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Research Article

Qualitative and Quantitative Assay of Hydroxycinnamates of *Prunus Spinosa* L.

Sergey Kondrashev[1], Nadezhda Nesterova[3], Alexey Luzin[1], Vitaliy Kochanov[1], Anna Luzina[4], Alexey Matyushin[2,*]

ABSTRACT

Background: Blackthorn (*Prunus spinosa* L.) is a plant commonly found in the Russian Federation on the roadsides, forest margins, and meadows. Despite lack of recognition by the official medicine, blackthorn fruits possess antioxidant properties and are used in homeopathic preparations. They may also demonstrate antibacterial and anticancer potential due to hydroxycinnamic acids. The aim of present study was to identify and assay hydroxycinnamates in fruits of *P. spinosa* cultivated in Moscow Region. **Materials and Methods:** Fresh and dried fruits of *P. spinosa*, gathered from plants cultivated in Moscow Region in the harvest maturity stage, were used in the study. Qualitative composition of hydroxycinnamic acids was assessed by high performance liquid chromatography, using reversed phase C18 column. Total hydroxycinnamic acids (THA) content was assessed spectrophotometrically. **Results:** Similar chromatographic profiles were obtained for both fresh and dried blackthorn fruits, the two most abundant compounds being epicatechin (2.91%) and chicoric acid (2.90%). Fruits gathered in Chekhovsky District had lower content of hydroxycinnamates (0.798 ± 0.89) than those coming from Klinsky District (0.886 ± 0.92). However, the THA content in dried fruits grown in both districts was found to be similar (0.540 ± 0.71 and 0.557 ± 0.74, respectively). **Conclusions:** It can be concluded that blackthorn fruits can be considered as a source of hydroxycinnamic acids, as both fresh and dried fruits contain at least eleven hydroxycinnamates. It was found that the dried fruits have similar content of hydroxycinnamic acids, independently of their origin. Future research should be aimed at drying method optimization.

Key Words: Blackthorn, Herbal raw material, HPLC, Hydroxycinnamic acids, *Prunus Spinosa* L., Spectrophotometry.

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INTRODUCTION

Blackthorn (*Prunus Spinosa* L., *P. stepposa* Kotov) is a woody plant or shrub belonging to the *Prunus* genus of the *Rosaceae* family. In the Russian Federation it is commonly found on the roadsides, forest margins, meadows, and on the slopes of mountains and hills.[1] Leaves and fruits of blackthorn are used in medicine since ancient times: in *De Medicina*, a medical treatise written by Roman physician Aulus Cornelius Celsus in the 1st Century AD, its fruits are mentioned numerous times as a remedy for gastrointestinal tract diseases, intertrigo, skin lesions, and wounds.[2] Hippocrates of Kos recognized different kinds of *P. spinosa* as part of a healthy diet, which fitted his famous concept "Let food be thy medicine, and let medicine be thy food". Flowers, fruits, and leaves of blackthorn were used by Claudius Galenus and Pedanius Dioscorides. A separate chapter of *The Canon of Medicine* by Abu Ali ibn Sino was devoted to plums, including blackthorn. Ibn Sino stated different characteristics of fruits, noting that they help to make bile flow and strengthens the stomach, and described how to obtain and use blackthorn gum, which was thought to be helpful in crushing gallstones.[3] In Russian traditional medicine blackthorn fruits decoction was prescribed to treat poisoning and later – for women suffering from gestational toxicosis.

With the advance of synthetic drugs *P. spinosa* was almost forgotten and is currently not recognized by modern regulatory documentation as herbal raw material; it is mentioned only in the "Nomenclature of single-component homeopathic medicines approved in the Russian Federation" listed in the Annex to the Order of the Ministry of Health and Medical Industry No. 335 dated 29.11.1995. However, recent studies have drawn attention of scientists to blackthorn fruits as they contain numerous biologically active substances that demonstrate wide range of pharmacological activities.

For example, phytochemical analysis of *P. spinosa* fruits gathered in Krasnoyarsk Krai, Russia, revealed presence of sugars (more than 7%), flavonoids (up to 5%), extractives (over 12%), allowing authors to consider blackthorn as a prospective raw material not only for food industry, but also for herbal drug development and manufacturing.[4] Blackthorn fruits also contain antioxidants, thus making them one of the possible means to fight oxidative stress – a key factor in ageing pathogenesis.[5] Chromatographic analysis revealed presence of phytosterols in various aerial parts of blackthorn: β-sitosterol – in fruits, leaves, and flowers, 3-O- β-D-glucopyranoside, γ-sitosterol, stigmasterol – in flowers.[6] Safonova *et al.* (2011) showed that leaves and fruits of *P. spinosa* have similar micro- and macroelemental composition, but the ratio between certain chemical elements varies.[7]

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The study by Kumarasamy *et al.* (2004) described antibacterial and antiphage activity of blackthorn fruit juice.[8] These findings suggest that blackthorn remains yet another prospective, but largely unstudied source of beneficial active substances. No study to date has revealed full therapeutic potential of blackthorn fruits in terms of their chemical composition.

Hydroxycinnamic acids are nonflavonoid phenols - prevalent plant phenolic acids that are generally derived from cinnamic acid. Some of them (caffeic, ferulic, p-coumaric) demonstrate antibacterial activity against widespread pathogens, such as *Escherichia coli*, *Staphylococcus aureus,* and *Bacillus cereus* at different pH,[9] whereas others were found to be effective in *in vitro* inhibition of low-density lipoprotein oxidation.[10] They possess strong antioxidant activity due to their ability to scavenge hydroxyl radicals, peroxyl radicals, and other radical species.[11] Moreover, hydroxycinnamic acids demonstrate antigenotoxic[12] and even anticancer properties.[13] Therefore, the aim of our study was to identify and assay hydroxycinnamates in fruits of *P. spinosa*, widely cultivated in Moscow Region.

MATERIALS AND METHODS

Fresh and isothermally dried fruits of *P. spinosa*, gathered from plants cultivated in Moscow Region in the harvest maturity stage, were used in the study. Average sample was pooled from 20-30 fruits obtained from the middle parts of at least three tree crowns, then divided into two parts: one was used immediately after gathering, the other was dried.

All reagents used in the study were of analytical grade.

Qualitative analysis of biologically active substances was performed using aqueous alcohol solution, prepared in the following manner: a 5.0 g sample was ground, then placed in a round bottom flask, 50 ml of 50% alcohol were added, the flask was attached to a reflux condenser and heated on a water bath for 30 min. After that the solution was quantitatively transferred into a 50 ml volumetric flask and diluted to volume with 50% alcohol. The flask was placed into an ultrasonic bath for 5 minutes, then 1 ml of the sonicated solution was centrifugated at 14000 RPM for 10 minutes. The supernatant was transferred into a chromatographic vial and analyzed using Gilson (Gilson, France) high performance liquid chromatography (HPLC) system consisting of degasser, pump, thermostated autosampler (samples temperature – 15 °C), diode-array detector, and thermostated chromatographic column (250 × 4.6 mm, 5 µm, Hypersil C18(2)). HPLC conditions were as follows:

Mobile phase: trifluoroacetic acid (pH=2.5) / acetonitrile

Flow rate: 1.0 ml/min

Detection: UV, λ=330 nm

Injection volume: 20 µl

Total hydroxycinnamic acids (THA) content in blackthorn fruits was assayed using spectrophotometric method and expressed as chlorogenic acid. Absorption spectrum of chlorogenic acid reference standard (Sigma-Aldrich, USA) in 70% alcohol demonstrated peak absorbance at 330 nm and a characteristic shoulder at 300 nm, and was found to be matching the absorption spectra of aqueous alcoholic extracts from fresh and dried fruits of *P. spinosa*.

About 2 g of fruits, accurately weighed and ground (passing through No. 10 mesh sieve), were placed into a 200 ml volumetric flask and 70 ml of distilled water were added. The flask was attached to a reflux condenser and heated on a water bath for 15 minutes. Extraction was performed three times. The extracts were cooled at room temperature, filtered through a paper filter, combined, and quantitatively transferred into another 200 ml volumetric flask. The solution was diluted to volume with distilled water and 1 ml aliquot was transferred into a 50 ml volumetric flask, and then diluted to volume with 20% alcohol. Absorbance was measured at characteristic wavelength of 330 nm.[9] Aqueous alcohol (20%) was used as reference solution.

THA content (X, %), expressed as chlorogenic acid, was calculated using the following formula:

$$X = \frac{A_x \times 200 \times 50 \times 100}{E_{1cm}^{1\%} \times m \times V_a \times (100 - W)},$$

where Ax – absorbance of test solution;

$E_{1cm}^{1\%}$ – specific absorbance of chlorogenic acid at 330 nm, equals to 507;

m – mass of ground fruits, g;

V_a – aliquot volume, ml;

W – moisture content, %.

Statistical analysis was performed according to the State Pharmacopeia's requirements (General Monograph 42-0111-09 "Statistical analysis of results of chemical experiments").

RESULTS AND DISCUSSION

Chromatographic analysis of alcoholic extracts, prepared from blackthorn fruits at various vegetative stages, demonstrated their identity in terms of biologically active substances qualitative composition (Figure 1 and Table 1).

Following statistical analysis of five parallel experiment results it was found that total hydroxycinnamic acids content in *P. spinosa* fruits, expressed as chlorogenic acid, was higher in fresh fruits than in dried ones (Table 2).

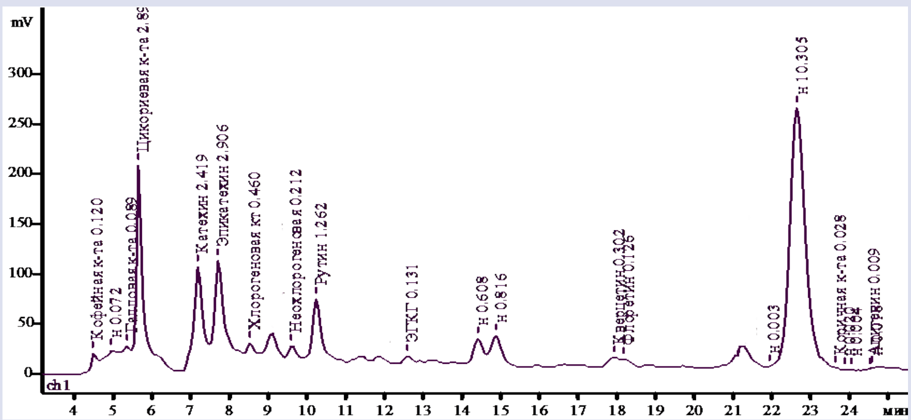

Figure 1: Sample HPLC chromatogram of an alcoholic extract from *P. spinosa* fruits.

Table 1: Phenolic compounds of dried and fresh *P. spinosa* fruits.

No.	Retention time, min	Area	Area, %	Compound
1	4.460	86.71	0.12	Caffeic acid
2	4.895	52.11	0.07	Unidentified
3	5.319	64.34	0.09	Gallic acid
4	5.633	2088.28	2.90	Chicoric acid
5	7.159	1741.89	2.42	Catechin
6	7.690	2093.01	2.91	Epicatechin
7	8.511	331.60	0.46	Chlorogenic acid
8	9.073	582.90	0.81	Unidentified
9	9.514	152.45	0.21	Neochlorogenic acid
10	10.22	908.78	1.26	Rutin
11	11.84	87.84	0.12	Unidentified
12	12.56	94.00	0.13	Epigallocatechin gallate
13	14.41	438.15	0.61	Unidentified
14	14.86	588.03	0.82	Unidentified
15	17.90	217.32	0.30	Quercetin
16	18.21	90.67	0.13	Unidentified
17	21.12	186.74	0.26	Unidentified
18	21.27	262.50	0.36	
19	21.38	205.58	0.29	Unidentified
20	21.98	2.27	0.00	Unidentified
21	22.63	7422.06	10.31	Unidentified
22	23.65	19.94	0.03	Cinnamic acid
23	23.86	14.28	0.02	Unidentified
24	24.3	2.96	0.00	Unidentified
25	24.51	6.54	0.01	
26	24.59	12.90	0.02	Unidentified
27	27.68	0.00	0.00	Unidentified
28	28.74	25.73	0.04	Unidentified
29	37.50	54243.52	75.31	Unidentified

Table 2: THA content in *P. spinosa* fruits, expressed as chlorogenic acid.

No.	Object, origin	THA, expressed as chlorogenic acid, %
1	Fresh fruits, Klinsky District of Moscow Region	0.886 ± 0.92
2	Dried fruits, Klinsky District of Moscow Region	0.540 ± 0.71
3	Fresh fruits, Chekhovsky District of Moscow Region	0.798 ± 0.89
4	Dried fruits, Chekhovsky District of Moscow Region	0.557 ± 0.74

It is worth noticing that the THA content in dried fruits grown in Klinsky and Chehovsky Districts are similar (0.540 ± 0.71 and 0.557 ± 0.74, respectively). However, despite the location of their growth, blackthorn fruits gathered to the south of Moscow (Chekhovsky District) had lower content of hydroxycinnamates, which may be attributed to natural variation in biologically active compounds content.

The results of the study confirm literature data on identification and quantitative assessment of hydroxycinnamic acids in different species of the *Rosaceae* family. Each plant has its own specific set of hydroxycinnamates. For example, coluria (*Coluria geoides* Pall.) contains gallic, *m*-coumaric, vanillic, and ellagic acids,[15] cherry laurel (*Prunus laurocerasus* L.) – vanillic, protocatechuic, *p*-hydroxybenzoic, 3 4-dihydroxybenzoic, caffeic, *p*-coumaric, and cinnamic acids.[16] Several kinds of hawthorn commonly contain neochlorogenic and chlorogenic acids, as well as caffeic and ferulic ones.[17] Taking this variability into account, it is feasible to include chromatographic profiles of hydroxycinnamic acids in raw material specifications for both pharmaceutical and food industry, as it provides opportunity to identify even finely cut material and avoid misgrading.

CONCLUSION

It can be concluded that blackthorn fruits might be considered as a source of hydroxycinnamic acids – substances with promising pharmacological activity. Based on HPLC data, both fresh and dried fruits contain at least eleven hydroxycinnamates; some of the compounds remained unidentified, suggesting further in-depth research of their nature. Spectrophotometric analysis of THA content demonstrated that dried fruits have similar content of hydroxycinnamic acids, independently of their origin, and the growth location itself does not necessary convey information about higher or lower content of these substances. Future research should investigate various drying methods and conditions, in order to minimize the loss of biologically active substances and prolong shelf life.

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CONFLICTS OF INTEREST

The authors declare no conflict of interests.

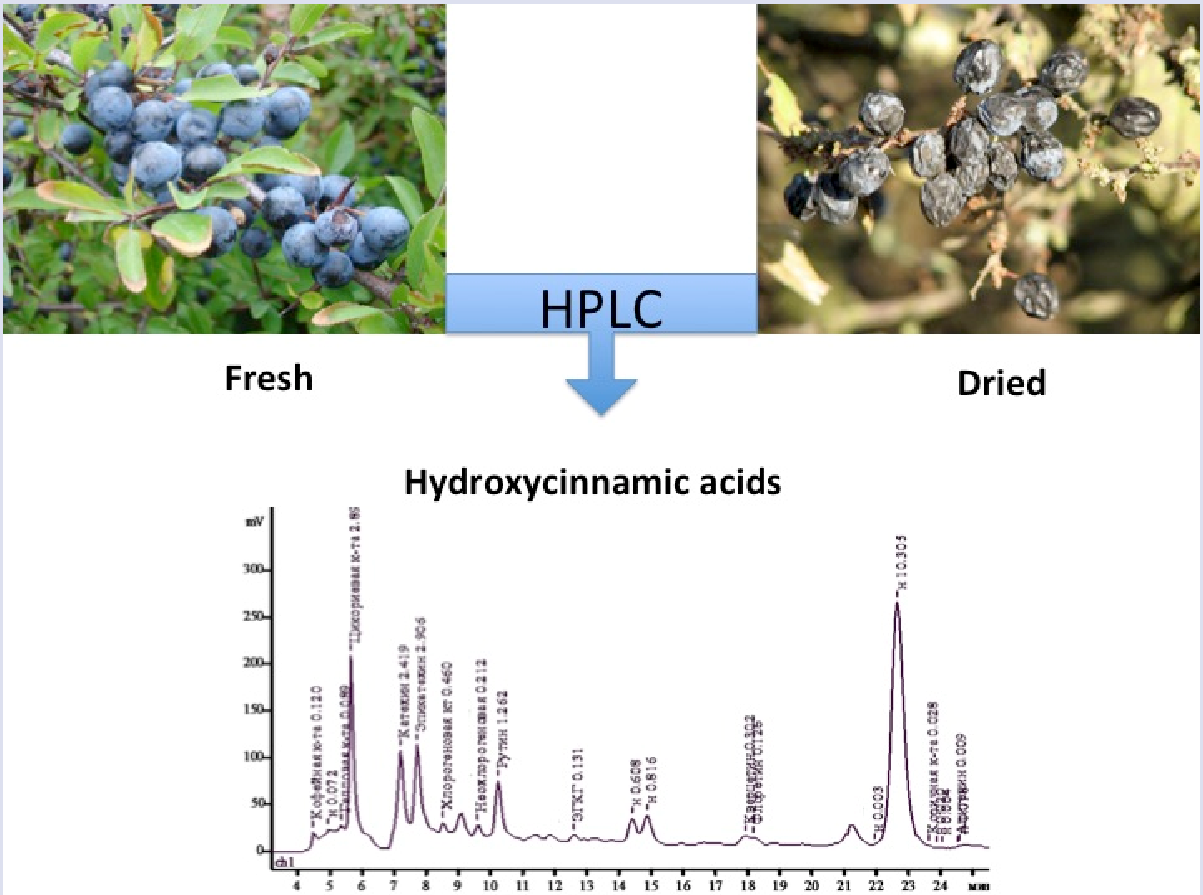
ABBREVIATIONS

HPLC: High performance liquid chromatography; **THA**: Total hydroxycinnamic acids.

REFERENCES

1. Russian Plant Resources: wild flowering plants, their composition, and biological activity. Moscow: Fellowship of Scientific Publishers, 2009;205.
2. *Aulus Cornelius Celsus.* De medicina. Translation from Latin. Moscow, 1959.
3. Abu Ali Ibn Sino. The Canon of Medicine. Vol. 2. Uzbek SSR: FAN, 1994;619.
4. Kokh JA, Kokh DA. Fruits of prunus spinosa of krasnoyarsk krai -The perspective source for receiving biologically the active materials. Agricultural Journal in the Far East Federal District. 2017;1(41):57-60.
5. Velickovic JM, Kostic DA, Stojanovic GS, Mitic SS, Radelovic SS, Dordevic AS. Phenolic composition, antioxidant and antimicrobial activity of extracts from *Prunus spinose* L. fruit. Hemijska Industrija. 2014;68(3):297-303.
6. Wolbis M, Olszewska M, Welowski W. Triterpens and sterols in the flowers and leaves of *Prunus spinose* L. (*Rosaceae*). Acta Poloniae Pharmaceutica. 2001;58(6):459-62.
7. Safonova IA, Yatzuk VY, Fatyanov AA, Safonov AA. Studiyng of elemental composition of *Prunus spinosa* (L.) fruits and leaves. Belgorod State University Scientific Bulletin. Medicine Pharmacy. 2011;4(13):165-9.
8. Kumarasamy Y, Cox PJ, Jasparrs M, Nahar L, Sarker SD. Comparative studies on biological activities of *Prunus padus* and *P. spinosa*. Fitoterapia. 2004;75(1):77-80.
9. Herald PJ, Davidson PM. Antibacterial activity of selected hydroxycinnamic acids. Journal of Food Science. 1983;48(4):1378-9.
10. Meyer AS, Donovan JL, Pearson DA, Waterhouse AL, Frankel EN. Fruit hydroxycinnamic acids inhibit human low-density lipoprotein oxidation *in vitro*. Journal of Agricultural and Food Chemistry. 1998;46(5):1783-7.
11. Shahidi F, Chandrasekara A. Hydroxycinnamates and their *in vitro* and *in vivo* antioxidant activities. Phytochemistry Reviews. 2010;9(1):147-70.
12. Ferguson LR, Zhu ST, Harris PJ. Antioxidant and antigenotoxic effects of plant cell wall hydroxycinnamic acids in cultured HT-29 cells. Molecular Nutrition & Food Research. 2005;49(6):585-93.
13. Rocha LD, Monteiro MC, Teodoro AJ. Anticancer properties of hydroxycinnamic acids - A Review. Cancer and Clinical Oncology. 2012;1(2):109-21.
14. Deineka VI, Khlebnikov VA, Sorokopudov VN, Anisimovich IP. Chlorogenic acid in fruits and leaves of some plants in the *Berberidaceae* family. Chemistry of Natural Compounds. 2008;1:57-61.
15. Myadelets MA, Dutova SV. A comparative assessment of phenolics composition of *Coluria geoides* (*Rosaceae*) dry extract. Proceedings of IX International Symposium "Phenolic compounds: fundamental and applied aspects". 2015. Moscow, Russia.
16. Ayaz FA, Reumanen M, Var M. Determination of carboxylic acids in the fruits of *Laurocerasus officinalis* Roem. and its cultivars. ChemInform. 1999;10(18):1083-6.
17. Goncharov NF, Mihailov IV, Goncharov NN. Hydroxycinnamic acids in flowers and leaves of non-pharmacopoeial hawthorn species. Fundamental Studies. 2011;9(1):146-8.

GRAPHICAL ABSTRACT



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