



INFLUENCE OF STEEPING TIME ON BIOLOGICAL ACTIVITY OF BLACK MULBERRY LEAVES TEA

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Black mulberry leaves teas (BMLTs) were prepared using boiled water and different steeping time (5, 10, 20, 35 and 45 minutes). In order to establish the connection between steeping time and tea quality, total phenolics content (TPC), total flavonoids content (TFC) contents were measured, as well as two antioxidant assays (DPPH and reducing power assays), along with antimicrobial and cytotoxic tests. The obtained results showed that TPC, TFC, IC₅₀ and EC₅₀ values increased with the increase in steeping time, while antimicrobial and cytotoxic activity exhibited different tendency. Based on the obtained results, the 10-minute steeping time was the optimum for tea preparation and this tea was used for the determination of polyphenolic profile using HPLC-MS technique. The results showed that the main compounds in BMLT were chlorogenic and caffeic acids with the contents of 7226.00 and 537.52 µg/g, respectively.

KEY WORDS: black mulberry leaves, tea preparation, biological activity, steeping time

INTRODUCTION

Different medicinal plants have been used as a source of health benefit compounds for a long time. Awareness of the presence of such compounds in plants lead to increased popularity of teas all over the world (1). Herbal teas are very popular merchandise in the stores due to their fragrance, antioxidant properties and therapeutic applications, and can be found as pure or blended samples (2, 3). As a consequence of such popularity, hundreds of different herbal teas are sold in stores. Teas have been used for treatment of infections, ailments and diseases for centuries in many countries (4). These beverages may be prepared from almost every part of plant such as dry and fresh fruits, leaves, flowers, and sometimes even seeds and stems, and the process of preparation of teas is quite simple. They are prepared by pouring boiling water over the herbal material and allowing the steeping for a certain period of time, usually 5-15 minutes (5). The preparation technique relies on tradition and medical purpose. Teas are often prepared in different ways in different parts of the world. For example, teas may be infused several times, prepared

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using water at different temperature or with addition of milk, lemon, honey or sugar (6). Studies have shown that medicinal plants exhibit antioxidant (7-9) and antimicrobial (10) activities. Due to such activity of the plant itself, preparation and usage of appropriate herbal tea can be very useful in the management of various diseases, such as Type 2 diabetes (11), various cardiovascular conditions (12), obesity (13), and Alzheimer's disease (14). Presence of polyphenolic compounds in the herbal beverages and teas is very significant due to biological activity of this class of compounds. They are known as potent antioxidants (3, 15) and are able to reduce the deleterious effects of reactive oxygen species (ROS) (16). Besides antioxidant activity, polyphenolics exhibit wide range of biological activities, such as antibacterial, anti-carcinogenic, anti-inflammatory, anti-viral, anti-allergic, estrogenic and immune-stimulating effects (17). Polyphenolics exert the antioxidant activity as a result of their redox properties. They are able to act as reducing agents, hydrogen atom donor and singlet oxygen quenchers, exhibiting also chelating potential toward metal ions (15).

Black mulberry (*Morus nigra*) belongs to the family *Moraceae*. It has been used for medicinal purposes for several centuries. It is cultivated commercially in China, India, Japan, and South Europe. A special characteristic of mulberries is that almost all the parts of the plant are pharmacologically used (18). Mulberry tea and its constituents exhibit a broad array of biological activities such as cytotoxic effects on cancer cell lines (19-21), reduce risk of atherosclerosis (22-24), anti-inflammatory effect (25, 26), hypolipidemic effect (27), neuroprotective effect (28), hypoglycemic effects (29,30) and antioxidant effect (31-34). To our best knowledge, black mulberry is not significantly presented in the Serbian market, with the exception of its fruit, which is used as food. Tea made from the leaves of this plant is still not popular in Serbia regardless of the presence and availability of the plant material itself. For such reasons, the study was conducted in terms of determining the leaves chemical composition and biological activity, in order to examine the possibility of using black mulberry leaves in the tea industry.

As polyphenolic compounds showed to be beneficial for human health, the process of tea preparation should be governed in such a way to ensure the highest possible content of these compounds in teas. Previous studies demonstrated that there was a correlation between tea preparation conditions and the content of extracted compounds from plant materials such as polyphenolic compounds (6, 35). In order to establish the connection between the time of steeping and content of polyphenols and flavonoids and biological activity of black mulberry beverages, total phenolic content (TPC) and total and total flavonoids content (TFC) were determined, as well as the antioxidant, antimicrobial and cytotoxic activities. HPLC analysis was also performed to evaluate polyphenolic profile of the obtained teas.

EXPERIMENTAL

Chemical reagents

Folin-Ciocalteu reagent, trichloroacetic acid, 1,1-diphenyl-2-picrylhydrazyl hydrate (DPPH), chlorogenic acid, gallic acid, dihydrobenzoic acid, sinapic acid, catechin, epica-



techin, vanillic acid, caffeic acid, quercetin, ferulic acid, resveratrol, ellagic acid, *p*-coumaric acid, caftaric acid, and rutin were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Aluminum chloride hexahydrate, sodium carbonate and sodium acetate trihydrate were purchased from Merck (Darmstadt, Germany). Potassium ferricyanide and ferric chloride were obtained from Zorka (Šabac, Serbia). Cirsimarin, resazurin, amaricin, nystatin, sabourand dextrose, Tween 80 and *cis*-diamminedichloroplatinum (*cis*-DDP) were purchased from Tedia Company (USA). Acetonitrile and water were products of Fisher Chemical (LC-MS and HPLC grade). Formic acid was purchased from Carlo Erba (Italy). All other chemicals and reagents were of analytical reagent grade.

Plant material

Dried plant material of the commercially available *Morus nigra* L. leaves (Adonis D.O.O., Sokobanja) was used. The samples of mulberry leaves were dried naturally (in the shade, on draft) during one month and ground in a blender before the extraction. Mean particle size was determined using sieve sets (Erweka, Germany). The mean particle size of the plant samples was 1.26 ± 0.07 mm.

Preparation of teas

Leaves of black mulberry (2 g) were topped with boiled water (200 mL). After certain steeping time (5, 10, 20, 35 and 45 minutes), the beverages were filtrated. The obtained black mulberry leaf teas (BMLT) were used for further analysis.

Determination of TPC and TFC

TPC in teas was determined using the Folin-Ciocalteu method (36). The reaction mixture was prepared by mixing 0.1 mL of the solution (concentration 50 mg/mL) of desired BMLT, 7.9 mL of distilled water, 0.5 mL of the Folin-Ciocalteu's reagent and 1.5 mL of 20% sodium carbonate. After 1 h, the absorbance at 750 nm (VIS spectrophotometer, Janway 6300, Germany) was measured against the blank solution which was prepared in a similar manner, by replacing the extract with distilled water. The TPC, expressed as mg of chlorogenic acid equivalents per 200 mL of tea, was calculated using calibration curve of chlorogenic acid as standard.

The TFC in BMLTs were estimated according to previously described method (37). The reaction mixtures were prepared by mixing 5 mL of BMLTs, 1 mL of distilled water and 2.5 mL of AlCl_3 solution (26.6 mg $\text{AlCl}_3 \times 6\text{H}_2\text{O}$ and 80 mg CH_3COONa dissolved in 20 mL distilled water). A blank solution was prepared by replacing the BMLT with distilled water. The absorbance of probes and blank probe were measured immediately at 430 nm. The TFC, expressed as mg of rutin equivalents per 200 mL of tea, was calculated from a calibration curve using rutin as standard.



Antioxidant activity

Antioxidant activity of the BMLTs was assessed using two methods: DPPH and reducing power assays. The free radical scavenging activity of BMLT was determined according to the method described by Espin et al. (38). A certain volume of diluted BMLTs was mixed with 95% methanol and 90 μ M solution of 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical in order to obtain different final concentrations of teas. The blank probe was prepared by using proper extraction solvent instead of BMLT. After 60 min of incubation at room temperature, the absorbance was measured at 515 nm. The radical scavenging capacity (%RSC) was calculated using the Equation [1], where A_s is the absorbance of BMLT solution and A_b is the absorbance of the blank sample.

$$\%RSC=100-\left(\frac{A_s \times 100}{A_b}\right) \quad [1]$$

This activity was expressed as IC_{50} , which is the concentration of the solution tested required to obtain 50% of radical scavenging capacity.

Reducing power of the BMLTs was determined according to the assay based on the reduction of Fe^{3+} by polyphenol antioxidants (39). Different dilutions of BMLT (1 mL) were mixed with phosphate buffer (1 mL, 0.2 M, pH 6.6) and 1% potassium ferricyanide (1 mL) in glass tubes. Tubes were incubated at 50°C for 20 min. After incubation, 10% trichloroacetic acid solution (1 mL) was added to the reaction mixture. The tubes were then centrifuged at 3000 rpm for 10 min and the supernatant (2 mL) was further mixed with double distilled water (2 mL) and 0.1% $FeCl_3$ solution (0.4 mL). Absorbance was measured at 700 nm. The blank probe was prepared by using proper extraction solvent instead of BMLT. The reducing power was expressed as EC_{50} value (concentration in mg/mL), which causes reduction of 50% Fe^{3+} ions in the reaction mixture. The EC_{50} value was determined from the generated curve which represented the correlation between the BMLT concentration and measured absorbance. All experiments were performed in triplicate, and results were expressed as mean value.

Antimicrobial activity

Antimicrobial activity was determined according to the method described by Sarker et al. (40). The minimum inhibitory concentrations (MIC) of the BMLTs and cirsimarin against the test bacteria were determined by microdilution method in 96 multi-well microtiter plates. All tests were performed in Muller–Hinton broth (MHB), with the exception of the yeast, in which case Sabouraud dextrose broth was used. A volume of 100 μ L stock solutions of oil (in methanol, 200 μ L/mL) and cirsimarin (in 10% DMSO, 2 mg/mL) was pipetted into the first row of the plate. A volume of 50 μ L of Mueller Hinton or Sabouraud dextrose broth (supplemented with Tween 80 at a 12 final concentration of 0.5% (v/v) for analysis of oil) was added to the other wells. The same volume (50 μ L) from the first test well was pipetted into the second well of each microtiter line, and then 50 μ L of scalar dilution was transferred from the second to the twelfth well. 10 μ L of re-



sazurin indicator solution (prepared by dissolution of a 270-mg tablet in 40 mL of sterile distilled water) and 30 μ L of nutrient broth were added to each well. Finally, 10 μ L of bacterial suspension (106 CFU/mL) and fungi suspension (3×10^4 CFU/mL) was added to each well. For each strain, the growth conditions and the sterility of the medium were checked. Standard antibiotic amracin was used to control the sensitivity of the tested bacteria, whereas nystatin was used as control against the tested fungi. The plates were wrapped loosely with cling film to ensure that bacteria, prepared in triplicate, did not become dehydrated, and then they were placed in an incubator at 37°C for 24 h for the bacteria and at 28°C for 48 h for the yeast. Subsequently, the colour change was assessed visually. Any colour change from purple to pink or colourless was recorded as positive. The lowest concentration at which colour change occurred was taken as the MIC value (mg/mL) for the tested BMLTs and standard drug.

Cytotoxic activity

The obtained BMLTs were evaluated for their cytotoxic activity through their influence on the growth of malignantly transformed cell lines using the MTT assay. Malignant cell lines used in this assay were cell line derived from human rhabdomyosarcoma (RD cell line), cell line derived from human cervix carcinoma (Hep2c cell line), and cell line derived from murine fibroblast (L2OB cell line). Cells were seeded (104 cell/mL; 100 μ L/well) in 96-well cell culture plates (NUNC) in nutrient medium (MEM Eagle supplemented with 5% (for Hep2c) or 10% (for RD and L2OB), and grown at 37°C in humidified atmosphere for 24 h. Then, the corresponding probe (stock solution: 5 mg of BMLT dissolved in 1 mL of absolute ethanol) and control (absolute ethanol), diluted with nutrient medium to desired concentrations, were added (100 μ L/well) and cells were incubated at 37°C in humidified atmosphere for 48 h. Pure nutrient medium (100 μ L) represented positive control for each cell line. After the incubation period, the supernatants were discarded and 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyltetrazolium bromide (MTT) (dissolved in D-MEM (Dulbecco's modification of Eagle's medium) in concentration of 500 μ g/mL) was added to each well (100 μ L/well). After addition all wells were incubated at 37°C in humidified atmosphere for 4h. The reactions were halted by the addition of 100 μ L of sodium dodecyl sulphate (SDS) (10% in 10 mM HCl). After an overnight incubation at 37°C, absorbance was measured at 580 nm. The number of viable cells (NVC) per well was calculated from a standard curve, presenting the cell numbers against absorbance at 580 nm. The corresponding cells (grown in flasks), after cell count by hemocytometer, were used as standards. Standard suspensions were plated in serial dilution, centrifuged at 800 rpm for 10 min, and then treated with MTT/D-MEM and 10% SDS/10 mM HCl solutions in the same way as the experimental wells. The number of viable cells in each well was proportional to the intensity of the absorbed light, which was then read in an ELISA plate reader at 580 nm. Absorbance (A) at 580 nm was measured 24 h later. Cell survival (%) was calculated by dividing the absorbance of the sample with cells grown in the presence of various concentrations of the investigated extracts with control optical density (the A of control cells grown only in nutrient medium), and multiplying by 100. The blank absorbance was always subtracted from the absorbance of the corres-



ponding BMLT with target cells. The IC_{50} concentration was defined as the concentration of an agent inhibiting cell survival by 50%, compared with a vehicle-treated control. The results of the measurements were expressed as the percentage of positive control growth taking the *cis*-diamminedichloroplatinum (*cis*-DDP), determined in positive control wells as 100% growth (41, 42). All experiments were done in triplicate.

HPLC-DAD-ESI/MS analysis

Quantification of individual phenolic compounds was performed using the Perkin Elmer PE200 HPLC system which was composed of a binary pump, a column thermostat and an autosampler. The mass spectrometer used was 3200 QTRAP MS/MS with ESI ionization (Applied Biosystems/MDS Sciex, Foster City, USA). The experimental conditions were: mobile phase A: 50% acetonitrile, 50% acetic acid (0.5%); mobile phase B: 2% acetic acid; gradient elution: 0 min 30% A, 70% B; 10 min 30% A, 70% B; 30 min 100% A, 0% B; 35 min 100% A, 0% B; 40 min 30% A, 70% B for reconditioning of the system; flow rate: 0.7 mL/min; injection volume: 20 μ L; ionization: ESI negative; dwell time 50 ms; MRM transitions: gallic acid 169/125, dihydro-benzoic acid 153/109, sinapic acid 223/164, catechin and epicatechin 289/245, vanillic acid 167/123, caffeic acid 179/135, quercetin 301/151, chlorogenic acid 353/191, ferullic acid 193/134, resveratrol 227/185, ellagic acid 301/145, *p*-coumaric acid 163/119, caftaric acid 311/179. All solvents were HPLC grade and were filtered and degassed before their use.

RESULTS AND DISCUSSION

TPC and TFC

The TPC and TFC values were determined in BMLTs in order to establish the correlation between the steeping time and contents of these classes of compounds. The obtained results are presented in Figure 1 and Figure 2.

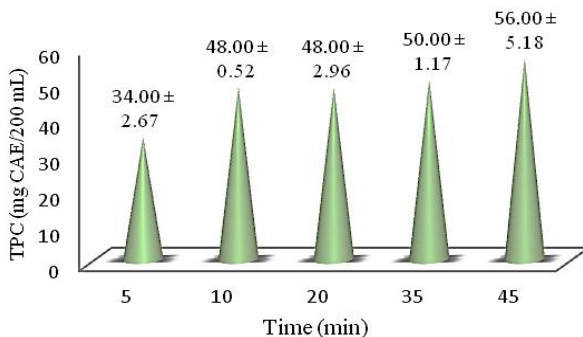


Figure 1. Dependence of TPC on steeping time



The presented results for TPC confirmed previous findings regarding the correlation between content of extracted compounds and the steeping time of plant material (6, 35). The histogram presented in Figure 1 clearly shows that TPC increased with the steeping time. It can also be noticed that the biggest difference in TPC was observed between the BMLTs obtained after 5 and 10 minutes.

The obtained results for TFC are presented in Figure 2. By taking a closer look at the result, it can be noticed that TFC followed the trend of TPC. The biggest difference is again seen between the BMLTs obtained after 5 and 10 minutes, while the increase in TFC after 10 minutes was negligible. Taking the TPC and TFC results into account, it might be concluded that 10 minutes would be optimal steeping time for preparing the tea with high content of biologically active compounds.

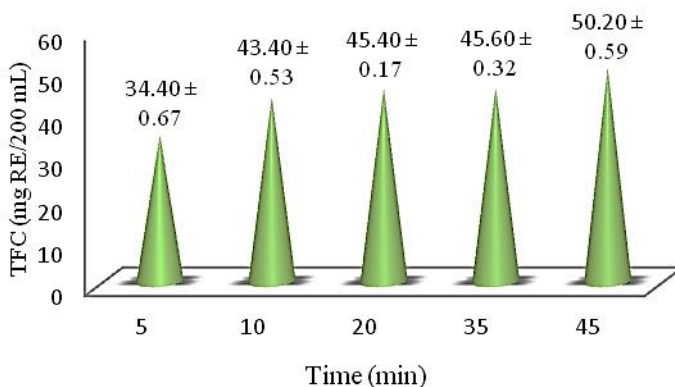


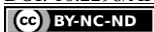
Figure 2. Dependence of TFC on steeping time

Antioxidant activity

The obtained results of antioxidant assays are presented in Table 1. The lower values of IC_{50} and EC_{50} indicate a higher activity. It can be noticed that the highest activity toward DPPH radical expressed BMLT obtained after 10 minutes, while BMLT obtained after 20 minutes showed slightly lower activity. The results of DPPH assay supported the previous conclusion that steeping time of 10 minutes should be sufficient for tea preparation. On the other hand, the highest reducing power exhibited the BMLT obtained after 20-minute steeping.

Table 1. Influence of steeping time on DPPH radical scavenging capacities and reducing power of BMLTs

Steeping time (min)	IC_{50} (mg/mL)	EC_{50} (mg/mL)
5	0.103 ± 0.005	2.668 ± 0.987
10	0.068 ± 0.015	2.406 ± 0.903
20	0.073 ± 0.002	2.205 ± 0.874



Vidović et al. (1) determined antioxidant activity of medicinal plant extracts using DPPH assays, and obtained IC₅₀ values in the range of 5.1-1943.7 mg/mL. By comparing these results with those presented in Table 1 it can be concluded that BMLT possessed a higher antioxidant activity. The presented results suggest that the EC₅₀ values exhibited similar tendency as the IC₅₀ values. Although the BMLT obtained after 20-minute steeping exhibited the strongest activity in this case, taking into account the results for TPC, TFC and IC₅₀, 10 minutes was taken as the optimum time.

The coefficients of correlation among the assays are presented in Table 2. It can be noticed that there is a very high correlation between TPC and TFC, TPC and IC₅₀ value, as well as between TFC and IC₅₀ values. The correlation between the IC₅₀ and EC₅₀ values is moderate, while the correlation among TFC and EC₅₀ values, as well as among TPC and EC₅₀ values, are rather weak.

Table 2. Pearson's correlation coefficients among TPC, TFC, IC₅₀ and EC₅₀

<i>r</i>	TPC	TFC	IC ₅₀	EC ₅₀
EC ₅₀	0.4532	0.2654	-0.6041	1
IC ₅₀	-0.9842	-0.9287	1	
TFC	0.9797	1		
TPC	1			

The negative correlation between TPC, TFC and IC₅₀ values indicates that the scavenging capacity of BMLTs toward DPPH radical increased with the increase in TPC and TFC. On the other hand, the negative correlation between IC₅₀ and EC₅₀ values indicates that these two tests are probably relying on different mechanisms, and that different compounds presented in obtained beverages exhibited different and specific activity under those assays. The difference of the presented correlation coefficients indicated the inefficiency of single assay to evaluate antioxidant activity of samples (43, 44), confirming the previous conclusion regarding the diversity and specificity in activity of different extracted compounds (45).

Antimicrobial and cytotoxic activities

The results of the measurement of the conducted antimicrobial activity, presented in Table 3 showed that BMLTs exhibited certain activity against all tested microbial stains. The highest activity regarding bacterial strains exhibited BMLT obtained after 20-minute steeping against *Escherichia coli* (19 µg/mL). It can be noticed that the activity against *Escherichia coli*, *Proteus vulgaris*, *Proteus mirabilis* and *Bacillus subtilis* increased with steeping time, while it was opposite in the case of the activities toward *Staphylococcus aureus* and *Klebsiella pneumoniae*. On the other hand, there is a difference in the activities against fungi. In the case of *Candida albicans*, the highest activity exhibited BMLT obtained after 10-minute steeping, while *Aspergillus niger* exhibited the highest sensitivity toward the BMLT obtained after 5 and 20 minutes of steeping.



Table 3. Antimicrobial activities of BMLTs

Microbial strains	MIC (µg/mL)				
	Steeping time			Standard	
	5	10	20	Amracin	Nystatin
<i>Staphylococcus aureus</i> ATCC 25923	39.100	78.125	156.250	0.970	/
<i>Klebsiella pneumoniae</i> ATCC 13883	39.100	39.100	156.250	0.490	/
<i>Escherichia coli</i> ATCC 25922	156.250	78.125	19.530	0.970	/
<i>Proteus vulgaris</i> ATCC 13315	312.500	156.250	78.125	0.490	/
<i>Proteus mirabilis</i> ATCC 14153	156.250	156.250	78.125	0.490	/
<i>Bacillus subtilis</i> ATCC 6633	156.250	78.125	39.100	0.240	/
<i>Candida albicans</i> ATCC 10231	78.125	19.530	39.100	/	1.950
<i>Aspergillus niger</i> ATCC 16404	19.530	78.125	19.530	/	0.970

The cytotoxic activities of BMLTs are presented in Table 4. In the case of Hep2 and RD cell lines, the highest activity exhibited the BMLT obtained after 5-minute steeping, while in the case of L2OB cell line the most potent BMLT was the one obtained after 5-minute of steeping. According to the American National Cancer Institute, the criterion of cytotoxic activity for plant extracts was $IC_{50} < 30 \mu\text{g/mL}$ (46). By taking a look at the presented results (Table 4), it can be noticed that the BMLT obtained after 5-minute steeping meets that requirement for all three cell lines, as well as the BMLT obtained after 10-minute steeping for Hep2 and RD cell lines.

Table 4. Cytotoxic activities of BMLTs

Steeping time	IC ₅₀ (µg/mL)		
	Hep2 cells	RD cells	L2OB cells
5	8.24 ± 0.85	21.25 ± 0.91	16.56 ± 1.05
10	22.25 ± 1.17	30.07 ± 0.63	101.25 ± 0.14
20	33.85 ± 0.64	51.36 ± 0.98	76.98 ± 0.25
(<i>cis</i> -DDP)	0.94 ± 0.55	1.40 ± 0.97	0.72 ± 0.64



Polyphenolic profile of BMLTs

Taking into account that the 10-minute steeping time appeared to be the optimal for BMLT preparation, the polyphenolic profile for that BMLT was obtained using HPLC-MS technique. The results of the performed analysis are presented in Table 5, and the corresponding chromatogram is shown in Figure 3.

Table 5. Polyphenolic profile of BMLT after 10-minute steeping

Compound	Content (µg/g)
Gallic acid	3.62
Sinapic acid	2.03
Vanillic acid	1.48
Caffeic acid	537.52
Chlorogenic acid	7226.00
Ferulic acid	4.87
<i>p</i> -Coumaric acid	11.87
Total	7787.39

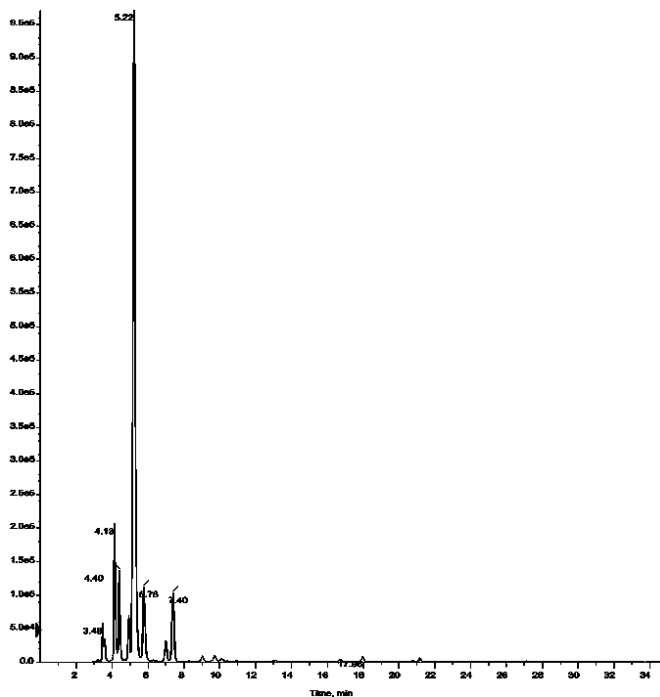


Figure 3. HPLC-DAD chromatogram of BMLT obtained after 10 minutes of steeping



The most abundant compounds in BMLTs were chlorogenic and caffeic acids. This result is in correlation with previously obtained ones, where the presence of these two compounds was found in high amounts (33, 47). Memon et al. (33) found that chlorogenic acid was the main polyphenolic compound in black mulberry leaves extract, while Sánchez-Salcedo et al. (47) also detected high contents of this compound.

It is well known that polyphenolic compounds exhibit wide range of biological activities such as antioxidant, antibacterial, anti-carcinogenic, anti-inflammatory, anti-viral, anti-allergic, estrogenic and immune-stimulating effects (3, 15-17). Furthermore, several studies also showed that the presence of polyphenolic compounds is very important because these compounds delay aging and regulate fat metabolism in *Caenorhabditis elegans*, which is used as a model (48) and lower hepatic lipid accumulation (49).

CONCLUSION

The awareness of the presence of biologically active compounds and the significance of tea consuming is a rising phenomenon. A well-known contribution of tea consuming to the health should provide the mandatory usage of this beverage as a dietary supplement. This study showed that black mulberry leaves possess numerous health-beneficial compounds which may be used in the everyday diet as a supplement. The investigation of the correlation between the steeping time and the biological activity of BMLTs revealed that the contents of health-beneficial compounds in tea increase with the steeping time, as well as the antioxidant activity. On the other hand, the influence of steeping time exhibited different effect on the antimicrobial and cytotoxic activities of BMLTs. This implies that during the tea preparation degradation and/or other transformations of the biologically active compounds occur due to prolonged exposure to the high temperature. Due to possible degradations, it is important to determine optimal steeping time to prevent or reduce the influence of this process on the tea quality. Taking all results into account, 10-minute steeping time should be optimal for tea preparation.

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УТИЦАЈ ВРЕМЕНА ПРИПРЕМЕ НА БИОЛОШКУ АКТИВНОСТ ЧАЈА ЦРНОГ ДУДА

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Узорци чајева листа црног дуда припремљени су преливањем листа биљке кључалом водом и стајањем током одређеног временског периода (5, 10, 20, 35 и 45 минута). Овако припремљени узорци чајева употребљени су у даљим истраживањима како би се утврдио утицај времена екстракција на биолошку активност узорака. Сама биолошка активност је одређена применом DPPH теста, теста одређивања редукционе моћи и одређивањем антимикуробне и цитотоксичне активности. Такође, спектрофотометријским методама одређени су и укупни садржаји полифенола (TPC) и флавоноида (TFC) у добијеним узорцима. На основу добијених резултата, утврђено је да је 10 минута оптимално време припреме чаја, при чему је тај узорак употребљен за одређивање шполифенолног профила чаја дуда применом HPLC-MS технике. Резултати HPLC анализе показали су да су хлорогенска и кафена киселина најдоминантнија једињења у узорку чаја са садржајима од 7226,00 и 537,52 µg/g, редом.

Кључне речи: лист црног дуда, чај, биолошка активност, време припреме

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