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Polyprenols of Grape *Vitis vinifera L.* Leaves

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Authors' contributions

This work was carried out in collaboration between all authors. Authors UTZ and NMM are extracted leaves; author KUK designed the study, wrote the protocol and the first draft of the manuscript; author KMS managed the analyses of the study; author NKK managed the literature searches. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: The study is aimed to isolate of polyprenols from grape *Vitis vinifera L.* leaves by different methods and to determine of polyprenol content of plant leaves growing in various regions of Uzbekistan.
Study Design: Isolation of polyprenols.
Place and Duration of Study: Department of Organic Chemistry, Institute of the Chemistry of Plant Substances (ICPS). The study was carried out between January, 2011 and December, 2012.
Methodology: We have studied isolation of polyprenols of grape leaves by using of simple, microwave and ultra sound extraction. Spectral, HPLC and HPTLC methods have used.
Results: It was determined that leaves polyprenols are in type polyprenyl homologues with 10-13 isoprene units where undecaprenol and dodecaprenol were dominant. Extraction carried out of grape leaves in various conditions (usual, microwave, ultrasound). Ethanol was high effective for microwave and ultrasound extraction. It was observed forming of polyprenol and high aliphatic alcohols esters (probably proceeding particularly esterification of polyprenols and aliphatic alcohols by organic acids which contain in extracts).
Conclusion: It was determined that extraction of grape leaves by alcohol in microwave

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and ultra sound stirring are high effective methods for isolation of polyprenols.

Keywords: Vitis vinifera L; Vitaceae; a nonsaponifiable fraction; polyisoprenoids; polyprenols; microwave; ultra sound extraction.

1. INTRODUCTION

Cultural grape - *Vitis L of Vitaceae* family is a woody climber in length of 20-40 m. It is cultivated in many countries: on Caucasus, in Moldova, in Ukraine, in Crimea and in Central Asia. Two main species are spread in Uzbekistan: *Vitis vinifera, V. tabrusca* leaves [1,2]. In traditional medicine extracts of leaves of a grape accept at violation of a swap of an oxalic acid, at a blood spitting, for gargles at anginas and out washings at dermal and hypertonic diseases [3]. Saccharides, quercetine, inosite, tannins, carotin, betaine, tocopherol, ascorbinic acid and other organic acids are discovered in grape leaves. They are rich on macro- and microelements. Among them there are potassium, calcium, sodium, magnesium, iron, aluminum, zirconium, silicium, phosphorous, sulfur, chlorine, etc. [1,4].

Polyprenols are known to possess anti-oxidative, hepatoprotective activities of an alcoholic extract of red leaves of a cultural grape *Vitis viniflora L.* [5].

It has not been studied polyisoprenoids of grape leaves till now. The wide spectrum of biological effect of these substances demonstrates expediency of study polyprenols of grape leaves. It is known that polyprenyl alcohols (PP) play a crucial role as lipophilic transmitting agents of saccharums in biosynthesis of bacterial polysaccharides and glycoproteins [6,7]. Plant polyprenols and their biological role is widely researched by many scientists of the world, but function endogenous PP plants while finally is not taped [8,9]. There are data about that they minister chemotaxonomic measure for plants [10] that is very relevant factor in probe of plants from various places of growth with miscellaneous soil-climatic conditions.

Besides, PP of plants introduce a great interest from the point of view of possibility of using for needs of national facility. On the basis of PP form flocks of pharmaceuticals and biologically active additives to nutrition and cosmetology [11,12]. Therefore researches of plant PP from domestic raw introduce certain interest.

2. MATERIALS AND METHODS

2.1 Plant materials

The grape leaves (five years' plant) used in this study was obtained from Tashkent, Bukhara and Samarkand areas in August, 2010, in a maturation starting period. The leaves were dried at room temperature in a shade, grinded with a level of 2-3 mm.

Cotton leaves polyprenols were used as the standard [13].

2.2 Methods

2.2.1 Isolation

It is known that extraction of polyprenol containing extracts are used different solvents, for example, methanol [14], isopropanol [15], ethanol [16,17], acetone [18], diethyl ether [19],

petroleum ether [20], or their mixtures as acetone-hexane [21], hexane-ethanol [22] and others. For comparing of completeness of an extraction of the total extractable compounds and finding of optimal conditions we have performed extraction of leaves with 96%-s ethanol: allowing that ethanol extracts as the pollution-free solvent most adequately model pharmaceutical drugs and biologically add activations to nutrition and cosmetology [23].

2.2.1.1 Isolation of the extractable sum (General method)

Plant leaves (1.0g) growing in Tashkent, Samarkand and Bukhara areas obtained to thickness of 2.0-3.0mm, extracted is triple 96%-s ethanol (100 ml) an insisting method. All alcoholic extracts were pooled, a solvent evaporated on a rotary evaporator at 40ºC. As a result were obtained the sum of extractable substances (SES) in number of 0.206, 0.197 and 0.188g with the content of polyprenols – 2.19%, 1.86 and 1.68% from ADW accordingly. Experiment repeated for 5 times.

2.2.1.2 Isolation of polyprenols from SES which gained by an insisting method

Grape leaves collected in a maturation (26.08.2010) in Tashkent area, were dried in a shade. Leaves of 1,0kg to freeness of 2.0-3.0mm, were extracted triple 96% ethanol at a water duty 8:6:6 (triply) an insisting method. All alcoholic extracts were collected, solvent ferried out on a evaporator at 40ºC. It was obtained of SES in number of 200.6g. The SES of 100.0g parted on a column. We have assembled on 250 ml of 100 fractions. Fractions 53-54-55 contained polyprenols, they were united. The yield was 9.15g (9.15% from the total with 73.5% content of polyprenols), the yields of fractions 56-58 were 1.8g – 0.36% from ADW (48-50% polyprenols3) homologues content was: deca -6.9, undeca-36.7, dodeca -46.8 and tridecaprenols- 9.6. by HPLC [24]. Overall yield of polyprenols is 1.53% from ADW. Experiment repeated for 5 times.

2.2.1.3 The isolation of Polyprenols from SES obtained by using microwave

The microwave with following engineering arguments are used for extraction: Model OM - 9925 E, the unit of a delivery - 230V, 50 Hz, power - 800 W, frequency of a microwave - 2450 MHZ, the time clock of 59 minutes of 90 sec, volume - 20 l.
Plant leaves (10.0g) extracted triple by 96% ethanol on 100 ml in microwave with a strain 450V at stirring within 2 minutes. We have collected of all alcoholic extracts (180 ml) and sampled of 100 ml an alcoholic extract, added 24 ml of 50% aqueous solution KOH and intermixed within 2 hours. To a reaction mixture added equivalent volume of water and extracted triply hexane. Hexane extensions collected to all, washed out by water to pH =7. Dried over anhydrous Na_2SO_4, solvent ferried out on a evaporator at 40ºC and have obtained a nonsaponifiable fraction 0.48 g which one parted on a column. Obtained polyprenols fraction in an amount 0.097 g is more than 92% purity (20.2% from a nonsaponifiable fraction or 1.36 g from ADW). Experiment repeated for 5 times.

2.2.1.4 Isolation of Polyprenols from SES obtained by ultra sound using

Ultra sound instrument is Varian aerograph, 220/240 V, 50/60 Hz. Plant leaves (10.0 g) were extracted triple 96% ethanol on 100 ml at ultra sound stirring within 30 minutes. We have collected of all alcoholic extracts (180 ml) and sampled of 100 ml an alcoholic extract, added 24 ml of 50% aqueous solution KOH and intermixed within 2 hours. To a reaction mixture added equivalent volume of water and extracted triply hexane. Hexane extensions collected to all, washed out by water to pH =7. Dried over anhydrous Na_2SO_4, solvent ferried out on an

evaporator at 40ºC and have obtained a nonsaponifiable fraction 0.55 g which one sharing on a column yields Polyprenols fraction 0.085 g more than 90% purity (15.5% from a nonsaponifiable fraction or 1.28 from ADW). Experiment repeated for 3 times.

2.2.2 Ultra sound acylation

2.2.2.1 Control experiment (ultra sound acylation of polyprenols by acetic acid)

To 100 mg (0.1305 mmol) Grape leaves Polyprenols of 95% purity were added 100 mg acetic acid (1.67 mmol) stirring in Ultra sound during 60 minute. Further a reaction mixture treated feeble solution sodium hydroxide and carbonate for deleting of excess of an acid and water before neutral reaction of washing waters. The rest terrified over anhydrous Na_2SO_4, ferried out a solvent, then filtrated through coarse grinding silica gel. It isolated by hexane at first, then system of solvents hexane-chloroform gradually enlarging polarity of system.

2.2.3 Chromatography

Sorbfil (Joint stock company Sorb Polymer Russian Federation) PTLC-AF-B-UV plates (10x10 cm), sorbent is silica gel, type of substrate aluminum, connecting silicazol and Silufol plates AL SIL G/UV (Germany, 20x20) were used for TLC, washed out by ethanol (96%), solvents system is benzene-ethylacetate 24:1 (A), hexane - chloroform 1:2 (B). Developing agent is $KMnO_4$ in sulfuric acid, 3%-s alcoholic solution of vanillin or an iodine pairs. Chromatography column sizes are 18x98 cm and 3.0x135 cm, adsorbent - silica gel 100/250 and 100/160 mesh, solvent system: petroleum ether - chloroform and petroleum ether – diethyl ether with gradual magnification of polarity were used.

The quantitative content of polyprenols was determined by HPTLC (Camag, Switzerland). Removal conditions are as in densitometry [25] on plates Sorbfil HPTLC-AF-UV (10x10cm), in a case of determining of homologues content of PP were used impregnated with paraffin (5%) Sorbfil. Sample application: 1 mg of the exaction mass is dissolved in 1 ml chloroform. Band wise with automatic TLC LINOMAT 5, up to 10 tracks, 3 mm band length, application volume 5 ml of the sample solution, 1 to 5 ml for the standard solution, track distance 7 mm, distance from the left side 15 mm, distance from lower edge 10 mm. Chromatography carry out in the twin-trough chamber 10x10 cm with tolyene- ethylacetate (19:1) (C) and acetone-methanol 5:1(D) Sorbfil HPTLC-AF-UV phases. The migration distance was 70 mm from the lower plate edge. Then the plate was dried in a stream of cold air for 10 min. Determining of qualitative content of polyprenols carried out by densitometry [25] with comparison standard sample. Homologues content was determined by comparison with mixture of polyprenols n=10-12 kindly transmitted from a collection of Institute Biochemistry and Biophysics PAS, Warsaw (Poland) and consists deca - 11.2%, undeca – 72.2%, dodecaprenol -16,6%. Absorption measurement made at 200-210 nm with TLC Scanner 3 and winCATS PP.

HPLC was performed on a reverse-phase column Lichrosorb RP-18 5 μm 6.3x0.2 cm, tocopherol palmitate were used as an internal standard. Samples were collected at a flow rate of 50 μl/min, eluation at 100 μl/min; detection at 210 nm, measurement time was 0.3 sec, sensitivity - 0.8-3.2. Rate of a paper was 12 mm/min. Analysis time of each probe was 12–18 min. As a screw taps were used individual polyprenols of sea buckthorn leaves [24].

2.2.3.1 Chromatographic purification of polyprenol fractions

Polyprenol fraction (9.0 g) parted on a column, an adsorbent ratio to an extract 40:1, a solvent is petroleum ether: diethyl ether. Takeoff of fractions was performed in bottles in 30 ml bulk, assembling on 15-20 ml. Join of fractions led according to results of TLC and in case of fraction polyprenol it is parallel from HPTLC on plates Silufol and Armsorb. There were obtained 16 fractions. Fractions 1-4 (0.12 g) represented a mixture of aliphatic hydrocarbons. Fraction 5 (0.09 g) is a mixture of unknown substances. Fractions 6-9 (0.98 g) are esters of sterols and triterpenols. Total 6.3 g content of 95% polyprenols were determined in fractions 10 (3.2 g), fraction 11 (3.1 g). Fractions of 12-14 (1.18 g) are a mixture of phytosterols and polyprenols. Fractions 15, 16 (0.32 g) contain β-sitosterin as the white crystals with m.p. 136-137ºC.

Homologous composition of fractions 10 and 11 were determined by HPTLC, using as the screw taps of cotton leaves polyprenols of lines L-4 [13].

Mixture of polyprenols is the light yellow oil. The molecular formulas are $C_{50}H_{82}O$, $C_{55}H_{90}O$, $C_{60}H_{98}O$, $C_{65}H_{106}O$.

2.2.4 Spectrums

IR spectra were obtained with IR Prestige-21 spectrophotometer (Shumadzu) in tablets of KBr (ν, cm^{-1}). ^{1}H NMR and ^{13}C NMR spectra were obtained with Bruker AM-300 absorption spectrometer at 300 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR. Measurements were made in $CDCl_3$, a dial δ, HMDS were used as internal standard.

3. RESULTS AND DISCUSSION

Polyprenols of grape of a vintage variety - “Buvaki”, on terrains of Tashkent, Bukhara and Samarkand areas are studied. SES is isolated from the grape leaves of a sort exsiccated in a shade “Buvaki”, collected in maturation on a procedure [26].

The greatest content of SES was found in grape leaves of Tashkent area samples and constitutes 20.60% from air-dry weight (ADW) (Table 1).

Table 1. The content of polyprenols in grape leaves of a sort “Buvaki”, growing in different regions of Uzbekistan (in % from ADW)

Growing area	The yield of SES	Content of Polyprenols
Tashkent region	20.6±0,21	2.19±0,074
Samarkand region	18.8±0,18	1.86±0,062
Bukhara region	19.7±0,19	1.68±0,055

The obtained data demonstrate that maximum content of polyprenols is grape leaves growing in the Tashkent area – 2.19%, and for plants of the Samarkand and Bukhara areas constitute 1.86% and 1.68% accordingly.

Further for obtaining polyprenol fractions of SES (Tashkent area) parted on a column with a silica gel in the ratio adsorbent: substance 15:1. Polyprenols were isolated in system of solvents of petroleum ether - chloroform 1:1. It is isolated 9.15 g polyprenol fraction which is

consists 73.5% polyprenols. The fraction which consists 48-50% (1.8 g) polyprenols were analyzed by HPLC, an eluent is a mixture of 1:3 methanol acetone (v/v) and found following homologues components of them: deca - 6.9, undeca-36.2, dodeca -46.8 and trideca- 9.6% (Fig. 1).

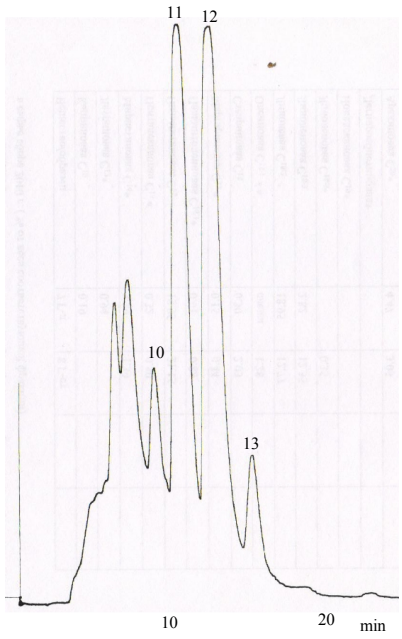

Fig. 1. HPLC analysis of polyprenol fraction of grape leaves

Polyprenols were isolated also. The further purification of polyprenol fraction was led on a silica gel column in the ratio adsorbent: substance 40:1. System of solvents as petroleum ether – diethyl ether has been used as a moving phase. There are obtained 16 fractions: 1-4 represented a compound of aliphatic hydrocarbons, 6-9 are esters of sterols and triterpenols. Fractions of 12-14 are a mixture of phytosterols and polyprenols. Fractions 15-16 contained β-sitosterin. Identification of fractions has carried out on TLC comparing by authentic standard samples [27] and data of IR-spectrums. The fractions 10-11 contained polyprenols (TLC). Homologous compositions of polyprenols were determined by HPTLC [25].

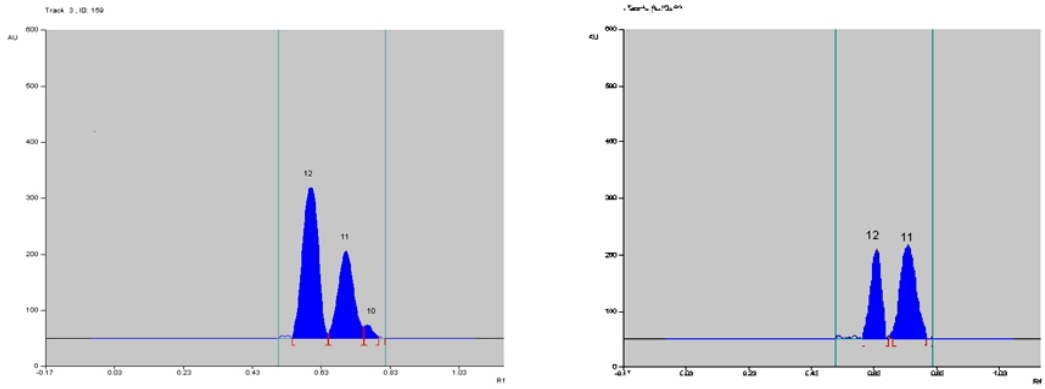

Fig. 2. HPTLC analysis of polyprenol fractions 10 (left) and 11 (right)

Polyprenol fraction HPTLC analysis demonstrates that the ratio of polyprenol homologues each fraction differs on the quantitative composition of homologues, i.e. in polyprenol fraction 10 dominates dodecaprenol (61%), content of undedeca - and decaprenols are 35.3 and 3.7% accordingly. In polyprenol fraction 11 content of undecaprenol was 53.5% (dodecaprenol 45%) (Fig. 2). It is possible to conclude that everyone polyprenol fraction differs from each other on a ratio of polyprenyl homologues on results of analysis.

Structure of grape polyprenols has studied by IR - and NMR spectroscopy.

IR-spectra: ν, cm^{-1}: 836.5 (C-H deformation oscillations of olefins), 1000 (C-O the valence oscillations of allyl alcohol), 1376 (C-H deformation vibrations of CH_3), 1448 (C-H deformation vibrations CH_3, CH_2), 1666.7 (C=C), 2853 (CH at CH_2), 2918.66 (CH at CH_2, CH_3), 2916.86 (SN CH_3), 3321.96 (the polymer associates).

^{1}H NMR-spectra, (300 MHz, δ, ppm, J/Hz): 1.19 (3H, s, CH_3), 1.51-1.53 (9H, s, three trans-C$\underline{H_3}$); 1.61-1.65 (20H, s, 7 cis-CH_3); 1.98, 2.02, 2.03 (39H, t, =CC$\underline{H_2}$); 3.89 (2H, dd, J=0.8, J=7.1 =CH-$\underline{CH_2}$OH); 5.05 (12H, t, J=5,7, =CH); 5.35 (1H, t, J=7,1 =CH at the end of unite).

^{13}C NMR-spectra, (75 MHz, δ, ppm, J/Hz): quartets at 16.21; 17.87; 23.56; 23.62 and 25.87. ($\underline{C}H_3$); triplets at 26.66; 26.89; 26.95; 27.04; 27.16; 29.91; 32.24; 32.46; 39.96; 39.996 ($\underline{C}H_2$); 59.25; doublets 124.41; 124.53; 124.68; 124.77; 125.15; 125.20; 125.28 ppm ($\underline{C}$H); singlets at 131.38; 135.10; 135.18; 135.41; 135.58; 136.26, and 140.03 ppm (C).

As a result of data of ^{1}H and ^{13}C NMR-spectrums it can be concluded that they contain in the molecule three trans-, 7-10 cis isoprene units and belong to the previously described ficaprenols [28].

We have performed extraction of leaves with 96% ethanol for comparing of completeness of extraction SES and finding of optimum conditions: 1) insisting at room temperature (method 1); 2) extraction in microwave (method 2); 3) ultra sound stirring (method 3). The results are given in Table 2.

Table 2. The yields of SES and content of grape leave polyprenols of a sort "Buvaki" depending on an extraction method (% from ADW)

Extraction type	Condition of extraction			The yield of SES	The yield of polyprenols
	Method	Number of extractions	Duration		
6% ethanol (method 1)	Insisting	3	24 hours	21.0±0,20	1.53±0.076
96% ethanol in microwave (method 2)	Stirring	3	2 min	39.0±0.04	1.36±0.001
96% ethanol (ultra sound) (method 3)	Stirring	3	30 min	44.0±0.14	1.28±0.007

The most effective method for isolation of SES is an extraction under the influence of ultra sound stirring where the yield constitutes 44.0% (Table 2). However, confrontation of content

polyprenols in SES (HPTLC) demonstrates that in case of a method 2 or 3 there is happen some structural changes of SES components, including polyprenols. It was possible that the using of ultra sound extraction in microwave contributes to particularly esterification of polyprenols and the high aliphatic alcohols with organic acids which are presented in extracts. Data of HPTLC affirms it. Polyprenols derived by methods 1, 2 and 3 were different on their UV spectra obtained using HPTLC; but their UV spectra were identical after hydrolysis. So the difference in the UV spectra of polyprenols was considered to be due to some modification of them at isolation procedure by ultra sound stirring and microwave extractions.

Besides, for improvement of this appearance we have discovered check experiments of acylation PP with an acetic acid with ultra sound stirring within 90 minutes. The yields of acyl-products was 66.2 mg. IR spectra:ν, cm^{-1}: 1723 (CO ester), 1269 (-C-O-C-), 1460, 2952 (CH of CH_3), 1666 (C=C) cm^{-1}. IR-spectral data are delivered affirm formations of acetate PP. It is known that separation solanesol from tobacco leaves with application ultra sound [22] where basic hydrolysis is performed. In case of extraction of foliage of a spruce and a fir with ultra sound stirring the aqueous solution of alkali [29] is used also. Therefore in these operations formations of connected PP was not determined. Probes in this direction will be prolonged, while we only guess a capability of PP modification.

Therefore for determination of the content of free polyprenols in SES (method 2 and 3) their basic hydrolysis on a procedure [30] was carried out. It was resulted nonsaponifiable fractions with 7.5% and 8.5% yields from ADW accordingly. The yields of polyprenols after sharing of nonsaponifiable fractions on a silica gel column were constituted by 1.36 and 1.28% accordingly.

4. CONCLUSION

Search of extractable compounds of grape leaves were demonstrated that polyprenols are introduced in a type polyprenyl homologues from 10-13 isoprene units where dodecaprenol and undecaprenol were dominate. It is found that microwave extraction and ultra sound stirring probably happen by particular esterification of polyprenols with acids which presented in extracts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Lavrenov VK, Lavrenova GV. Modern encyclopedia of drug plants. Neva, Sankt-Petersburg, Moscow; 2006.
2. Flora of Uzbekistan, “Science”, Tashkent. 1959;4.

3. Maznev NI, Drug Plants. The reference manual. Moscow: Martyn; 1999.
4. Dul VN, Chuparina EV, Dargaeva TG, Kopitkov YF, Sokolskaya TA. Cultural grape - *Vitus Vinifera L*. – is a new source of macro- and microelements. Bulletin VSU, Series: Geography, Geoecology. 2010;2:76-78. Russian.
5. Pari L, Suresh A. Effect of Grape (*Vitus vinifera L.*) Leaf Extract on Alcohol Induced Oxidative Stress in Rats. Food Chem Toxicol. 2008;46(5):1627-1634.
6. Sharma CB, Babczincki P, Lehle L, Tanner W. The role of dolichol monophosphate in glycoprotein biosynthesis in Saccharomyces cerevisiae. Eur J Biochem Polon. 1974;46:35-41.
7. Jankowski W, Chojnacki T. Enzymic formation of polyisoprenol phosphate sugars. Acta Biochimica Polonica. 1972;19:51-69.
8. Jack WR, Anthony CR, Kothapalli R, Kenneth KC. Distribution, metabolism and function of dolichol and polyprenols. Progress in Lipid Research. Oxford, New York. 1985;24(4):270-309.
9. Swiezewska E, Sasak W, Mankowski T, Jankowski W, Vogtman T, Krajewska I, et al. The search for plant polyprenols. Acta Biochimica Polonica. 1994;41:221-260.
10. Ranjan R, Swiezewska E, Chojnacki T. On the occurrence of polyprenols in plants. Acta Biochimica Polonica. 2001;50.
11. Sultanov VS, Lapteva UI, Roshin VI, Trofimova TN, Gavrisheva IA. Clinical search hepatoprotector “Ropen” at deceases of hepatobiliar systems. Gastroenterology of Sankt-Petersburg. 2010;(4):7-11. Russian.
12. The certificate with State registration №77.99.11.3.U.1829.3.10 from 12.03.2010. Available: http://sitopren.ru/index.htm.
13. Rashkes AM, Saitmuratova OKh, Khidyrova NK, Shakhidoyatov KhM, Leontev VB. Natural undecaprenol and its influence to biochemical reactions of cotton plantlet nuclear. Chem Nat Compounds. 1998;65-69. Russian.
14. Suga T, Ohta Sh, Nakai A, Munesada K. Glycinoprenols: Novel Polyprenols Passessing a Phytyl Residue from the leaves of Soybean. J Org Chem. 1989;54:3390-3393.
15. Roshin VI, Fragina AI, Solovev VA. Polyprenols and tocoferols from needles Picea Abies (L.) karst // Plant resources. Sankt Petersburg. 1986;4(22):530-537. Russian.
16. Vasilev SN, Roshin VI, Virodov VA. Content of extractable substances wood greens of a pine ordinary. Review Inform. Moscow: VNIPIEI Wood industry. Russia, 1991;72:57-62. Russian.
17. Kuchin AV, Karmanova LP, Koroleva AA, Moiseenkov AM. Search content of less molecular components wood greens Coniferous breeds *Pinus, Picea* и *Abies.* Chemistry of Woods and Woodchemistry. Works of Komi Research Center Ural Departure of Russia AS. – Syktyvkar. 1993;129:47-61. Russian.
18. Sasak W, Mankowski T, Chojnacki T, Daniewski WM. Polyprenols in *Juniperus communis* needles. FEBS Lett. -Elsevier Science. 1976;64:55-59.
19. Salenko VA, Kukina TP, Karamishev VN, Sidelnikov VN, Pentegova VA. Chemical search *Hippophae Rhamnoides*. II. Basic components of neutral parts hydrolysis products of berries leave extracts. Chem Nat Compounds. 1985;4:514-519. Russian.
20. Vasilev SN, Abiev RSh, Roshin VI. Fractionation biological active substances wood greens by liquid extraction method in apparatus pulse-resonance type. 2nd Meeting Wood chemistry and organic synthesis: thesis, Syktyvkar. 1996;35. Russian.
21. Marczewski A, Ciepichal E, Le XC, Tran TB, Swiezewska E, Chojnacki T. The search for polyprenols in dendroflora of Vietnam. Acta Biochimica Polonica. 2007;54(4):727-732

22. Machado PA, Fu H, Kratochvil RJ, Yuan Y, Hahm TS, Sabliov CM et al. Recovery of solanesol from tobacco as a value-added byproduct for alternative applications. Bioresource Technology. 2010;101:1091-1096 .
23. Patent of RUz № IAP 04396 from 08.07.2011. Preparation improuving exchange processes in skin and its obtaining method. Shakhidoyatov KhM, Khidyrova NK, Mamatkulova NM, Sirov VN, Khushbaktova ZA, Shakhidoyatov RKh et al. Russian.
24. Khidyrova NK, Rahmatova MJ, Kukina TP, Shakhidoyatov RKh, Shakhidoyatov KhM. Polyprenols and triterpenoids from leaves of Alcea nudiflora. Chem Nat Compounds. 2012;48(2):180-184.
25. Mamatkulova NM, Khidirova NK, Makarramov NI, Shakhidoyatov KhM. Polyisoprenoids of leaves *Morus alba* and *M.nigra*. Chem Nat Compounds. 2012;98-99. Russian.
26. Khidyrova NK, Van EV, Shakhidoyatov RKh, Babakulov KhM, Abdullaev ND, Shakhidoyatov KhM. Polyprenols from leaves and stems of the plant *Althaea officinalis*. Chem Nat Compounds. 2012;48(3):358-360.
27. Kiyamova SE, Khidyrova NK, Kukina TP, Shakhidoyatov KhM. Neutral constituents from leaves of plants of the family *Malvaceae I. Abutilon theophrasti*. Chem Nat Compounds. 2012;48(2):297-298.
28. Stone KJ, Wellburn AR, Hemming FW, Morton RA. The characterization of ficaprenol-10,11 and -12 from leaves of *Ficus elastica* (decorative rubber plant). Biochem J. 1967;102:313-124.
29. Koroleva AA, Karmanova LP, Kuchin AV. Method isolation of polyprenols from plant raw. Information of high schools. Chemistry and Chemical technology. 2005;48(3):97-99. Russian.
30. Rashkes AM, Rashkes YaV, Nadjimov UK, Khidyrova NK, Shakhidoyatov KhM. Comparison analysis of chemical content of leaves of normal on altitude and the dwarf saltant of cotton. Dokl AS UzSSR. 1989;53-54. Russian.

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