Pharmaceutical sciences

ASSESSMENT OF AVAILABLE PHYLLOCHINON IN THE LEAVES OF BERBERIS THUNBERGII DC

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Family Berberidaceae contains approximately 15 genera and 650 species. Specimen of this family is grown up practically in all continents but mostly they occur in temperate and subtropical areas in the northern hemisphere [32, 34].

Chemical composition of Berberis vulgaris is the most explored in Berberidaceae. All organs of Berberis vulgaris contain alkaloids (berberin is the main one), flavonoids (rutin, isoquercetin, quercetin, campferol, apigenin, lutein),cinnamomic acids (coffee acid, chlorogenic acid), tannins. Berberis fruit, except substances above-mentioned, contain organic acids, glycosides, pectin. Berberis leaves contain vitamins K and E, essential oil, flavonoids. [19, 29]

Berberis fruit, bark, leaves, roots are used in folk medicine. Medicinal teas based on Berberis tonic organism, increase blood coagulation, intensify bile secretion, cause angiostenosis and uterus contractility [29].

Nowadays cultivation of different species of Berberis is wide-spread all over the world. Berberis thunbergii DC due to its adequate acclimatization is cultivated according to special techniques of cultivation [14]. But availability and accumulation of biologically active substances including vitamin K, in Berberis species cultivated in Ukraine are not studied yet.

Materials and methods.

Research object: leaves of Berberis thunbergii DC, brand “Atropurpureanana”, stored up in Zaporizhzhya within the vegetation period 2014. The plants of this brand have been cultivated as ornamental plants. Purveyance of leaves has been carried out in different vegetation periods from May to September. The raw-materials gathered have been dried by thin layer turning it from time to time in the shade under shed at temperature 30 – 35 C.

To identify and isolate individual compounds techniques TCX have been used on the plates “Sorbfil АФ-А” in system benzol-petroleic ether (1:1). Standard samples have been used in the research: reagents, solvents according to requirements of the State Pharmacopoeia of Ukraine.

1,0 raw-materials crushed into 1mm fragments were placed into 25ml retort, then 10 ml hexane were added and all it was intermixed for 120 minutes, then it was filtrated, solvent was distilled in the hood at temperature not above 45 C to 2 – 3 ml volume. 0.1 ml extraction was brought upon the plate “Sorbfil AF-A”. The plate was dried a little and submitted to chromatography, after it was dried out and sustained under UV for 2 minutes.

To carry out adequate reaction 1.0 Berberis air – dry plant raw-materials was extracted three times with petroleic ether (1:5) for 5 minutes; 5ml extract was shaken up with 2 ml aniline. Aniline layer had yellow color.

Vitamin K amount in plant raw material has been assessed by spectrophotometry. For this procedure analytic sample of raw material had been crushed to fragments capable to penetrate through sieve with mesh diameter 1mm. Accurate amount (0.5) was placed in 100 ml conic retort and extracted three times. 25 ml of 96% spirit solution were heated on boiling water bath for 15 minutes. Hot extracts were filtrated into 100 ml retort. 4ml of lead acetate solution 10% was added to hot extraction by heating on boiling water bath for 2 minutes till obtaining precipitation coagulation, then it was cooled and filtrated into 100 ml measured retort, 96% spirit was added up to the mark. Solution obtained 5 ml was transferred in 50 ml measured retort and 96% spirit was added up to the mark. Solution optic density was measured on spectrophotometer Specord-200 Analytic Jena UV-vis at wave length 236 nm in cuvette with 10mm layer. Spirit 96% was used as solution for comparison.

Simultaneously optic density for phyllochinone standard solution (Sigma, 95%) was measured. Accurate amount of phyllochinone (about 0.005 gr) was placed into 100 ml measured retort, 50ml 96% spirit was added and intermixed to complete dissolution. Solution volume was completed with spirit to the mark (solution 1). 2.5 ml solution 1 were transferred in 25 ml measured retort, solution was completed with spirit 96% to the mark and was intermixed (solution 2).

Results.

When vitamin K was revealed by TCX method on the plate “Sorbfil AF-A” a spot with yellow-green fluorescence appeared displaying the presence of vitamin K (Rf= 0.67). While spraying with 5% solution phosphoro-molybdenic acid chromatograms obtained the brown-brick-red spots were observed.

Method of spectrophotometric analysis concerning vitamin K in Berberis plant raw material had been elaborated due to using intensive maximum absorption revealed at 236 nm (figure 1). The data obtained are presented in table 1.

The data obtained indicate that the highest concentration of vitamin K in the leaves of Berberis thunbergii-DC is observed in blossoming and right away after it

(May, June) and reaches 5.59+-0.03%. Gradually vitamin K content is decreased and in September forms 2.62+- 0.08%.

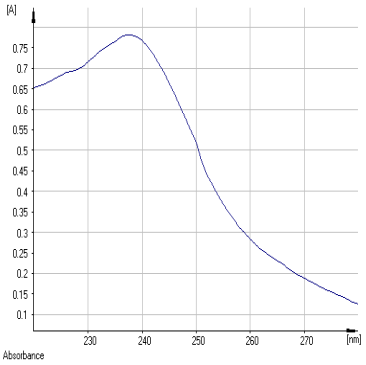

Figure1. UV-spectrum for absorption of ethyl extraction from the leaves Berberis thunbergii DC.

Table1.

Amount of vitamin K in the leaves Berberis thunbergii DC.($\bar{x} \pm \Delta \bar{x}$ %), n= 5

Season of purveyance	Amount
Berberis thunbergii DC., May	4,84 ± 0,02
Berberis thunbergii DC., June	5,59 ± 0,03
Berberis thunbergii DC., July	3,27 ± 0,06
Berberis thunbergii DC., August	3,17 ± 0,04
Berberis thunbergii DC., September	2,62 ± 0,08

Conclusions

Phyllochinone amount in the leaves Berberis thunbergii DC, species “Atropurpureanana” has been investigated by spectrophotometry. Maximum concentration for vitamin K has been observed during flowering and at once after it. It is 5.59 +- 0.03%.