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ANALYSIS OF FERULIC ACID IN PLANTS CONTAINING PHENYLPROPANOIDS

In this work the qualitative and quantitative chromatographic analysis of ferulic acid in plants, cultivated in the Republic of Belarus have been carried out. The most amounts of ferulic acid have been found by HPLC method in beetroot peel (147.34 mg/100 g dry material). It have been found that the kind of beetroot "Prygazhunja" contains the most amounts of ferulic acid, it could be used as raw material for isolation of ferulic acid in preparative amounts.

Introduction. Interest in phenylpropanoids as the basis for creating drugs with therapeutic effect, increases continuously. In this regard, one of the most important tasks is to study the content of plant facilities to determine the richest source of phenylpropanoids, growing on the territory of the Republic of Belarus.

One of the representatives of ordinary phenilpropanoids is ferulic acid (FA), 3-(4-hydroxy-3-methoxyphenyl)-2-propionic acid. It is bioavailable and has a number of therapeutic properties such as antiinflammatory, anti-diabetic, anti-cancer, hepatoprotective, antiatherosclerotic.

According to researchers indicated properties stipulate mainly antioxidant properties, as it can be seen from the structure of the compound (Fig. 1) [1].

The mechanism of antioxidant action of ferulic acid is accomplished through interaction of the hydroxyl group with organic free radicals or reactive oxygen species, formation of a stable phenoxyl radical and chain stopping by forming complexes with free radicals, as well as dimers of ferulic acid (curcumins). It should be noted that due to the stabilization of free radical ferulic acid cannot initiate a chain, and therefore it cannot manifest prooxidant effect and from this point of view, it has some advantages over the conventionally known antioxidants such as ascorbic acid [2].

Ferulic acid was found in a number of plants: rice (0.9%), wheat (0.66%), barley (0.14%), citrus fruits, vegetables, berries. In maize bran its content reaches 3.1% based on the dry matter [1, 3].

Ferulic acid in plants is present as the trans-isomer by 70% [1].

Main part. The aim of this work is to define qualitative and quantitative content of ferulic acid in plants growing on the territory of the Republic of Belarus, and the choice of the richest raw material source for its further isolation from plant material.

For this purpose were analyzed dried herbs of echinacea, chamomile, mint, violet, nettle (purchased at pharmacies of Minsk), cultivar flax seeds "Sunny" (CBG NASB), seeds of maize and beans, root crops of potatoes, beets, cultivar carrots "Riga" (purchased at retails of Minsk) as well as cultivar beets of Belarusian selection "Vesta", "Gaspadynya", "Prygazhunya" of 2013 harvest grown in RUE "Institute of vegetable-growing".

Qualitative and quantitative assessment of ferulic acid in these plants was produced by thin-layer and high performance liquid chromatography respectively. To do this, the preliminary allocation of the substance of the plant facilities was performed. The dried material (2 g) was hydrolyzed and extracted with ethyl acetate, the extract was then separated from the biomass, and was concentrated under reduced pressure and at a temperature not exceeding 60ºC.

Before the extraction, alkaline hydrolysis using 4 M NaOH solution (50 ml) was carried out for 24 hours followed by acid hydrolysis (pH <2, HCl).

Combining the two types of hydrolysis provides complete release of ferulic acid from cooligomeric state in which it is present in the plant associated with polysaccharides mainly by arabinose and lignin (Fig. 2).

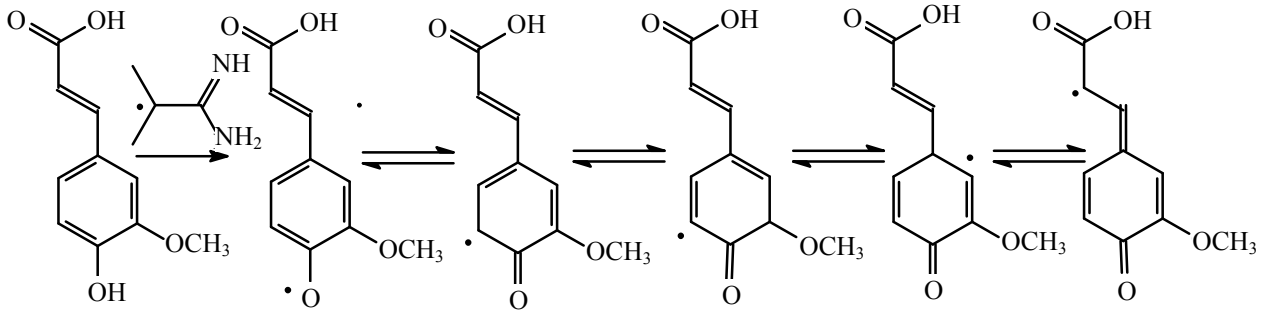

Fig. 1. Structural formulae of ferulic asid
and resonance forms of its phenoxyl radical

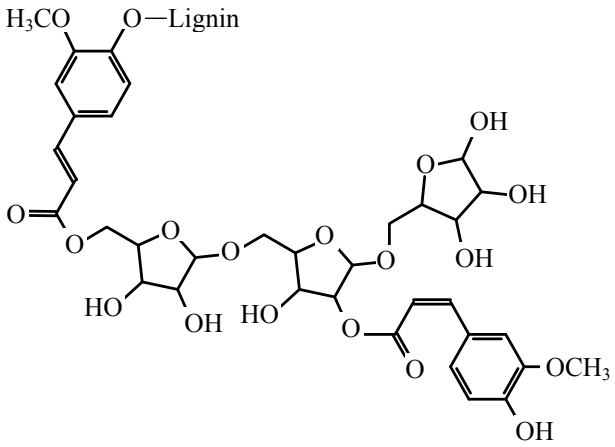

Fig. 2. Structure of oligomer of ferulic acid and arabinoses

Alkaline treatment substantially provides hydrolytic cleavage of ester bonds, and acid treatment provides cleavage of ether bonds [4].

Concentrated extracts were dissolved and subjected to TLC analysis. For this mobile phase was selected. As the stationary phase silica gel plates Kiselgel 60 F254 (Merck, USA) were used. Detecting of substances on the plates was accomplished by irradiating UV light at 254 and 365 nm and visible light after the treatment with iodine vapor and 2% $FeCl_3$ solution in methanol. Identification of ferulic acid was performed by comparing the color spots and R_f value in appropriate mobile phase with color and indication of the standard sample of ferulic acid (Sigma, USA). The results of mobile phase selection and R_f values for the ferulic acid in different conditions are shown in Table 1.

As it is seen from Table 1, in the systems A and B throwing substances to the front line occured, and in the system B ferulic acid rose slightly from the starting line.

Table 1

Values R_f for ferulic acid in different mobile phases

Ratio of solvents, % vol.	R_f	Stained spots under UV irradiation
A. Ethyl acetate : acetic acid: water (3 : 1 : 1)	0.95	Darkblue
B. Toluol : acetic acid: water (8 : 2 : 1)	0.36	Blue-violet
C. Toluol : ethyl acetate : formic acid : water (10 : 100 : 100 : 10)	0.80	Blue-violet
D. Water : propanol-2 : 25% aqueous solution of ammonia (1 : 8 : 1)	0.50	Bright bluish-violet

As it is described in reference [5], the resolving power of the solvent system is maximum in the region where R_f = 0.5 and decreases towards both the start and the front side. In this regard, for the chromatographic separation of the mixture of substances one should be used such a system of solvents, in which area of the component under study would be located near the line with R_f = 0.5. Thus, mixture G was the optimal mobile phase for the TLC analysis of extracts containing ferulic acid.

The result of TLC analysis revealed that in herbs of chamomile, nettle, mint, violet, echinacea and in flax and bean seeds ferulic acid is found in trace amounts.

HPLC analysis on a chromatograph Shimadzu with UV detector at a wavelength of 320 nm was carried out on the extracts from seeds of corn, carrot, root, potatoes and beet, where the apparent presence of ferulic acid was detected. As the stationary phase, column with silica gel (5 microns) was used which was associated with octadecylsilane, Symmerty C18 (250 × 4.6 mm). Elution was carried out with a mixture of acetonitrile and bidistilled water (20 : 80), acidified with formic acid up to pH = 2.45, in isocratic mode at room temperature at a flow rate of eluent of 0.5 ml / min [6].

Identification of ferulic acid in the chromatogram of the extracts was performed by retention time of ferulic acid, which coincided with the retention time of the standard sample and was 21.88 min (Fig. 3). Quantitative determination of the compounds in the extracts was performed by the calibration curve constructed according to standard solutions of ferulic acid (y = 123,814x + 12,670,782, R^2 = 0.92).

The content of ferulic acid in plant extracts under study is presented in Table 2.

Table 2

The results of quantitative determination of ferulic acid in plants under analysis

Plant matter	Content, mg /100 g of dry matter	Content of FA on references
Maize seeds	48.79	3.1 wt % on dry matter [3]
Root crop of carrot	43.18	1.5 mg / 100 g of dry matter [7]
Root crop of potatoes (skin)	8.36	0.6 mg / 100 g of damp matter [8]
Root crop of beet	50.16	25 mg / 100 g of dry matter [7]; 9.9 mg / 100 g of damp matter [8]; 0.8 wt % on dry matter [1]
Root crop of beet (peeling)	147.34	

As it can be seen, the largest number of ferulic acid is contained in the beet and the content in the peel of root is almost 3 times as much as the content in the pulp. These data are adjusted well with the results of [9], where the total amount of phenolic compounds in acid equivalent of gallic acid (GAE) is investigated.

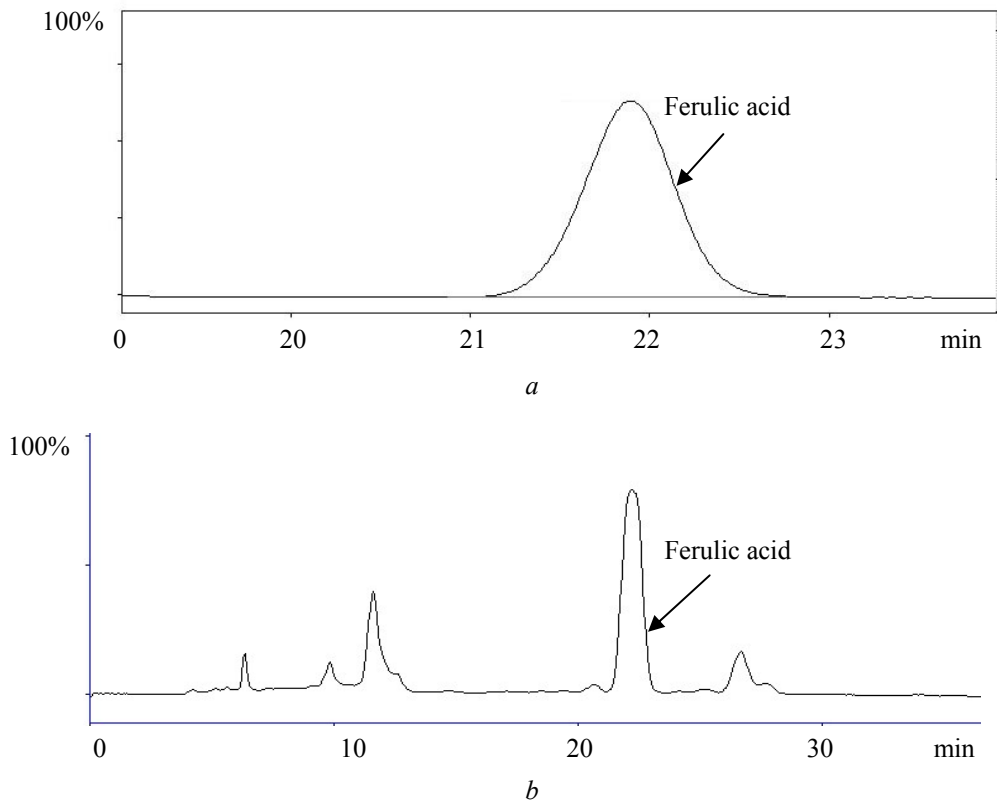

Fig. 3. A typical HPLC chromatogram of a standard sample of ferulic acid (Sigma, USA) (*a*) and extract (*b*)

According to the source [9], the least amount of phenolic compounds contained in above-ground part of the beet (4.2 mg/g dry matter) in the root pulp the content was 11.4 mg/g of dry matter, and the greatest amount of phenolic compounds presented in the peel of beet roots (15.5 mg/g of dry matter).

Such distribution of phenolic compounds, including ferulic acid, in various organs of the plant may be associated with the physiological function of the protective substance for the plant, as ferulic acid has an antimicrobial effect. Disagreement with the literature data on the content of ferulic acid can be explained by the difference of plant cultivars, culture conditions, diversity in reporting on the content (on a dry or wet material), as well as the fact that in some cases, as objects for analysis were not fully plant organs (seeds, roots) but their parts (bran, peeling of the root).

Table 3

The results of determining of ferulic acid in the peel of beet cultivars of Belarusian selection

The name of the cultivar	Content of FA, mg/100 g of dry matter
Vesta	374.88
Gaspadynya	335.00
Prygazhunya	391.96

In connection with the data obtained, the quantitative content of ferulic acid in the skin of beet cultivars of Belarusian selection was analyzed, the results of determining are presented in Table 3.

As can be seen from Table 3, the maximum content of ferulic acid is present in the cultivar "Prygazhunya", so it can be used as feedstock for the ferulic acid release and creating on its basis pharmaceuticals.

Conclusion. Thus the selection of optimal conditions for qualitative TLC analysis was implemented, as well as qualitative determination of ferulic acid in facilities growing in the Republic of Belarus by HPLC. It was determined that the optimal raw material for the isolation of ferulic acid is peeling of the root of beet. Among the grades of the Belarusian selection the richest with ferulic acid is "Prygazhunya", exactly it can be used for the isolation of ferulic acid in preparative amounts to further creation on its basis medicinal agents.

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