EXPLORING THE PHYTOCHEMICAL PROFILE AND QUALITY CONTROL OF *Hippophae rhamnoides* (SEA BUCKTHORN) BRANCHES

Oana Teodora APREUTESEI[1],
Carmen Elena TEBRENCU[2], Elena IONESCU[3]

Abstract. *Hippophae rhamnoides* L. (sea buckthorn) is increasingly consumed worldwide as home remedies and used as raw material for the pharmaceutical and food industry. An important step in the quality control of *H. rhamnoides* is establishing the appropriate analytical method to identify the specific marker profile. This study aimed to establish a chemical fingerprint for extract from *H. rhamnoides* branches as a reference for quality control and explore chemical variation in derived herbal formulations using thin-layer chromatography (HPTLC). The HPTLC analysis evidenced chlorogenic acid (*Rf* 0.52), kaempferol (*Rf* 0.98), and other polyphenolic acids (*Rf* 0.16, 0.25, 0.31, 0.48). A characteristic phytochemical fingerprint was established for *H. rhmanoides* branches that can be further used for exploring phytochemical profile and could serve as a valuable tool in the quality control of herbal formulations derived from *H. rhamnoides*.

Keywords: *Hippophae rhamnoides* L., herbal formulations, HPTLC fingerprint, quality control

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1. Introduction

H. rhamnoides, commonly known as sea buckthorn, is a perennial shrub from the *Elaeagnaceae* family, native to temperate regions of Europe, Asia and North Africa. Known for centuries for its medicinal and nutritional properties, the plant has gained significant attention in modern times for its bioactive compounds that contribute to health benefits. Today, extracts and derivatives of sea buckthorn are

[1] PhD, Bioeng., Researcher, Stejarul Research Centre for Biological Sciences, National Institute of Research and Development for Biological Sciences, Piatra Neamt, Romania (oanaciuperca@yahoo.com)
[2] PhD, Eng, Senior Researcher, The Research and Processing Centre for Medicial Plants PLANTAVOREL SA, Piatra Neamt, Romania, Associated Member of the Academy of Romanian Scientists (carmen@plantavorel.ro)
[3] PhD, Eng, Senior Researcher, The Research and processing Centre for Medicial Plants PLANTAVOREL SA, Piatra Neamt, Romania, Member of the Academy of Romanian Scientists, Corresponding Author (elenaionescu@plantavorel.ro)

utilized in dietary supplements, functional foods, and cosmetic products due to their antioxidant, anti-inflammatory, immunomodulatory and regenerative effects [1][2][3]. The fruits and other parts of *H. rhamnoides* such as leaves, branches are rich in various bioactive compounds, each contributing to its therapeutic properties. Key components include vitamins, essential fatty acids, flavonoids, carotenoids, phenolic acids.

Sea buckthorn is an excellent source of essential vitamins such as vitamin C (which supports the immune system), vitamin E (with antioxidant properties), B vitamins (including B1, B2, B6, and B9), and provitamin A (carotenoids). These vitamins play vital roles in protecting the body from oxidative stress, supporting immune function, and promoting skin health [1][2][4].

Sea buckthorn contains a unique combination of polyunsaturated fatty acids, particularly omega-7 fatty acids (palmitoleic acid), which have beneficial effects on skin health, cell membrane integrity, and possess anti-inflammatory properties [5][6]. Phenolic compounds like quercetin and kaempferol contribute to sea buckthorn's antioxidant and anti-inflammatory actions. These compounds help reduce the risks of chronic diseases, such as cardiovascular and neoplasic diseases [7][8]. Sea buckthorn contains carotenoids such as beta-carotene and lycopene, which provide potent antioxidant effects and support eye health [9]. Caffeic acid and chlorogenic acid are among the phenolic compounds found in sea buckthorn. These compounds have anti-inflammatory and cellular protection properties [7][9].

The bioactive compounds from *H. rhamnoides* provide a multitude of health benefits. Sea buckthorn has been shown to reduce oxidative stress and inflammation in the body. These properties are beneficial for mitigating chronic inflammatory conditions, such as arthritis and cardiovascular diseases [1][2][5]. Sea buckthorn oil is widely recognized for its regenerative and moisturizing properties, making it a popular ingredient in skincare products. The oil promotes wound healing, reduces scars, and moisturizes the skin [3][4]. The high levels of vitamin C and flavonoids found in sea buckthorn help strengthen the immune system, aiding the body's ability to combat viral and bacterial infections [2][10]. Scientific studies suggest that the flavonoids and essential fatty acids in sea buckthorn can support cardiovascular health by reducing inflammation, improving lipid profiles, and protecting blood vessels [1][6][7].

Due to its rich nutritional composition, *H. rhamnoides* is widely used in dietary supplements. These supplements come in various forms, including capsules, tablets, oils, and powders, and are marketed for various health benefits: immune support, skin care, antioxidant properties, cardiovascular health. Due to these remarkable properties, *H. rhamnoides* is a popular ingredient in dietary supplements, functional foods, and cosmetics, contributing significantly to modern health and wellness.

H. rhamnoides L. (sea buckthorn) is increasingly consumed worldwide as home remedies and used as raw material for the pharmaceutical and food industry, representing a substantial proportion of the global plant-based products market. An important step in the quality control of *H. rhamnoides* is establishing the appropriate analytical method to identify the specific marker profile. However, the complexity and the large variation of phytochemicals in the herbal-based formulation challenge the authentication and quality control procedures. Establishing cost-efficient analytical techniques is an essential step to bypass these problems.
This study aimed to establish a chemical fingerprint for extract from *H. rhamnoides* branches as a reference for quality control in derived herbal formulations using high thin layer thin-layer chromatography (HPTLC).

2. Materials and methods

The quality control of *H. rhamnoides* (sea buckthorn) refers to the systematic process of ensuring that the plant, its extracts, or any herbal formulations derived from it meet predefined requirements for quality, safety, and efficacy. This involves several steps: (1) **identification and authentication** - ensuring that the plant material used is indeed *H. rhamnoides*, not a different species, which could affect the properties of the product; (2) **phytochemical analysis -** examining the chemical compounds in the plant (e.g., flavonoids, carotenoids, fatty acids, and vitamins) applying techniques like HPTLC (High-Performance Thin Layer Chromatography), HPLC (High-Performance Liquid Chromatography) or GC-MS (Gas Chromatography-Mass Spectrometry); (3) **purity and contaminant testing -** checking for contaminants such as heavy metals, pesticides, or microbial contamination, ensuring that the product is safe for consumption; (4) **standardization -** ensuring that the bioactive ingredients from vegetal material or its extract are present in consistent amounts, so each batch of the formulation has the same therapeutic effect; (5) **physical and chemical properties -** assessing properties like moisture content, pH, and solubility to confirm that the formulation is stable and can be used effectively; (6) **shelf-life and stability testing -** ensuring that the product retains its quality over time, under various storage conditions.
High-Performance Thin-Layer Chromatography offers several advantages for fingerprinting extract from sea buckthorn (*H. rhamnoides*) branches in order to satisfy the quality requirements. HPTLC ensures simultaneous analysis of multiple samples under identical chromatographic conditions, enhancing throughput and consistency [11]. Regarding cost-effectiveness, this technique is economically viable, reducing both solvent consumption and operational costs, making it suitable for routine quality control applications [12]. Standardized HPTLC procedures ensure reproducibility, consistent and reliable results, which is crucial for

establishing chemical fingerprints of natural products [13]. HPTLC enables the detection of a wide range of compounds, including flavonoids, tannins, and other phytochemicals present in sea buckthorn branches, facilitating a thorough chemical analysis [14]. These benefits make HPTLC a valuable tool for the quality control and authentication of sea buckthorn branch extracts and plant-derived products based on *H. rhamnoides*.

Raw material

Branches of *H. rhamnoides* L. were collected from Siret Valley, Bacău region, Romania, in September 2023. Plant material was dried in a well-ventilated room, in a single layer, protected from direct solar light. The dried branches were grounded using a laboratory mill. The samples were stored in a clean dessicator until were used for HPTLC investigation.

Chemicals

All the standards were of analytical grade or pure and the following were used: chlorogenic acid, hyperoside, caffeic acid, neochlorogenic acid, ferulic acid, rutin hydrate, quercetin, protocatechuic acid, kaempherol. All the reagents used as mobile phase were of analytical grade or pure. HPTLC plates G60 F254, 200x100mm (Merck, Darmstadt, Germany) were used as stationary phase for HPTLC identification.

Method

The sample was prepared by extraction with methanol - vegetal material/solvent rate -1/50 m/v for 24 hours at room temperature. HPTLC identification was performed according to literature [15]. Reference solutions were prepared with a concentration of 0.2 mg/mL in methanol for each and stored at 4°C until use. The sample was applied on plate using using Camag Linomat IV automatic sample applicator.

The HPTLC plate was developed in a saturated vertical developing chamber at room temperature (20-22°C) for 30 min, using as mobile phase a mixture of ethyl acetate: formic acid: acetic acid: distilled water (20:2,2:2,2:5,4). The development distance was 7 cm. After the developing, the plates were air dried at room temperature. A visualising agent was selected and helped in detection and confirmation of the identity of raw material. A 1% diphenylboric acid 2-aminoethyl ester methanolic solution and a 5 % polyethylene glycol 400 ethanolic solution were used for spraying the plate, for visualising of polyphenols and flavonoids in the extract, followed by heating it at a temperature of 100°C for 10 min. The processing of the chromatogram was carried out using CAMAG Reprostar 3 with digital video camera, on derivatized plate in visible light (Fig. 1). The *Rf* values of phytochemicals in raw material extracts of *H. rhamnoides* are presented in

Table 1. HPTLC plate with methanolic extract was scanned under UV at 254 nm and 366 nm.

3. Results and discussions

The HPTLC analysis revealed the presence of polyphenols, which were identified as distinct blue fluorescent bands under the specified conditions, while flavonoids were visualized as clear yellow bands. Chlorogenic acid was detected with a retention factor (*Rf*) of 0.52, kaempferol at *Rf* of 0.98, and several other polyphenolic acids, with *Rf* values ranging from 0.16 to 0.48. These findings not only confirm the diversity of polyphenolic compounds present in *H. rhamnoides* branches but also suggest that specific interactions between the components and the stationary phase that might influence their retention times. The variation in *Rf* values among the polyphenolic acids could indicate differences in their polarity or molecular structure, providing valuable insight into the chemical complexity of the sample. These results also highlight the potential of the method for the detailed profiling of bioactive compounds in raw material belonging of *H. rhamnoides.*

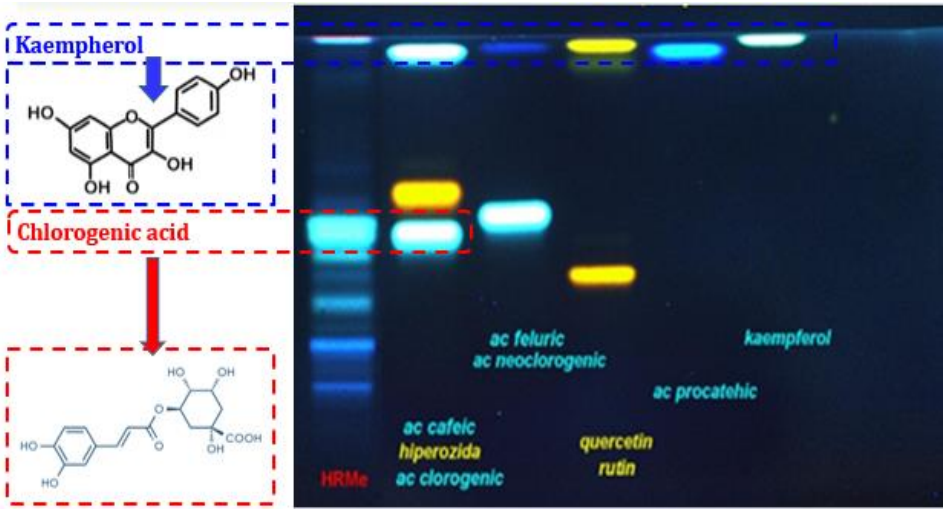

Figure 1. HPTLC fingerprint for extract from *H. rhamnoides branches* (HRMe) / after derivatization, 366 nm

A distinct phytochemical fingerprint with clearly distinguishable fluorescent blue bands was successfully established for *H. rhamnoides* branches, providing a reliable and reproducible method for characterizing the chemical composition of this raw material. The identification of these unique blue fluorescent bands not only highlights the presence of specific bioactive compounds but also enables a more

comprehensive exploration of the phytochemical profile of *H. rhamnoides* branches. This fingerprinting technique can be further utilized for quality control purposes, ensuring the authenticity of *H. rhamnoides* in raw plant material and finished herbal formulations. Moreover, the specific compounds corresponding to the fluorescent blue spots may serve as potential quality markers, offering a means of evaluating the purity of *H. rhamnoides* products. Such markers are invaluable in standardizing *H. rhamnoides*-based products, ensuring their autenticity for consumers.

Table 1. The bioactive compounds identified in *H. rhamnoides* extract using HPTLC

RF VALUE	REFERENCE SUBSTANCE	METHANOLIC EXTRACT OF *H. RHAMNOIDES* BRANCHES
0.16	-	Other polyphenolic acid
0.25	-	Other polyphenolic acid
0.31	-	Other polyphenolic acid
0.43	Rutin	-
0.48	-	Other polyphenolic acid
0.52	Chloroenic acid	Chlorogenic acid
0.58	Neochlorogenic acid	-
0.62	Hyperoside	-
0.95	Caffeic acid	-
0.96	Protocatechuic acid	-
0.97	Ferulic acid	-
0.97	Quercetin	-
0.98	Kaempherol	Kaempherol

4. Conclusions

H. rhamnoides is a plant with significant ecological and economic importance, widely recognized for its ability to grow in diverse environmental conditions, particularly in areas with poor soil quality and extreme climates. Due to the growing consumer demand for natural, health-promoting dietary options, *H. rhamnoides* has attracted attention in the field of nutrition and herbal medicine. Its rich content of bioactive compounds, such as vitamins, fatty acids, flavonoids, and polyphenolic compounds, makes it as a valuable plant with numerous health benefits, ranging from antioxidant properties to skin regeneration and immune support.

In order to maximize the potential of *H. rhamnoides* and ensure its authenticity and quality, it is crucial to establish a reliable phytochemical fingerprint for the plant. This fingerprint serves as a means to identify specific quality markers for each part of the plant, which can vary in their chemical composition. The chromatographic fingerprint of *H. rhamnoides*, obtained at 366 nm, reveals the presence of key bioactive compounds, including chlorogenic acid at an *Rf* of 0.52, kaempferol at *Rf*

0.98, and several other polyphenolics. These compounds are important for the plant's therapeutic effects and are essential for its standardization in both raw material and formulated products.

Our study provides valuable insights into the selection and identification of these bioactive compounds, offering a scientific basis for the quality control of *H. rhamnoides*. The detailed chromatographic data obtained here can help in distinguishing between different sources of *H. rhamnoides*, ensuring that the plant's medicinal properties are preserved and standardized in herbal formulations. Based on these preliminary results, we suggest that the applied chromatographic analysis will serve as a valuable tool for the quality control of *H. rhamnoides*-derived products, ultimately contributing to the development of safe and effective herbal medicines.

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