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Accumulation Dynamics of Polyprenols in *Alcea nudiflora*: Identification and Comparative HPTLC Analysis

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M. J. Rahmatova, N.M. Mamatkulova, Kh.U. Khodjaniyazov, N.I. Mukarramov, N.K. Khidyrova

S.Yu. Yunusov Institute of the Chemistry of Plant Substances AS
Uzbekistan, Tashkent city, Uzbekistan.

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ABSTRACT

Alcea nudiflora polyprenols accumulation dynamics in different parts of the plant and ontogenesis were studied. Polyprenols in the total neutral substances analyzed quantitatively by applying HPTLC. It was found that the plants contain deca-, undeca- and dodecaprenols with domination of undecaprenol. *Alcea nudiflora* distributed in Namangan region is rich for polyprenols comparing with Tashkent region.

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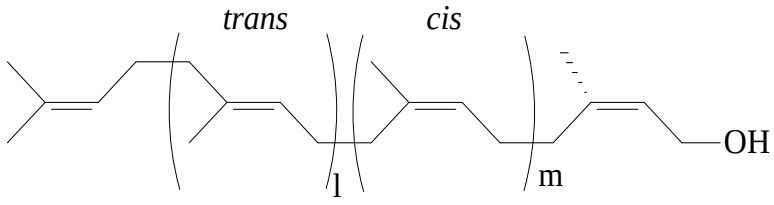
1. INTRODUCTION

Alcea nudiflora L. (Malvaceae) is widely distributed in the Pamir-Alai (Alai, Turkestan, Nuratau, Zarafshan) and Tien Shan ranges (Chatkal, Kuramin, Ugham, Mogoltau), and Tashkent region (Pskem, Buruchmulla, Sukok, Nanai and Ertash). Their various forms are differed in flower colors: white, pink, red, purple, and dark red (Fig.1).

Fig.1. *Alcea nudiflora*

Plant Polyprenols (PPre) are the major group of biologically active substances. They represent the big interest because these natural sources are used as drugs of various purposes, furthermore, application as biologically active food supplements and cosmetics. Function of Polyprenols consists in linkage and carriage of oligosaccharides from nucleotide phosphosaccharides to polypeptides and in formation of their complexes [1-3]. This is a general process for cells of all alive essences; its infringement leads to frustration of life ability of an organism in general. The most important natural function of Polyprenols is participation in glycosylation process of proteins [3].

In the nature, Polyprenols are widespread in green parts, basically in leaves of plants; in a human body they are concentrated in pancreas, brain, heart, spleen and other parts. Polyprenols differed by quantity of isoprene units and a geometrical configuration of double bonds and this difference is specific to different families of plants [2, 4, 5].



$l = 3, m \geq 3$

The wide range of biological activity of Polyprenols is probably due to their membrane-active properties and their ability to bind PPre fragments with biologically active groups [6-8].

It was also found that Polyprenols isolated from 108-F cotton leaves have the unique property of the opening calcium channels in membrane bilayers [9]. Plant biostimulator Uchkun was created on the basis of cotton polyprenols [10].

Earlier we have investigated Polyprenols of different parts of cotton plant belonging to *Malvaceae* family. It is earlier found that quantitative composition of Polyprenols consists 0.7-4.0% from depending on cotton grade and line [11, 12].

The purpose of the present research was to study accumulation dynamics of PPre in different plant organs (leaves, flowers, stems and seeds) of *Alcea nudiflora* collected from Tashkent and Namangan regions of the Republic of Uzbekistan by applying of HPTLC. Furthermore, comparative analysis of Polyprenols composition of *Alcea nudiflora* (Malvaceae) leaves with different color flowers was investigated.

2. MATERIALS AND METHODS

2.1. Isolation of Polyprenols

The plant raw material of *Alcea nudiflora* pink (leaves, epicalyx, stem, flowers and seeds) was collected from the Tashkent region on August 15, 2013 and dried in the dark at room temperature (20-25^{0}C). The *Alcea nudiflora* leaves (flowers of white, pink, red and dark red colors) native for Namangan region were collected in different phases of development-budding (June), the beginning of flowering (July), mass flowering and fruiting (August), fruiting (September).

At the first stage, solution system and TLC plates were chosen for obtaining the reliable amounts of Polyprenols as the optimum separation conditions.

The solvent system used is given below: benzene-ethyl acetate, 24:1(A); $CHCl_3$–hexane, 2:1(B); toluene-ethyl acetate, 19:1(C); and acetone–hexane, 9:1(D) by application of different plates of firms as Merck and Watchman: Silufol-254 UV, Sorbfil HPTLC-AF-UV. Thus, the best results were obtained when the solvent system C and plates of Sorbfil HPTLC-AF-UV were used. Cotton leaves polyprenols used as standard samples, calibrated earlier with samples kindly presented of members of the Institute of Biochemistry and Biophysics (Warsaw, Poland) [13].

Isolation of total neutral substances

It was earlier determined that polyprenols of this plant occurred as free or connected forms [14]. The dried up plant material (leaves, epicalyx, stem, flowers and seeds with a crushing degrees of 2.0-3.0 mm, each 10g) was extracted by ethanol (3x). Ethanol extract was concentrated and treated with aqueous potassium hydroxide solution, subsequently extracted with benzene. Benzene fraction treated by water and 5% sodium carbonate solution. The residue was dried over Na_2SO_4. The solvent was removed. The yield of total neutral substances was 4.0-0.2% of air-dry weight (ADW) (table 1). The sum of Polyprenols obtained was analyzed on HPTLC. Polyprenols distribution in different parts of *Alcea nudiflora* was studied at the first step (table 1).

Table 1: Composition of polyprenols in different organs of *Alcea nudiflora*, Tashkent region (% from ADW)

Plant part	Yield of neutral substances (NS)	PP composition	
		From total NS	From ADW
Leaves	4.0	45.6	1.82
Epicalyx	1.6	23.9	0.38
Stem	0.8	38.1	0.31
Flowers	0.7	20.5	0.14
Seeds	0.2	9.0	0.018

Method HPTLC had earlier successfully used by us for the control of transformation of deoxypeganine alkaloid to peganol and bromopeganol [15], and at determination of the homologous polyprenols composition of *Morus alba* and *M. nigra* (Moraceae) leaves [16].

2.2. Sample preparation: The total neutral substances (1 mg) were dissolved in chloroform (1 ml). Chromatogram layer: HPTLC plates Sorbfil HPTLC-AF-UV (10x10cm, Russian Federation), alternative HPTLC–Platten Kieselgel 60F 254 (20x20cm, Merck, Germany), DC-Folien Al Sil G/UV (20x20cm, Whatman, UK), and Silufol-254 UV (15x15cm, Czechoslovakia).

2.3. Sample application: Band wise on LINOMAT 5, upto 10 tracks, band length 3 mm, the sample solution application volume 5 ml, track distance 7 mm, distance from the left side 15 mm, distance from lower edge 10 mm.

2.4. Chromatography: In the twin-trough chamber 10x10cm with toluene-ethyl acetate (19: 1) for the Merck, Whatman, Silufol-254 UV, and 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.

2.5. Densitometry: Absorption measurement made at 200 nm with TLC Scanner 3 and winCATS for Polyprenols.

3. RESULTS AND DISCUSSION

It is known, that for the qualitative and quantitative analysis of Polyprenols, in the majority cases, is used on HPLC [9, 17, 18]. We applied for this purpose an express method of HPTLC which can estimate in parallel at once is a little bit exemplary for comparison of the contents of Polyprenols in *Alcea nudiflora* leaves from various origin. The best results were obtained at usage of solvent system: toluene-ethyl acetate (19:1) and Sorbfil HPTLC-AF-UV plates. Elution made in the glass chamber (distance-7cm).

It can be seen from table 1, that the maximum of polyprenols content found in leaves and its share was 4.8 times more compared to epicalyx, 9.8 then in stems, 13 times more than in flowers and more than 100 times in the seed, which is in agreement previously obtained data for other plants [19, 20]. Further investigations are carried out on the leaves of *Alcea nudiflora.*

The influence of geographical factors for polyprenols accumulation in *Alcea nudiflora* (pink) leaves was studied. The amount of polyprenols in plant leaves collected from Tashkent and Namangan regions was compared. The total neutral substances isolated by yields of 4.0 and 5.1 % from air dried weight (ADW), respectively, were analyzed. Figures 2-4 presents the UV-spectrum and chromatograms.

The analyses are showed that the polymer homologies of PPre with isoprene units of n=10-12 presented, where undecaprenol (n=11) dominated (peaks with a big area) regardless of germination region (fig. 3, 4).

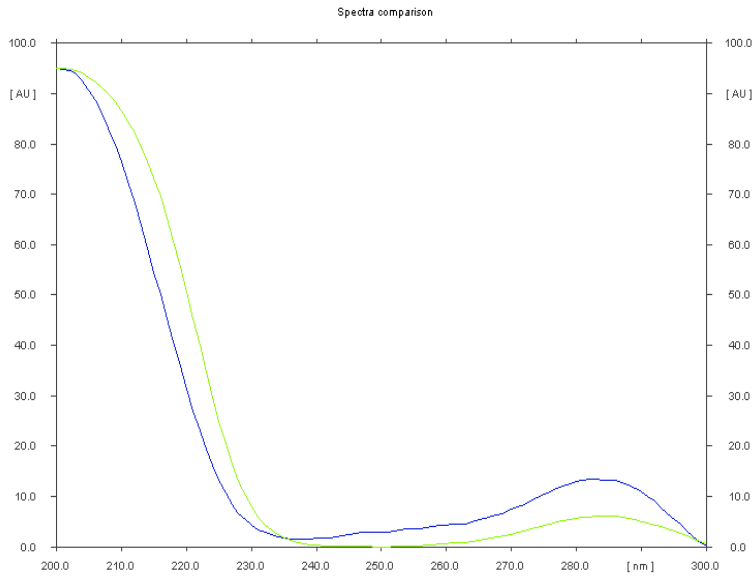


Fig. 2. The UV spectrum of the reference sample and the leaf sample of *Alcea nudiflora*

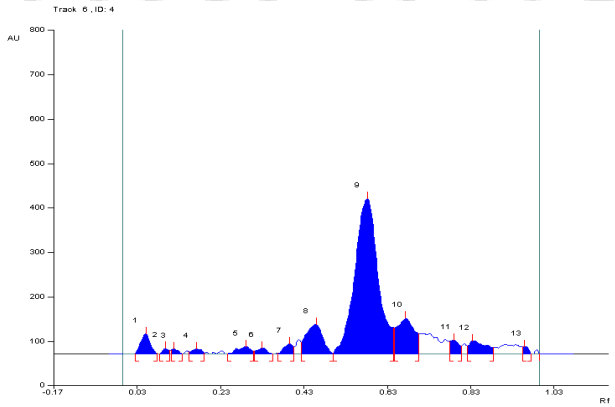


Fig. 3. HPTLC of the total NS of *Alcea nudiflora* from Namangan region

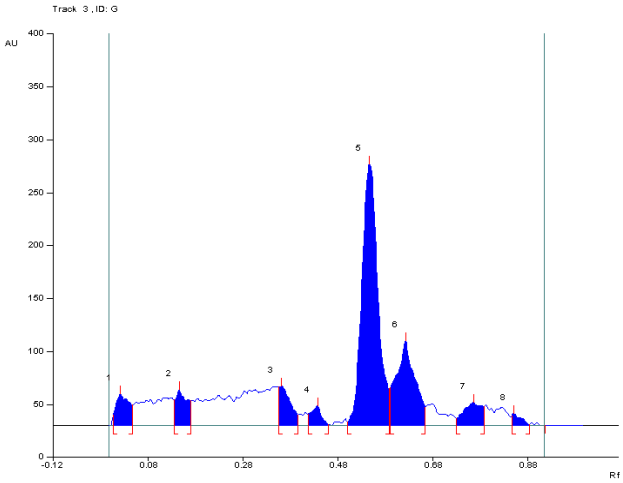


Fig. 4. HPTLC of the total NS of *Alcea nudiflora* from Tashkent region

Polyprenols composition in *Alcea nudiflora* from Namangan region was 51.7% and for Tashkent region 46.7% of the total neutral substances.

Accumulation dynamics of polyprenols in leaves of the Namangan origin plant was studied in different periods of plant development, i.e. at budding, beginning of flowering, the beginning of full flowering and fruiting, fruiting. The results are given in table 2.

Table 2: Polyprenols accumulation in different color leaves of *Alcea nudiflora* at vegetation phase of June - September, *from ADW, %*

Sr. No.	Sample	Vegetation period	Yield of NS	PP composition
1	*Alcea nudiflora White flowers*	June	2.2	1.09
		July	3.3	1.48
		August	4.6	1.88
		September	3.4	1.59
2	*Alcea nudiflora Pink flowers*	June	2.3	1.12
		July	3.9	1.82
		August	5.1	2.32
		September	4.7	1.97
3	*Alcea nudiflora Red flowers*	June	2.1	1.02
		July	3.3	1.46
		August	3.6	1.66
		September	3.3	1.44
4	*Alcea nudiflora Dark red flowers*	June	2.1	1.00
		July	3.2	1.43
		August	3.7	1.78
		September	3.3	1.45
5	*Alcea nudiflora purple flowers*	June	2.1	0.99
		July	3.0	1.33
		August	4.2	1.78
		September	3.6	1.52

Determination of homologous polyprenols composition in leaves showed that decaprenols occurred at a ratio 2.7/8.6 (Tashkent/Namangan), undecaprenols 53.8/63.9, and decaprenols 43.5/27.5, respectively (Fig. 5).

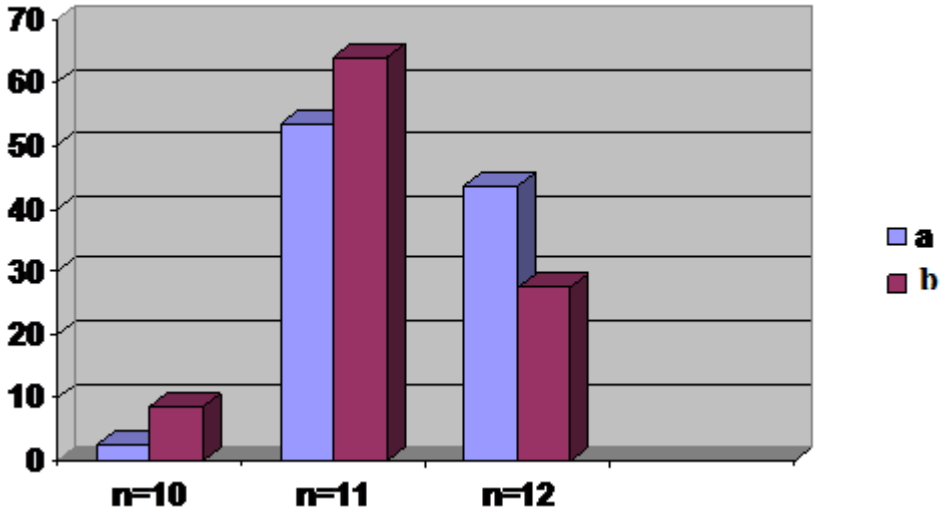


Fig. 5. Polyprenyl homologs composition of *Alcea nudiflora*:

a) Belonging to Tashkent region b) belonging to Namangan region.

Decaprenols' (n=10) quantity in *Alcea nudiflora* from Namangan region are 3.2 times more than Tashkent origin, undecaprenols 1.2, while against to it dodecaprenols are 1.6 times less.

CONCLUSION

Alcea nudiflora could be applied as a source for obtaining of polyprenols. Polyprenols distributed in all parts of the plant, whereas the maximum polyprenol accumulation takes place at flowering stage on August for Namangan region plants and its value is 2.32% from ADW.

Alcea nudiflora Polyprenols from Tashkent and Namangan regions and their homological structures quantitatively differ.

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