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Chromatographic Profile of Polyphenols in the *Agastache foeniculum* (Pursh) Kuntze Herb: Evaluation of Optimal Extraction Efficiency

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'The HPLC analysis revealed the contents of polyphenols in the anise hyssop (*Agastache foeniculum* (Pursh) Kuntze) herb during its triple extraction. Several hydroxycinnamic acids (rosmarinic, chlorogenic, ferulic and caffeic) and flavonoids (apigenin, apigenin-7-O-glucoside, hyperoside, quercitrin, rutin and quercetin) were identified in the *A. foeniculum* herb. It was established that rosmarinic acid followed by apigenin-7-O-glucoside and apigenin were the predominant compounds of the A. foeniculum raw material. The content of rosmarinic acid as the major compound during the primary, secondary and tertiary extraction decreased in the following order: 37.563>15.435>0.642 (mg/g); the content of apigenin-7-O-glucoside was 24.508>9.107>0.945 (mg/g) and apigenin was 19.547>9.676>1.816 (mg/g), respectively. Generally, the third extraction was determined to be inefficient in terms of low content of polyphenols as well as excessive analysis time and solvent costs.

Keywords: Aerial part; Anise hyssop; High-performance liquid chromatography; Multiplicity of extraction; Polyphenols.

In the process of developing new medicines from herbal raw materials, it is important to determine the spectrum of major bioactive compounds. Scientists have proven a significant influence of genetic prerequisites (depending on the chosen subspecies, chemotype/variety, and age of the plant), climatic conditions and cultivation features on the accumulation of polyphenols in plants' raw material[1]. Undoubtedly, the choice of the extraction method, type of solvent, the raw material-extractant ratio, time, temperature, rate, and multiplicity of extraction play key roles in the phytochemical analysis[2].

The use of water-methanol and water-ethanol mixtures is quite effective due to their ability to extract both hydrophilic and hydrophobic compounds of a polyphenolic nature. Besides, these two solvents are the most compatible with the principles of green extraction among a whole range of organic solvents[3]. The pharmaceutical and food industries increasingly prefer green solvents (water, ethanol, deep eutectic solvents, etc.) for extraction due to their safety and recycling[4,5].

It should be noted that the extremity of extraction of bioactive compounds from plant raw material can be associated with risks through various factors. Thus, the high extraction levels may elevate the concentration of active compounds, potentially increasing the risk of undesired effects or toxicity. However, the low extraction efficiency can result in inadequate isolation of beneficial compounds, limiting therapeutic potential and

raising the risk of insufficient drug efficacy. Thus, achieving an optimal balance in extraction levels becomes crucial for minimizing risks and attaining the desired pharmacological effect of developed herbal substances[6].

The Giant hyssop (*Agastache* Clayton ex Gronov, Lamiaceae Martinov family) genus comprises 22 species native mostly to North America, and only *A. rugosa* (Fisch. & C.A. Mey.) Kuntz) originates from East Asia[7,8]. Some *Agastache* species are used in traditional medicine as natural remedies against pain, bronchitis, hypertension, and gastrointestinal disorders [8,9]. The Korean mint *(A. rugosa*) is the most studied species of this genus regarding its chemical composition and biological activity while other species of this genus (*A. foeniculum* (Pursh) Kuntze, *A. mexicana* (Kunth) Lint & Epling, etc.) have attracted much less attention of researchers in the area of pharmacognosy[8,9]. The biological activities of *Agastache* species were related mainly to the valuable compounds of their essential oils [7,8,10,11]. Such groups of valuable secondary metabolites of the *Agastache* representatives as polyphenols or triterpenoids were investigated much less [7,8,12-15].

This study aimed to conduct the chromatographic analysis of polyphenols and determine the influence of extraction frequency on the efficiency of extracting hydroxycinnamic acids and flavonoids from raw material of anise hyssop *(A. foeniculum)* under its cultivation in Ukraine.

MATERIALS AND METHODS

Plant material

The aerial part of *A. foeniculum* (the variety with white flowers) was harvested during the flowering period from the plots (Fig. 1, *a*) in the Ternopil region (Ukraine). The collected flowering shoots were cut into pieces up to 15 cm long and dried at 25–35°C. Dried raw material (Fig. 1, *b*) were sifted through a sieve with a hole diameter of 2.5 mm before the extraction.

HPLC analysis

The chromatographic analysis of phenolic compounds was performed by the validated method of high-performance liquid chromatography (HPLC)[16] in 70% ethanol extracts of the ground raw material. The triple extraction of raw materials was used was used in this study. The raw material-solvent ratio was 1:10. The extractions were performed in an ultrasonic bath for 30 min each time (at 40°C).

A Shimadzu LC20 Prominence chromatograph with column Phenomenex Luna C18 (250 mm×4.6 mm with silica gel as sorbent) was used for HPLC analysis. The UV absorption spectra of the reference standards of polyphenols and the test samples were recorded in the range of 190–400 nm. The gradient elution was carried out with two solvents: 1) 0.1% aqueous solution of trifluoroacetic acid; 2) 0.1% solution of trifluoroacetic acid in acetonitrile[17]. The time of HPLC analysis was 60 min. The HPLC analysis was carried out in triplicate.

RESULTS AND DISCUSSION

Several hydroxycinnamic acids (rosmarinic, ferulic, caffeic and chlorogenic) and flavonoids (apigenin, apigenin-7-*O*-glucoside, hyperoside, quercitrin, rutin, and quercetin) were revealed in the *A. foeniculum* herb by HPLC method (Table 1, Fig. 2-4).

It was established that the content of rosmarinic acid as the main identified dominant compound during the primary, secondary and tertiary extraction of the *A. foeniculum* raw material decreased in the following order: 37.563>15.435>0.642 (mg/g). Regarding the predominant flavonoids, the content of apigenin-7-*O*-glucoside was 24.508>9.107>0.945 (mg/g) and apigenin 19.547>9.676>1.816 (mg/g), respectively.

Thus, it was revealed that during the secondary extraction of *A. foeniculum* raw material, a 2-3 times lower quantitative content of dominant polyphenols was extracted compared to the primary extraction. As for the tertiary extraction of plant raw materials, it was determined to be inefficient in terms of excessive time and solvent consumption because HPLC analysis showed that an order of magnitude less polyphenolic compounds are compared to primary extraction.

Rosmarinic acid, the predominant component of the studied *A. foeniculum* herb, possesses noticeable antioxidant, anti-inflammatory, antiviral, antimicrobial hepatoprotective, anti-nociceptive and immunomodulatory properties[5,18-20]. Apigenin and apigenin-7-*O*-glucoside as other

predominant compounds of the investigated raw material demonstrate the prominent antioxidant, anti-inflammatory and anticancer effects[21,22]. As it is known, among a wide range of exogenous antioxidants, polyphenols are one of the most effective classes of compounds possessing antioxidant properties.

Numerous data of scientific literature regarding the polyphenolic profiles of the different *Lamiaceae* species demonstrated that rosmarinic acid is quite often their common major compound[14, 23-30]. Thus, the ultra-performance liquid chromatography analysis of methanolic extracts from *A. rugosa* roots revealed the predominance of rosmarinic acid among 24 identified polyphenols[25]. Its level was 3.82–9.16 mg/g, depending on the used *in vitro* culture system. The content of rosmarinic acid in the 70% ethanolic extract from *A. foeniculum* herb grown in Romania fluctuated in the range of 6.45-8.12 mg/g, depending on the harvesting period of the plant raw material[26]. The other study revealed that the content of rosmarinic acid in the ethanolic extract of *Origanum vulgare* (*Lamiaceae*) herb

Table 1. The content of phenolic compounds in the *Agastache foeniculum* herb (HPLC analysis)

Compound	Retention time, min	Content, mg/g First extraction	Secondextraction	Third extraction
Chlorogenic acid	19.9	2.124	0.935	0.096
Caffeic acid	22.3	5.563	2.872	0.301
Rutin	31.5	0.926	0.319	0.045
Ferulic acid	32.3	1.296	0.456	0.060
Hyperoside	32.8	9.713	5.483	0.432
Quercitrin	34.9	0.469	0.128	<0.01
Rosmarinic acid	37.8	37.563	15.435	0.642
Apigenin-7-*O*-glucoside	38.2	24.508	9.107	0.846
Quercetin	46.6	0.317	0.143	<0.01
Apigenin	52.4	19.547	9.676	1.816

a b

Fig. 1. Appearance of *Ocimum sanctum* plants on the experimental plot (during flowering) (a) and dried raw material (b)

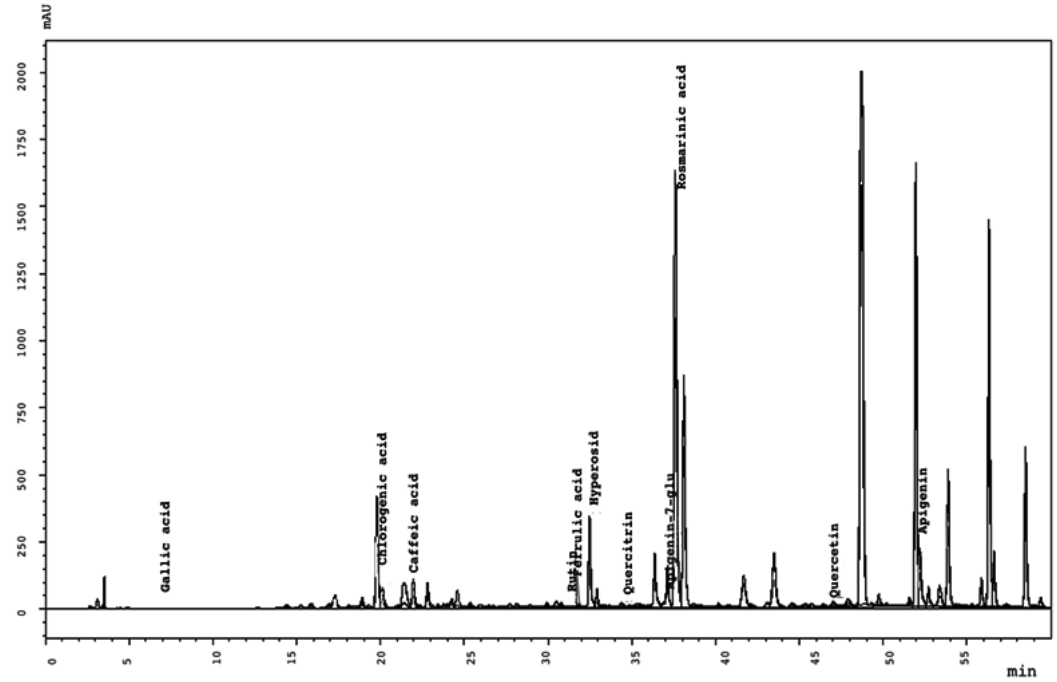

Fig. 2. HPLC chromatogram of hydroxycinnamic acids and flavonoids in the *A. foeniculum* herb after the first extraction (at 280 nm, 330 nm and 350 nm)

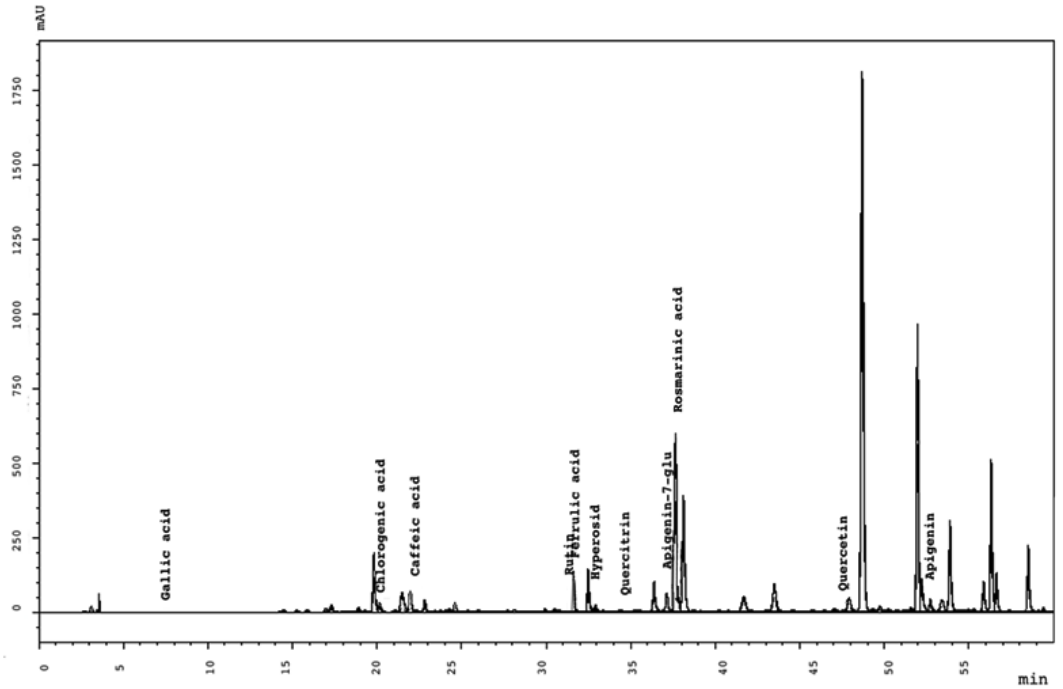

Fig. 3. HPLC chromatogram of hydroxycinnamic acids and flavonoids in the *A. foeniculum* herb after the second extraction (at 280 nm, 330 nm and 350 nm)

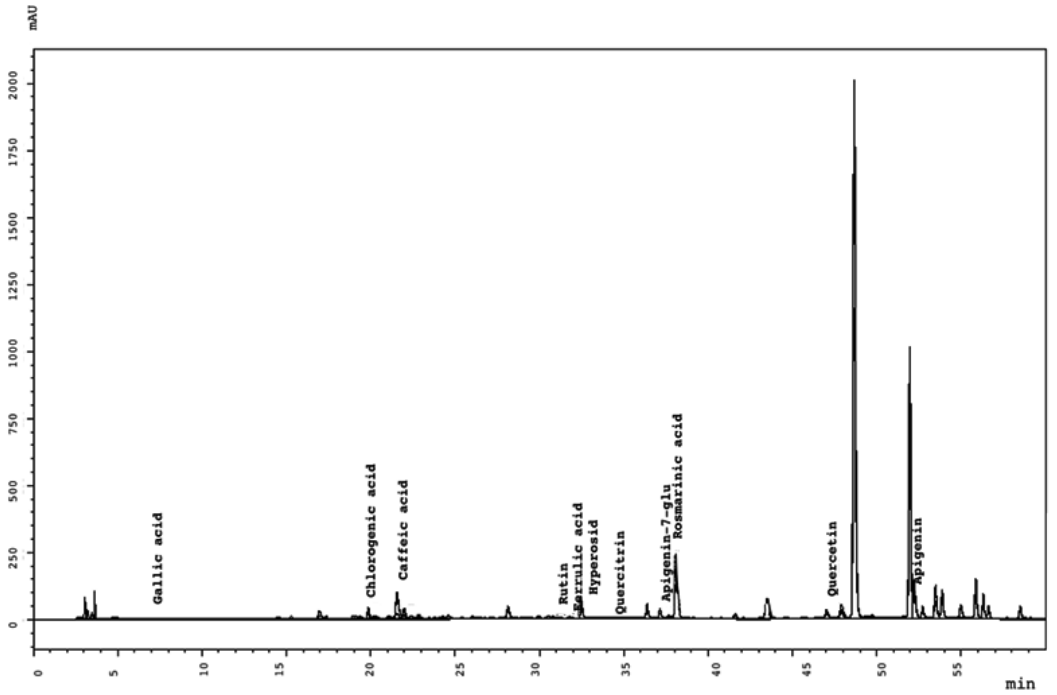

Fig. 4. HPLC chromatogram of hydroxycinnamic acids and flavonoids in the *A. foeniculum* herb after the third extraction (at 280 nm, 330 nm and 350 nm)

was 12.40 mg/g[27]. Rosmarinic acid (21.42 mg/g) was also the main predominant hydroxycinnamic acid of the *Betonica peraucta* (*Lamiaceae*) herb collected in Ukraine[28]. The experimental results showed that the content of rosmarinic acid in the aerial parts of several *Lamiaceae* species from different genera collected in Ukraine was in the ranges of 12.61–24.83 mg/g in the methanolic extracts obtained by maceration[29,30] which is consistent with our data regarding *A. foeniculum* herb.

The experimental studies demonstrated that the concentrations of polyphenols were higher in the methanolic extract of *A. rugosa* compared to the ethanolic one[24]. The content of flavon genistein as the main predominant compound of *A. rugosa was* 3.17 mg/g in methanolic extract and 2.23 mg/g in ethanolic extract which is much less than in the *A. foeniculum* herb studied by us. Researchers found significant correlations between the total phenolic contents and the antioxidant activity of the studied extracts.

Recently, it was revealed by Korean researchers[31] the high bioactive potential of rosmarinic acid and flavons tilianin and acacetin as key phenolic compounds of *A. rugosa* in humans and a Caco-2 cell model. Another validated bioanalytical method was developed for the simultaneous quantification of the dominated bioactive phenolic compounds (rosmarinic acid and flavons) from the *A. rugosa* aerial part in human plasma using UHPLC-MS/MS[32]. These clinical studies showed that the concentration of rosmarinic acid was the highest among other polyphenols in plasma when applied the dry extract obtained from the *A. rugosa* aerial part with 50 % ethanol. This technique offered the precise, accurate, and repeatable method for analyzing *A. rugosa* biocompounds in human plasma samples and detecting the analytes at very low concentrations (the lower limit of quantitation for both tilianin and rosmarinic acid was 0.5 ng/mL and 0.1 ng/mL for acacetin). The anti-inflammatory effects of flavones and two phenylpropanoid glucosides isolated from the *A. rugosa* aerial part were demonstrated in the *in vitro* studies using macrophages[33].

CONCLUSION

The contents of hydroxycinnamic acids and flavonoids under the influence of triple extraction of the *A. foeniculum* herb were revealed using HPLC analysis. The main polyphenolic compounds in the *A. foeniculum* raw material during all stages of extraction were rosmarinic acid, apigenin-7-*O*-glucoside and apigenin. It was concluded that the third extraction was inefficient in terms of low content of polyphenols as well as excessive analysis time and solvent costs compared to the first and second extractions.

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Conflict of Interests

There were no commercial or financial links that may be deemed a potential conflict of interest during the research.

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