that these products have a lower price and no side effect comparable with synthetic drugs (Stoia and Oancea, 2013).

On the national market there is an important number of plant derived products for both internal and external administration. For the producers is very important to have reproducible raw material in the terms of chemical composition.

Therefore, is very important for the production process to have simple and feasible quality control methods for raw material and for finished products. In the context of complex chemical composition of vegetal raw material, depending on the cultivating region, the climate (temperature, humidity, light and wind), the harvest time and plants part used, the chromatographic fingerprint can certify the species, being an important step in plant-derived products quality control.

The High performance thin layer chromatography (HPTLC) has become one of the most important tools for quality control of plant-derived products on basis of its simplicity and accurately, fingerprint chromatography being accepted by the World Health Organization as an identification and quality evaluation technique for vegetal raw material (Alaerts, et al 2007).

Through, HPTLC technique, depending on the chromatographic conditions, can be identify numerous classes (16) of bioactive compounds. These bioactive compounds are often secondary metabolites, commercially important and find use in numerous plants - derived products. Among these, phenolic compounds occupy one of the first places due to their health promoting properties (Ghasemzadeh and Ghasemzadeh, 2011). The consumption of products that contain phenolic compounds has been often associated with decreased risk of developing several diseases. Phenolic compounds are known for their action in an important number of biological activities with antibacterial, antioxidant and anti-inflammatory properties. Most of the literature data regarding the biological activity of phenolic compounds refers to antioxidant properties witch may be due mainly redox properties, that allow them to act as a reducing agent and as a hydrogen donor (Kamatou et al 2010; Samec, et al 2010; Rice-Evans al 1996).

Given the importance of the these compounds, HPTLC chromatographic fingerprint of raw material can be an important step in quality control of production process.

The species selected for this study are some of the most valuable Romanian species, which are present in the plant derived products (Table 1).

Table1. Plant material

Latin name	***Common name***	***Family***	***Part used***
Juglans regia L	walnut	*Juglandaceae*	*Juglandis folium et pericarpium*
Morus nigra L.	mulberry tree	*Moraceae*	*Mori folium*
Althaea officinalis L	marshmallow	*Malvaceae*	*Althaeae folium*
Carum carvi L.	caraway	*Apiaceae*	*Carvi fructus*
Crataegus monogyna Jacq.	hawthorn	*Rosaceae*	*Crataegi folium cum flores*
Tilia cordata Mill.	linden	*Tiliaceae*	*Tiliae flores*
Achillea millefolium L.	yarrow	*Asteraceae*	*Millefolii flores*

MATERIALS AND METHODS

Raw material - was purchased from the local store in the form of tea products.

Sample preparation: the samples were prepared by extraction with ethanol 50 % (v/v) - vegetal material/ solvent rate -1/15 m/v for 5 minutes at boiling temperature of the solvent. The solution was filtered and frozen until analysis.

HPTLC Analysis for phenols: According to TLC Atlas - Plant Drug Analysis (Wagner, H. and Bladt S. 1996) was performed a densitometric HPTLC analysis for the development of characteristic fingerprint profile for phenolic compounds. 3-3.5µl of the samples and 1-3µl of references substances (10^{-3} M quercetin, rutin, hyperoside, chlorogenic acid, caffeic acid, rosmarinic acid, ferulic acid, apigenin - 7-glucoside - Sigma-Aldrich) were loaded as 10 mm band length in the 20 x 10 Silica gel 60F254 TLC plate using Hamilton-Bonaduz, Schweiz syringe and CAMAG LINOMAT 5 instrument. The mobile phase was constituted of ethyl acetate-acetic acid-formic acid-water 100:11:11:27 (v/v/v/v). After development, plates were dried and derivatized in Natural products–polyethylenglycol reagent (NP/PEG) (Sigma-Aldrich) reagent. The fingerprints were evaluated at 366 nm in fluorescence mode with a WinCats and VideoScan software.

RESULTS AND DISCUSSIONS

Figure 1 shows chromatographic phenolic fingerprint of S1-*Juglandis folium et pericarpium,* S2-*Mori folium,* S3-*Althaeae folium*, S4-*Carvi fructus,* S5-*Crataegi folium cum flores*, S6-*Tiliae flores,* S7-*Millefolii flores* and references substances, S8-ferulic acid, S9-hyperoside, S10- apigenin-7- glycoside, S11-caffeic acid, S12-chlorogenic acid, S13-rutin, S14-rosmarinic acid, S15-quercetin.

Juglans regia, walnut, is a large, deciduous tree that grows to 25-35 m in high. *Juglandis folium et pericarpium* consists in the leaves of the tree and the green husk of the nuts of *Juglans regia.* The tree was brought from Persia by the Romans. Now, grows in the South-East Europe,

East Asia, Himalaya and China. The chemical composition of the leaves and green husk includes phenolic compounds and juglone. The active principles have bactericidal, astringent, slightly hypotensive, hypoglycemic, soothing, healing, emollient, antidiarrheal properties (Istudor 1998; Pârvu, 1997; Cosmulescu and Trandafir, 2011). All parts of *Juglans regia* specie (as green walnuts or husk, shells, kernels, seeds, bark and leaves) are used in plant-derived products, for healthcare improvement (Stampar, et al., 2006).

In this study, the HPTLC fingerprint (S1) of *Jugladis folium et pericarpium* revealed the presents of flavonoid glycosides as orange spots, with hyperoside (rate of flow values Rf~0.67) and avicularin (Rf~0.89) as major compounds. Neochlorogenic acid, as blue spot (Rf~0.58) and kaempferol-3-arbinoside (Rf~0.92), as green spot were also present. (Figure 1, Figure 2). The obtained results were compared with literature data (Wagner and Bladt, 1996).

Ten phenolic compounds contained by leaves, determined by High-performance liquid chromatography with photodiode, were reported: 3- and 5-caffoylquinic acids, 3- and 4-p-coumaroylquinic acids, p-coumaric acid, quercetin 3-galactoside, quercetin 3-pentoside derivative, quercetin 3-arabinoside, quercetin 3-xyloside and quercetin 3-rhamnoside, (Pereira et al., 2007).

Morus nigra, mulberry tree, is a large, deciduous tree that grows to 12-15 m in height. *Mori folium* consists in the leaves of the tree. It is found in East, West and South East Asia, South Europe, South of North America and in some areas of Africa. In medicinal, economical, industrial and domestic fields, mulberries have enormous importance. *Mori folium* is commonly used for sudorific, antidiarrheal, adjuvant in the treatment of diabetes, myocardial dystrophy activities (Watson and Dallwitz, 2007; Pârvu, 1997).

This qualitative study determined that *Mori folium* chromatographic profile (S2) shows the presence of rutin as orange spot (Rf~0.41), chlorogenic acid as blue spot (Rf~0.51), hyperoside as orange spot (Rf~0.68), apigenin-7-glycoside as orange spot (Rf~0.75) (Figure 1, Figure 3).

Mori folium phenolic compounds identify by HPLC are p- hydroxybenzoic acid, vanillic acid, chlorogenic acid, syringic acid, p-coumaric acid, m-coumaric acid (Memon et al., 2010).

Althaea officinalis, marshmallow, is a perennial plant with erect, woody stems, 60-120cm high. The plant is indigenous to western Asia and Europe, and is naturalized in the USA. *Althaeae folium* are the leaves of marshmallow that contain as major chemical compounds, phenols. The leaves are used for emollient, antidiarrheal and soothing actions (Pârvu, 1997; WHO Monograph, 2003).

In our study, in marshmallow leaves (S3) were identified apigenin-7-glucoside (Rf~0.75), ferulic acid (Rf~0.94) and another two blue major spots that according to Wagner and Bladt, (1996) and based on the relationship spot color - Rf are caffeic acid derivates (Figure 1).

Carum carvi L. is one of the oldest spices cultivated in Europe. Caraway is growing on 20-30 cm stems. *Carvi fructus* are the fruits of the plants that have as active principles volatile oils and phenolic compounds. Carvi fructus acts as a carminative, against spasmodic gastro-intestinal complains, irritable stomach, indigestion, lack of appetite and dyspepsia and relieving flatulent colic (Pârvu 1997; Thippeswamy, et al., 2013).

In our results phenolic compounds revealed in *Carvi fructus* (S4) are chlorogenic acid (Rf~0.51), rosmarinic acid (Rf~0.89) as blue spots and hyperoside (Rf~0.67) as orange spots (Figure 1, Figure 4).

Determined by HPLC, caraway contained a mixture of phenolic acids including gallic acid, catechuic acid, caffeic acid, cinnamic acid, ferulic acid and flavonols such as quercetin and kaempferol (Thippeswamy and Rajeshwara 2014).

Crataegus monogyna Jacq., hawthorn, is a shrub or a small tree of 5-14 m height. It is found in Europe, North Africa and western Asia. *Crataegi folium cum flores* are the leaves and flowers of the hawthorn that have as active principles phenolic compounds. The plant is used for cardiac insufficiency (Pârvu, 1997).

On our hydroalcoholic extract (S5) only vitexin -2-O-rhamnoside (Rf~0.43) as yellow - green spot, hyperoside (Rf~0.68) as orange spot,

caffeic acid (Rf~0.93) as blue spot were identified.

Methanolic extracts of hawthorn were reported to have as phenolic compounds rutin, vitexin - 2-O-rhamnoside, caffeoyl quinic acids, hyperoside, luteolin-5-O-glucoside, vitexin and cafeic acid, determined by TLC (Wagnera and Baldt, 1996). The different results between the extracts may be due to the different extraction solvent and also because of the extraction time (Figure 1).

Tilia cordata, linden, is a deciduous tree that grows to 20-40 m in height. Linden is found in Europe from England to Scandinavia, Russia, Spain, Italy, Greece, Romania, Bulgaria, Turkey and western Asia. *Tiliae flores* are the flowers of the tree, having as major active principles volatile oil and phenolic compounds. Flowers have diaphoretic, antipyretic, emollient, expectorant, sedative, anxiolytic, decongestant, anti-inflammatory and diuretic activities (Rodriguez-Fragoso et al., 2008; Pârvu 1997).

In Figure 1 (S6) - fingerprint of *Tilia cordata* flowers are present flavonoids as rutin (Rf ~0.41) and hyperoside (Rf~0.68) as well asflavonoid glycoside derived from quercetin, myricetin and kaempferol (Wagner and Bladt 1996) and ferulic acid (Rf~0.94) (Figure 5). The major flavonoids found in the flowers extract by HPTLC were: quercetin-3,7-di-O-rhamnoside, kaempferol-3,7-di-O-rhamnoside and kaempferol 3-O-(6"-p-coumaroyl glucoside) or tiliroside (Negri et al., 2013).

Achillea millefolium, yarrow, is a perennial herb, 30–90 cm in height, with aromatic odor and grayish- green colour. *Millefolii flores* consists in the dried flowering tops of *Achillea millefolium L*. The plant is Native to Asia, Europe and North America, now being widely distributed and cultivated in the temperate regions of the world. Major chemical constituents of the flowers are essential oil (0.2-1.0 %) and phenolic compounds.

It is used for internal administration for loss of appetite, dyspeptic ailments, common cold, such as mild spastic discomfort of the gastrointestinal tract, as a choleretic and for the treatment of fevers and for external administration for skin inflammation and wounds (WHO Monograph, 2003; Pârvu, 1997).

In (S7) we have found the following compounds: rutin (Rf~0.41), hyperoside (Rf~0.68), as well as flavonoid glycoside (yellow - orange spots Rf~0.19-0.25) and chlorogenic acid (Rf~0.51) (Figure 1, 6).

Eight phenolic compounds were identified by HPLC in extracts from yarrow flowers: chlorogenic acid and flavonoids, namely vicenin-2, luteolin-3',7-di-O-glucoside, luteolin-7-O-glucoside, rutin, apigenin-7-O-glucoside, luteolin, and apigenin (Benetis et al., 2008).

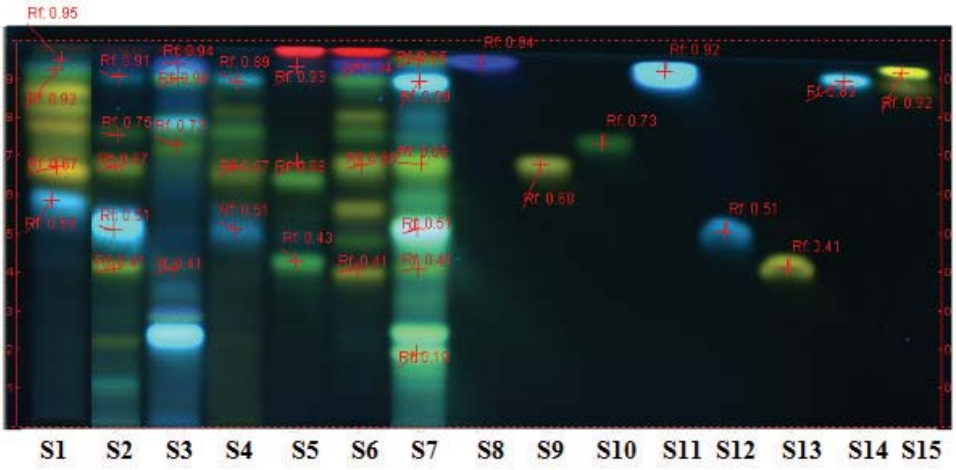

Figure 1. Chromatographic fingerprint of the species comparative with references substances

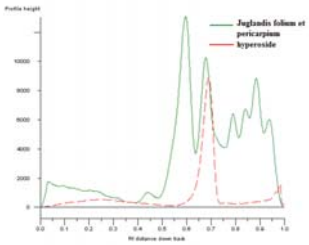

Figure 2. Profile comparison *Juglandis folium et pericarpium*/references substances

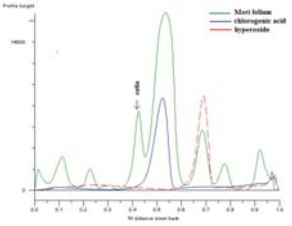

Figure 3. Profile comparison *Mori folium*/ references substances

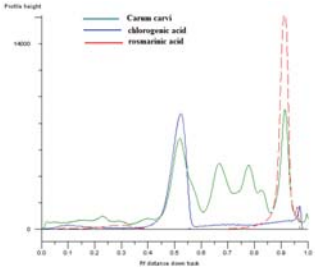

Figure 4. Profile comparison *Carum carvi*/ references substances

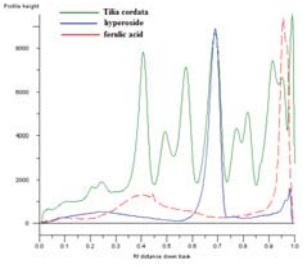

Figure 5. Profile comparison *Tilia cordata*/ references substances

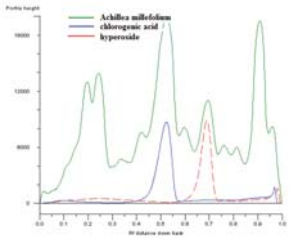

Figure 6. Profile comparison *Achillea millefolium*/ references substances

CONCLUSIONS

To ensure consumer health protection, the quality and safety of vegetal raw material, particularly those used for plant-derived products, must be determined.

With a market in continuous expansion, the competition between the producers is getting stronger every day. Therefore, in quality control management are needed safe, easy and not very expensive methods as HPTLC, especially as this method is accepted at international level.

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