

ANTIOXIDANT PROPERTIES OF FOOD SUPPLEMENTED WITH MEDICINAL PLANTS

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ABSTRACT: The aim of this work was to prove the antioxidant activity of the mixture of selected medicinal herbs, composed to stimulate digestion, in cookies in order to create new functional food formulations. Initially, the composition and quantity of plant phenolics of *Petroselinum fructus*, *Frangulae cortex*, *Mentha piperitae folium*, *Carvi fructus*, *Betulae folium* and the "Vitalplant" mixture were tested, as these compounds had been shown to be the most responsible for the antioxidant activity of plant materials. Antioxidant activities and thermal stability of above mentioned medicinal plant extracts were tested by six direct and indirect tests. In the final part of the thesis, the potential of "Vitalplant" mixture to retard the process of lipid oxidation and to elevate the antioxidant potential of cookies were tested. According to the obtained results, all tested plant drugs are rich source of plant phenolics, and at the same time possess antioxidant activity in all applied tests. Referring to the thermal stability of extracts, significant changes in antioxidant activities were not found after the thermal treatment. "Vitalplant" addition improved antioxidant activity and oxidative stability of the cookies dose-dependently, indicating its potential as an ingredient for functional food formulations.

Key words: medicinal plants, antioxidants, lipid oxidation, cookies

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INTRODUCTION

Spices and herbs have been added to foods since ancient times, not only as flavoring agents, but also as folk medicines and food preservatives (Milovanović et al., 2009; Šarić et al., 2009; Škrinjar et al., 2009). Although they can be contaminated by pesticides, heavy metals and microorganisms (Mišan et al., 2007; Matić et al., 2008; Milovanović et al., 2008), medicinal plants are generally recognized by the consumers to be safe. Presently, there is an increasing interest both in the industry and in the scientific research of spices and aromatic herbs because of their strong antioxidant properties (Kabić et al., 2008). Phenolic substances have been shown to

be the most responsible for the antioxidant activity of plant materials.

In this study, a selection of medicinal plants with proven pharmacological action and also rich in plant phenolics was made. Parsley fruit (*Petroselinum fructus*) has been known for its diuretic action due to the presence of apiole in its essential oil. Buckthorn bark (*Frangulae cortex*) contains anthraquinone glycosides with purgative effects. Mint leaves (*Mentha piperitae folium*) and caraway fruit (*Carvi fructus*) essential oils are frequently used in herbal drugs for treatment of abdominal discomfort and pain. Birch leaves (*Betulae folium*) have been known for its diuretic effects.

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The objective of this research was to evaluate antioxidant activity of the ethanolic extracts of above mentioned herbal drugs and of the mixture of these medicinal herbs "Vitalplant" (*Frangulae cortex* (35%), *Menthae piperitae folium* (20%), *Carvi fructus* (20%), *Petroselinii fructus* (25%)) and how it was affected by thermal treatment, as well as the potential of "Vitalplant" mixture extract and pulvis to retard the process of lipid oxidation in cookies enriched with the mixture.

MATERIAL AND METHODS

Ethanolic extracts of commercially available herbal drugs (*Petroselinii fructus*, *Frangulae cortex*, *Mentha piperitae folium*, *Carvi fructus*, *Betulae folium*) and "Vitalplant" mixture were compared by: 1) identification and quantification of main phenolic acids and flavonoids, the content of total plant phenolics and flavonoids, and 2) estimation of antioxidant activity by different direct and indirect methods (through measuring scavenging activity on 1,1-diphenyl-2-picrylhydrazyl (DPPH[•]), hydroxyl ([•]OH) and superoxide anion (O₂^{•-}) radicals, reducing power, chelating activity on Fe²⁺ and antioxidant activity based on coupled oxidation of β -carotene and linoleic acid (AOA) (Mišan *et al.*, 2009). Thermal stability of extracts was determined by measuring the residual antioxidant activity after heating (simulated baking process) (Mišan *et al.*, 2008).

Chemical composition of extracts

In the first part of this thesis, a special focus on identification and quantification of plant phenolics in selected herbal drugs was made, since phenolic acids and flavonoids may significantly contribute to their overall antioxidant activity. Referring to the obtained amounts of total phenolics, significant differences ($P < 0.05$) were found among extracts in the following order: mint > buckthorn > "Vitalplant" > parsley > caraway. Total flavonoid content varied from 0.510% (parsley) to 2.05% ("Vitalplant"). Determination of the amounts and species of polyphenols in crude extracts is one of the greatest challenges for analysts because of the number of natural phenolics belonging to different classes. As a part of the research, a new rapid resolution HPLC

method was developed to enable a rapid separation of a mixture which consisted of hydroxybenzoic acids, hydroxycinnamic acids, flavones, flavonols, flavanone, flavonol-glycoside and anthraquinone, in a single run, within 22 minutes (Mišan *et al.*, 2011). According to the obtained results, crude ethanolic extract of mint was the most abundant in rosmarinic acid. Birch extract is rich in glycosides of quercetin and myricetin, chlorogenic acid and various other low-molecular phenolics, while caraway extract in kaempferol and quercetin glycosides. Quantitative analysis of phenolic compounds in the ethanolic extract of parsley has shown the presence of gallic acid, protocatechuic acid, caffeic acid, trans-cinnamic acid, luteolin, kaempferol and apigenin. In the buckthorn extract which contains up to 8% anthraquinone glycosides, the presence and content of numerous phenolic acids and flavonoids was confirmed and determined. "Vitalplant" mixture contains wide range of phenolic compounds, free phenolic acids and phenolic acids derivatives. Flavonoids are mainly present in glycosidic form and therefore hydrolysis resulted in the increased content of aglycones.

Antioxidant activity of medicinal plants

In the second part of the thesis, due to a lack of a protocol for the *in vitro* estimation of the antioxidant activity of medicinal plants, six tests ((ESR) spectroscopy and spectrophotometry) were used for testing the efficiency of the extracts. Referring to the obtained results, the mint extract was shown to possess the highest antioxidant capacity in all but in the AOA test, in which buckthorn bark extract had the highest activity. Birch leaves possessed a relatively high antioxidant capacity in all of the tests, while parsley extracts had a relatively low antioxidant activity in comparison to the other tested samples. Caraway fruit had the lowest content of total phenolics, but the highest scavenging activity towards [•]OH. Commercial mixture "Vitalplant" exhibited a relatively high antioxidant activity in most of the tests, which can be explained by synergistic effects of its components. Referring to the thermal stability of extracts, significant changes in antioxidant activities were not found after thermal treatment (Table 1).

Table 1.

Scavenging activity against DPPH[•] of the ethanolic extracts before and after thermal treatment.

Sample	Scavenging activity against DPPH [•]	
	Non treated	Thermally treated
	IC ₅₀ (mg/ml) ± SD	IC ₅₀ (mg/ml) ± SD
Mint leaves	0.172 ± 0.002 ^b	0.167 ± 0.003 ^b
Buckthorn bark	1.18 ± 0.210 ^c	0.920 ± 0.180 ^{ac}
Birch leaves	0.632 ± 0.005 ^{ab}	0.580 ± 0.016 ^{ab}
Caraway fruit	2.06 ± 0.137 ^d	1.82 ± 0.045 ^d
Parsley fruit	4.65 ± 0.820 ^e	4.79 ± 0.005 ^e
Vitalplant	0.893 ± 0.022 ^{ac}	0.792 ± 0.076 ^{ac}

Values followed by different literals indicate significant differences according to Duncan's test ($P < 0.05$).

Supplementation of cookies with "Vitalplant" mixture

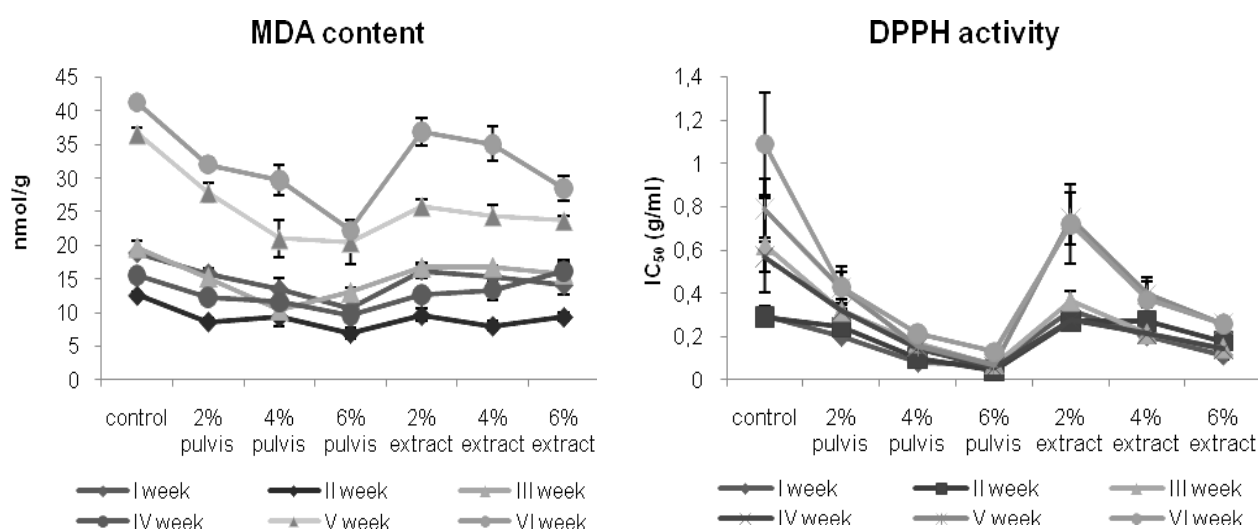


Figure 1. MDA content and DPPH radical scavenging activities of "Vitalplant" enriched cookies during 6 weeks of storage

Third part of the thesis refers to the investigation of the potential of "Vitalplant" mixture to retard the process of lipid oxidation in cookies, investigated by malonyldialdehyde (MDA) and DPPH tests.

As a part of a study, new wire-cut cookie formulations were developed by supplementing basic cookie formula with "Vitalplant" mixture under the laboratory conditions. By adding pulvis and ethanolic extract of the herbal mixture "Vitalplant" to the basic formulation, each at three levels, apart from the control sample, cookies were prepared to provide six variations: 2%, 4%, and 6% addition.

Vitalplant" addition improved antioxidant activity and oxidative stability of the cookies in a dose-dependent manner (Figure 1). Addition of pulvis was more efficient in retarding oxidative changes than the addition of extract.

CONCLUSION

According to the obtained results, all of the tested plant drugs are rich source of plant phenolics, and at the same time possess antioxidant activity in all applied tests. Referring to the thermal stability of extracts, significant changes in antioxidant activities were not found after the thermal treatment. Regarding the obtained results, "Vitalplant"

mixture has a potential as an ingredient for functional food formulations.

Further research needs to be done in order to confirm the functionality of the "Vitalplant" supplemented cookies through *in vivo* studies and acceptability of the products through consumer's studies.

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АНТИОКСИДАНТНА СВОЈСТВА ХРАНЕ СА ДОДАТКОМ ЛЕКОВИТОГ БИЉА

Александра Мишан

- Извод из докторске дисертације -

Циљ овог рада био је да се докаже антиоксидантна активност смесе лековитог биља у кексу, наменски компоноване да стимулише дигестију, што би била полазна основа за креирање нових функционалних производа. Прва фаза рада је обухватила испитивање састава и садржаја фенола биљних дрога: *Petroselinum fructus*, *Frangulae cortex*, *Mentha piperitae folium*, *Carvi fructus*, *Betulae folium* и смесе "Vitalplant", јер је познато да су ова једињења носиоци антиоксидантне активности у биљкама. У наставку истраживања, испитана је антиоксидантна активност и термичка стабилност екстракта горенаведених биљака применом шест директних и индиректних тестова. У последњој фази испитана је способност смесе "Vitalplant" инхибирања процеса оксидације липида и повећавања антиоксидантне активности кекса. Резултати су показали да су испитиване биљке богат извор биљних фенола и да поседују антиоксидантну активност у свим примењеним тестовима. Након термичког третмана, није дошло до значајне промене у антиоксидантној активности. Обогаћивање кекса "Vitalplant" смесом, управо пропорционално њеном садржају, доводи до повећања антиоксидантне активности кекса и смањења степена липидне пероксидације, чиме је доказан њен потенцијал за развој функционалних производа.