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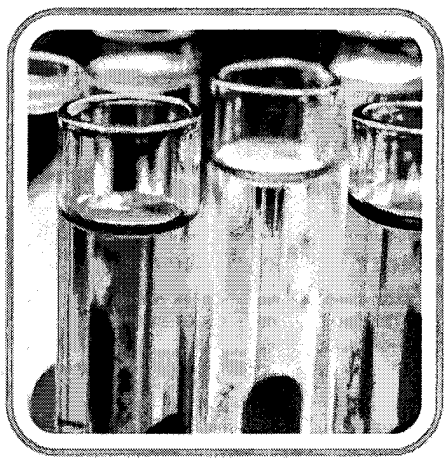

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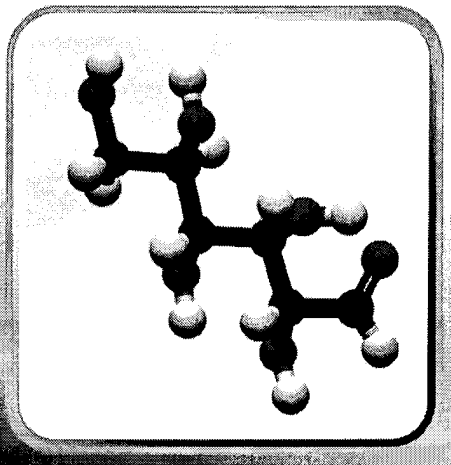

СОДРЖИНА

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DW (average dry biomass 5.68±4.08 per explant). The root dry weight recorded in *G. elegans* was 0.92±0.54 g. The growth was similar for *G. scorzonerifolia* and *G. paniculata*; the dry weight reached 0.29 g and 0.34 g per explant, respectively. Minimum growth was observed in *G. trichotoma* cultures. Generally, the dry weights were 2.9–5.9% of the fresh weights, which closely paralleled the fresh weights responses. The fingerprints of the saponin HPLC profiles of the root *in vitro Gypsophila* cultures were drastically different with at least up to 30 different saponins detected for some of them. The roots of *G. elegans* accumulated saponins (expressed as gypsogenin 3-*O*-glucuronide) up to 65 mg/g DW (mean value 31.36±31.83 mg/g DW). These amounts were higher than in *G. paniculata* and *G. trichotoma* roots found in this study and as the best producing ones (30.2–40 mg/g). The cell lines of *G. glomerata* showed a smaller quantitative amount of saponins (between 1.3 and 7.10 mg/g DW) than those of *G. elegans* but nearly the same HPLC profiles as for root extracts of intact *G. paniculata*. The other species showed a smaller production of saponins after 3 months of subculture. However, individual lines (each line cultures representing one phenotype) of *G. scorzonerifolia, G. paniculata* and *G. trichotoma* showed variation in gypsogenin 3-*O*-glucuronide content: in some cases the levels were below LD or LQ. Generally, the content of gypsogenin 3-*O*-glucuronide was not related to the growth rate of the cultures: the maximum average content recorded in *G. elegans* cultures in liquid medium represented 8-fold increase compared with that found in *G. glomerata*, but the mean dry biomass was 6 times lower.

In conclusion, the biosynthetic behavior of *Gypsophila* species assayed was different but we found gypsogenin saponins in all excised root cultures which prove that *in vitro* root culture can contribute in producing saponins from *Gypsophila*. It could be suggested that selection of cell lines of *G. elegans* and *G. glomerata* may be effective for enhancing the saponin production in *Gypsophila* root cultures.

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CONTENT OF TOTAL ANTIOXIDANTS IN INFUSIONS PREPARED FROM MEDICINAL PLANTS FREQUENTLY USED IN REPUBLIC OF MACEDONIA

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BACKGROUND

Free radicals are highly reactive and unstable transitional chemical compounds that have potential to damage the chemical structure and impair the function of lipids, proteins and nucleic acids. It is well known that free radicals are normally produced during the metabolic processes and that, in physiological conditions, their harmful effect over bio-molecules is disabled by the activity of endogenous (represented mainly by antioxidant enzymes) and exogenous (represented by dietary antioxidants) antioxidant barrier. In conditions of extremely high production of free radicals within the biological system and/or lack of endogenous and/or exogenous antioxidants, a state of oxidative stress occurs, which has been proven to be the basis of many chronic diseases, primarily atherosclerosis, various cancers and neurodegenerative diseases. Considering the involvement of oxidative stress in the pathogenesis of above mentioned chronic diseases, numerous trials were conducted to study the possible preventive effect of antioxidant (pro)vitamins: beta-carotene, vitamin C and E. Inconsistent and mainly negative results of these trials, as well as positive results from research on the effect of diet rich in fruits and vegetables and of green tea on chronic diseases associated with oxidative stress, favored the use of natural versus synthetic antioxidants. It is believed that one of the possible reasons for such results is that natural products, despite the known antioxidant (pro)vitamins, contain numerous other antioxidants that have strong physiological effect. It is also believed that antioxidants contained in the natural products are important both because of their individual functions and their synergistic effects in the body.

AIM OF THE STUDY: Knowing the positive effects of green tea on the prevention of some of the chronic diseases that are associated with high oxidative stress, our aim was to measure the level of total antioxidants in infusions prepared from some frequently used medicinal plants in Republic of Macedonia and to compare the obtained values with that of green tea.

MATERIAL AND METHODS

The content of total antioxidants was measured in infusions prepared from the following medicinal plants: lemon balm (Balm leaf), St. John's wort (Hyperici herba), linden (Tiliae flos), nettle (Urticae folium) and green tea (Camellia sinensis). Medicinal plants from Good Nature, Alkaloid Skopje, were purchased in the local market, packed in filter bags.

Infusions prepared from lemon balm, St. John's wort and nettle picked in Maleshevo Mountains were also analyzed.

Preparation of infusions: 0,5g dry plant was poured with 50mL boiling water and let stand for 15 minutes, covered.

The content of total antioxidants was measured by FRAP method, according to Benzie and Strain. The method is based on the principle

that ferric to ferrous ion reduction at low pH causes production of colored ferrous-tripyridyltriazine complex that absorbs at 593nm. Solutions of $FeSO_4$ with known concentrations were used as standards. The concentration of total antioxidants is expressed as mmol/L $FeSO_4$.

RESULTS

Results from our measurements once again confirmed the high content of total antioxidants in the infusion prepared from green tea: 25,2mmol/L. From the other studied medicinal plants, the infusion prepared from lemon balm (Good Nature, Alkaloid) had particularly high value of total antioxidants, very close to that of green tea: lemon balm – 19,2mmol/L. Total antioxidants content in the infusions prepared from other Macedonian medicinal plants under study, from Good Nature, Alkaloid, were: St. John's wort – 8,5mmol/L; linden – 6,1mmol/L; nettle – 2,9mmol/L.

Infusions prepared from lemon balm and nettle picked in Maleshevo Mountains gave similar results as commercial medicinal plants: lemon balm – 19,5mmol/L; nettle – 3,7mmol/L. Exception was the infusion prepared from St. John's wort picked in Maleshevo Mountains that had higher content of total antioxidants compared with commercial medicinal plant: St. John's wort – 12,6mmol/L.

CONCLUSION: Besides well known health effects of our frequently used medicinal plants: lemon balm, St. John's wort, linden and nettle, of particular importance is their content of total antioxidants that has potential to contribute in lowering of free radicals and improvement of oxidative status in the body.

In this context it is important to remind of our previously published data about total antioxidants level in herbal infusions suitable for unrestricted everyday use (4) that could also contribute in reducing of oxidative stress.

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СОДРЖИНА НА ВКУПНИ АНТИОКСИДАНСИ ВО ИНФУЗИ ПОДГОТВЕНИ ОД НЕКОИ НАЈЧЕСТО КОРИСТЕНИ ЛЕКОВИТИ РАСТЕНИЈА ВО РЕПУБЛИКА МАКЕДОНИЈА

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ВОВЕД

Слободните радикали претставуваат високореактивни и нестабилни преодни хемиски компоненти кои имаат потенцијал за нарушување на хемиската структура и функцијата на липидите, протеините и нуклеинските киселини. Познато е дека слободните радикали нормално се создаваат во текот на метаболичките процеси и дека, во физиолошки услови, нивното штетно дејство врз биомолекулите е оневозможено со активноста на ендогената (претставена главно со антиоксидантните ензими) и егзогената (претставена со антиоксидансите примени со храната) антиоксидантна бариера. Во услови на особено висока продукција на слободни радикали во биолошкиот систем и/или недостиг на едогени и/или егзогени антиоксиданси, се јавува состојба на оксидативен стрес за која е докажано дека претставува основа на голем број хронични заболувања, во прв ред атеросклероза, различни карциноми и невродегенеративни заболувања. Имајќи ја предвид инволвираноста на оксидативниот стрес во патогенезата на претходно споменатите заболувања, направени беа бројни испитувања за потенцијалниот превентивен ефект на антиоксидантните (про)витамини: бета-каротен, витамин С и Е. Неконзистентните и претежно негативни резултати од ваквите студии, како и позитивните резултати од истражувањата за ефектот на исхраната богата со овошје и зеленчук и на зелениот чај врз хроничните заболувања поврзани со оксидативниот стрес, ја фаворизираат употребата на природните антиоксиданси наспроти синтетичките препарати. Се смета дека една од можните причини за ваквите резултати од истражувањата е таа што природните продукти, покрај познатите антиоксидантни (про)витамини, содржат и бројни други антиоксиданси кои имаат силно физиолошко дејство. Се смета исто така дека антиоксидансите содржани во природните продукти се значајни како поради своите поединечни функции, така и со нивното синергистичко дејство во организмот.

ЦЕЛ НА ТРУДОТ

Знаејќи за позитивните ефекти на зелениот чај во превенцијата на некои од хроничните заболувања кои се поврзани со висок оксидативен стрес, наша цел беше да го измериме нивото на вкупни антиоксиданси во инфузи подготвени од некои најчесто употребувани лековити растенија во Република Македонија и да ги споредиме добиените вредности со онаа од зелениот чај.

МАТЕРИЈАЛ И МЕТОДИ

Содржината на вкупните антиоксиданси беше одредувана во инфузи подготвени од следните лековити растенија: маточина (Balm leaf), кантарион (Hyperici herba), липа (Tiliae flos), коприва (Urticae folium) и зелен чај (Camellia sinensis). Лековитите растенија од Good

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Nature, Алкалоид Скопје, беа набавени во слободна продажба, пакувани во филтер врекички.

Анализирани беа исто така и инфузи од маточина, кантарион и коприва подготвени од лековити растенија собрани во Малешевските Планини.

Подготовка на инфузите: 0,5g суво исситнето растение се прелива со 50mL врела вода и се остава да стои 15 минути во покриен сад, после што се процедува и се анализира.

Вкупната содржина на антиоксиданси беше одредувана со FRAP методата, според Benzie и Strain. Методата е базирана на принципот на редукција на фери- во феро-јон при ниска pH, при што се продуцира обоен феро-трипиридилтриазин комплекс кој апсорбира на 593nm. Раствори од $FeSO_4$ со позната концентрација беа користени како стандарди. Концентрацијата на вкупните антиоксиданси е изразена како mmol/L $FeSO_4$.

РЕЗУЛТАТИ

Резултатите од нашите мерења уште еднаш ја потврдија високата содржина на вкупните антиоксиданси во инфузот подготвен од зелениот чај: 25,2mmol/L. Од останатите испитувани лековити растенија, инфузот од маточина (Good Nature, Алкалоид) исто така покажа особено висока вредност на вкупни антиоксиданси, многу блиска со таа од зелениот чај: маточина – 19,2mmol/L. Содржината на вкупните антиоксиданси во инфузите подготвени од останатите испитувани македонски лековити растенија од Good Nature, Алкалоид, изнесуваше: кантарион – 8,5mmol/L; липа – 6,1mmol/L; коприва – 2,9mmol/L.

Инфузите подготвени од маточина и коприва собрани од Малешевските Планини дадоа слични резултати како и комерцијалните лековити растенија: маточина – 19,5mmol/L; коприва – 3,7mmol/L. Исклучок претставуваше инфузот подготвен од кантарион од Малешевските Планини, кој имаше нешто повисока содржина на вкупни антиоксиданси во однос на комерцијалниот: кантарион – 12,6mmol/L.

ЗАКЛУЧОК

Покрај добро познатите лековити дејства на нашите многу често користени лековити растенија: маточина, кантарион, липа и коприва, од особено значење е и нивната содржина на вкупни антиоксиданси која има потенцијал да придонесе за намалување на содржината на слободните радикали, а со тоа и за подобрување на оксидативниот статус на организмот.

Во овој контекст значајно е да се истакнат и нашите претходно објавени податоци за нивото на вкупни антиоксиданси во хербални инфузи погодни за неограничена секојдневна употреба (4), кои исто така би можеле да придонесат за намалување на оксидативниот стрес.

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VARIATIONS IN THE ESSENTIAL OIL CHARACTERISTICS OF *SATUREJA MONTANA* L.(LAMIACEAE) DEPENDING ON ENVIRONMENTAL CONDITIONS AND PHENOLOGICAL STAGE

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Satureja montana L. is a perennial semi-shrub growing wild in southern Europe, the Caucasus and northern Africa [1]. This aromatic species is frequently used as a traditional medicinal plant. With regard to the presence of phenolic compounds, *S. montana* is known to posses certain pharmacological activities [2-5]. The present study describes the content, chemical composition and compositional variations of the essential oil at different phenological stages of *Satureja montana* L. transferred from natural habitat to Belgrade. Two subspecies *S. montana* subsp. *montana* and *S. montana* subsp. *pisidica* (Wettst.) Šilić were included in the analysis.

The essential oil was obtained from the aerial parts of the plant by hydrodistillation and analyzed by gas chromatography (GC/FID and GC/MS). *S. montana* subsp. *montana* exhibited the lowest oil content in the pre-flowering stage (0.5%) and the highest in the flowering stage (1.2%). With the development of fruit and seeds the oil content dropped from 0.9% to 0.6%. Plants gathered in natural habitats exhibited the same trend. The main constituent in the oil of the cultivated plants in the pre-flowering stage was thymol (48.9%), whose concentration in the remaining phenological stages varies between 2% and 3%. Linalool (50%-75.6%) was the dominant component in the oils isolated from plants in the flowering, fruiting and full fruiting stages. The concentration of linalool in oil of plants in the pre-flowering stage was only 1.1%. Thymol methyl ether was the second most important component in oil of cultivated plants in the pre-flowering stage (12.4%), flowering stage (16.6%) and full fruiting stage (23.6%). This component was missing in the oils of plants in the fruiting stage, which contained a high concentration of cis-sabinene hydrate. In the oil obtained from plants in other stages, cis-sabinene hydrate varies between 0.7% and 1.2%. Oils of plants from the natural populations were rich in thymol (37.6%), carvacrol (13.2%) and p-cymene (11.1%) in the pre-flowering stage. The amount of p-cymene rises sharply in the oil of plant in post fruiting stage (57.14%), while the amount of thymol and carvacrol drops significantly (1.3%; 0.4%).

S. montana subsp. *pisidica* contained much more essential oil in the pre-flowering stage (0.8%) compared to the post-flowering stage (0.3%). The plants from the natural population were very rich in oil (1.2%). In the pre-flowering stage the oil obtained from cultivated plants contained mostly carvacrol (43.5%), γ-terpinene (17.5%) and thymol (11.1%). In the post-flowering stage the concentration of these components decreases (19.3%; 5.6%; 5.2%), while p-cymene (30%) and linalool (13.7%) emerge as dominant. In the oil of plants in the pre-flowering stage the p-cymene content amounted to 5.3%, while the concentration of linalool was very low, 0.85%. Plants collected from natural habitat exhibited a domination of p-cymene (24.9%), cis-sabinene hydrate (15.4%) and thymol (11.1%). Cis-sabinene hydrate in oil of cultivated plants appeared in a 1% concentration in pre-flower-