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ANTIOXIDANT ACTIVITIES OF HERBS, FRUIT AND MEDICINAL MUSHROOM *Ganoderma lucidum* EXTRACTS PRODUCED BY MICROFILTRATION PROCESS

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Abstract: This paper presents kinds of extraction and cross-flow filtration of composition of 46 healthful and aromatic herbs, 8 fruits and fungi *Ganoderma lucidum*. Those extracts are part of Bitter 55, which have significant antioxidant capacity. Antioxidative activities of plant extracts have been determined by DPPH test using method of Blois. Bitter 55 which was kept at the green bottle in the dark has EC_{50} = 141.07 µl/ml and it was stable during 150 days. Synthetic anti-oxidants BHT (ditertbutil-hydroxytoluen, EC_{50} = 6.2 µgml^{-1}), trolox (vitamin E analog soluble in water, EC_{50} = 6.8 µgml^{-1}) were used for comparison. EC_{50} values were calculated as concentration of the extract necessary to decrease DPPH radical concentration for 50 %. Bitter 55 contents 35% vol of alcohol (wheat origin), 88.22 g/l total extract and slice of medicinal mushroom *Ganoderma lucidum* (1 % w/v) which was extracted 30 days before analyses. The main problem in practical applications of MF is the reduction of permeate flux with time, caused by the accumulation of feed components in the pores. During microfiltration bitter herbal liquor, the function of filtrate flux is decreased with VCR. Dependence of decreasing flux with VCR can be

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separated in three periods. For the first, starting period, rapid decrease of filtrate flux is characteristic. Second period is defined with much smaller decrease of the flux than in the first phase. Third period has as characteristic minor decrease of flux and can be defined as steady state. Steady state emerges after τ_s = 80 min.

Key words: antioxidant activity, bitter, extraction, herbs, fruits, *Ganoderma lucidum.*

Introduction

Products made from herbs named Bitters are products which consumers very often drink, not only because of their refreshment properties, but also healthy reasons. Active ingredients of these products are different bioactive compounds such as different phenols, polyphenols, phenol carboxylic acids, flavonoids, tannins etc. Different products labeled as bitter herbal liquors can be found at the market. They are made from 25, 32 or even 56 extracts obtained by alcohol or water extraction from different parts of herbs, fruits, seeds, spices, roots, etc [1]. It was shown that bitter made from herbs extract, fruits and extract of fungi *Ganoderma lucidum* is a very interesting healthy product. These drinks are better solution then other standard spirits. Additives and other ingredients of bitters provide good sensor quality and refreshment. Those facts are very interesting from the aspect that at Balkan Peninsula about 6600 kind of plants can be found. The half of them grows in Serbia, over 800 have healthful properties, and about 220 are rare and endemic.

Official journal of EU regulations for spirit drinks appoint that Bitters are spirits made from ethyl alcohol of agricultural origin, sugar, water, fruit juices and bitter aromatic herbal extracts. Bitters have to take next requirements: minimum 25 % vol of alcohol, minimum 10 g/l total extract and predominantly bitter taste [2].

Bitters made from herbs and fruits are very interesting as safe and healthful drinks. These drinks are better solutions then other standard spirits. Fruit content and herbal extract provide intake of bioactive compounds. Herbal or fruit extracts imply extracts, which are permitted to human consumption. Herbs extracts as a major compounds of bitters, can be produced by alcohol-water maceration at the ambient temperature. Traditional methods, such as maceration, percolation, boiling with water, extraction with cold (enfleurage) or with hot fat, which have been used for many decades, are very time-consuming and require relatively large

quantities of solvents [3]. There is an increasing demand for new extraction techniques with shortened extraction time, reduced organic solvent consumption, and increased pollution prevention. New extraction methods including supercritical fluid extraction, vertical (turbo) extraction, ultrasound-assisted extraction, microwave-assisted extraction are fast and efficient for extracting chemicals from solid plant matrixes [3]. Water maceration at high temperature is used for the production of teas. The benefits of extractions at the low temperatures are known. Alcohol–water maceration has advantages against water maceration in regard to microbiology stability.

Bitter herbal liquor, Bitter 55 contains extract of 46 types of carefully selected herbs, 8 fruits and medicinal mushroom *Ganoderma lucidum.* Bitter 55 belongs to a class of bitter herbal liqueurs that can be used as aperitif (tonic) or digestive drink. Before meals, Bitter 55 is used as aperitif to stimulate the appetite, whereas after meals, Bitter 55 is used to improve digestion. Bitter 55 can be served with a slice of lemon or orange and ice. Most of the beneficial health effects of bitter, such as: decreasing of stress, tiredness and exhaustion, positive effects on the regulation of the gastrointestinal tract and metabolism, on the glandular secretion, immuno-stimulation, as well as preventive role in the development of cancer and heart diseases, could be ascribed to its antioxidant activity.

The latter properties, which are linked to its ability to increase the secretion of gastric juices, could derive from the high content of phenol-related substances found in the liquor and thus from their antioxidant capacity. The antioxidant activity proved to be directly correlated with the content of total phenols. Bitter 55 is appreciated for its bitter, sophisticated and appealing flavour, for its very special aroma, its dark brown color and its properties as a tonic and digestive aid. Industrial production coexists with preparations at home.

Phenol compounds are naturally occurring substances in herbs, fruits, vegetables, nuts, seeds and flowers, and are integral part of human nutrition. Epidemiological studies have shown that consumption of phenol-rich beverages, such as bitter herbal liquers, teas, wines, correlates with reduced coronary heart disease mortality [7]. The strikingly low incidence of coronary heart disease in France as compared with other western countries with comparable dietary intake has been regarded as ‘‘French paradox’’. Although several hypotheses have been proposed, there is strong believe that lower risk of heart disease is associated with the increased wine consumption in France [8,9]. Antioxidant capacity of red wine is five times

higher then white wines [7,10]. The same effect can be reached consumption bitter herbal liquors made from different herbs and fruits. The protective effects of herbs, fruits, and red wine consumption against coronary artery disease and certain types of cancer are partly attributed to the flavonoid content of these foods. It has been demonstrated both in vitro and in vivo that these phenol compounds can offer significant anti-oxidant effects in human body [11]. The same authors study provides additional support for protective effects of polyphenol antioxidants on cardiovascular disease.

Plants contain a wide variety of free radical scavenging molecules [23]. The phenol compounds in herbs and fruits are in the range from relatively simple compounds to complex tannin type substances. Phenolic substances isolated from a wide range of medicinal plants and fruits acts as antioxidants and exhibit suppression activities on cancerogenesis and mutagenesis in human and animal organisms, which is due to their ability to reduce free radical formation and to scavenge free radicals (hydroxyl, peroxyl, alkoxyl radicals) [12]. Many studies have suggested that flavonoids exhibit biological activities, including antioxidants, antiallergenic, antiviral, anti-inflammatory, anticancer, vasodilating actions, as well as, enzymatic inhibition, photo sensibilisations [13] or UV-protection [14].

The composition of phenol substances in bitter depends on the type of herbs and fruits used for extraction and distillation, procedure of fruit pressing and processing, and the chemical reactions that occur during the aging of herbal and fruit extract in oak barrels or inox tanks [24].

Ganoderma lucidum (Ganodermataceae) in basidiomycetous fungi has been used to treat various human diseases such as hepatopathy, chronic hepatitis, nephritis, gastric ulcer, hypertension, bronchitis and tumorigenic diseases in oriental folk medicine. *G. lucidum* has also been reported to contain polysaccharides and protein bound polysaccharides which have antitumor and antihypertensive activities and decrease the blood glucose level [25]. Due to its ability to cure many different diseases it received names like “Elixir of life”, “Food of Gods”, “Mushroom of Universe” [26,27]. G. lucidum was reported to contain some intensively bitter components including lucidenic acid and ganoderic acid, which are known to inhibit histamine release from most cells, an angiotensin converting enzyme that is produced in response to hypertension in the rennin system of blood control, and growth of liver cancer cells. It was isolated strong antihypertensive triterpene compounds such as ganoderal, ganoderol and ganoderic acids from a 70% methanol extract of G. lucidum [26]. Extracts

from dry fruit body of *Ganoderma lucidum* is preformed as a hot water extraction and ethanol extraction. Ethanol extraction has obtained with 50 % and 70 % vol. solution of grain ethanol and wine distillate during 21 days of maceration. [27].

The production phases of essentially consists maceration (infusion) in water-ethanol solution six different compositions selected herbs. It follows thermal treatment of fruits, pressing to juice and filtration of juice. Mix of juices and herbal extracts are blend with sugar syrup, alcohol and additives, and final clarification using "cross-flow" microfiltration (MF).

Compared to the conventional filtration process (kiselgur and filter sheets T-2600 and K-300, Seitz–Germany), MF can bring the following benefits: eliminate the use of diatomaceous earth or/and filter sheets, thereby, reducing production cost; improve the clarity of the extract; increase the product yield; reduce labor costs etc. The main problem in practical applications of MF is the reduction of permeate flux with time, caused by the accumulation of feed components in the pores (membrane fouling) and on the membrane surface (concentration polarization and gel formation). At present, microfiltration is used to purify the herbal extracts as a new advanced technology, but the membrane fouling leads to the dramatic decline of the permeate flux of membrane [4,5]. Application of microfiltration to the clarification of fruit juices has been extensively studied during the last 25 years [6]. Membrane technology provides an economical and reliable separation in many fields. Ceramic membrane filtration is an advanced method for separating substances from the liquid extracts due to their excellent selectivity, permeability and thermal and chemical stability. However, the permeate flux decreases dramatically during filtration process because the colloids are adsorbed on membrane surface and into the inner pores. In order to control membrane fouling during bitter clarification, several flux enhancement methods have been proposed such as periodic gas backwashing with air or N_2, use of static mixer or high velocity of feed solution.

According to a constant pressure MF theory, the steady-state permeate flux (J_s) is expressed by the resistance-in-series model:

$$J_s = \frac{\Delta p}{\mu_0 R} = \frac{\Delta p}{\mu_0 (R_m + R_p)} = \frac{Q_m}{\rho_0 A_m} \qquad (1)$$

where Δp is the transmembrane pressure, μ_o the permeate viscosity, and R the total hydraulic resistance, R_m is the hydraulic resistance of clean membrane and R_p the total (overall) fouling resistance, Q_m is the mass flow rate of permeate, A_m is the effective cross-sectional membrane area and ρ_o is the permeate density. Permeate flux decline with time can be explained by the volume concentration ratio (VCR):

$$VCR = \frac{V_0}{V_0 - V_p} \tag{2}$$

where V_o is volume of bitter at the start and V_p is permeate volume (clear bitter) [6].

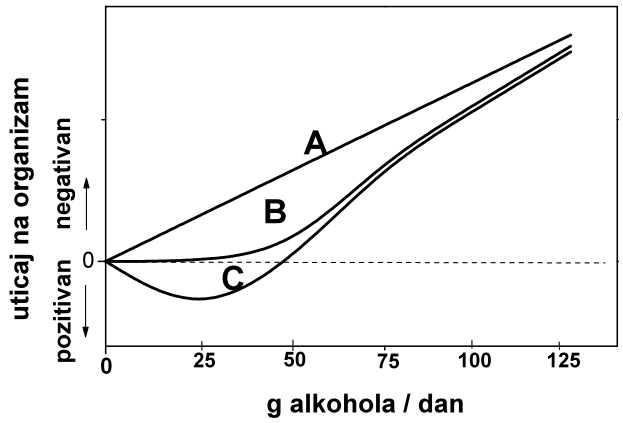

Fig. 1. – The influence of alcohol on human health

Bitters contain alcohol, which can affect health. Several theories are known about the effect of alcohol on human health [15,16]. Old theories are based on the damage effect of alcohol. A new one is based on the influence of maximum daily intake of alcohol. However, the most of authors confirm that alcohol daily intake under some level can have positive influence on human health (Fig. 1). But also, over that level, daily intake of alcohol has bad influence. The positive influence has daily intake of alcohol less then 20 – 25 g [16,17]. 50 ml of Bitter 55 contains 13.8 g.

The definition of a “drink” varies considerably from country to country, but it is usually agreed to be a can/glass of beer (250–350 cc), a glass of

wine (150 cc) or a tot/measure (30–50 cc) of spirits. Thus, a "drink" may contain anywhere between 10 and 15 g of alcohol and it is important that all alcohol studies should be explicit about the methods used to estimate intake in grams of alcohol per unit time (day, week, months) [15].

The liquor - Bitter 55 has never been scientifically investigated, thus the main aim of this work was to examine antioxidant capacity of it. This paper presents kinds of extraction of 46 healthful and aromatic herbs and 8 fruits, which are part of Bitter 54. The aim was to investigate the antioxidant capacity of bitter herbal extract made from 54 plants, and bitter herbal extract made from the same 54 plants with extract of medicinal mushroom *Ganoderma lucidum* (1 % w/v). The second aim was to determine stability during storage of bitters in different kind of bottles and different light conditions. The research was focused on its antioxidant properties.

Materials and Methods

Herb and fruit extracts

Herbal and fruit extract (Bitter 54) is natural product made from 46 different extracts of healthful and aromatic herbs and 8 fruits, permitted for human nutrition. Bitter 54 is commercial product in Serbia, contains 35 % vol of alcohol and 88.22 g/l total extract. Food-grade ethanol (96 % vol, wheat origin) was used. Next 46 different herbs were extracted, or extracted and distillated: Linnean herbarium (*Paris quadrifolia L.*), Prostrate knotweed or knotgrass (*Polygonum aviculare L.*), Mountain germander (*Teucrium montanum L.*), Common sage (*Salvia officinalis L.*), Common Yarrow (*Achillea millefolium L.*), Peppermint (*Mentha piperita L.*), Wild Thyme or Creeping Thyme (*Thymus serpyllum L.*), Common Thyme (*Thymus vulgaris L.*), Camomile (*Matricaria chamomilla L.*), Wall germander (*Teucrium chamaedrys L.*), Grand Wormwood (*Artemisia absinthium L.*), Mellisa (*Melissa officinalis L.*), Hibiscus (Hawaiian hibiscus*)*, Eugenia (*Eugenia caryophyllata L.*), Anise (*Pimpinella anisum L.*), Cinnamon (*Cinnamomum div.)*, Vanilla (*Vanilla planifolia)*, Dog Rose or rosehip (*Rosa canina L.*), Common Juniper (*Juniperus communis L.*), Carob tree (*Ceratonia siliqua L.*), Oregano or pot marjoram (*Origanum vulgare L.*), St John's wort (*Hypericum perforatum L.*), Ribwort Plantain (*Plantago lanceolata*), Uva (*Arctostaphylos uva ursi*), Mulberry (*Morus alba L.*), Rosemary (*Rosmarinus officinalis L.*), Lady's mantle (*Alchemilla vulgaris L.*), Basil (*Ocimum basilicum L.*), Elder or Elderberry (*Sambucus*

nigra L.), Horsetails (*Equisetum arvense L.*), Shepherd's Purse (*Capsella bursa-pastoris L.)*, Senna alexandrina (*Cassia officinalis)*, Blackberries leaf((*Rubus fruticosus L.*), Birch (*Betula L.*), Hawthorn (*Crataegus oxyacantha L.*), European Mistletoe or Common Mistletoe (*Viscum album L.*), Fennel (*Foeniculum vulgare Mill.*), Centaury (*Erythraea centaurium Pers.*), Heartsease or Johnny Jump Up (*Viola tricolor L.*), Oak wood (*Quercus*), Pot Marigold or English Marigold (*Calendula officinalis L.*), Stinging nettle (*Utrica dioica L.*), Coltsfoot (*Tussilago farfara L.*), Pimpernel (*Anagallis arvensis L.*), Common Dandelion (*Taraxacum officinale Web.*), Cypress Spurge (*Euphorbia cyparissias L.*). It has been used juice or extract next 8 fruits: Common fig (*Ficus carica L.*), Grape (*Vitis vinifera)*, plum (*Prunus domestica L.*), apple (*Pirus malus L.*), raspberry (*Rubus idaeus L.*), orange (*Citrus aurantium L.*), lemon (*Citrus limonum Risso*), grapefruit (*Citrus paradisi)*. The additive E160d (caramel) has been used to gain dark-brown color, and sugar (sucrose) in amount of 75 g/l.

Bitter 55 is natural product made from Bitter 54 and medicinal mushroom *Ganoderma lucidum*. Two sets of samples were considered: Commercial brand (Bitter 54) made from the same herbs and fruits and home-made Bitter 55, which was made from Bitter 54 and slice of medicinal mushroom *Ganoderma lucidum* (1 % w/v). Slice of *Ganoderma lucidum* was extracted 30 days before analyses, at the ambient temperature. All of samples were kept at the green bottle in the dark.

Membrane, experimental set-up for cross-flow microfiltration and procedure

The final composition of herbal extracts and fruit juices were classified at the laboratory equipment for cross-flow microfiltration. The experiments were performed with inorganic tubular microfiltration ceramic (Kerasep membrane type W5 - pore size 0.2 μm, Tech-Sep, Miribel, France). Ceramic Kerasep membrane has 19 channels with 4 mm diameter, effective length 270 cm and effective membrane area of 0.0644 m^2. This membrane was installed inside a plastic module with a stopper tire. The membrane was cleaned after experiment with a hot 1% (w/w) aqueous solution of NaOH and 1 mg/l NaClO solution for about 30 min. The acid cleaning was not used because no improvement in permeate flux recovery was observed.

The schematic view of the experimental setup is shown in Fig. 2. The feed herbal extract with fruit juice content (in the amount of Vo = 8.5 liters) was recycled between the retention reservoir and the module by a membrane

pump and the feed flow velocity was controlled with a laboratory made rotameter (υ = 0.6 m/s). Temperature in the system (20°C) was adjusted by passing the retention stream from a bypass line through the thermostat bath. Transmembrane pressure (Δp = 200 kPa) was controlled by the back-pressure valve. The permeate (clear bitter) was collected in a reservoir placed on a digital balance. The mass of permeate collected was measured with an accuracy of 0.1 g every 1 min for period of 120 min.

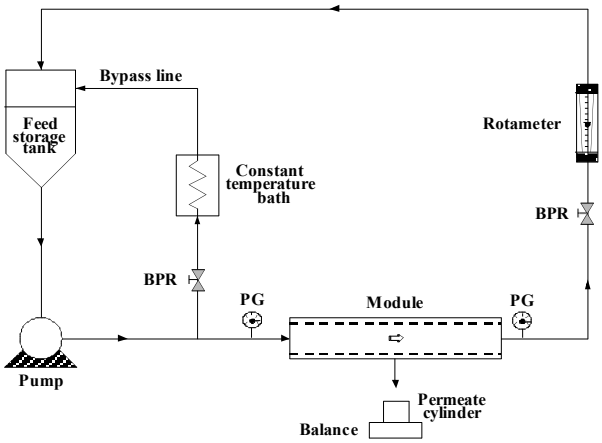

Fig. 2. – Schematic view of the experimental setup
(PG pressure gauge, BPR back-pressure regulator)

DPPH photometric assay

The free radical-scavenging activity of the plant extracts was evaluated using the stable radical DPPH (Aldrich Chemical Co, $C_{18}H_{12}N_5O_6 \cdot xH_2O$) [18,19]. Series of extracts with different concentrations were prepared in methanol (111.11 µl/ml, 176.47 µl/ml, 250 µl/ml, 333.33 µl/ml). Then, 200 µl of each concentration were mixed with 1800 µl of DPPH solution (40 mg/l in methanol) and placed in the dark at the room temperature for 30 min. The absorbance of each sample of the plant extract containing DPPH (*A*s) was measured at 517 nm using a GBC Cintra 40 spectrophotometer. Methanol (1800 µl) plus the plant extract solution (200 µl) was used as the blank (A_b), while the DPPH solution (1800 µl) plus methanol (200 µl) was used as the control (A_c). All determinations were performed in triplicate. The DPPH anti-radical-scavenging activity, DPPH (%), of each plant extract was determined using the following equation:

$$DPPH\,(\%) = 100\left[1-\left(\frac{A_s - A_b}{A_c}\right)\right] \quad (3)$$

where A_s is the absorbance in the presence of the plant extract in the DPPH solution, A_c is the absorbance of the control solution (containing only DPPH) and A_b is the absorbance of the sample extract solution without DPPH. Synthetic anti-oxidants BHT (ditertbytil-hydroxytoluen, EC_{50} = 6.2 $\mu g ml^{-1}$) and trolox (vitamine E analog soluble in water, EC_{50} = 6.8 $\mu g ml^{-1}$) were used for comparison [20]. EC_{50} values were calculated as concentration of the extract necessary to decrease DPPH radical concentration for 50 %. Series of bitters, which were used for determining of storage influence during 150 days, were prepared in concentration of 250 µl/ml methanol as solvent.

Results and Discussion

The most problem in bitter production is stabilization i.e. clarification and filtration. Conventional filtration process using filter sheets can be stopped due to the accumulation of feed component at the sheets surface. The reason is high concentration of suspended solids and colloids in feed solution (Bitter 54). However, microfiltration is good solution.

During »cross-flow« microfiltration, mass flow rate of permeate and permeate flux decrease with time (tab.1, fig. 3A). It's caused by the accumulation of feed compounds in the pores (membrane fouling) and concentration polarization i.e. gel formation at the membrane surface. Second reason of flux decreasing with time is increase of feed flow viscosity due to high colloids concentration in feed solution. Steady-state mass flow rate of permeate has been occurred after some time (τ_s = 80 min). The permeate flux decreases dramatically during filtration process because the colloids are adsorbed on membrane surface and into the inner pores. After time (τ_s), at which the steady-state was established, membrane fouling and gel formation at the membrane surface are constant. It's consequence of transmembrane pressure (Δp = 200 kPa) and applying »cross-flow« velocity (υ = 0.6 m/s). The total hydraulic resistance is collect of hydraulic resistance of clean membrane and the total (overall) fouling resistance ($R = R_m + R_p$).

During microfiltration of bitter, function of filtrate flux decreases with VCR (fig. 3B) similar as function of flux filtrate with time. Dependence of flux decreasing with VCR can be separated in three periods.

For the first, starting period, rapid decrease of filtrate flux is characteristic. Second period (VCR 3 – 7) is defined with much smaller decrease of the flux than in the first phase. Third period has as characteristic minor decrease of flux, so minor that can be defined as steady-state. Behavior of flux is direct proportional to resistance of secondary layer on membrane. Boundaries between these periods are very specific and are specially determined for every system of investigation.

Tab. 1. – Change of mass flow rate of permeate and permeate flux in correlation with time (transmembrane pressure Δp = 200 kPa, feed flow velocity υ = 0.6 m/s, temperature t = 22°C, volume of bitter at the start V_0 = 8.5 liters, final permeate volume V_p = 7.95 liters)

Time (min)	VCR	Mass flow rate of permeate, Q_m (g/min)	Permeate flux, J x 10^6 (m/s)
1	1.06	500	124.42
10	1.53	200	49.77
20	2.06	115	28.62
30	2.80	85	21.15
40	3.82	50	12.44
50	4.76	48	11.94
60	5.86	30	7.46
70	7.23	20	4.98
80	8.37	15	3.73
90	9.66	10	2.48
100	11.12	11	2.73
110	13.01	11	2.73
120	16.65	11	2.73

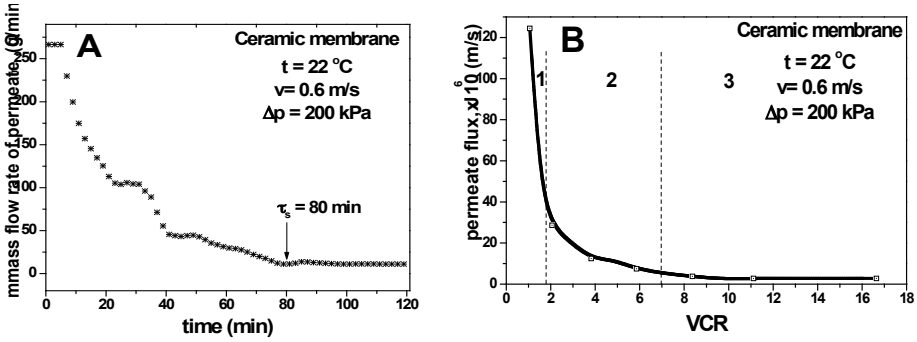

Fig. 3. – Change of mass flow rate of permeate in correlation with time (the time τ_s at which the steady-state was established is shown) – A and effect of volume concentration ratio on permeate flux - B

It was determined that bitter herbal liquors (Bitter 54 and Bitter 55) posse's significant antioxidant potential. Antioxidant activity, i.e. dependence of percentage of absorption inhibition of methanol solution DPPH (%) on 517 nm from concentration of bitters (µl/ml) is showed in figure 4. Decrease of absorption of DPPH solution has a coefficients of correlation r = 0,9955 from linear regressive analysis in investigated diapason of concentration of bitters (111,11 µl/ml of solvent, 176,47 µl/ml, 250 µl/ml, 333,33 µl/ml). EC_{50} of sample Bitter 54 is 139,47 µl/ml of solvent, i.e. 0,387 µl bitter/µg DPPH radical. EC_{50} of sample Bitter 55 is 141,07 µl/ml of solvent, i.e. 0,392 µl bitter/µg DPPH radical. EC_{50} values are concentration of the extract necessary to decrease DPPH radical concentration for 50%. (determined via absorption inhibition of DPPH solution on 517nm).

Synthetic antioxidants, BHT (ditertbutil-hydroksytoluen) and trolox (water soluble analog vitamin E) has EC_{50} values 6,2 and 6,8 µg/ml, respectively. L-ascorbic acid, towards some authors has EC_{50} = 3,9 µg/ml [21], but towards other ones 8,3 µg/ml [22] dependent of the method used. Towards, concentration of Bitters 54 and Bitters 55 has the same antioxidant effect as these concentrations of BHT and trolox is 139,47 µl/ml and 141,07 µl/ml. Ingesting 50 ml of Bitter 55 drink in orgasm ensure the same antioxidant effect as 2222 µg synthetic antioxidant BHT and 2474 µg synthetic antioxidant trolox.

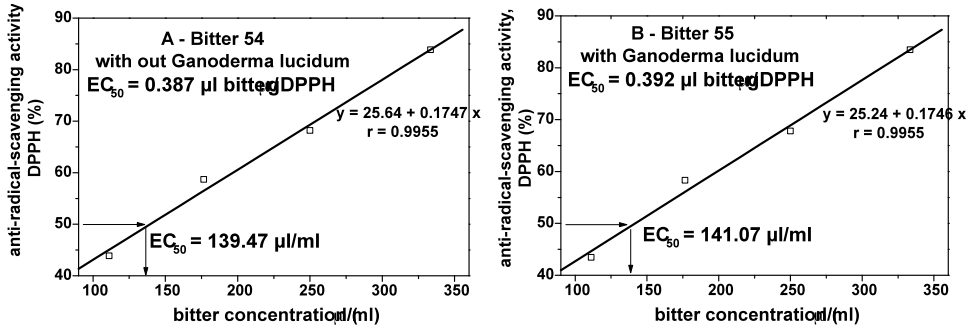

Fig. 4. – Anti-radical-scavenging activity (DPPH %) vs. bitter concentration (µl/ml), A – Bitter 54 with out Ganoderma lucidum, B - Bitter 55 with slice of Ganoderma lucidum 10 g/l

Antioxidant capacity (EC_{50} in µl/µg DPPH) of different samples is shown in figure 5. Comparing antioxidant effect of Bitter 55 with one

traditional bitter which can be found in pharmacy as a medicament, it can be seen that Bitter 54 and Bitter 55 possess almost three times higher antioxidant activity. On the other hand, comparing activity of Bitter 54 and Bitter 55 with one commercial bitter liquor in supermarket, it can also be seen almost six time higher antioxidant activity. Bitter from pharmacy comprises 0,4 g of herbs in extract in 50 ml. Bitter 55 comprises 0,76 g of herbs in extract and around 7,5 ml juice in the same volume. Lower value of EC_{50} means higher antioxidant capacity (figure 5). Bitter 54 and Bitter 55 possess the highest antioxidant activity of investigated liquors and that can be assigned to high concentration of herbal extract (50 ml of Bitter 55 comprise 35 % v/v alcohol extract 0,76 g herbs) and high content of juice (12,5 %). Moreover his main function as drink, Bitter 55 can have therapeutic effect. Bitter 55 could be used as dietary supplements or even medicaments

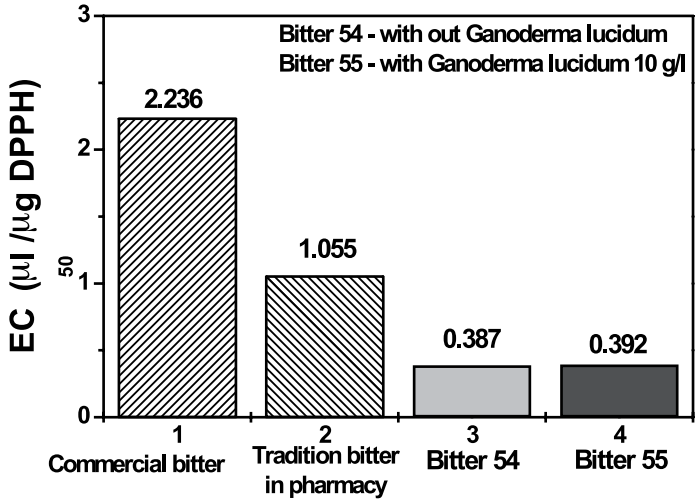

Fig. 5. – EC_{50} values (µl/µg DPPH) as concentration of the different bitters necessary to decrease DPPH radical concentration for 50 %

Dependence of percentage absorption inhibition of DPPH solution on 517 nm from time of storage (in days) of Bitter 54 and Bitter 55 in green bottle in dark, on ambient temperature is shown in figure 6. There is no change of antioxidant capacity under these conditions during 150 days of storage.

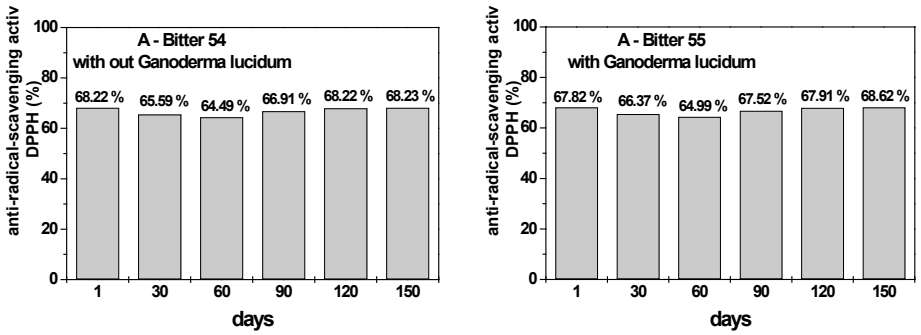

Fig. 6. – Change of anti-radical-scavenging activity (DPPH) during storage bitters in green bottles at the dark place and ambient temperature

Conclusion

During microfiltration bitter herbal liquor, the function of filtrate flux decreases with VCR. Dependence of decreasing flux with VCR can be separated in three periods. For the first, starting period, rapid decrease of filtrate flux is characteristic. Second period is defined with much smaller decrease of the flux than in the first phase. Third period has as characteristic minor decrease of flux and can be defined as steady state. Steady state emerges after τ_s = 80 min.

Comparing of antioxidant capacity of Bitter 55 with traditional herbal extract from pharmacy (EC_{50} = 1,055 µl/µg DPPH) and with commercial bitter liquor from supermarket (EC_{50} = 2,236 µl/µg DPPH) it can be concluded that Bitter 55 (EC_{50} = 0,387 µl/µg DPPH) possesses significantly higher antioxidant effect. The main reason for this fact arises from high concentration of herbal extract and fruit juice content. During storage of Bitter 55 in green bottle in dark during 150 days no change of antioxidant activity was observed.

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MIKROFILTRACIJA I ANTIOKSIDATIVNI KAPACITET KOMPOZICIJE EKSTRAKTA LEKOVITIH BILJAKA, VOĆA I MEDICINSKE GLJIVE *Ganoderma lucidum*

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R e z i m e

Sastojci lekovitog bilja i voća koji pokazuju potencijalnu antioksidativnu sposobnost su različite bioaktivne komponente, kao što su različiti fenoli, polifenoli, fenolne kiseline, flavonoidi, tanini i dr. Na tržištu se nalaze biljni ekstrakti od 12, 25 pa sve do 56 lekovitih i aromatičnih biljaka i voća. Dodatak medicinske gljive *Ganoderma lucidum*, biljnom ekstraktu od 54 lekovitih i aromatičnih biljaka, predstavlja dobro rešenje. Ekstrakcijom gljive Ganoderma (*Ganoderma lucidum)* zajedno sa lekovitim i aromatičnim biljem, zadržavaju se sve aktivne vrednosti biljaka, uz postizanje dodatnih lekovitih svojstava gljive. Ovakav proizvod, na bazi ekstrakata lekovitog i aromatičnog bilja, voća i gljive Ganoderma, je interesantan sa aspekta mogućih lekovitih svojstava. On može biti deklarisan ili kao gorki biljni liker u komercijalnoj prodaji ili čak kao dijetetski suplement za prodaju u apotekama. Takav proizvod u svakom slučaju predstavlja, kako zdravstveno tako i nutritivno, bolje rešenje od drugih alkoholnih pića.

U radu su prikazani načini ekstrakcije i mikrofiltracije 46 lekovitih i aromatičnih biljaka, gljive *Ganoderma lucidum* i 8 voćnih vrsta koji čine kompoziciju Bittera 55. Utvrdjeno je da gorki biljni liker – Bitter 55 sadrži značajan antioksidativni potencijal. Potencijalna antioksidativna aktivnost određivana je DPPH testom, metodom po Blois-u.[1] Vrednost EC_{50} uzorka BITTER-a 55 koji je stajao u zatamnjenoj boci u mraku je 139,47 µl/ml rastvarača i nije se promenila tokom 150 dana čuvanja. Sintetički antioksidansi BHT (ditercbutil-hidroksitoluen) i troloks (analog vitamina E

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rastvoran u vodi) imaju vrednosti EC_{50} redom 6,2 i 6,8 μg/ml. Vrednost EC_{50} predstavlja koncentraciju ekstrakta koja za 50% smanjuje koncentraciju DPPH radikala (praćeno preko inhibicije apsorpcije rastvora DPPH na 517nm).

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