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CORRELATION BETWEEN CAFFEINE CONTENT, ANTIOXIDANT POWER, TOTAL POLYPHENOL AMOUNT AND GROWTH HEIGHT OF ARMENIAN MOUNTAINOUS HERBS

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The amount of caffeine and the dependence of its content on the growth height of Armenian mountainous herbal infusions such as *Serpylli herba*, *Menthae piperitae folium*, *Mentha spicata*, and *Matricaria chamomilla* were studied by virtue of UV-Vis absorption spectroscopy after performing liquid-liquid extraction and multiple Gaussian curve fitting procedure to resolve the overlapping absorption bands. The obtained results were compared with those of Chinese green tea. The height of the plant growth significantly affects the caffeine content. The amount of caffeine in the infusions increases with the increase of the growth height of plants. Antioxidant activity of herbal infusions was studied using 1,1-diphenyl--2-picrylhydrazyl (DPPH) and new developed p-nitroso-N,N-dimethylaniline (PNDMA) assays. The IC_{50} values for DPPH and the rate constant of reaction between antioxidants derived from infusions and hydroxyl radicals were determined and compared with those of the well-known antioxidant vitamin C. Herbal infusions exhibited significant antioxidant activity comparable to that of green tea. Moreover, two assays revealed some differences, which are explained in the terms of hydrophobic and hydrophilic nature of the antioxidants. The concentrations of some flavonoids and flavonoid glycosides, such as quercetin and rutin, were determined by HPLC. Moreover, the content of total polyphenols was determined using Folin-Ciocalteu method.

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Introduction. Caffeine (1,3,7-trimethylxanthine) is in a class of molecules with conjugated planar ring systems that constitute the most widely distributed naturally occurring methylxanthines. It is found in various foods and drinks regularly consumed by human beings (e.g., coffee, tea, cola beverages, chocolates). The amount of caffeine varies according to species and origin of plants [1]. It is a well-established fact that caffeine has a number of physiological activities. It acts as a stimulant to the central nervous system and heart, and increases the activity of brain through its

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adenosine antagonist action. Caffeine is used in the treatment of mild respiratory depression caused by narcotics and for the treatment of circulatory failure [2]. It is used with aspirin in some preparations for the treatment of headache and with ergotamine in antimigraine preparations in order to produce a sense of alertness [3]. It can cause the relaxation of bronchial muscle, gastric acid secretion and diuresis [4]. Its concentration *in vivo* is important for various disorders such as heart disease, carcinogenesis, kidney malfunction and asthma [5]. However, in excess caffeine causes unpleasant symptoms, as well as a state of excitement and anxiety [6, 7]. The tea, we generally drink is made from the leaves of an Asian evergreen known as *Camellia sinensis*. White tea, green tea, red tea, and black tea all come from this plant, and all contain caffeine [8].

The amount of caffeine in green tea and its extraction have been extensively studied due to the high content of various polyphenolic compounds with pronounced antioxidant, antiviral, and anti-inflammatory activities. Synergistically with caffeine, these antioxidants are used for obesity management via control of metabolic rate and fat oxidation [9–11]. Different physicochemical methods have been developed for the measurement of caffeine content in various natural products, such as HPLC, FTIR, Raman spectroscopy, etc. [12, 13]. Although the UV-Vis spectroscopy cannot be used directly, because of spectral overlap, however this method has number of advantages: it is fast, sensitive, cheap, and easy regarding both the measurement procedure and the data analysis [14]. The proposed protocol may allow replacing the use of expensive equipment, such as those required to apply standard HPLC methods, which limit the application in small industrial laboratories [6].

Determination of caffeine amount in various natural products is important both from biomedical and practical points, as the byproduct of decaffeination from natural products can be used for preparation of different drugs. The measurement of caffeine in green tea is well documented, however the determination of caffeine amount in mountainous herbal leaves collected at 1600 *m* a. s. l. and higher is limited.

On the other hand, one of the main challenges in food manufacturing process is the oxidation of lipids leading to the reduction of shelf life as well as to the change of quality and nutritional value of food products [15]. Therefore, the use of antioxidants is gaining more interest in order to preserve food from the development of rancidity, deterioration as well as discoloration caused by lipid oxidation. Moreover, the antioxidant activity of fruits, vegetables and various beverages has biomedical significance, as these components may reduce oxidative damage in human body caused by free radicals and reactive oxygen species, which is related to the development of coronary heart disease, neurodegenerative disorder, cancer etc. [16]. Antioxidant properties of various types of tea (green, white, oolong, and black) are extensively studied [15, 17–21]. It is well known that polyphenolic compounds present in tea leaves that are responsible for antioxidant activity are mainly flavonoids-flavanols and their gallic acid derivatives known as catechins. Moreover, these compounds have not only antioxidant, but also antimicrobial activity [22]. Besides polyphenolic compounds caffeine also has a low contribution to the overall antioxidant activity. However, it is reported that antioxidant activity of caffeine is several orders lower in magnitude compared with flavan-3-ols [23]. On the other hand, there are relatively few studies of free-radical scavenging activity of herbal

infusions such as *Serpylli herba*, *Menthae piperitae folium*, *Mentha spicata*, and *Matricaria chamomilla*. It is known that infusions of these herbs also possess an antioxidant activity. Several studies were performed to measure the total antioxidant activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay [22–24].

In this work, the amount of caffeine is determined in mountainous herbal infusions depending on the height of the growth of plant, and the obtained data are compared with those of commercial Chinese green tea leaves. The antioxidant activity of herbal tea leaves is also studied using two different assays – DPPH and p-nitroso-N,N-dimethylaniline (PNDMA). HPLC technique is used for qualitative and quantitative analysis of herbal infusions, particularly to determine the amount of some flavonols, such as rutin and quercetin. Moreover, the content of total polyphenols is determined using the standard spectroscopic Folin-Ciocalteu method.

Serpylli herba, *Menthae piperitae folium*, *Mentha spicata* and *Matricaria chamomilla* were collected in high mountains of Armenia localized from 1600 to 2200 *m* a. s. l. It is well known that herbal infusions have various physiological effects. Infusions of *Serpylli herba* can be used for acute and chronic respiratory diseases, has and have germicidal, anti-inflammatory, sedative and analgesic effects. They can be used as diuretics and antihypertensive agent [25–27]. *Menthae piperitae folium* leaves have anti-inflammatory, analgesic, sedative, as well as stimulant and mood increasing activities [28–30]. Infusion of *Matricaria chamomilla* lowers the sugar level in the blood and can be used as a protective agent in diabetic complications. It can be used for the prevention of osteoporosis and cancer. Moreover, it has sedative and anti-inflammatory effects [31, 32]. Therefore, the measurement of biologically active compounds in herbal infusions such as caffeine, total polyphenols, quercetin, rutin, and the evaluation of their antioxidant power are in the focus of interest both from pharmaceutical and scientific viewpoints.

Materials and Methods.

Chemicals and Samples. Green tea (*Camellia sinensis*) was purchased from specialized tea store, and four herbs – *Serpylli herba*, *Menthae piperitae folium*, *Mentha spicata*, and *Matricaria chamomilla* were collected from different mountainous areas of Armenia localized from 1600 to 2200 *m* a. s. l. Dichloromethane, Folin-Ciocalteu reagent, ethanol, DPPH and hydrogen peroxide aqueous solution (35%) were purchased from Sigma, Germany, and were used without further purification. PNDMA (purity 98%) was obtained from Alfa Aesar. Recrystallized sodium sulfate was dried at 160°C within 1 day and stored in a desiccator over sulfuric acid. Recrystallized sodium carbonate was dried at 320°C within one day and stored in a desiccator over sulfuric acid. The completeness of dehydration was checked via IR spectroscopy. Deionized double distilled water was used (conductance less than 2 $\mu S \cdot cm^{-1}$ at 25°C), and the desired concentration of the solutions was obtained by dilution.

Liquid–Liquid Extraction of Caffeine. Several extraction protocols were employed in order to determine the quantity of caffeine in tea leaves, and it was reported that the most widely used solvent for extraction of caffeine is dichloromethane with an efficiency of 98–99% [33].

Extraction was done according to the following procedure: tea and herbal infusions were prepared by mixing 6 *g* of each plant and 45 *mL* of water and heated to dissolve the water soluble compounds into the hot water. The hot solution was allowed to cool for 10 *min* and was mixed with dichloromethane for the extraction

of caffeine from the infusion. The solution was mixed using a separatory funnel, and the caffeine was extracted by dichloromethane from the aqueous solution. The extraction was performed two more times with new amounts of dichloromethane. However, tannins are present in plant leaves, which are slightly soluble in dichloromethane. To separate the caffeine from the tannins, sodium carbonate was added to convert polyphenolic compounds into their salts. The latter are insoluble in organic solvent, but soluble in water. The extraction of caffeine proceeded 3 times, and the dichloromethane solution was dried using anhydrous sodium sulfate. The salt was filtered, and the solvent was evaporated on a hot plate to gain caffeine. Deionized double distilled water was added to the dry residue, and the UV-Vis absorption spectrum was recorded.

Extraction for Measurement of Antioxidant Activity and Total Polyphenol Content. For DPPH measurements the infusions were prepared using aqueous extraction procedure. 1 *g* of each plant was mixed with 30 *mL* of double distilled water and stirred with a glass rod at 80°C for 10 *min*. All samples were filtered and cooled to room temperature, after which all samples were diluted 14-; 25-; 50- and 100-fold using a 100 *mL* volumetric flask. For PNDMA assay the extraction procedure was the same, and the stock solution with a concentration of 4 *mg*/*mL* was used, which was then diluted to get 10%, 20%, 50% and 80% (v/v) solutions.

Free Radical Scavenging Ability Using DPPH Radical. For the study of free-radical scavenging activity of green tea and herbal infusions a DPPH solution in ethanol was used. According to the DPPH method of antioxidant assay [34], the reaction with the antioxidant leads to the stabilization of the DPPH radical, and as a result of which the purple color of the DPPH radical solution changes to bright yellow. This change can be detected by spectrophotometric method. 1900 μL of DPPH ethanolic solution (C_{DPPH}=5.4·10^{-5} *M*) was placed in a quartz cuvette and 100 μL of sample was added. The absorption was measured during time at 517 *nm* wavelength until the absorption value remains constant. To measure the absorption of the DPPH solution at $t = 0$ *min*, 100 μL of double distilled water was added instead of the sample. The obtained results were expressed as a percentage of inhibition of DPPH according to equation [16, 24]:

$$Inh\% = \frac{A_0 - A_f}{A_0} \cdot 100\%,$$

where A_0 and A_f are the initial and final values of absorbance after the reaction reaches steady state.

The percentage of inhibition was plotted against concentration in *mg*/*mL*, and the equation for the linear dependence was used to obtain the IC_{50} value. A lower IC_{50} value indicates greater antioxidant activity [16].

Free Radical Scavenging Ability Using PNDMA Assay. In the PNDMA assay, the antioxidant properties are estimated by studying the kinetics of competitive reaction between hydroxyl radicals and PNDMA. Hydrogen peroxide is radiated by UV light at 313 *nm*. As a result, hydroxyl radicals are formed that react with PNDMA, causing decolorization of dye. The rate of the reaction between hydroxyl radicals and PNDMA is determined from the absorption at 440 *nm* using spectrophotometric method. Addition of the sample affects this reaction due to the competitive reaction between polyphenols and hydroxyl radicals. As a result, the rate of decolorization of the radical target PNDMA decreases. By the examination of the

rate of decolorization of dye the rate constant of reaction between antioxidant and hydroxyl radical may be determined according to the following equation [35–39]:

$$k_{\mathrm{OH+polyphenol}} = 1.25 \cdot 10^{10} \frac{[\mathrm{PNDMA}]}{[\mathrm{polyphenol}]}\left(\frac{W_1}{W_2} - 1\right), \quad (1)$$

where [polyphenol] and [PNDMA] are the molar concentrations of polyphenol and PNDMA, respectively; $1.25 \cdot 10^{10}$ is the rate constant of the reaction between hydroxyl radical and PNDMA ($mol^{-1}L \cdot s^{-1}$); W_1 and W_2 are the slopes of the plots of PNDMA absorption versus time of hydrogen peroxide radiation in the absence and presence of an antioxidant, respectively.

The same method was used to determine the rate constant of the reaction between hydroxyl radicals and PNDMA in the presence of well-known antioxidant ascorbic acid which is $9.45 \cdot 10^9$ $M^{-1} \cdot s^{-1}$ [36].

HPLC Analysis of Phenolic Compounds. Phenolic compounds were determined using a HPLC Waters Separation module e2695 system consisting of UV detector (Waters, USA). The separation was performed on a Nucleosil C18 column (250×4.6 *mm*, 5-μm particles) at a column temperature of 30°C. As a mobile phase isocratic elution was used consisting of an aqueous solution of 2% acetic acid and 50% methanol. The flow rate of mobile phase was 0.9 *mL*/*min* with an injection volume of 10 μL. HPLC chromatograms were detected using a UV detector at 272 *nm*. The herbal infusions were prepared according to the standard extraction process described above at a concentration of 0.4 *mg*/*mL*.

7.5 *mg* and 11.6 *mg* of rutin and quercetin standards were accurately weighed and the volume was fixed to 10 *mL* with mobile phase to prepare the standard stock solutions with concentrations of 0.75 *mg*/*mL* and 1.16 *mg*/*mL*, respectively. The standard stock solutions were mixed by Vortex mixer for 5 *min*, and then filtered by an organic filter membrane of 0.22 μm. For the calibration curve the stock solutions were diluted, and standard solutions with concentrations of 0.65 *mg*/*mL*, 0.52 *mg*/*L*, 0.44 *mg*/*L*, 0.35 *mg*/*L* and 0.95 *mg*/*L*, 0.85 *mg*/*L*, 0.75 *mg*/*L*, and 0.6 *mg*/*L* were prepared for rutin and quercetin, respectively. The analysis test was carried out according to the chromatographic conditions. The phenolic compounds were characterized according to their retention time and comparison with standards.

Determination of Total Polyphenols. The total polyphenol content was determined using Folin-Ciocalteu method described in [40]. For the analysis 2 *mL* of 0.5 *mg*/*mL* herbal infusion was applied to 2 *mL* Folin-Ciocalteu reagent and 10 *mL* of distilled water was added. After incubation for 1 *min* 5 *mL* of 20% sodium carbonate solution was added and the final volume was made up to 50 *mL*. The samples were kept in darkness for 1 *h*, after which the absorbance was measured at 765 *nm*. The concentration of total polyphenols as gallic acid equivalents (GAE) in *mg*/*L* was calculated using calibration curve $y = 0.0043+0.002C$ (R^2=0.994) [40].

Instrumentation. The absorption spectra were recorded using PG Instruments T60 spectrophotometer. For determination of caffeine the spectra were recorded in the range of 190–600 *nm*, and for antioxidant activity in the range of 300–800 *nm* with a path length of 1 *cm*. UV-Vis measurements were carried out at 293.15 *K*. Overlapping bands were resolved using a multiple Gaussian curve fitting procedure using Origin software [41]. The positions were obtained from the second derivative spectra [42, 43].

Results and Discussion.

Determination of Caffeine Content. The amount of caffeine was determined in mountainous herbal infusions, particularly in *Serpylli herba*, *Menthae piperitae folium*, *Mentha spicata*, and *Matricaria chamomilla* collected in different mountainous areas of Armenia localized 1600 *m* a. s. l. and higher. Sample 1 (ST1) is the *Serpylli herba* collected at 1600–1800 *m* a. s. l., sample 2 (ST2) is *Serpylli herba* collected at 1700 to 1900 *m* a. s. l., and sample 3 (ST3) is *Serpylli herba* collected at 2000 *m* to 2200 *m* a. s. l. Sample 4 (SP4) is the *Menthae piperitae folium* collected at 1600–1800 *m* a. s. l., and sample 5 (SM5) is *Mentha spicata* collected at 1700–1900 *m* a. s. l. Sample 6 (SC6) and sample 7 (SC7) are *Matricaria chamomilla* samples collected at 1700–1900 and 2000–2200 *m* a. s. l., correspondingly.

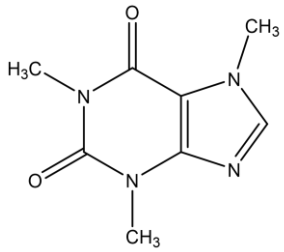

Fig. 1. Molecular structure of caffeine.

Fig. 1 shows the molecular structure of caffeine. It is well known that green tea leaves are significant sources of antioxidants, mainly polyphenols. Among these polyphenols mainly catechin species such as (+)-catechin, (−)-epicatechin, (−)-epigallocatechin, (−)-epicatechin gallate, (−)-gallocatechin, (−)-gallocatechin gallate and (−)-epigallocatechin gallate make up 30% of the mass of green tea leaves [10, 11, 44]. Among the polyphenolic compounds, chlorogenic acids also have significant antioxidant properties [6, 12, 45]. The UV-Vis absorption spectra of caffeine is characterized by an absorption band in the range 220–400 *nm*, which is related to the carbonyl group on structure of caffeine [6, 46].

Table 1

Results of the Curve fit for the UV-Vis absorption spectra for ST1, ST3, SP4 and SM5

Peak	Center / *nm*	Height	Width	Area
ST1				
1	194.36	1.66383	13.14327	27.40763
2	200.62	0.2959	6.52842	2.42109
3	212.56	0.51856	25.73263	16.72426
4	264.88	0.15664	32.48122	6.37678
ST3				
1	190.88	0.65752	7.3864	6.08695
2	197.95	1.2589	10.73832	16.94283
3	210.09	0.55822	28.67893	20.06435
4	262.48	0.17587	68.30419	15.05573
SP4				
1	193.82	0.58149	25.96892	18.9258
2	226.73	0.19422	31.71005	7.71877
3	267.80	0.1577	37.9561	7.50177
4	328.76	0.03733	66.53292	3.11256
SM5				
1	193.58	1.26807	20.60953	32.75461
2	204.95	0.24203	19.29186	5.85208
3	205.10	0.06515	6.18627	0.50511
4	220.79	0.19276	26.32191	6.35902
5	234.13	0.59782	41.4004	31.01952
6	280.50	0.22508	30.21226	8.52265
7	332.41	0.27164	56.51387	19.23998

It was reported that mainly caffeine and chlorogenic acids contribute to the absorption profile in this region [6, 12]. The absorption of chlorogenic acids is characterized by two maxima located at about 217 and 330 *nm* [6, 47], whereas the absorption of caffeine is observed at 272 *nm* [6, 12, 45]. The absorption bands of chlorogenic acids interfere the caffeine absorption having the effect on the maximum peak of caffeine. To resolve the overlapping bands, a deconvolution procedure was used starting from the frequencies obtained by the second derivative method.

Fig. 2 shows the band-resolved UV-Vis absorption spectra of aqueous solutions of ST1, ST3, SP4 and SM5, and the results of curve fit are presented in Tab. 1.

Table 2

The concentration of caffeine in aqueous solutions derived from extraction and its amount in mg per 100 g of plant leaves

Sample	Concentration of caffeine in stock solution, *mol/L*	Amount of caffeine in leaves, *mg*/100 *g*
Green tea	7.1610^{-3}	115.91
ST1	$1.50 \cdot 10^{-4}$	2.54
ST2	$1.53 \cdot 10^{-4}$	2.52
ST3	$2.63 \cdot 10^{-4}$	4.26
SP4	$1.61 \cdot 10^{-5}$	0.23
SM5	$4.06 \cdot 10^{-5}$	0.66
SC6	–	–
SC7	–	–

The obtained data show that deconvolution of the absorption spectra of *Serpylli herba* and *Menthae piperitae folium* gives four components independent on the location of herbs. The absorption spectrum of *Mentha spicata* consists of seven components, whereas the absorption spectra of *Matricaria chamomilla* extracts show the absence of absorption band characteristic for caffeine (figure is not shown). From the absorption at 272 the concentration of caffeine was calculated using the molar extinction coefficient of 11150 $L \cdot mol^{-1} \cdot cm^{-1}$ taken from the literature [12, 48]. Using the concentrations values, the amount of caffeine in 100 *g* of herbal tea leaves was calculated and the results are presented in Tab. 2 with the value of commercial Chinese green tea leaves for comparison. In the literature, the amount of caffeine in green tea leaves prepared from *Camellia sinensis* plant varies from 16 *mg* [2] to 75 *mg* [8] and more [44] per 100 *g* of tea leaves. However, it is well known that the caffeine content in the extract depends largely on the variety of tea plant, its growing conditions and the way it is processed and brewed. In our experiments, we have used commercially bagged green tea and the amount of caffeine was obtained 116 *mg* per 100 *g* of tea leaves. From the Tab. 2 it is obvious that the caffeine content in herbal tea leaves is about 50 times less than in *Camellia sinensis* leaves. Moreover, the amount depends on the location of the plant. Particularly the amount of caffeine in the *Serpylli herba* samples collected at 1600–1800 and 1700–1900 *m* a. s. l. is almost the same, whereas it increases almost twice above 2000 *m* a. s. l. In *Menthae piperitae folium* the content of caffeine is almost 10 times less compared with that of *Serpylli herba*. In the *Mentha spicata* leaves, the amount of caffeine is higher than that of *Menthae piperitae folium*. Both samples of *Matricaria chamomilla* show the absence of caffeine or negligible amount that cannot be detected by UV-Vis absorption.

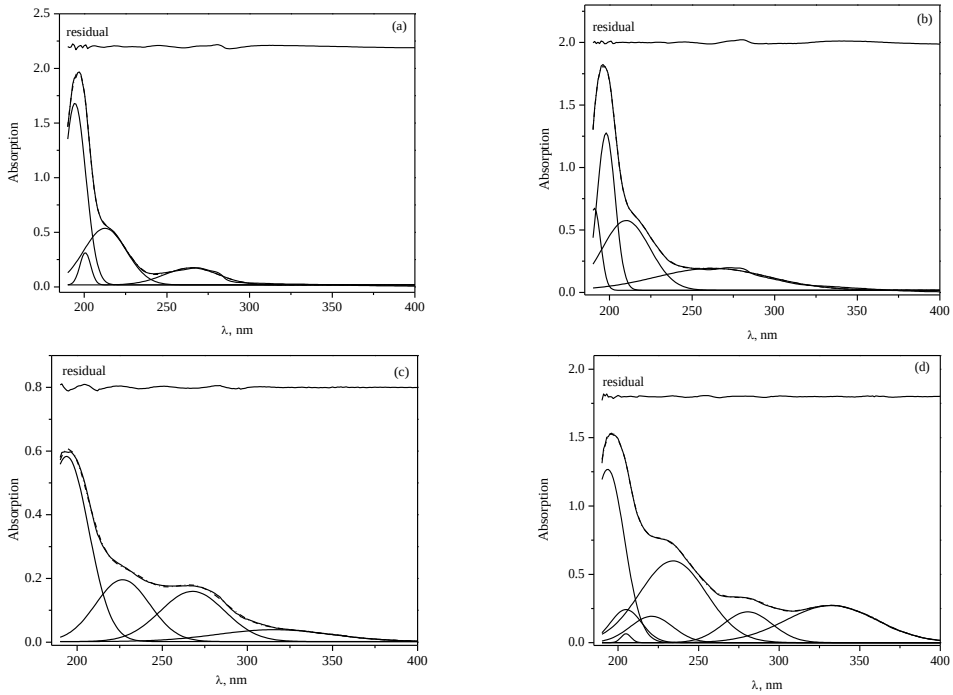

Fig. 2. Band-resolved spectra of aqueous solutions of caffeine extracted from ST1 (a), ST3 (b), SP4 (c) and SM5 (d) with fitted bands and residual.

From Fig. 2 it can be seen that in the absorption spectra of *Serpylli herba* (ST1, ST3) the characteristic absorption band of chlorogenic acids at 330 *nm* is not observed (the second-derivative spectra also do not show this band). In the spectrum of *Menthae piperitae folium* (SP4) the absorption band at 330 *nm* is observed. In the spectrum of *Mentha spicata* (SM5) absorption band at 330 *nm* is also observed, moreover absorption is significantly higher compared with that of *Menthae piperitae folium*. These data suggest that the content of chlorogenic acids in *Serpylli herba* is negligible regardless its location, whereas *Menthae piperitae folium* and *Mentha spicata* leaves contain chlorogenic acid. Moreover, in *Mentha spicata* leaves the amount of chlorogenic acids is much higher compared to *Menthae piperitae folium*.

Antioxidant Properties of Herbal Infusions. DPPH Assay. Antioxidant properties of herbal infusions were determined using both DPPH and PNDMA assays. For these measurements, the plants were collected at 1700–1900 *m* a. s. l. It is well known that DPPH assay is one of the widely used methods to study the free-radical scavenging ability of compounds as this is a rapid, simple, and inexpensive method [34, 49]. The absorption of DPPH during time was measured for green tea and herbal infusions at four different concentrations. The percententage of DPPH inhibition was calculated for each sample, and the percentage inhibition was plotted against concentration to calculate IC_{50} (figure not shown). The IC_{50} value for vitamin C in MeOH is 7.9 *mg*/*mL* [16]. The obtained IC_{50} values (the concentration in *mg*/*mL* of phenolic compounds required to reduce 50% of the free DPPH radical) is given in Tab. 3. The value of IC_{50} for green tea sample obtained in our study was 0.023 *mg*/*mL*, whereas in the literature it was reported 23.26 μg/*mL* for green tea and

36.07 $\mu g/mL$ for white tea [50], which is in good well agreement with our results. Lower IC_{50} value indicates greater antioxidant activity, which decreases in the following order: *Mentha spicata* > green tea > *Serpylli herba* > *Matricaria chamomilla* > *Menthae piperitae folium*, indicating that among all measured infusions *Mentha spicata* infusion possesses the highest free-radical scavenging activity, which is even greater than that of green tea. Moreover, *Menthae piperitae folium* shows minimal antioxidant activity compared to others.

Table 3

IC_{50} of DPPH, reaction rate constant between total antioxidants and hydroxyl radicals, amount of the quercetin and total polyphenolic content in GAE for green tea and aqueous herbal infusions

Sample	IC_{50}, *mg/mL*	*k*, $L \cdot mol^{-1} \cdot s^{-1}$	Quercetin, *ppm*	GAE, *mg/L*
Green tea	0.023	$1.34 \cdot 10^9$	0.50	63.5
Mentha spicata	0.011	$8.5 \cdot 10^8$	0.31	17.5
Serpylli herba	0.044	$4.4 \cdot 10^8$	0.16	26
Menthae piperitae folium	0.089	$2.4 \cdot 10^8$	0.13	18
Matricaria chamomilla	0.084	$1.1 \cdot 10^8$	1.55	2

On the other hand, it is known that the rate of reaction with DPPH depends on the reducing potential of the compound. Moreover, DPPH reacts only with lipophilic antioxidants [23], therefore, we have measured antioxidant activity of mountainous herbal infusions using PNDMA assay, which reacts mainly with hydrophilic antioxidants.

Antioxidant Properties of Herbal Infusions. PNDMA Assay. To estimate the antioxidant activity of herbal infusions caused by hydrophilic antioxidants the competitive reaction between hydroxyl radicals and free radical target PNDMA was studied. The reaction with free radicals causes decolorization of PNDMA and, by measuring the absorption at 440 *nm* it is possible to calculate the rate of reaction. Fig. 3 shows the dependence of absorption of PNDMA on the period of radiation of hydrogen peroxide in the absence and presence of green tea and herbal infusions.

From the Fig. 3 it can be seen that in all cases the addition of herbal infusion causes the decrease in the rate of decolorization of PNDMA, which means that polyphenols react with hydroxyl radicals, and as a result, the competitive PNDMA-hydroxyl radical reaction becomes slower. The latter indicates that herbal infusions have significant antioxidant activity. From the slopes of the plots for different contents of infusions the rate of hydroxyl radical-PNDMA reaction is determined and the plots of slopes versus volume percentage of infusion are depicted in Fig. 3 (f). The greater is the decrease in the rate of the competitive reaction, the stronger is the antioxidant activity.

The total concentration of polyphenols in *mol/L* in infusions was not determined in our study, as herbal infusions contain various antioxidants, therefore to determine the rate constants we used the same concentrations of vitamin C and its rate constant. The latter was determined by the same method [36]. Using this approach, it is possible to calculate the rate constants for total antioxidants present in herbal infusions, and the results are presented in Tab. 3 as vitamin C equivalents. Tab. 3 shows, that among the studied herbal infusions green tea exhibits the strongest

antioxidant activity. Moreover, the rate constant of the reaction between antioxidants present in green tea and hydroxyl radicals is only 10 times lower than that of vitamin C. The obtained results show that antioxidant activity decreases in the following order: green tea > *Mentha spicata* > *Serpylli herba* > *Menthae piperitae folium* > *Matricaria chamomilla*. The value of rate constant for *Matricaria chamomilla* infusion is almost 100 times lower than that of vitamin C.

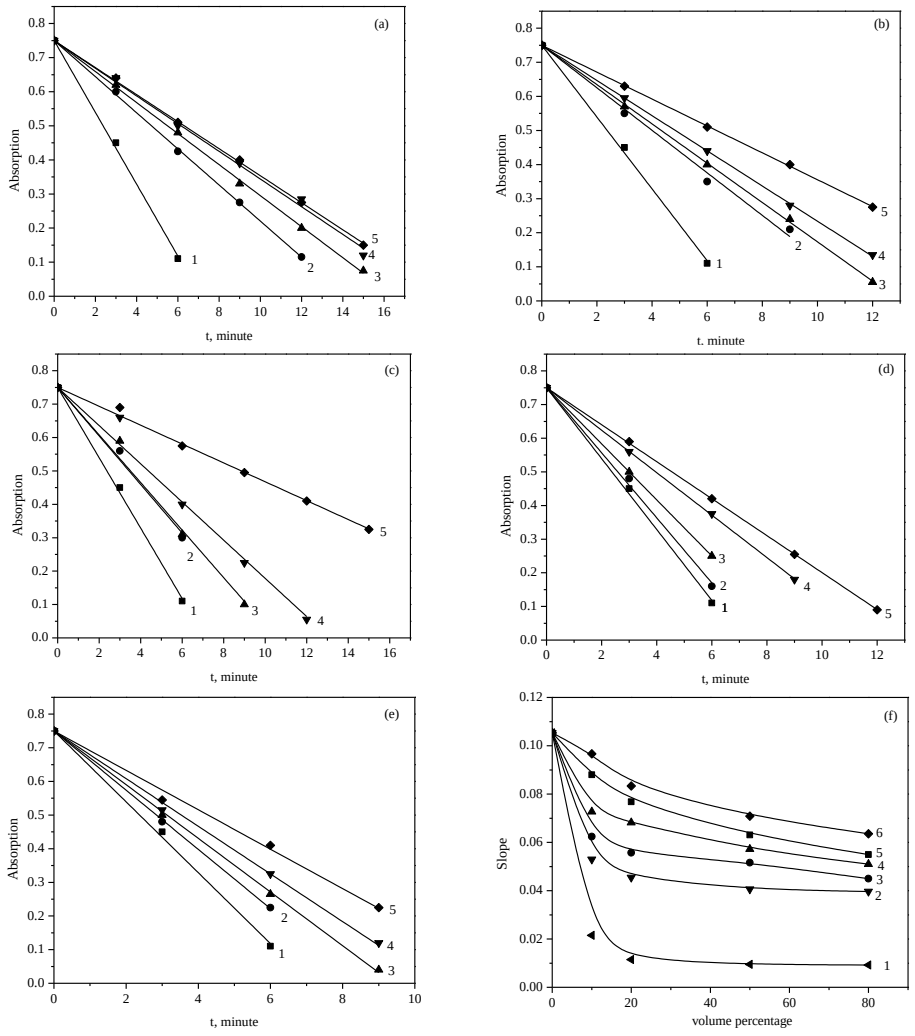

Fig. 3. Absorption of PNDMA vs time of irradiation of hydrogen peroxide in the presence of green tea (a), and herbal infusions – *Mentha spicata* (b), *Serpylli herba* (c), *Menthae piperitae folium* (d), and *Matricaria chamomilla* (e). The volume percentage of the infusions is 1 – 0; 2 – 10%; 3 – 20%; 4 – 50%, and 5 – 80%. (f) represents the dependence of the slopes of plots on the volume percentage of infusions: 1 – vitamin C; 2 – green tea; 3 – *Mentha spicata*; 4 – *Serpylli herba*; 5 – *Menthae piperitae folium*; 6 – *Matricaria chamomilla.*

The results obtained from DPPH assay show that *Mentha spicata* is more powerful antioxidant than green tea, as well as *Matricaria chamomilla* has higher

antioxidant activity than *Menthae piperitae folium*. The obtained difference may be explained in terms of the nature of the antioxidants present in the infusions. As it was mentioned above DPPH reacts with lipophilic antioxidants, whereas PNDMA assay reveals the antioxidant activity of hydrophilic compounds. Therefore, it can be concluded that the amount of hydrophilic antioxidants is higher in green tea, whereas *Mentha spicata* contains a higher concentration of lipophilic antioxidants. The comparison of free-radical scavenging activity of *Menthae piperitae folium* and *Matricaria chamomilla* reveals a similar situation.

On the other hand, the obtained results show that regardless the absence of caffeine *Matricaria chamomilla* infusion possesses significant antioxidant activity.

High-performance Liquid Chromatography (HPLC). The chromatogram of standard solutions of rutin and quercetin were recorded at 272 *nm* (figure not shown). The resolution and analysis time were improved by adjusting the elution program until a satisfactory result was achieved. The study found that isocratic elution gave the best result. From the standard solutions, it was revealed that rutin was eluted at 6 *min* and quercetin at 12.3 *min*.

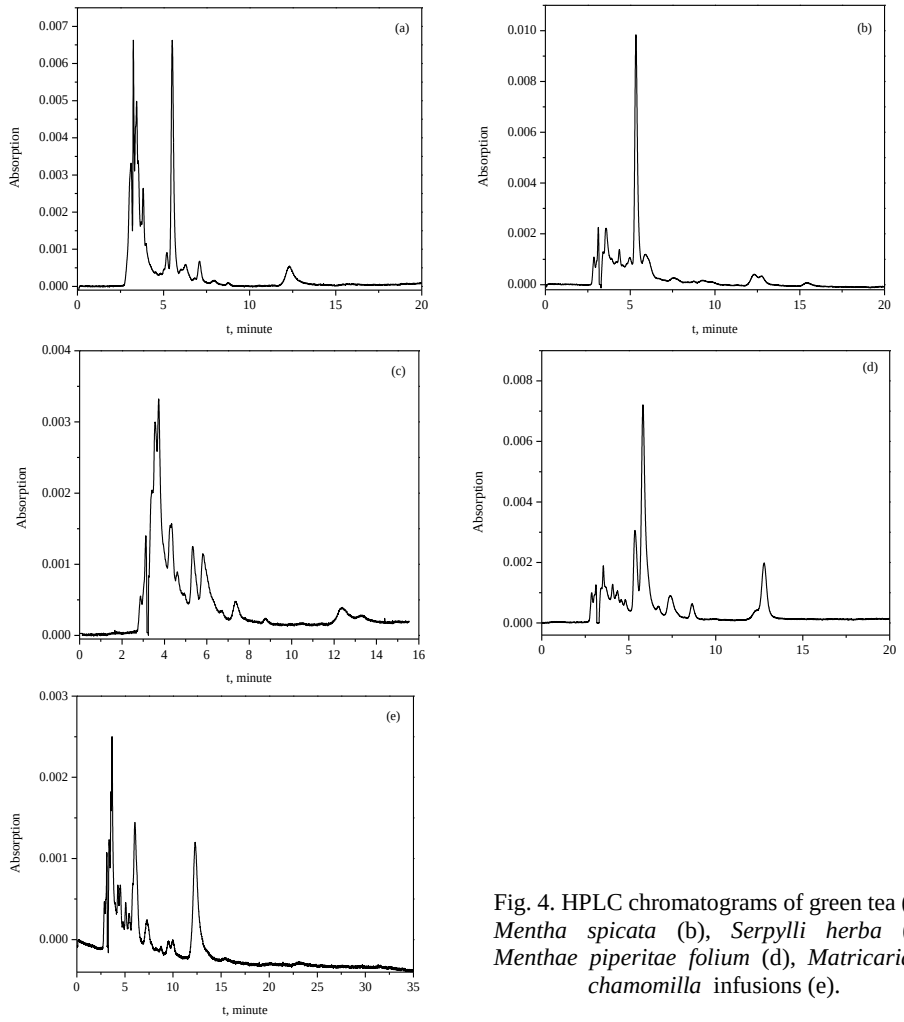

Fig. 4. HPLC chromatograms of green tea (a); *Mentha spicata* (b), *Serpylli herba* (c), *Menthae piperitae folium* (d), *Matricaria chamomilla* infusions (e).

The chromatograms of herbal infusions along with green tea extract are depicted in Fig. 4. From the chromatograms it is obvious that rutin is absent in all samples. The amount of flavonols along with the chromatographic parameters are given in Tab. 3. From the table it can be seen that the highest content of quercetin is present in the infusion of chamomile (1.55 *ppm*), while the others contain very low amounts. The amount of quercetin in Napier grass samples was reported 0.39 *ppm* [51], whereas other researchers report that depending on the tea variety, origin, and bud packing time not all green tea samples contain quercetin [52]. These results are in good agreement with those obtained in our study, where green tea sample contains very low amount of quercetin (0.5 *ppm*) and does not contain rutin. From the obtained results it is obvious that the amount of quercetin decreases in the following order: *Matricaria chamomilla* > green tea > *Mentha spicata* > *Serpylli herba* > *Menthae piperitae folium*, which can partially explain the antioxidant activity of these herbal infusions.

Determination of Total Polyphenols Content. The total polyphenolic content was determined using Folin-Ciocalteu method. To verify if the calibration curve given in [40] can be used in our experiments we have performed the experiments for both green tea and black tea. The obtained values of GAE were 63.5 *mg*/*L* and 49 *mg*/*L*, correspondingly, which are in good agreement with the results reported in [40]. The content of polyphenolic compounds is given in Tab. 3. From the Table it can be seen, that *Serpylli herba* infusion contains the highest amount of total polyphenols among the studied infusions, whereas the values for *Mentha spicata* and *Menthae piperitae folium* are almost the same. The total pholyphenolic content is much lower in *Matricaria chamomilla*. The obtained values are higher than those reported in [53, 54]. The obtained deviation may be explained in terms of the height of the plant growth, which may affect the content of various compounds, as we have obtained in the case of caffeine content. The total polyphenolic content decreases in the following order: green tea > *Serpylli herba* infusion > *Menthae piperitae folium* infusion ≈ *Mentha spicata* infusion > *Matricaria chamomilla* infusion.

Conclusion. The amount of caffeine, quercetin and rutin was determined in the infusions of *Serpylli herba*, *Menthae piperitae folium*, *Mentha spicata*, and *Matricaria chamomilla* using UV-Vis absorption spectroscopy and HPLC. The obtained results were compared with those for green tea. The results show that, among herbal infusions, *Serpylli herba* contains the highest amount of caffeine. Moreover, the height of the growth of plant affects caffeine content. On the other hand, *Matricaria chamomilla* infusions were caffeine-free. HPLC studies show that rutin is absent from all samples, whereas there are few amounts of quercetin in all samples, which is maximum in *Matricaria chamomilla* infusion. The antioxidant activity of the herbal infusions was also studied using both DPPH and PNDMA assays. It was shown that herbal infusions exhibit significant antioxidant activity. IC_{50} for DPPH as well as the rate constant of the reaction between antioxidants derived from the infusions and hydroxyl radicals were determined. The results obtained from DPPH assay show that *Mentha spicata* is more powerful antioxidant compared with green tea, as well as *Matricaria chamomilla* has higher antioxidant activity than *Menthae piperitae folium*, whereas PNDMA assay shows that among the studied herbal infusions green tea exhibits the strongest antioxidant activity.

The obtained difference may be explained in terms of the nature of antioxidants present in the infusions. As it is well known DPPH reacts with hydrophobic antioxidants, whereas PNDMA assay reveals the antioxidant activity of hydrophilic compounds. The content of total polyphenols was also determined using Folin-Ciocalteu method. The obtained results show that, among herbal infusions, *Serpylli herba* contains the highest amount of total polyphenols, whereas the minimum amount is observed in *Matricaria chamomilla* infusion.

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ՀԱՅԿԱԿԱՆ ԼԵՌՆԱՅԻՆ ԲՈՒՅՍԵՐԻ ԱՃԻ ԲԱՐՁՐՈՒԹՅԱՆ, ԿՈՖԵԻՆԻ ՔԱՆԱԿՈՒԹՅԱՆ, ՀԱԿԱՕՔՍԻԴԻՉ ԱԿՏԻՎՈՒԹՅԱՆ ԵՎ ԸՆԴՀԱՆՈՒՐ ՊՈԼԻՖԵՆՈԼՆԵՐԻ ՔԱՆԱԿՈՒԹՅԱՆ ՄԻՋԵՎ ԿՈՌԵԼՅԱՑԻԱ

Հայկական լեռնային բուսական թուրմերում կոֆեինի քանակությունը և բույսի աճի բարձրությունից դրա կախվածությունն ուսումնասիրվել է էլեկտրոնային կլանման սպեկտրոսկոպիայի մեթոդով՝ նախապես իրականացնելով հեղուկ-հեղուկ լուծահանում և բազմակի Գաուսյան կորի մշակում՝ վերադրված կլանման շերտերի բաժանման նպատակով: Որպես լեռնային բույսեր կիրառվել են *Serpylli herba*, *Menthae piperitae folium*, *Mentha spicata* և *Matricaria chamomilla*: Ստացված տվյալները համեմատվել են չինական կանաչ թեյի համար ստացված արդյունքների հետ: Բույսի աճի բարձրությունը էական ազդեցություն ունի դրանում կոֆեինի քանակության վրա՝ թուրմերում կոֆեինի քանակությունն աճում է բույսի աճի բարձրությանը զուգընթաց: Բուսական թուրմերի հակաօքսիդիչ հատկություններն ուսումնասիրվել են 1,1-դիֆենիլ-2-պիկրիլհիդրազիլ (DPPH) ռադիկալի և վերջին տարիներին մշակված պ-նիտրոզո-N,N-դիմեթիլանիլինի (PNDMA) մեթոդների միջոցով: Որոշվել են DPPH-ի հետ ռեակցիայի IC_{50}-ը, ինչպես նաև թուրմից ստացված

հակաօքսիդանտների և հիդրօքսիլ ռադիկալների միջև ռեակցիայի արագության հաստատունը: Ստացված արդյունքները համեմատվել են հայտնի հակաօքսիդիչ վիտամին C-ի տվյալների հետ: Բուսական թուրմերը ցուցաբերում են կանաչ թեյին համեմատական հակաօքսիդիչ հատկություններ: Ավելին, երկու մեթոդները ցույց են տվել որոշակի տարբերություններ, որը կարելի է բացատրել հակաօքսիդիչների հիդրոֆիլ և հիդրոֆոբ բնույթով: ԲԱՀՔ մեթոդով իրականացվել է որոշ ֆլավոնոիդների և ֆլավոնոիդների գլիկոզիդների, մասնավորապես կվարցետինի ու ռուտինի անալիզ: Ավելին, Ֆոլին-Չոկոլտի մեթոդով որոշվել է ընդհանուր պոլիֆենոլների քանակությունը:

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КОРРЕЛЯЦИЯ МЕЖДУ СОДЕРЖАНИЕМ КОФЕИНА, АНТИОКСИДАНТНОЙ АКТИВНОСТЬЮ, ОБЩИМ КОЛИЧЕСТВОМ ПОЛИФЕНОЛОВ И ВЫСОТОЙ РОСТА АРМЯНСКИХ ГОРНЫХ ТРАВ

С помощью спектроскопии УФ-видимого поглощения было изучено количество кофеина и зависимость его содержания от высоты роста армянских горных травяных настоев, таких как *Serpylli herba*, *Menthae piperitae folium*, *Mentha spicata* и *Matricaria chamomilla*. Исследованиям предшествовали жидкостно-жидкостная экстракция и процедуры многократной аппроксимации Гауссовых кривых для разложения перекрывающихся полос поглощения. Полученные результаты были сопоставлены с результатами, полученными для китайского зеленого чая. Высота роста растений существенно влияет на содержание кофеина. Количество кофеина в настоях увеличивается с увеличением высоты роста растений. Антиоксидантная активность травяных настоев изучалась с использованием методов 1,1-дифенил-2-пикрилгидразил (DPPH) радикала и недавно разработанного п-нитрозо-N,N-диметиланилина (PNDMA). Были определены значения IC_{50} для DPPH и константа скорости реакции между антиоксидантами, полученными из настоев, и гидроксильными радикалами. Полученные данные сравнивались с аналогичными показателями известного антиоксиданта витамина С. Травяные настои демонстрируют значительную антиоксидантную активность, сопоставимую с активностью зеленого чая. Кроме того, два метода выявили некоторые различия, которые объясняются гидрофобной и гидрофильной природой антиоксидантов. Концентрации некоторых флавоноидов и флавоноидных гликозидов, таких как кверцетин и рутин, были определены методом ВЭЖХ. Кроме того, методом Фолина-Чокальтеу было определено общее количество полифенолов.