Bioactive compounds and their health-promoting capacity of some Caucasian endemic and rare medicinal plants.

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Bioactive Compounds and Their Health-promoting Capacity of Some Caucasian Endemic and Rare Medicinal Plants

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Abstract

Despite the growth of the synthetic medicine production, there is a growing demand for herbal remedies for the world market. At the same time, in the last decades there has been a sharp increase of anthropogenic influence on the flora; particularly some rare and endangered herbs are more and more collected from wild population in many regions of the world, also in Caucasian region, for local demand and export. The Caucasus is one of the richest and most threatened mountainous reservoirs of plant and animal life on Earth. The aim of this work is to study the content, the composition of biologically active compounds (essential oils, carotenoids, polyphenols, alkaloids and dianthrone derivatives) and their antioxidative capacity, as well as the lipid peroxidation potential of different eco races of some Caucasian endemic, rare, endangered and valuable medicinal plants - thyme (*Thymus transcaucasicus*), St John's-wort (*Hypericum eleonorae*) and knapweed (*Centaurea hajastana*). The metabolite profiles of these herbs were analyzed by GC/MS and HPLC. Bioactive compounds were quantified and their health-promoting properties were analyzed in bioassays. It was shown that the content, composition, antioxidative activity and lipid peroxidation potential of studied biologically active compounds significantly depends on plant provenances (eco races). Such research can provide new important knowledge about the bioactive compounds and their pharmacological effects of the most promising provenances of selected Caucasian endemic and rare species. It can also make significant contribution in discovering new effective drugs.

INTRODUCTION

Medicinal plants are grown and used since ancient times. In recent years a growing demand for herbal remedies and natural products can be observed worldwide, despite the booming production of synthetic substances. At the same time, in the last decades there has been recorded strong increase of anthropogenic influence on flora; particularly some rare, endangered and valuable herbs are more and more collected from wild population in many regions of the world, also in the new independent states of the Caucasian region of the former Soviet Union, for local demand and export. The Caucasus is a biological crossroads, where species from Central and Northern Europe, Central Asia, the Middle East and North Africa mingle with endemics. Conservation International has designated the Caucasus as one of its 34 “biodiversity hotspots” around the world. Biodiversity of the Caucasus is being lost at an alarming rate (Caucasus Protected Areas

Fund, 2008). Armenia is a small mountainous country on the Armenian Plateau in the South of the Caucasus between the Black and Caspian Seas. The mountainous nature of Armenia results in a series of highly diverse landscapes - seven distinct landscape types are described in Armenia - from deserts to alpine lands. The flora of Armenia includes about 3.500 species, a great number being medicinal and aromatic plants (Takhtajyan, 1954-2001). The local endemic flora comprises more than 120 species. Theoretically, about 800 species of Armenian flora can be used as medicinal plants. However, actually the natural resources of most of these plants, do not allow collecting them from natural ecosystems in industrial or commercial quantities (Global environmental conventions-Biodiversity of Armenia, 2001). Although the species diversity of useful plants in Armenia has been investigated rather well, the biologically active compounds of some important, rare and endemic species, as well as the intra-specific categories (like chemo or eco races) are not adequately investigated. This is not equal, as the value of herbs, in particular, is determined by biologically active stuff. In spite of this fact, till now there is no complete information on chemical composition and pharmacological differences between different ecological provenances and chemo-races of endemic and rare valuable herbs of Armenia. Possible new bioactive compounds from Caucasian endemic species can make significant contribution in discovering of new effective drugs improving the public health.

MATERIAL AND METHODS

Following medicinal plants which are included in the regional Red Data Books for endangered and rare plants are chosen as research objects: transcaucasian thyme (*Thymus transcaucasicus* Ronn.) - Lamiaceae, armenian St John's-wort (*Hypericum eleonorae* Jelen.) - Clusiaceae (formerly also Hypericaceae) and armenian knapweed (*Centaurea hajastana* Tzvel.) - Asteraceae. The plant material of selected species was collected from different ecological provenances in Armenia (Aragatsotn, Kotayk, Syuniq, Shirak, Tavush provinces) (Fig. 1, 2).

Essential oils from fresh and dry herbal material of selected species were extracted by steam distillation and pentane extraction for 1 h (SDE). Identification of essential oil main compounds was performed with a GC-MS system (HP 5890 Series II/HP 5971 A). Mass spectra were evaluated by comparison of retention times and mass spectra (Wiley, 1990) and with an own terpenoid mass spectra database. Carotenoids were extracted from 0.1 g dry material (powder) with MeOH/THF (50:50) followed by hexane and separated by HPLC (Sander et al., 1994). Additionally the content of carotenoids in fresh herbal material was determined photometrically (Wetstein, 1957). Polyphenols were extracted from 0.4 g dry material (powder) with 80% MeOH and separated by HPLC. Folin-Ciocalteu-test applied to determinate the total content of polyphenolics in plant material (Singleton et al., 1998). The method developed by Poutaraud et al. (2001) enabled the assay of hypericins and hyperforins by HPLC (*Hypericum* species). Alkaloids of *Centaurea* species were extracted with 10% ammonium hydroxide and ethanol followed by HCI and trichloromethane and separated by HPLC. The ABTS-system was used to evaluate the antioxidative capacity of essential oils, carotenoid-rich and polyphenol-rich extracts (Arnao et al., 1999). The influence of bioactive substances on lipid peroxidation in human blood plasma was analyzed according to Schnitzer et al. (1995) and Atkin et al. (2005).

RESULTS AND DISCUSSION

Thymus transcaucasicus

It was found that different ecological provenances have significant influence on quantity of bioactive compounds of thyme (Table 1). Plants collected from Kotayk province provided maximum content and yield of essential oil, aw well as high content of carotenoids and polyphenols. It was shown that the main compounds of thyme essential oil were α-terpineol (11.91-41.87 %), linalool (3.51-40.20 %), borneol (16.79-40.07 %), carvacrol (3.80-29.55 %), geraniol (1.59-16.59 %), geranyl acetate (1.24-13.00 %) and thymol (0.33-5.23 %) (Fig. 3). The composition of essential oil and tested eco races of thyme reached different relationships. The main identified carotenoids of thyme were astaxanthin, chlorophyll a, chlorophyll b, α-carotene and β-carotene. The influence of eco races on composition of carotenoids was considerable as well. HPLC analysis of polyphenolics provided differences in sensitivity of main polyphenols of thyme to the ecological origin of plants. It was found that polyphenolics mainly consisted of phenolic acids and flavonoids. Essential oil of thyme showed no significant differences in antioxidative capacity (Fig. 4), while carotenoids and polyphenols have enhanced antioxidative capacity when plants grown in Kotayk province (Fig. 5, 6). Essential oils led to a strong decrease in lipid peroxidation in case of Kotayk ecological provenance (Fig. 7). Carotenoid-rich extracts of thyme showed no essential differences in lipid peroxidation (Fig. 8), while for polyphenolic extracts, the samples from Kotayk and Syuniq provinces provided strong decrease of lipid peroxidation (Fig. 9).

Hypericum eleonorae

It was shown that different ecological provenances have significant impact on amounts of bioactive compounds of St John's-wort (Table 1). Plants collected from Tavush province provided maximum content and yield of essential oil, while in case of carotenoids and polyphenols there were no essential differences between two eco races. It was cleared up that the major compounds of essential oil were β-caryophyllene (23.25-38.49 %), d-germacrene (13.38-18.07 %), β-farnesene (11.21-13.32 %) and α-pinene (3.26-10.89 %) (Fig. 10). The composition of essential oil and ecological origin of St John's-wort reached different relationships. The main carotenoids were found to be the followings: neoxanthin, violaxanthin, zeaxanthin, luteolin, chlorophyll a, chlorophyll b, α-carotene and β-carotene, while the main polyphenolics were hyperoside, quercetin and rutin. We have recorded some effect of eco races on composition of carotenoids and polyphenolics; however this impact was less critical than in case of essential oil. Hypericin and hyperforin, which characterize the *Hypericum* genus, are thought to be the most important active components of St John's-wort plant; therefore we have concentrated on analyses of those particular dianthrone derivatives. We have found that *Hypericum eleonorae* species contents 10-20% more hypericin and hyperforin than common St John's-wort plant (*Hypericum perforatum*) from the same provinces. Such high concentration of dianthrone derivatives was registered probably because *Hypericum eleonorae* normally grows in comparatively higher altitude (2000-2200 meters), consequently with a high exposure to UV light. The effect of plant's ecological origin on quantity of dianthrone derivatives in *Hypericum eleonoraea* was remarkable too with high results in Tavush region (2.5 % hypericin and 2.7 % hyperforin). It was found that different eco races have stronger impact on antioxidative capacity and lipid peroxidation of carotenoid-rich and polyphenol-rich extracts than on antioxidative capacity and lipid peroxidation of essential oil (data not shown).

Centaurea hajastana

For knapweed we have only one known ecological provenance in Armenia (Table 1). It was shown that the essential oil of *Centaurea hajastana* consisted manly of the following compounds: β-caryophyllene (13.79-19.07 %), γ-elemene (5.11-9.18 %), d-germacrene (13.38-18.07 %), β-eudesmol (11.21-25.35 %) and caryophyllene oxide (9.44-10.26 %) (Fig. 11). The major carotenoids were chlorophyll a, chlorophyll b, α-carotene and β-carotene. Flavonoid aglycones (quercetin, kaempferol, isorhamnetin, apigenin, luteolin, hispidulin) and their glycosides, and caffeic, chlorogenic, neochlorogenic acids, as well as anthocyanins have been isolated from the *Centaurea hajastana*. HPLC analysis afforded some indole alkaloids in knapweed: moschamine, cis-moschamine, centcyamine and cis-centcyamine. Antioxidative capacity and lipid peroxidation potential of essential oil, carotenoid-rich and polyphenol-rich extracts of unique *Centaurea hajastana* eco race was tested as well (data not shown).

CONCLUSIONS

Screening of bioactive compounds, as well as analyses of their health-promoting capacity of Caucasian endemic and rare medicinal plants - *Thymus transcaucasicus, Hypericum eleonorae, Centaurea hajastana* showed their unique metabolic profiles and differentiated pharmacological impacts. The quantity, quality, as well as antioxidative activity and lipid peroxidation potential of studied biologically active compounds significantly depends on plant provenances (eco races).

ACKNOWLEDGEMENTS

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Tabels

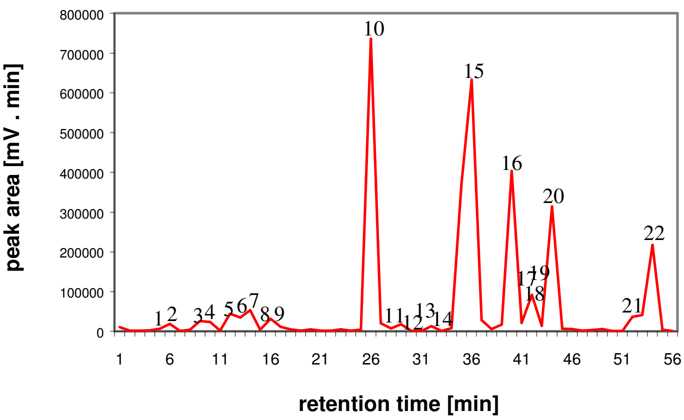
Table 1. Bioactive compounds of *Thymus transcaucasicus, Hypericum eleonorae* and *Centaurea hajastana* grown in different ecological provenances of Armenia

Species (ecological provenance)	Essential oil		Carotenoids	Polyphenols
	content, %	yield, ml/plant	mg%	Gallic acid equivalents-GAE [mM]
Thymus (Aragatsotn)	2.3 b	0.490 b	58.8 b	0.693 ± 0,02 b
Thymus (Kotayk)	2.8 a	0.857 a	80.3 a	0.825 ± 0,01 a
Thymus (Syuniq)	1.8 c	0.338 c	59.8 b	0.569 ± 0,02 c
Thymus (Tavush)	1.9 c	0.363 c	73.3 a	0.576 ± 0,01 c
LSD_{05}	0.25	0.095	9.3	0.092
Hypericum (Syuniq)	0.25 b	0.0638 b	69.8 a	0.519 ± 0,02 a
Hypericum (Tavush)	0.31 a	0.1020 a	73.3 a	0.524 ± 0,01 a
LSD_{05}	0.03	0.01	8.3	0.062
Centaurea (Shirak)[1]	0.01	0.0003	68.9	0.443 ± 0,01

[1]*Centaurea hajastana* growing only in Shirak province

Figures

Fig. 1,2. Maps of Armenia (1.Aragatsotn, 2.Ararat, 3.Armavir, 4.Gegharkunik, 5.Kotayk, 6.Lori, 7.Shirak, 8.Syunik, 9.Tavush, 10.Vayots Dzor, 11.Yerevan provinces) Source: Wikipedia



1=α-pinene; 2=sabinene; 3=β-myrcene; 4=α-terpinene; 5=limonene; 6=1,8-cineole; 7=γ-terpinene; 8=p-cymene; 9=α-terpinolene; 10=linalool; 11=linalyl acetate; 12=bornyl acetate; 13=β-caryophyllene; 14=pulegone; 15=α-terpineol; 16=borneol; 17=d-germacrene; 18=geranyl acetate; 19=nerol; 20=geraniol; 21=thymol; 22=carvacrol

Fig. 3. Chromatogram of a typical *Thymus transcaucasicus* essential oil sample

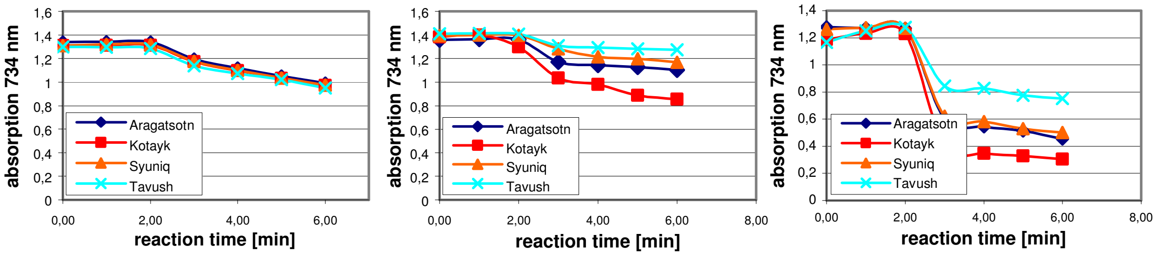


Fig. 4,5,6. Antioxidative capacity by ABTS model system as affected by essential oil, carotenoid-rich and polyphenol-rich extract of thyme grown in different ecological provenances of Armenia (from left to right)

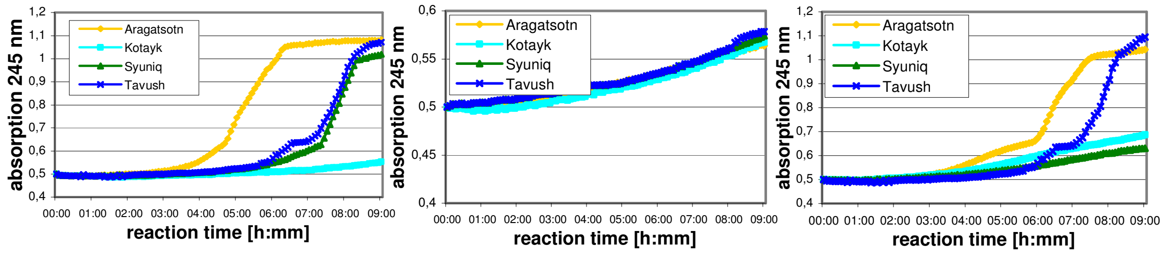
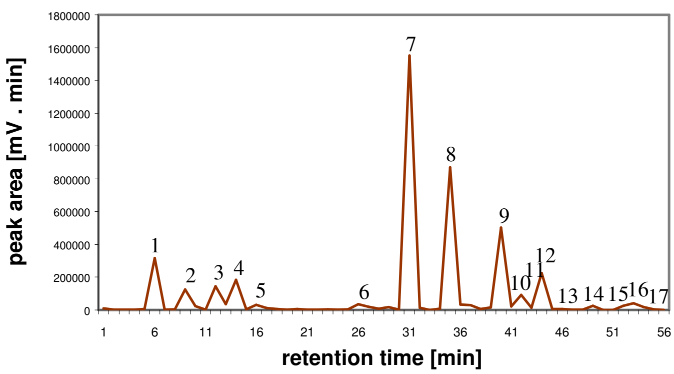
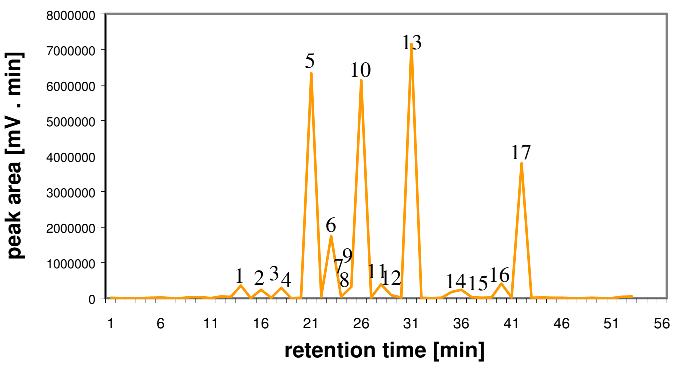


Fig. 7,8,9. Lipid peroxidation in human blood plasma as affected by essential oil, carotenoid-rich and polyphenol-rich extract of thyme grown in different ecological provenances of Armenia (from left to right)



1=α-pinene; 2=β-pinene; 3=2-methyl-decane; 4=2-methyl-dodecane; 5=tridecane; 6=α-copaene; 7=β-caryophyllene; 8=d-germacrene; 9=β-farnesene; 10=β-chimachalene; 11=cadinene; 12=1-hexyl-2-propyl-cyclopropane; 13=cyclododecane; 14=6,10,14-trimethyl-2-pentadecanon; 15=ciclotetradecane; 16=nonadecane; 17=eicosane

Fig. 10. Chromatogram of a typical *Hypericum eleonorae* essential oil sample



1=1-octen-3-ol; 2=δ-elemene; 3=α-copaene; 4=β-elemene; 5=β-caryophyllene; 6=γ-elemene; 7=aromadendrene; 8=α-humulene; 9=β-farnesene; 10=d-germacrene; 11=β-selinene; 12=γ-cadinene; 13=β-eudesmol; 14=δ-cadinene; 15=α-cadinene; 16=b-germacrene; 17=caryophyllene oxide

Fig. 11. Chromatogram of a typical *Centaurea hajastana* essential oil sample