Caucasian endemic medicinal and nutraceutical plants: *In vitro* antioxidant and cytotoxic activities and bioactive compounds

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Introduction

Medicinal plants have been grown and used since ancient times. Despite the growth of the synthetic medicine production, there is a growing demand for herbal remedies for the world market. Currently, at least 25% of medicines used in the world are derived from plants and many synthetic analogues are built on prototype compounds isolated from plants. Approximately two-thirds of new drugs in the past 25 years have originated from the discovery of particular secondary metabolites derived from natural biodiversity.[1] Historically, screening of natural materials for biological activity has worked well, but until recently, fewer than 15% of higher plant species have been examined for bioactivity.[2] There can be no doubt that observational knowledge about the effect of a plant on other organisms offers ideal opportunities to limit the huge diversity of possible leads to more promising ones (knowledge-based drug discovery).[3] Identification of plants with pharmacological activity can be successfully based on information gained through knowledge on their traditional use.[4] This is particularly true for biodiversity-rich regions of the world such as the Caucasus. The Caucasus hotspot is home to about 6.400 plant species (including a great number of medicinal, aromatic and spice plants), more than 1.600 of which (25 percent) are restricted to the region. Armenia is a small mountainous country on the Armenian Plateau in the South of the Caucasus between the Black and Caspian Seas. Vavilov identified Armenia as one of the centers of biodiversity for wild relatives of cultivated plants and as one of the world minor centers of origin of cultivated plants.[5] The flora of Armenia includes about 3.500 species, a great number being medicinal and nutraceutical plants. Theoretically, about 800 species of Armenian flora can be used as medicinal and nutraceutical plants.[6] Although the species diversity of useful plants in Armenia has been investigated rather well, the metabolic profiles and biological activities of important species are not adequately investigated.[7] Until now, there is no complete information on bioactive compounds and pharmacological potential of endemic, rare and valuable medicinal and nutraceutical plants of Caucasus in spite of their long-time traditional (ethnobotanical) uses.[8–10] **As far as the authors are aware, there is no work carried out on cytotoxic activities of selected Caucasian endemic plant species, while antioxidant properties and some bioactive compounds of a few species are only generally described in available literature.**

The aim of this research is to study the pharmaceutical potential (antioxidant and cytotoxic / pro-apoptotic activities) and biologically active compounds (terpenoids and polyphenols) of selected

Caucasian endemic medicinal and nutraceutical plants. Antioxidant effects were assessed using two different assays: ABTS-system, and lipid-peroxidation. In addition total phenolic content was determined by the Folin-Ciocalteau method. Cytotoxicity tests were carried out using human liver cancer (HepG2) cells. Terpenoids of selected species were extracted and analyzed by GC and GC-MS, and polyphenols were extracted and separated by HPLC.

Materials and methods

Selected species

The following medicinal and nutraceutical plants were chosen as research objects having large application in traditional medicine of regional communities in Caucasus:[11–17]

Armenian knapweed (*Centaurea hajastana* Tzvel.) – Asteraceae; Armenian hawthorn (*Crataegus armena* Pojark.) – Rosaceae; Transcaucasian Hogweed (*Heracleum transcaucasicum* Manden.) – Apiaceae; Armenian St John's-wort (*Hypericum eleonorae* Jelen.) – Clusiaceae; Armenian blackcurrant (*Ribes armenum* Pojark.) – Grossulariaceae; Transcaucasian rose (*Rosa sosnovskyana* Tam.) – Rosaceae; Armenian raspberry (*Rubus takhtadjanii* Mulk.) – Rosaceae; Armenian rowan (*Sorbus hajastana* Gabrieljan) – Rosaceae; Transcaucasian thyme (*Thymus transcaucasicus* Ronn.) – Lamiaceae.

The selection criteria for these endemic plants are based on their long-time local (ethnobotanical) uses in combination with the local biodiversity issues. Voucher specimens are deposited at the Herbarium of the Institute of Botany of National Academy of Sciences of Armenia.

Plant collection

Fresh medicinal plant materials (herbal material, shoots, flowers and fruits) from different ecological provenances in Armenia grown under natural soil conditions were collected (Table 1). The plants were gathered during July-September 2015. The fresh medicinal material was dried at 35 ^{o}C for 5-7 days. The plants were identified by comparison with the plant specimens at the Herbarium of the Institute of Botany of National Academy of Sciences of Armenia. The collection of samples is fully compliant with the international rules on the sustainable use of biodiversity and was performed in cooperation with the Institute of Botany of National Academy of Sciences of Armenia. According to the Armenian national CBD rules, the Institute of Botany of National Academy of Sciences, as state non-profit research organization, has full rights to collect and use the endemic plants for research purposes.

Measurement of antioxidant activities and lipid-peroxidation

The ABTS-system was used to evaluate the antioxidative capacity of essential oils and polyphenol-rich extracts of selected species.[18,19] In this system, myoglobin (Sigma) and H_2O_2 (Merck) oxidise ABTS (Merck) to the green ABTS•+ radical cation. 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) radical cation (ABTS•+) is a stable chromophore which absorbs strongly at 734 nm. This reaction can be followed photometrically. The lower rate of ABTS•+ formation indicates reducing or Fe-chelating properties of the extract. Prior to use, the ABTS•+ working solutions for essential oil (1:20) and polyphenols (1:5) were prepared by diluting the stock solution with EtOH (Merck).

The influence of bioactive substances on lipid peroxidation in human blood plasma was analyzed according to Schnitzer et al.[20] and Atkin et al.[21]. ~~The analyses were performed with 3-4 replications.~~

Measurement of cytotoxic / pro-apoptotic activities

One gram of powdered dry plant material was kept in 50 mL conical flask and added 10 mL of solvent (80% ethanol). This extract was used for two reasons: (1) to obtain one extract covering a broader lipophilicity range of chemical constituents; (2) to use an extraction protocol analogous to decoction preparations employed by traditional healers or the general public. Plant materials were extracted in a shaking incubator (Grant OLS 200, UK) at 40ºC for 4 hr. The residue was re-extracted under the same condition three times. Then, extracts were filtered by using Millex (Merck KGaA, Germany) sterile syringe filter (0.22 µm). The solvent from the extract was removed by using rotary vacuum evaporator (BÜCHI, Switzerland) with the water bath temperature of 40°C. Finally, all freeze dried extracts were then kept in glass bottles in a freezer (−20 ºC) until used for the pharmacological testing. Thereafter, the extracts were redissolved in 100% DMSO (Sigma-Aldrich) at a concentration of 50mg/ml and stored in amber glass bottles for bioassays.

HepG2, human hepatocellular carcinoma cell line was purchased from Sigma-Aldrich (ACC No 85011430, Lot 11C013). The cells were maintained in Minimum Essential Medium Alpha + Glutamax (MEM) (Gibco) supplemented by 10% Foetal Bovine Serum (FBS) (Gibco) and 1% Penicillin-Streptomycin (10,000 U/mL) (Gibco) in TPP 75 cm^3 cell culture flasks at 37ºC in 5% CO_2/ 95% air. The fresh medium was replaced every two days. The cells were sub-cultured when they reached about 70-80% confluent. Cytotoxic activities were examined against human liver cancer HepG2 cells using Alamar Blue assay (AB). The assay was performed according to the company (AbD Serotec) instruction with some modifications. The cells were seeded at the density of 5,000 cells per well in 96-well black microplates

(Greiner Bio-One) and allowed to attach for 24 hours. The extracts (6.25 – 200 µg/ml), epigallocatechin gallate (EGCG) (Sigma-Aldrich) as positive control (0.1 – 10 µM), and control were then added.[22] After 48 hours of extracts treatment, the medium was replaced with 100 µl of diluted AB solution (1:10 in complete medium) and the plates were incubated for 2 hours at 37ºC. Then the fluorescence intensity (FI) was measured at 560 nm excitation and 590 nm emission by using a microtiter plate reader (Infinite M200, Tecan). The %cell viability was calculated as follows:

$$\%\text{Viability} = \frac{\text{mean FI}_{\text{sample}} - \text{mean FI}_{\text{blank}}}{\text{mean FI}_{\text{control}} - \text{mean FI}_{\text{blank}}} \times 100$$

This assay was carried out using three independent assays performed in triplicate. ~~The average of %Viability and S.D. were calculated using Microsoft Excel 2016 and IC_{50} was calculated using GraphPad Prism 7 software (GraphPad Software, Inc.).~~

Analysis of bioactive compounds

Essential oils

Essential oils from dry plant materials of selected species were extracted by steam distillation and pentane (Carl Roth) extraction for 1 h (SDE). The extracts were dried over sodium sulfate (Merck) and concentrated under a stream of nitrogen. The amount of essential oil was determined gravimetrically. The composition of the essential oil was analyzed by GC and GC-MS. The oil was diluted in acetone (Carl Roth) (split 1:40) with separation of the compounds by GC (Fisons Instruments Mega 5360, Italy) on a Supelco-Wax capillary column (60 m, i.d. = 0.32 mm, 0.25 µm film thickness) with helium as carrier gas (0.8 ml/min) and a temperature program: 50 °C (3 min), 10 °C/min, 120 °C (2 min), 2 °C/min, 155 °C (0 min), 8 °C/min, 240 °C. Identification of essential oil main compounds was performed with a GC-MS system (HP 5890 Series II/HP 5971 A) on the same column and with the same temperature program by electron impact ionization at 70 eV. Mass spectra were evaluated by comparison of retention times and mass spectra[23] and with an own terpenoid mass spectra database.

Polyphenols

Polyphenols were extracted from 0.4 g dry material (powder) with 80% MeOH (Carl Roth) followed by ethylacetate and separated by HPLC. Extracted polyphenols were resolved in 500 µl CH_3CN (Merck) and

500 µl EtOH (Merck), injection volume was 100µl. The analytical HPLC was carried out on a dionex system (pump P580, autosampler Gina 50) using a 250 mm x 4,6 mm RP-18 column (phenomenex Hydro-RP) with guard column. A gradient sequence using (A) water, acetonitrile and acetic acid (Merck) (97:2:1) and (B) acetonitrile in the following proportions: 0 % B (0min), 50 % B (5min), 95 % B (10min), 0 % B (14min) at a flow rate of 1 ml/min. Detection and quantification were performed using a Diode Array detector (Dionex UVD 340S) and chromeleon (Dionex) software. Folin-Ciocalteu-test (Merck) applied to determinate the total content of polyphenolics in plant material.[24]

Statistical analysis

GraphPad Prism 8 software (GraphPad Software, Inc., San Diego, CA) was used for statistical analysis. Data were subjected to the Kruskal-Wallis test followed by Dunn's multiple comparison test. A value of P<0.05 was considered statistically significant. The average of %Viability and S.D. were calculated using Microsoft Excel 2016. All the experiments were conducted in triplicates.

Results

Antioxidant activity and lipid-peroxidation of plant extracts

The highest antioxidant activities by ABTS model system were found in *Thymus transcaucasicus*, *Heracleum transcaucasicum, Ribes armenum* and *Crataegus armena* followed by *Centaurea hajastana* and *Hypericum eleonorae*. We found also that all endemic species studied showed comparatively strong inhibition and overall enhanced antioxidative capacity in the ABTS system (Figure 1).

On the other hand, *Rubus takhtadjanii*, *Crataegus armena* and *Thymus transcaucasicus* showed the most potent inhibition of lipid-peroxidation, followed by *Rosa sosnovskyana and Hypericum eleonorae* (Figure 2).

Cytotoxicity of plant extracts

During the pre-screening for cytotoxic / pro-apoptotic effects, four plant extracts exhibited interesting activity by inhibiting the cell proliferation for more than 50% at 200µg/ml. *Crataegus armena* showed the strongest effect followed by *Thymus transcaucasicus*, *Rubus takhtadjanii* and *Centaurea hajastana*

(Figure 3). Therefore, these four species were selected for further studies on the cytotoxic / pro-apoptotic activity as a basis for potential anticancer effects.

Crataegus armena strongly inhibited the cell proliferation with IC_{50} of 8.66 µg/ml. *Thymus transcaucasicus* moderately inhibited the cell proliferation with IC_{50} of 44.25 µg/ml, while *Rubus takhtadjanii* and *Centaurea hajastana* exhibited weak cytotoxicity with IC_{50} of 158.43 and 164.44 µg/ml, respectively. For example, at the concentration of 25 µg/ml the ethanolic extracts of *C. armena* expressed the highest cytotoxicity followed by extracts of *T. transcaucasicus*, *R. takhtadjanii* and *C. hajastana* (Table 2).

Bioactive compounds of selected species

The content of essential oils and polyphenols of selected species (*Centaurea hajastana, Crataegus armena* and *Thymus transcaucasicus*) was analysed. The Folin-Ciocalteu expressed as gallic acid equivalents showed remarkably high amounts of phenolics in *C. armena* followed by *C. hajastana* and *T. transcaucasicus,* while the results showed that the highest content of essential oils were provided by *T. transcaucasicus* and *C. hajastana* (Table 3).

Centaurea hajastana

It was found the main constituents of *C. hajastana* essential oil were β-eudesmol (24.65 %), β-caryophyllene (19.12 %), d-germacrene (17.33 %), caryophyllene oxide (10.12 %) and γ-elemenel (9.13 %). 17 compounds representing about 92 % of the total oil were identified (Table 4). Flavonoid aglycones, such as kaempferol, quercetin, isorhamnetin, apigenin, and caffeic, chlorogenic, neochlorogenic, protocatechuic, ferulic, chicoric acids, as well as other polyphenols have been isolated from the *C. hajastana* (Table 5).

Crataegus armena

The main components of *C. armena* essential oil are butyraldehyde (15.21 %), hexanol (14.38 %), benzaldehyde (13.86 %), capronaldehyde (8.14 %), β-myrcene (4.75 %) and β-caryophyllene (3.38 %). 17 volatile compounds representing about 72 % of the total essential oil were identified (Table 6). Polyphenolics of *C. armena* mainly consisted of kaempferol, apigenin, quercitrin, isovanillic acid, hyperoside, ursolic acid and arbutin (Table 7).

Thymus transcaucasicus

T. transcaucasicus essential oil consisted mainly of borneol (19.70 %), α-terpineol (17.37 %), thymol (11.18 %) carvacrol (10.05 %), linalool (9.34 %), geraniol (8.93 %), 1,8-cineole (7.10 %) and geranyl acetate (4.57%). 22 compounds representing about 99 % of the total oil were identified (Table 8). We found that polyphenolics of *Thymus transcaucasicus* mainly consisted of phenolic acids and the major compound was rosmarinic acid. At the same, we identified considerable amounts of other polyphenols, such as quercetin, caffeic acid and cryptochlorogenic acid (Table 9).

Discussion

The region of Caucasus remains unexplored in terms of bioactive compounds and pharmacological potential of many endemic, rare and valuable medicinal and nutraceutical plants in spite of their long-time traditional uses. This study confirms the antioxidant and antiproliferative capacity of some studied Caucasian endemic medicinal and nutraceutical plants, most importantly *Crataegus armena* and *Thymus transcaucasicus*. ~~Correlation of metabolite profiles with biological activities showed the nature of biologically active compounds of medical interest.~~

The antioxidant properties of *Crataegus armena*, *Ribes armenum*, *Rosa sosnovskyana*, *Rubus takhtadjanii* and *Sorbus hajastana* have not been reported before, while antioxidant activities of other species of *Crataegus, Ribes, Rosa, Rubus and Sorbus* genera have been well documented. A significant *in vitro* antioxidant potential of different extracts and essential oil of *Thymus transcaucasicus, Centaurea hajastana* and *Hypericum eleonorae* was already reported by Manukyan[7] and Bektas et al.[25], while antioxidant activity of essential oil of *Heracleum transcaucasicum* was described by Torbati et al. [26] Although in the last years the pharmacological relevance of antioxidant assays of plant extracts is under controversial discussion, the antiproliferative activities of medicinal plants are increasingly in the focus as potential source of new anticancer drugs from natural sources.

Cytotoxic activities of *Crataegus armena*, *Thymus transcaucasicus, Rubus takhtadjanii* and *Centaurea hajastana* have not yet been reported previously. Cytotoxicity of other species from *Crataegus, Thymus, Rubus* and *Centaurea* genera have been reported elsewhere. In particular, two *Crataegus* species – *C. pinnatifida* Bunge [27-29] and *C. cuneata* Sieb et. Zucc.[30] showed cytotoxicity against human cancer cells.

The essential oils of *Thymus caespititius* Brot., *Thymus mastichina* L., *Thymus pulegioides* L. and *Thymus villosus* subsp. *lusitanicus* (Boiss.) Cout. showed antiproliferative activity by preventing the growth of THP-1 leukemia cells, [31] while *Thymus munbyanus* subsp. *coloratus* (Boiss. & Reut.) Greuter & Burdet showed noteworthy cytotoxicity on A-375 human melanoma cells[32]. The essential oil of *T. alternans* Klokov exhibited significant antiproliferative effects on melanoma (A375), breast (MDA-MB 231), colon (HCT116) cell lines.[33] The antiproliferative activity of *T. vulgaris* L. essential oil as well as thymol and carvacrol against THP-1 cells was reported by Aazza et al. [34] The essential oil of *T. vulgaris* inhibits head and neck squamous cell carcinoma (HNSCC) cell growth.[35] *T. parnassicus* Halácsy was found to have cytotoxic activities against Caco2, HepG2 and MCF7 cell lines.[36] In this study, we found similar activities against HepG2 cells in case of *T. transcaucasicus*. Furthermore, essential oils from different *Thymus* species induced cell death in both human epitheloid cervix carcinoma and histiocytic leukemia cell lines. [37]

A number of *Rubus* species showed cytotoxic activities. For example, the fruits of *R. chingii* Hu showed cytotoxic activity against HepG2, Bel-7402, A549 and MCF7 human cancer cell lines, [38,39] while *R. idaeus* L. revealed higher cytotoxic activity towards the human leukemia cell lines: J45 and HL60.[40,41] In the current study, *R. takhtadjanii*, like *R. chingii*, showed similar cytotoxic activity against HepG2 cell lines. ~~Ellagitannins from *R. idaeus* were found to be active against human colon adenocarcinoma cell line Caco2.[42] It was also found that colon adenocarcinoma (SW 480) cells are more susceptible to *R. idaeus* leaf extract in comparison with human laryngeal carcinoma (HEp2) cells.[43] Phenolics from *R. fairholmianus* Gardner induces cytotoxicity and apoptosis in human breast adenocarcinoma (MCF7) cells.[44]~~ *R. fairholmianus* inhibits human melanoma (A375) and lung cancer (A549) cells.[42] ~~and induced toxic effects in human colorectal cancer (Caco2) cells.[43]~~ *R. ellipticus* Sm. extracts showed potent antiproliferative activity against human cervical cancer (C33A) cells.[43] *R. phoenicolasius* Maxim. was found to be active against MCF7 and NCI-H460 tumour cell lines.[44] *R. rosaefolius* Sm. showed selective activity against the multidrug-resistant ovary cancer cell (NCI-ADR/RES) line,[45] while *R. parvifolius* L. was found to be active against leukemia K562 cells.[46]

Different *Centaurea* species showed cytotoxic activities against diverse types of cancer cells. It was reported that *C. schischkinii* Tzvelev exhibited promising *in vitro* cytotoxic activity against CaCo2 colon cancer cell lines.[47] Sesquiterpene lactones isolated from the aerial parts of *C. zuccariniana* DC. und *C. Achaia* Boiss. & Heldr. exhibited growth inhibiting effect against a number of human cell lines (i. e., DLD1, SF268, MCF7, H460 and OVCAR3).[48] Cytotoxic activity of *C. calolepis* Boiss. was observed toward pig kidney epithelial (LLC-PK_{11}), human malignant melanoma (SK-MEL) and human ductal carcinoma (BT-549) cells.[49] *C. africana* Lam. showed cytotoxicity against the human myeloid leukaemia cell line HL-60,[50] while *C. bruguierana subsp. belangeriana* (DC.) Bornm. demonstrated significant

cytotoxicity against colon adenocarcinoma and breast ductal carcinoma cell lines.[51] *C. aegyptiaca* L. demonstrated outstanding results against HepG2, MCF7, HCT-116 and HELA cell lines,[52] as well as against Hep-2 cell line.[53] In case of HepG2 cell lines we found weak cytotoxic activity of *C. hajastana* in our study as well. ~~*C. nerimaniae* Kültür had a significant antiproliferative effect on HeLa and MDA-MB-231 cells.[58] *C. albonitens* Turrill showed potential cytotoxic effects in NALM-6, REH, NB4 and KMM-1 cell lines[59] and *C. scoparia* Sieber ex Spreng. demonstrated strong cytotoxicity against HeLa cells.[60] *C. ragusina* L. showed significant cytotoxic activity against human bladder (T24) and human glioblastoma (A1235) cancer cell lines.[61] It was found that *C. drabifolia* subsp. *detonsa* (Bornm.) Wagenitz showed a potent activity against two cancer cell lines, namely acute lymphoblastic leukemia (CCRF-CEM) and its multidrug-resistant subline CEM/ADR5000.[62] It is shown that *C. solstitialis* L. ssp. *solstitialis* exhibited very high antiproliferative activity on C6 and HeLa cells.[63] *C. kilaea* Boiss. showed fairly strong activity against MCF7 and PC-3 human cancer cell lines,[64] while *C. arenaria* M.Bieb. ex Willd. demonstrated antitumour effects against HeLa, MCF7 and A431 cell lines[65] and *C. deflexa* Wagenitz showed antiproliferative activity against human pancreatic and colonic cancer cells.[66]~~

The results on bioactive compounds in this study are in good accordance to previous investigation with *C. hajastana*,[7] although in our study we have recorded some significant variations in percentage of terpenes and polyphenols in this Caucasian endemic medicinal plant. The composition of essential oil and polyphenols of *C. armena* have not yet been reported, so we could not find any literature that is relevant to our results. Our results on *T. transcaucasicus* are also mainly in line with the findings reported by Manukyan,[7] although the composition of terpenes in *T. transcaucasicus* varies. Kutzner et al.[54] found that one year old full blooming plant of *T. transcaucasicus* from controlled greenhouse soilless culture was predominantly (more than 90% in the overall intensity of all detected peaks) composed of monoterpenes including thymol, γ-terpinene, α-pinene, 1,8-cineol and borneol. Sesquiterpenes were only detected at minor amounts, e.g. caryophyllene, germacrene D, α-bisabolene and β-ocimene. In another study, researchers found that thymol was the only dominant component in *T. transcaucasicus*.[25] Ezzatzadeh et al.[55] reported that the main volatile constituents from leaf, flower, stem and root of *T. transcaucasicus* were thymol, α-terpineol, geraniol, p-cymene, carvacrol, pentacosane and 1.8-cineole.

Although in our study we used the whole extracts of medicinal plant materials, correlation of metabolite profiles with biological activities showed the nature of biologically active compounds of medical interest. In this respect, terpenes and polyphenols as bioactive compounds are in particular interest as potential antioxidant and cytotoxic agents. From nine plant species only *Crataegus armena* and *Thymus transcaucasicus* showed comparatively strong antioxidant, as well as high to moderate cytotoxic activities. Some of identified main terpenes and polyphenols, such as β-caryophyllene,[56] kaempferol,[57] quercitrin,[57] apigenin,[58] hyperoside,[59] and ursolic acid[60] in *C.*

armena and borneol,[61] carvacrol,[62] thymol,[62-63] linalool,[64] geraniol,[65] 1,8-cineole,[66] rosmarinic acid,[67] quercetin[57] and caffeic acid[68] in _T. transcaucasicus_ were found to have antioxidant and cytotoxic activities. It should also be mentioned that borneol, linalool and rosmarinic acid, like plant extracts of _C. armena_ and _T. transcaucasicus_ in this study, showed similar cytotoxic activity against HepG2 cell lines. It can be hypothesized that these bioactive constitutions are some of the major compounds responsible for antioxidant and cytotoxic activities in our medicinal plant mixtures. At the same time, synergistic effects of different bioactive compounds could be a significant factor related to bioactivities.

Conclusion

Ethnopharmacological knowledge is beneficial in guiding which plants may have potentials to yield antioxidant and/or anticancer products. Based on this study, *Thymus transcaucasicus, Heracleum transcaucasicum, Ribes armenum, Rubus takhtadjani* and *Crataegus armena* could be developed further based on their strong *in vitro* antioxidant effects, while *Crataegus armena* and *Thymus transcaucasicus* might yield novel natural compounds with anticancer effects. This study also suggests that the plant extracts might yield valuable adjuncts for use in standard chemotherapy. The study also showcases the tremendous phytochemical potential of the Armenian flora. However, further detailed phytochemical, pharmacological and *in vivo* studies should be the next step in the identification of other active compounds of the lead plants, +particularly *Crataegus armena*, *Thymus transcaucasicus* and *Heracleum transcaucasicum*, which are currently ongoing.

Declarations

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Conflict of interest

The authors report no conflicts of interest.

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Table 1 Collection sites of selected Caucasian endemic medicinal and nutraceutical species and percentage yield of ethanol solvent **(values represent means ± S.D., n=3)**

Species (plant material)	Locality (province in Armenia)	Yield of ethanolic extracts (g/100 g of dry weight)
Centaurea hajastana (flowers)	Shirak	6.89 **± 0.15**
Crataegus armena (fruits)	Syunik	9.22 **± 0.22**
Heracleum transcaucasicum (shoots)	Vayots Dzor	11.41 **± 0.34**
Hypericum eleonorae (herbal material)	Tavush	1.99 **± 0.05**
Ribes armenum (fruits)	Vayots Dzor	4.82 **± 0.13**
Rosa sosnovskyana (fruits)	Kotayk	5.89 **± 0.14**
Rubus takhtadjanii (fruits)	Syunik	2.77 **± 0.07**
Sorbus hajastana (fruits)	Yerevan	9.89 **± 0.32**
Thymus transcaucasicus (herbal material)	Kotayk	3.58 **± 0.12**

Table 2 Cytotoxic activities of ethanolic extracts of Caucasian endemic medicinal and nutraceutical species in the Alamar Blue assay (values represent means ± S.D., n=3)

Species	HepG2 cells	
	IC_{50} (µg/ml)	(% viability at 25 µg/ml)
Centaurea hajastana	164.44 ± 2.15*****	95.68 ± 0.98*****
Crataegus armena	8.66 ± 0.87*****	21.25 ± 0.42*****
Rubus takhtadjanii	158.43 ± 1.09*****	66.98 ± 0.7*****
Thymus transcaucasicus	44.25 ± 1.14*****	57.04 ± 0.55*****

*** P<0.0001**

Table 3 The content of essential oils and polyphenols of selected Caucasian endemic medicinal and nutraceutical plants (values represent means ± S.D., n=3)

Species	Essential oils (% / dry weight)	Polyphenols (mg gallic acid equivalent / g dry weight)
Centaurea hajastana	0.46 ± 0.01	54.9 ± 1.64
Crataegus armena	0.04 ± 0.001	93.8 ± 2.85
Thymus transcaucasicus	0.55 ± 0.02	29.7 ± 0.64

Table 4 Chemical composition of *Centaurea hajastana* essential oil

Constituents*	Retention Time (min)	Content in essential oil, %
1-octen-3-ol	9.41	1.53**
δ-elemene	11.11	0.62
α-copaene	11.67	0.75
β-elemene	18.64	1.18
β-caryophyllene	21.68	19.12
γ-elemene	24.20	9.13
aromadendrene	24.30	1.02
α-humulene	27.52	0.72
β-farnesene	28.69	0.45
d-germacrene	30.60	17.33
β-selinene	32.37	0.24
γ-cadinene	32.50	0.18
β-eudesmol	36.99	24.65
δ-cadinene	39.74	1.69
α-cadinene	40.05	1.12
b-germacrene	40.62	1.67
caryophyllene oxide	40.97	10.12
Total		91.52%

* Only identified compounds are presented
**Data are expressed as the mean ± S.D., n=3

Table 5 Polyphenolic composition of *Centaurea hajastana*

Compound*	Retention Time (min)	Crude extract peak area (%)	Concentration (mg/g dried product)
caffeoyl hexoside	1.63	1.18**	0.67 ± 0.02
quinic acid	3.64	1.32	0.82 ± 0.02
chlorogenic acid	12.51	21.82	15.16 ± 0.3
protocatechuic acid	21.64	4.95	3.12 ± 0.06
ferulic acid	21.88	2.06	1.31 ± 0.03
chicoric acid	41.65	3.46	2.42 ± 0.05
isorhamnetin	44.26	7.46	5.12 ± 0.1
kaempferol	44.59	5.69	4.26 ± 0.08
quercetin	45.87	6.28	4.84 ± 0.8
apigenin	48.24	2.41	1.82 ± 0.03
apigenin C-glucoside	48.63	2.74	2.14 ± 0.04
patuletin	49.33	2.98	2.42 ± 0.05
isorhamnetin 3-O-hexoside	49.56	5.94	4.65 ± 0.08
quercetin hexoside	49.89	5.82	4.57 ± 0.08

* Only identified compounds are presented
**Data are expressed as the mean ± S.D., n=3

Table 6 Chemical composition of *Crataegus armena* essential oil

Constituents*	Retention Time (min)	Content in essential oil, %
butyraldehyde	2.32	15.21**
valeraldehyde	8.66	2.56
capronaldehyde	9.52	8.14
hexanol	10.31	14.38
α-pinene	10.56	2.82
benzaldehyde	10.78	13.86
β-myrcene	10.94	4.75
α-terpinene	11.32	0.52
cymene	11.46	0.28
limonene	11.59	0.69
1,8-cineole	11.97	0.32
γ-terpinene	13.65	2.19
α-terpinolene	20.82	0.18
β-bourbonene	24.18	0.84
β-caryophyllene	27.34	3.38
α-humulene	27.81	0.74
α-farnesene	28.89	1.38
Total		72.24%

* Only identified compounds are presented
**Data are expressed as the mean ± S.D., n=3

Table 7 Polyphenolic composition of *Crataegus armena*

Compound*	Retention Time (min)	Crude extract peak area (%)	Concentration (mg/g dried product)
ursolic acid	2.69	4.09**	1.12 ± 0.02
arbutin	3.36	3.92	1.03 ± 0.02
gentisic acid	6.54	0.15	0.04 ± 0.001
chlorogenic acid	7.04	1.06	0.29 ± 0.006
isovanillic acid	8.22	7.09	1.94 ± 0.04
vitexin	11.05	1.39	0.38 ± 0.006
hesperidin	17.33	0.29	0.08 ± 0.002
isovitexin	17.67	1.55	0.42 ± 0.008
hyperoside	18.55	4.97	1.36 ± 0.02
isoorientin	20.45	0.81	0.22 ± 0.004
myricetin	31.89	0.23	0.06 ± 0.001
rutin	41.56	1.02	0.28 ± 0.004
apigenin-7-O-glucoside	47.08	0.22	0.05 ± 0.001
hesperetin	48.19	0.16	0.04 ± 0.001
kaempferol	53.23	33.45	9.15 ± 0.2
quercetin	58.29	0.41	0.11 ± 0.002
quercitrin	63.36	8.18	2.21 ± 0.04
apigenin	76.63	23.32	6.53 ± 0.01

* Only identified compounds are presented
**Data are expressed as the mean ± S.D., n=3

Table 8 Chemical composition of *Thymus transcaucasicus* essential oil

Constituents*	Retention Time (min)	Content in essential oil, %
α-pinene	6.76	1.82**
sabinene	6.84	0.54
β-myrcene	9.42	1.15
α-terpinene	10.33	0.88
limonene	11.67	0.92
1,8-cineole	13.03	7.10
γ-terpinene	15.70	0.06
p-cymene	16.73	1.14
α-terpinolene	20.61	0.15
linalool	24.30	9.34
linalyl acetate	26.69	0.34
bornyl acetate	26.9-1	0.10
β-caryophyllene	28.46	0.02
pulegone	29.03	1.60
α-terpineol	36.99	17.37
borneol	39.74	19.70
d-germacrene	40.05	1.99
geranyl acetate	40.62	4.57
nerol	42.92	0.22
geraniol	44.56	8.93
thymol	51.82	11.18
carvacrol	54.25	10.05
Total		99.17%

* Only identified compounds are presented
**Data are expressed as the mean ± S.D., n=3

Table 9 Polyphenolic composition of *Thymus transcaucasicus*

Compound*	Retention Time (min)	Crude extract peak area (%)	Concentration (mg/g dried product)
chlorogenic acid	6,31	3.65**	1.91 ± 0.05
cryptochlorogenic acid	15.84	4.61	2.41 ± 0.06
caffeic acid	20.35	5.32	3.52 ± 0.05
p-coumaric acid	35.54	3.82	1.03 ± 0.03
ferulic acid	39.14	3.85	1.26 ± 0.03
rutin	41.65	3.88	1.19 ± 0.03
quercetin-3-O-glucoside	43.96	2.68	0.95 ± 0.02
apigenin-7-O-glucoside	47.03	2.23	1.54 ± 0.04
rosmarinic acid	49.93	28.67	14.95 ± 0.4
kaempferol	53.12	3.31	0.69 ± 0.02
quercetin	58.23	10.01	5.43 ± 0.2
apigenin	76.53	2.82	1.24 ± 0.03
carnosic acid	88.97	2.28	0.74 ± 0.02

* Only identified compounds are presented
**Data are expressed as the mean ± S.D., n=3

Figure legends

Figure 1 Antioxidant capacity by ABTS model system as affected by ethanolic extracts of Caucasian endemic medicinal and nutraceutical plants.

Figure 2 Lipid peroxidation in human blood plasma as affected by ethanolic extracts of Caucasian endemic medicinal and nutraceutical plants.

Figure 3 %Viability of HepG2 cells after the treatment with 200µg/ml of extracts of Caucasian endemic medicinal and nutraceutical plants **(values represent means ± S.D., n=3).**