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CHEMICAL COMPOSITION AND BIOACTIVITY OF EXTRACTS FROM LEAVES AND BRANCHES OF ARMENIAN *PISTACIA ATLANTICA* DESF.

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Biologically active compounds in aqueous and ethanol extracts from branches and leaves of Armenian *Pistacia atlantica* were determined. The presence of flavonoids, floroglucides, coumarins, tannins, anthracene derivatives, sugars, saponins, phenolic glycosides and alkaloids are shown. Flavonoids in ethanol extracts from leaves and branches were obtained more (1.81 and 0.95 *mg*/*g*) than in aqueous extracts (0.95 and 0.3 *mg*/*g*). The most amounts of coumarins (2.2 *mg*/*g*) and anthracene derivatives (8.5 *mg*/*g*) were determined in ethanol extract from leaves. The aqueous extract from leaves was more effective in inhibiting of dipeptidyl peptidase IV and adenosine deaminase activities.

Keywords: *Pistacia atlantica*, branches and leaves extracts, dipeptidyl peptidase IV, adenosine deaminase.

Introduction. *Pistacia* L. is a genus of angiosperm plants belonging to *Anacardiaceae* family. According to the last study the genus contains nine species and five subspecies [1]. Only one species of *Pistacia atlantica* Desf. exists in Armenia. It is represented by its northern (Ijevan floristic region) and southern (Yerevan, Darelegis, Zangezur and Meghri floristic regions) populations [2]. The plant is monoecious shrubs or small trees growing to 5–15 *m* tall. These plants bloom from March to May, fruiting from June to September. The species grows on dry, steep and rocky southern slopes, in sparse forests on the height of 373–1500 *m* above sea level. The common names of Pistachio tree in Armenia are: pistakeni, khankeni, khankatsar, pastgheni.

Since ancient times, it has been used in Armenia as a medicine plant and incense source [3]. Different parts of *Pistacia* species (aerial part, leaves, fruit and resin) have been traditionally used for several therapeutic properties such as abdominal discomfort and pain, dyspepsia and peptic ulcer, as diuretic and stimulant agents.

Amirdovlat Amasiatsi in his “Useless for Ignoramuses” [4] cited the resin of *P. atlantica* as an antiseptic, inflammation and wound healing drug. Several studies have reported the antimicrobial, antioxidant and analgesic activity of *P. atlantica* [5–9].

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In Iran pistachio is used in folk medicine for problems in digestive, nervous and cardiovascular systems, liver and pediatric diseases, as well as against infection, atrophy and fatigue. The resin of *P. atlantica* has been used for treating digestive, hepatic and kidney diseases [10]. The oil from pistachio has an application in functional dyspepsia. It is used against allergic asthma and as prevention of penetrating cytokines during chronic diseases in respiratory system. Its rough oil extract shows wound healing feature [11–14]. The seeds contain fat and nitrogen compounds; the leaves contain tannins, ascorbic acid, and the wood contains fragrant gum. It has antidepressant and antistress properties, counteracts the appearance and development of chronic fatigue [15]. Pistachio fruits are very nutritious and contain more than 50% fat, carbohydrates and proteins. They are used in confectionery industry. Its dense wood is used in furniture manufacturing, as well as for getting oil and aromatic gum. The gum is used as a chewing gum in pharmaceutical, confectionery, cosmetics and varnish industry. The extract of its leaves inactivates acetylcholinesterase [16]. The extract of the gum possesses an antibacterial effect against both Gram-positive and Gram-negative bacteria due to its linalool and carvacrol activity [17]. Ether oils from its leaves and gum have antibacterial and antifungal activity [7, 18]. 3-metoksikarpakromen extracted from *P. atlantica* is used as a suppressor of growth of *Helicobacter pylori* cells, preventing many infectious diseases [19]. The active substances of the oil of almost all Pistachio species have antitumor activity. The 50% ethanol extract from gum inhibits the proliferation of human cancer cells HCT116, causes a cascade of cellular reactions that interrupt the matrix adhesion and promote cell death *in vitro* [20]. The mastic gum is used in the treatment of prostate cancer [21].

This article describes the results of the study of biologically active compounds in the aerial parts of *P. atlantica,* a representative of the southern population of the species, growing in Vayots Dzor province of Armenia. Their influences on the activities of adenosine deaminase (ADA) and dipeptidyl peptidase IV (DPPIV), activities of which deviate from the norm in a wide variety of pathologies [22, 23], are studied.

Materials and Methods.

Chemicals and Equipment. The enzymes DPPIV and ADA were isolated and purified from a kidney cortex and lung respectively, in the laboratory of H.Ch. Buniatian Institute of Biochemistry, as described earlier [24, 25]. The substrates Gly-Pro p-nitroanilide toluene sulfonate salt (GPN-Tos) and adenosine were purchased from "Sigma Ltd" (USA). The other reagents were of the highest purity.

Spectral measurements were carried out on Specord M-40 UV-VIS spectrophotometer (Germany) and Perkin-Elmer MPF-44A spectrofluorimeter (USA) in quartz cuvettes with light path of 0.5 and 1 *cm*.

Plant Material. The appropriate parts of the plant were collected in Vayots Dzor province of Armenia. Control specimens were deposited at the herbarium of the Department of Botany and Mycology of YSU.

Extraction. The collected parts of plants were dried in shade. A 10% (w/v) extract of dry materials in distilled water was prepared by incubating in a water bath at 96°*C* for 30 *min*. Ethanol extracts (70% ethanol) were prepared by incubating 4 days at the room temperature by periodical shakes. The extracts were filtered through sterile gauze and a proper glass filter, dried to a constant weight and kept

at –18ºC. Before using, the dried extracts were weighed and dissolved in 70% ethanol and water solution.

Identification of Chemical Constituents of Plant Extracts. The constituents of the extracts were characterized by a qualitative chemical analysis [26]. The indicating colors used in the following specific reactions were: bright yellow was used for flavonoids in a basic medium (5% ethanol solution of $AlCl_3$ was also used); dark violet was used for phenolic glycosides (or of a precipitate) in the presence of $FeSO_4$ crystals; black-green or black-blue was used for tannins at the reaction with 1% iron ammonium alums; pink-red was used for anthracene derivatives in the presence of 5% ethanol solution of sodium hydroxide; bright red-cherry was used for coumarins, added some Pauli's reagent after boiling in the basic medium. Then, for phloroglucides was used Pauli's reagent; for alkaloids was used Dragendorff's reagent; for carbohydrates was used Benedict's test and for cardio glycosides reactions of Legal and Keller–Kiliani were used. The presence of saponins was checked by formation of a frothing after boiling and vigorously.

Thin-Layer Chromatography for Anthracene Derivatives and Flavonoids. The anthracene derivatives of the extracts were characterized by TLC analysis on silica gel sheets (silica gel, glass support, Fluke) in the appropriate solvent system EtOAc–MeOH–H_2O (100:17:13) revealing by 5% solution of KOH in methanol. For flavonoids the solvent system was EtOAc–CH_2O_2–H_2O (8:3:1), revealing by 1% solution of $AlCl_3$ in ethanol.

The Quantitative Chemical Analysis. To evaluate the quantity of flavonoids, the qualitative reaction with $AlCl_3$ (5% in ethanol) was standardized by commercial quercetin ("Sigma"). To evaluate the quantity of coumarins, the above noted qualitative reaction was standardized by commercial ethoxycoumarin ("Sigma").

The quantity of anthracene derivatives was evaluated by the standard curve derived for istizin. It is given that the absorption of 1% solution of cobalt chloride at 515 *nm* is equal to the absorption of 3.6 $\mu g/mL$ of an alkaline-ammonia solution of istizin, different concentrations of $CoCl_2$ solution were prepared, their absorptions were measured, and the standard curve was derived.

Enzymes Assay. ADA activity was assayed by determination of ammonia liberated in the adenosine deamination reaction, using phenol-hypochlorite colorimetric method [24]. The activity of DPPIV was assayed, using GPN-Tos as a substrate, as is described in the work [25]. Briefly, 0.5 *mL* of an assay mixture contained 40 *mM* K,Na-phosphate buffer, pH 7.4, and an aliquot of enzyme. The reaction was initiated by adding the substrate up to the final concentration of 0.24 *mM*, and stopped in 30 *min* by cooling the mixture in an ice bath. The differential absorption at 390 *nm* was registered against an identical mixture without enzyme, and the amount of depleted p-nitroaniline was evaluated from its extinction coefficient at the wavelength of 9.9 $mM^{-1}cm^{-1}$ [27].

To evaluate the influence of a plant extract on the enzymatic activity, the enzymes were pre-incubated with a respective extract for 15 *min* before the initiation of the reaction. The activity in the presence of the plant extract was expressed as a percentage against the activity without any extract taken as 100%.

Evaluation of IC50. The activity of enzymes was determined in the presence of different amounts of plant extracts. The dependence of the inhibition value on

the concentration of the inhibitor in the assay mixture was defined and the IC50 value was determined as the concentration of the dry material (in $\mu g/mL$) necessary for inhibiting the enzyme activity down to 50% of the initial. For calculation the computer program GraFit Version 5 (Leatherbarrow, 2001, Erithacus Software Ltd., Horley, UK) was used.

Statistical Analysis. The statistical analyses of the data were carried out using the computer program InStat (Version 3 for Windows GraphPad Software, Inc., San Diego, CA, USA). The data are presented as the average of triplicate assays ± the standard error (SE).

Results and Discussion. There is little scientific information on the chemical composition of branches and leaves of *P. atlantica.* The results of qualitative chemical analysis of aqueous and ethanol extracts of these parts of the plant are shown in Tab. 1.

Table 1

Chemical composition of aqueous and ethanol extracts of P. atlantica

Bioactive compounds	Extract from branches		Extract from leaves	
	aqueous	ethanol	aqueous	ethanol
flavonoids	+ +	++	+++	+++
phloroglucides	+ + + +	++++	+++	++
coumarins	+	++	++	+++
phenolic glycosides	++++	++	++++	+
tannins	++++	+++	++++	++++
anthracen derivatives	+++	+++	+	+++
antocianins	–	–	–	–
alkaloids	++	–	–	–
sugars	+++	+++	+	+
cardiacglycosides	–	–	–	–
saponins	+++	++++	+	++

+ existence of compounds; – absence of compounds.

As it can be seen, the quantities of some compounds are more in the ethanol, others are in aqueous extracts. Both the leaves and the branches are rich in tannins. It is known that the gall developing on leaves contain an increased percentage of tannins (up to 20%). They serve as raw material for obtaining tannin extracts in leather manufacturing.

The branches and leaves are also rich in phloroglucides and phenolic glycosides. The amount of saponines in both extracts from branches are higher compared with coumarins. The high content of the latter was in the extracts from leaves. The extracts from these both parts of the plant contain a sufficient amount of flavonoids, in accordance with the data of other researchers [16]. The alkaloids were found only in the aqueous extract from branches. We observed anthracene derivatives in ethanol extracts from both branches and leaves. Cardiac glycosides and anthocyanins were not found in these extracts. In Tab. 2, the quantities of anthracene derivatives, coumarins and flavonoids in aqueous and ethanol extracts from branches and leaves of *P. atlantica* are expressed in *mg* of the corresponding commercial compound, used as a standard, per 1 *g* of the dry extract.

Table 2

The content (mg/g of dry extract) of anthracene derivatives, coumarins and flavonoids in aqueous and ethanol extracts from branches and leaves of pistachio

Compound	Extract from branches		Extract from leaves	
	aqueous	ethanol	aqueous	ethanol
anthracene derivatives (as istizin)	3.48 ± 0.05	8.53 ± 0.09	1.74 ± 0.01	4.22 ± 0.03
coumarins (as ethoxycoumarin)	1.098 ± 0.04	1.86 ± 0.02	1.82 ± 0.03	2.2 ± 0.12
flavonoids (as quercetin)	0.30 ± 0.01	0.95 ± 0.02	0.75 ± 0.01	1.81 ± 0.01

The total content of flavonoids in ethanol extract from branches in terms of quercetin was 0.95 *mg* per 1 *g* of the dry extract, which is 3 times more than that in the aqueous extract. The highest content of flavonoids was found in the ethanol extract from leaves (1.81 *mg/g*), which is also approximately 3 times greater than that in aqueous extract. As seen from Tab. 2, both ethanol and aqueous extracts of *P. atlantica* are rich in coumarins. The highest content of coumarins is obtained in the ethanol extract from leaves, 2.2 *mg/g* of the dry extract. The results of quantitative analyses of these constituents are in a good agreement with the results of qualitative chemical analysis (Tab. 1).

Besides quantitative and qualitative analyses, the existence of flavonoid compounds and anthracene derivatives in ethanol and aqueous extracts from branches and leaves of the *P. atlantica* was confirmed by TLC analysis, applying the appropriate systems of organic solvents. Higher content of flavonoids in leaves compared with branches was confirmed (Fig. 1).

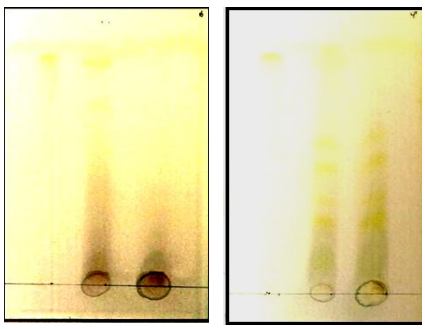

Fig. 1. TLC pictures for ethanol (points 2) and aqueous (points 3) extracts from branches (A) and leaves (B) in the solvent system for revealing flavonoid compounds, EtOAc–CH_2O_2–H_2O (8:3:1). Commercial quercetin, as a standard, is placed in points 1.

Fig. 2 illustrates a thin-layer chromatography picture for anthracene derivatives. In accordance with the results of the quantitative analyses, these compounds are presented poorly in the aqueous extract from leaves. The presence of different type compounds in flavonoid and anthracene derivative fractions of ethanol and aqueous extracts is evidenced by TLC pictures (Figs. 1 and 2).

It is known, that activities of DPPIV and ADA increase in a number of pathologies. As a result, the levels of their substrates, the vitally important peptides and anti inflammatory adenosine decrease. Hence, to decrease various pathological processes, the inhibition of activity of these enzymes can be considered as a

therapeutic approach. We studied the ability of ethanol and aqueous extracts from branches and leaves of *P. atlantica* to inhibit the activities of ADA and DPPIV, purified from bovine lung and kidney respectively.

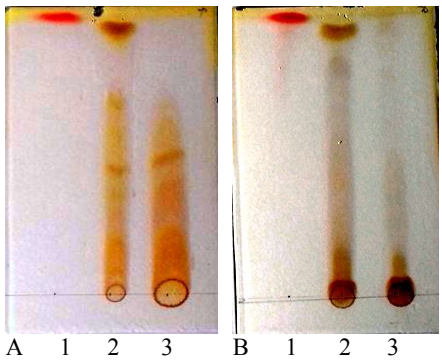

Fig. 2. TLC pictures for ethanol (points 2) and aqueous (points 3) extracts from leaves (A) and branches (B) in the solvent system for revealing anthracene derivatives, EtOAc–MeOH–H_2O (100:17:13). Emodin, as a standard, is placed in points 1.

Fig. 3 presents the concentration dependence of ADA activity suppression by aqueous extract from pistachio leaves. It resembles a hyperbola.

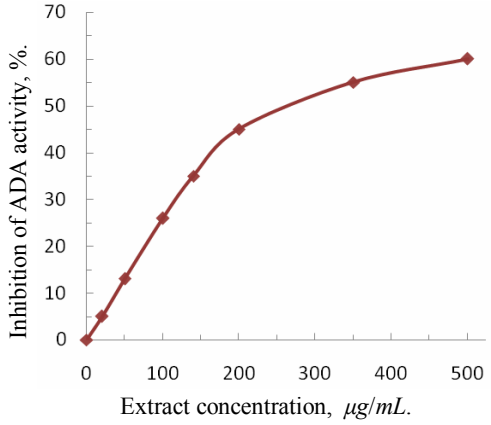

Fig. 3. Concentration dependence of ADA activity inhibition by aqueous extract from leaves of *P. atlantica.*

From the corresponding concentration dependences, the IC50 values for ethanol and aqueous extracts from branches and leaves of *P. atlantica* the inhibition of the activities of DPPIV and ADA were calculated (Tab. 3).

Table 3

IC50 values (μg/mL, ±SE) for the Pistachio extracts in inhibition of DPPIV and ADA

Enzyme	Extract from branches		Extract from leaves	
	aqueous	ethanol	aqueous	ethanol
ADA	438 ± 6.3	315 ± 4.16	276 ± 16.9	290 ±14.1
DPPIV	–	–	350 ± 36.0	390 ± 34.0

The inhibition of ADA activity by the extracts from *P. atlantica* branches was a little worse compared with the extract from leaves. In Tab. 3 the values for

the inhibition of DPPIV activity by the extracts from plant branches are not presented, because of their low efficiency.

Conclusion. The chemical analyses of aqueous and ethanol extracts from branches and leaves of *P. atlantica* evidenced the presence of flavonoids, floroglucides, coumarins, phenolic glycosides, tannins, anthracene derivatives, sugars, saponins in the plant and only alkaloids in an aqueous extract from branches. TLC analyses indicated the existence of different types of flavonoids and anthracene derivatives in the extracts. The compounds of the last family were not shown in the extracts from *P. atlantica* grown in other places. The contents of flavonoid compounds in ethanol extracts from leaves and branches (1.81 and 0.95 *mg*/*g* of dry extract) were more than that in the aqueous extracts (0.95 and 0.3 *mg*/*g*).

For the first time, anthracene derivatives and coumarins in branches and leaves of *P. atlantica* were found. The most quantity of coumarins (2.20 *mg/g*) was evaluated in ethanol extract from leaves. In the case of anthracene derivatives, the most quantity of (8.50 *mg*/*g*) was obtained in ethanol extract from branches. In aqueous extracts from branches and ethanol extract from leaves the contents were nearly the same (3.48 and 4.2 *mg*/*g*). The extracts from leaves were rather efficient in inhibition of enzymatic activities of both DPPIV and ADA.

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