Comparative Fingerprint and Extraction Yield of Medicinal Herb Phenolics with Hepatoprotective Potential, as Determined by UV-Vis and FT-MIR Spectroscopy

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Abstract

The present study was aimed to compare the polyphenolic composition of six medicinal herbs, from wild flora of Romania. The plants investigated, Cynara scolimus (artichoke), Taraxacum officinalis (dandelion), Chelidonium majus (celandine), Hypericum perforatum (St. John’s wort), Silybum marianum (Mary thistle) and Lycopodium clavatum (Wolf’s claw) are known, to have hepatoprotective action. Using in parallel glycerol-water, ethanol-water and methanol, the solvent-dependence of the extract fingerprint and composition in bioactive molecules was studied by UV-Vis and Infrared (FT-MIR) spectrometry. The extraction yields, calculated as an extraction factor (EF) were superior in acidic methanol comparative to glycerin and ethanol, favourising the increase in phenolic acids against flavonoid derivatives. Based on the differences of polarity between the three solvents used, higher EF values were obtained for dandelion, artichoke, celandine and St. John wort, more rich in phenolic acids than flavonoids. Mary thistle and Wolf’s claw had lower concentrations of phenolics, but higher content of lignans and terpenoids. Based on the FT-MIR peaks from 8 regions, for each plant extract, has been determined the fingerprint region between 900 and 1500 cm$^{-1}$ and identified the specific functional groups. A good, significant correlation was found between the concentration of total phenolics calculated by UV-Vis spectrometry and FTIR methods, after calibration with gallic acid. The value of the MIR signal at 1743 cm$^{-1}$ may be considered a good indicator of phenolics concentration in such extracts. Combined UV-Vis and FTIR spectroscopy are recommended as rapid and reliable tools to investigate the fingerprint and to predict the composition of medicinal plants or to evaluate the quality and authenticity of different standardized formulas.

Keywords: FT-MIR, hepatoprotection, medicinal herbs, quality and authenticity, UV spectrometry

Introduction

The traditional herb medicines showed, since centuries, beneficial effects on health promotion, out of side effects, as compared with synthetic drugs. It is also known that their composition is dependent on many ontogenic or genotypic factors influenced by their environment, age, time of harvesting, drying and storage, as well the solvent used to obtain extracts. Due to their natural heterogeneity, the quality of herbs from wild environments shows great fluctuations, so their standardization has been extensively promoted during the last years, the following three attributes being verified: authenticity, purity and assay of their action (Hussain et al., 2009; Yadav and Dixit, 2008). The identification of phytochemicals’ fingerprint by chromatography and spectroscopy may provide effective information about qualitative and quantitative composition of herbal medicines and their pattern recognition can be achieved by chemometry (Bender, 2005; Maloney, 2004) and used to discriminate among different herbs and extracts, with noticeable role in the development of standardized formulas, like other pharmaceuticals to make these remedies evidence-based medicines, (Giri et al., 2010; Liang et al., 2004; McGuffin et al., 1997; Yadav and Dixit, 2008), according to WHO requirements (WHO, 2003)

The evaluation of a herbal product by its metabolomic fingerprinting can be accomplished by appropriate methods, including HPLC with UV (DAD), ELSD, MS detection or GC-MS, HPTLC-densitometry, FT-MIR, NIR, NMR or a combination of these techniques (Fan et al., 2006; Giri et al., 2010; Gong et al., 2006; 2009; Hashimoto and Kameoka, 2008; Li et al., 2008; Mattoli et al., 2006).

The UV-Vis spectroscopy offer a simple, cheap and easy-to-use technique to identify and quantify the main phytochemicals, discriminating between the lipophilic and hydrophilic phytochemicals, in relation to the polarity of the extraction solvent. Fourier transform infrared spectroscopy (FTIR) offers a rapid and non-destructive investigation, easy to
use to fingerprint herbal extracts or powders, is relatively uncommon compared with chromatographic and classical methods (Hussain et al., 2009a; Li et al., 2004b; Liu et al., 2006). The use of attenuated total reflectance (ATR) device evolved rapid FTIR measurements of liquids such as oils and plant extracts, allowing the identification and quantification of valuable plant biomarkers (Schultz and Baranska, 2007).

The herbs, known traditionally, to have hepatoprotective action are generally rich in polyphenols with high antioxidant potential (Mary thistle, arthichoke, dandelion, greater celandine, St. John’s wort, etc.) since major liver diseases are related to oxidative stress and cellular necrosis (Negi et al., 2008; Utrilla, 1996).

Dandelion (Taraxacum officinalis) is an old remedy for granular unbalances, used in the therapy of liver diseases for the biliary stimulation, normalization of blood circulation, diuresis and toxins release. Its bitter substances (eudesmanolides and germacranolides) named generically “taraxacin”, are anti-vomitives and antioxidants (Jeon et al., 2008). Dandelion contains as well flavonoids (7-D glucosides of apigenol and luteoline) and steroids (stigmasterol, sitosterol, ergosterol) which block the de novo synthesis of cholesterol and are competitors of cholesterol deposition (Williams et al., 1996). It contains also inulin with protective action against diabetes and liver diseases (Schütz et al., 2006), similarly to artichoke.

Since many years it was reported that artichoke (Cynara scolymus) induces regeneration of rat liver (Maros et al., 1966), mainly due to cynarin, a phenolic derivative which stimulates liver cell excretion (Li et al., 2004a; Wang et al., 2003). Luteolin, a flavone which inhibit the de novo cholesterol synthesis as well sesquiterpenes lactones has choleretic and chologogue effects (Saenz Rodriguez et al., 2002). The leaf extract of artichoke reduces mild dispesia (Marakis et al., 2002) and have antioxidant potential, as demonstrated on leukocytes (Perez-Garcia et al., 2000).

Greater celandine (Chelidonium majus) is a medicinal poppy (Bone, 1996) with beneficial antipatotoxic and meanwhile controversial effects on liver (Duke, 1985; Mitra et al., 1992; 1996). It is rich in alkaloids (Gu et al., 2010), mainly chelidinon, a naphtofenantridin derivative which stimulates the enzymatic production in liver and pancreas, it is colecytokinetinc and antispastic (Gilca et al., 2010; Hriscu et al., 1980). It was demonstrated experimentally to be antiinflamator, anticancerogenic şi antimicrobian (Vavrekova et al., 1996) been recommended in cyrosis and chronic hepatitis therapy. Using a standardized extract (4 mg chelidinon for 6 weeks) for patients with digestive syndroms, a significant reduction of symptoms was reported (Ritter and Schatton, 1993). Sanguinarine is another alkaloid found in celandine which acts as a colchicine-like cytostatic (Lopus and Panda, 2006; Malikova et al., 2006) with antiviral, antiinflamatory and antibacterial potential (Zdariľová et al., 2006). Meanwhile, overdoses are recommended to avoid, due to its alkaloid charge and hepatotoxicity (Benninger et al., 1999).

Mary thistle (Silybum marianum) is known to have hepatoprotective action and to be a hepatocyte activator (Ferenci et al., 1989; Salmi and Sarna, 1982), being used not only as tea but also in some standardized drugs such as Silimarina (generic name of lignan flavonoids, a mixture of silibine, silibinine, silicristine and silidiane). Silibine is the most active derivative, the extracts being standardized at 70-80% silbine. The mechanisms involved in its hepatoprotective action are diverse and include antioxidant and antiperoxidation effects (Basaga et al., 1997; Bosio et al., 1992), detoxifying effect (Baer-Dubowska et al., 1998; Miguez et al., 1994), antiviral action (McPartland, 1996) and glutathion protection (Cabrera, 1996). Silimarina protects hepatocytes against toxic effects of acetaminophen, ethanol, carbon tetraclorine (Bosio et al., 1992; Favari and Perez-Alvarez, 1997; Muriel et al., 1992), decrease the fibrosis by III-peptide procolagen inhibition (Feher et al., 1989) and inhibit cytochrome P 450 (Baer-Dubowska et al., 1998).

St. John’s wort (Hypericum perforatum) contains several compounds with hepatoprotective properties, by synergistic action of hypericins and phenolics. Hypericins are reddish-violet condensed anthraquinones found (0,1-0,2%) in leaves and flowers. Due to their UV fluorescence, are photoactivated in sun, being good candidates for tumor photodynamic therapy. It has also anti-depressive and antiviral, has chologague and choleretic properties (Ali and Olivo, 2002; Kurth and Spreeman, 1998). Flavonoid glucosides (rutoside) protects the liver against oxidative stress, especially by its action on superoxid dismutase (Istudor, 2003). The condensed tannins and hyperforin, have antibiotic effects, inhibits cytochrom P450 CYP3A4 şi CYP2C9 (Barnes et al., 2001; Gitea et al., 2007; Kurth and Spreeman, 1998; Nahrstedt and Butterweck, 1997).

Wolf’s claw (Lycopodium clavatum) is a less studied herb, which contains more than 35 alkaloids and other biomolecules, whose structure and pharmacologic effects are not yet elucidated. The bioactive molecules are lycopodine, clavatoin, clavoloin, clavatin, licanitin, annapodin, izolzcopodin, as well nicotine, triterpenoids and sterols, flavonoids, radium (known as an antitumor mineral). Triterpenoids and sterols are stimulators of digestive system, antialergic, prevent the cholesterol and fats deposition, may have good effects on liver tumor inhibition, anti-inflammatory and antimicrobial action (Bentarcor-Fernandez et al., 2003).

The present study aimed to compare the fingerprint of different extracts of the above-mentioned medicinal herbs, collected from wild flora of Romania. The dependence of the extract composition on the solvent polarity (glycerol-water vs ethanol-water vs acidic methanol) was studied by UV-Vis spectrometry and Infrared (FT-MIR) fingerprints.
Materials and Methods

Medicinal plants and preparation of extracts

Six types of medicinal plants, from wild flora of different areas of Transylvania, Romania were investigated. The plants were numbered as follows: 1- *Cynara solimius* (artichoke), 2- *Taraxacum officinalis* (dandelion), 3- *Chelidonium majus* (celandine), 4- *Hypericum perforatum* (St. John’s wort), 5- *Silybum marianum* (Mary thistle), 6- *Lycopodium clavatum* (Wolf’s claw). Aliquots of 15 g from each dried and ground plant (selected from 100 g mix of leaves, stems and flowers) were extracted in 85 ml solvent: methanol 90% in water, acidulated with 1% hydrochloric acid (M) or ethanol 93% in water (E) or 30% glycerin in water (G). After sonication 30 min, centrifugation and filtration, the clear extracts were kept in the deep freezer until analysis.

UV-Vis spectra and calculation of extraction factors

The UV-Vis spectra were recorded (700-200 nm) for each extract (M, E or G) using a Jasco V 530 Spectrophotometer. There were identified the maxima wavelengths specific for phenolics (280 and 330 nm), carotenoids (420-470 nm) and/or chlorophylls (663 nm).

To compare the yields of extraction in different solvents it has been calculated the Extraction Factors (EF) of bioactive molecules from each extract, considering the absorption maxima from UV-Vis spectra and the mean values calculated (X ± SD) for extraction factors (EF).

Results and discussions

Extraction factors of bioactive molecules, based on UV-Vis spectra

The comparative UV-Vis spectra of the ethanol (E) and glycerin (G) extracts of the six medicinal plants were recorded (data not shown), as well in methanolic extracts (M) (Fig. 1), methanol being considered a “reference” solvent known to extract phenolics and terpenoids from these plants.

Based on their specific spectra, the absorption maxima of each plant extract and the mean values of extraction factors (EF) were calculated for each solvent (E, G and M) (Tab. 1).

To have an integrated image of the differences between plants, solvent type and concentrations of bioactive molecules extracted, the EF mean values at 270-290 nm (for phenolic acid derivatives extracted in E, G, M) (EFE1, EFG1, EFM1) and at 317-340 nm (for flavonoid derivatives) (EFE2, EFG2, EFM2) for each of the 6 plants were represented (Fig. 2).

According to Tab. 1 and Fig. 2 data, it has been noticed that extraction factors in acidic methanol were superior to glycerin and ethanol, especially for phenolic acids (EFE1, EFG1, EFM1) comparing to flavonoid derivatives (EFE2, EFG2, EFM2). Based on the differences of polarity between the three solvents used (glycerin the most polar, followed by methanol and ethanol) it has been noticed high-
er EF values for dandelion (1), celandine (3) and St. John’s wort (4), more rich in polar molecules, such as phenolic acids and flavonoids. Plants 1 and 3 had similar EF in M and G, plant 2 (artichoke) was better extracted in ethanol and was more rich in phenolic acid derivatives. St. John’s wort (4) components were two times better extracted in methanol than glycerin, and low EF values in ethanol, an indication of polar active molecules. Mary thistle (5) and Wolf’s claw (6) contained reduced concentrations of phenolics, but high absorptions in methanol at 280 and 233 nm, respectively, which might be attributed to higher concentrations of lignans and terpenoids.

For therapeutic reasons it has been consider that ethanol extracts or evaporated methanolic extracts can provide higher concentrations of bioactive molecules from these plants. Anyway, as it was reported recently, methanolic extracts showed hepatoprotective activity against Carbon Tetrachloride-Induced Hepatotoxicity in Rats (Ahsan et al., 2009). Of course for humans the total elimination of methanol (under vacuum) is a condition to have a safe standardized extract.

**FT-MIR fingerprint**

The FT-MIR spectra (4000-900 cm⁻¹) of E and G extracts of each plant were registered and the specific wave numbers and intensities were considered (data not shown). Fig. 3 presents the FT-MIR spectra of M extracts and Tab. 2 includes the corresponding absorption peak areas for specific regions (1-8). In Tab. 2, is included the total phenolics concentration in methanol (M) extracts determined by FTIR and by Vis spectrometry.

The functional groups identification was based on the FTIR peaks attributed to stretching and bending vibrations. Eight areas (marked from 1 to 8) (Fig. 3) were identified in the MIR domain and the fingerprint region was localized between 900 and 1500 cm⁻¹ (areas 1-4).

Area 1 (< 1000 cm⁻¹) corresponds to C-H bending vibrations from isoprenoids, area 2 (997-1130 cm⁻¹) to stretching vibrations C-O of mono-, oligo- and carbohydrates, with signals at 1030, 1054, 1104, and 1130 cm⁻¹, while area 3 (1150-1270 cm⁻¹) corresponds to stretch-
Fig. 3. The FTIR fingerprint of the metanolic (M) extracts of the studied plants: *Cynara scolimus* (1); *Taraxacum officinalis* (2); *Chelidonium majus* (3); *Silybum marianum* (4); *Hypericum perforatum* (5); *Lycopodium clavatum* (6). The specific regions are numbered 1 to 8.
recorded between the IR areas 1, 2, 4, 6, 7 corresponding to each plant extract (E, G or M).

Looking to region 1 (specific to terpenoids) it has been noticed that plants 6, 5 and 4 had higher peak areas in ethanol, similarly to results from UV-spectra. In the other IR regions (4 and 6) no significant differences between the three solvents extracts were noticed, but in regions 2 (corresponding to glucosides) and 7 (lipids), in all plant extracts, the M extract was significantly more charged in molecules that E or G extracts.

Finally it has been compared the phenolic concentrations, determined by FTIR method, based on the peak intensity at 1743 cm\(^{-1}\) and total phenolics calculated from Vis spectrometry. A significant (p<0.05) correlation factor was obtained, as shown in Fig. 5. It is known that the measurement by Vis spectrometry is not specific to phenolics and can overestimate concentrations, while the FTIR method using the peak area (950-1900 cm\(^{-1}\)) estimation can give also false results. It can be consider in this case that measurements based on FTIR absorption intensity at 1743 cm\(^{-1}\) offer the best evaluation of the phenolics concentration in these plants. Generally the concentrations oscillated between 15 to 20 mg GA/ml methanolic extract (15000-20000 ppm), in agreement with other determinations.

Conclusions

The data presented in this study showed that UV-Vis spectrometry and FT-MIR spectroscopy are adequate techniques to fingerprint comparatively and to evaluate the extraction yield of medicinal herbs with hepatoprotective potential. Based on UV spectrometry, the extraction yields were superior in acidic methanol comparative to glycerin and ethanol, increased in phenolic acids comparative to flavonoid derivatives. Based on the differences of polarity between the three solvents used, higher extraction yields were obtained for dandelion, artichoke, celandine and St. John wort, more rich in phenolic acids than flavonoids. Mary thistle and Wolf’s claw had lower concentrations of phenolics, but higher content of lignans and terpenoids. Based on the FT-MIR spectroscopy, for each plant extract it was determined the fingerprint region
to be located between 900 and 1500 cm⁻¹ and it has been identified the specific functional groups.

All FTIR data will be correlated and further validated with the detailed HPLC analysis of the same extracts, in order to validate the FTIR method as a good tool to investigate the fingerprint and to predict the composition of medicinal plants or to evaluate the quality and authenticity of different standardized formulas.

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References


