e-ISSN: 2687-6698 **Research Article**

Turkish Journal of Analytical Chemistry
www.turkjac.org
2019
TurkJAC

Comparison of different extraction methods for total phenolic content and antioxidant activity of dried *Diospyros lotus* L fruits

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Abstract

The yield, total phenolic content, and antioxidant activity values of the extracts obtained from the dried fruits of the Diospyros lotus L. plant by using Soxhlet (SXE), ultrasound-assisted extraction (UBE), and hot solvent extraction (HME) techniques with methanol were investigated. The highest extraction yield was obtained from HME experiments with 50.67 ± 0.63% and UBE with 49.50 ± 1.05%, respectively. While the extract obtained by the UBE technique showed a lower TPC value (1464 ± 57 mg GAE/100 g original sample) compared to the extracts obtained from the other two techniques, it showed higher antioxidant activity values than that of the HME technique. While these values were determined as 192.53 ± 4.45 and 273.10 ± 34.79 mg/mL (SC_{50}, lower is better) for the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity assay, 0.65 ± 0.04 and 0.25 ± 0.04 g TEAC/100 g air-dried sample for cupric ion reducing antioxidant activity (CUPRAC) assay, respectively. Both the UBE and HME techniques have clearly demonstrated that they are more advantageous than conventional Soxhlet extraction for simplicity of application, reduced solvent consumption, extraction of thermally sensitive compounds, and shortened extraction times.

Keywords: *Diospyros lotus* L., total phenolic content, antioxidant activity, Soxhlet extraction, ultrasound-assisted extraction, hot methanol extraction

1. Introduction

In recent years, there has been an increasing number of studies on the extraction of antioxidant compounds from natural origins, isolation of active ingredients, or the use of these extracts directly instead of synthetic antioxidants in order to extend the shelf life of foodstuffs. The driving force behind this situation is the research results that synthetic antioxidants may have some health-related drawbacks [1].

Compounds with antioxidant effects are usually found in low concentrations in their natural sources, so the number of antioxidants supplied with the amount of food taken in normal dietary meals is not able to reach the desired levels. Numerous studies have been brought to the literature by various research groups in order to eliminate this deficiency and to increase the concentration of antioxidant compounds by extracting them from natural products by various methods, to calculate the amount of the original product required for the intake of sufficient antioxidants, and to determine the optimum conditions for extraction. One of the pillars of the studies carried out in this direction is the development and use of new and more effective extraction techniques. Some of the new techniques used alongside the traditional Soxhlet technique (which is considered as a reference technique) in bioactive compound extraction from natural products are (1) Supercritical Extraction (SCE), (2) Ultrasound-Assisted Extraction (UAE), (3) Hot Solvent Extraction (HSE) and Microwave-Assisted Extraction (MAE) [2-6].

Locally, dried fruits of *D. lotus* L are consumed directly (especially in Artvin-Yusufeli) or used to sweeten tea in Eastern provinces (Erzurum, Kars). Jam is also made from ripe fruits in these regions. It is known to have a constipation effect [7]. The fruits of the plant have been investigated by different research groups in terms of antioxidant [8-10], anticancer properties [8], phenolic content [10], fatty acid composition [11]. However, none of these studies is a comparative study

Citation: F. Akdeniz, İ. Dursun, K. Tepebaş, H. Özbay, L. Kekeç, S. Yıldız, Comparison of different extraction methods for total phenolic content and antioxidant activity of dried *Diospyros lotus* L fruits, Turk J Anal Chem, 3(2), 2021, 64-69.
https://doi.org/10.51435/turkjac.1032908

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Received: December 06, 2021
Accepted: December 26, 2021

based on extraction techniques. Almost all of them were carried out by using traditional percolation and/or refluxing techniques, and no studies were found with new extraction techniques. Thus, the aim of the study is to compare the effects of three different extraction techniques (Soxhlet, ultrasound-assisted and hot solvent extractions) applied to dried and ground fruits of the *D. lotus* L. plant using methanol solvent on the total phenolic content and antioxidant activities of the extracts.

2. Materials and methods

2.1. Material and sample preparation

D. lotus (Black Date Persimmon) is generally grown in the North and North-East regions of Turkey for its edible fruit. The plant is a 10-15 m high tree with simple leaves, reddish or greenish-white flowers, and deciduous in winter. Its fruit is up to 15 mm in diameter, yellowish or bluish-black in color, and it is a spherical-shaped drupe (Fig. 1).

Figure 1. Air-dried fruits of *D. lotus* L (from Dr. F. Akdeniz archive)

Plant material was obtained as dried fruit from commercial sources of Trabzon city in Turkey for this study. Dried fruits were pitted, ground in an IKA A11 basic model laboratory mill, and stored in colored storage bottles at 4°C.

2.2. Extraction procedures

In this study, two techniques that have recently attracted attention in the extraction of antioxidant active substances, Ultrasound-Assisted Extraction (UAE) (an ultrasonic bath was used in the study, thus, it is referred to as UBE) and Hot Solvent Extraction (HSE) techniques were used and the results were compared to those of obtained in the traditional Soxhlet Extraction (SXE) technique. Methanol, which is known to be effective in dissolving phenolic antioxidant compounds, was used as the extraction solvent. Total phenolic contents (TPCs) of the extracts were determined according to Folin Ciocalteu's method [12]. Antioxidant activities were determined according to the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method [13] and the Cupric Ion Reducing Antioxidant Capacity (CUPRAC) determination method which is a relatively new method developed by Apak et al. [14].

For Soxhlet extraction experiments, approximately 5 g of the air-dried matter was weighed with 0.1 mg precision and placed in the extraction cartridge, then it was extracted for 6 hours (approximately 36 siphons) using 150-200 mL of HPLC grade methanol (Sigma-Aldrich, Germany). In ultrasonic bath extractions (UBE), 5 g of substance was weighed with 0.1 mg precision and placed in a conical flask and 100 mL of solvent (methanol) was added each time. After they were mixed thoroughly, it was placed in a Bandelin Sonorex (Germany) model ultrasonic bath and extracted for 20, 10, and 5 min (in total 35 min), respectively, at a frequency of 35 kHz. In hot methanol extractions (HME), 0.5 g sample weighed with 0.1 mg sensitivity was placed in an autoclave with a volume of 75 ± 1 mL made of 316 stainless steel (autoclave described in detail elsewhere [15]. 15 mL of solvent was added to it and extracted at 100 ± 3°C for an hour. All experiments were carried out in triplicate.

The solvent of the extracts obtained was removed in a rotary evaporator, and the extract yields were calculated in percentage. Later, stock solutions were obtained by dissolving all extracts in methanol again and these solutions were kept at + 4°C in a refrigerator for total phenolic substance and antioxidant activity determination experiments.

2.3. Total phenolic content (TPC) and antioxidant activity assays

TPCs of the extracts were determined according to Slinkard and Singleton's method [12] with slight modifications applying the Folin-Ciocalteu reagent. For this purpose, 2.5 mL of distilled water was added to 50 µL of the solution with a concentration of 1 mg/mL prepared by diluting with the solvent used from the stock solutions. From 0.2 N solution prepared from original purchased Folin-Ciocalteau solution of 2 N by diluting at a volume ratio of 1:10, 250 µL was added to it, vortexed, and kept at room temperature for 3 minutes. An aliquot of 750 µL of a 7.5% (w/w) Na_2CO_3 solution prepared by dissolving 7.5 g of Na_2CO_3 in 92.5 mL of water was added. The vortexed mixture was incubated at room temperature for 2 hours and absorbance values were measured at 765 nm using a Thermospectronic Heliosα brand UV-Visible spectrophotometer. The experiments were repeated in triplicate and distilled water was used as blank. Being the blank absorbance A1 and the average absorbance of the three parallels A2,

absorbance differences (ΔA) were calculated from the following Equation 1.

$$\Delta A = A2\text{-}A1 \qquad (1)$$

The same procedure was repeated using seven standard gallic acid solutions with concentrations of 15.63, 31.25, 62.5, 125, 250, 500, and 1000 µg/mL. The absorbance differences were plotted against concentration to obtain a calibration graph. The total phenolic content of the extracts was given as mg gallic acid equivalent (GAE)/100 g air-dried sample.

The extracts were examined for DPPH radical scavenging antioxidant activities using the method of Cuendet et al. [13], with slight modifications. For this purpose, extracts with concentrations of 15.63, 31.25, 62.5, 125, 250, 500 and 1000 µg/mL prepared from stock solutions were used (in some cases, samples with concentrations of 7.81 and 3.9 µg/mL were also added to the procedure). Aliquots of 750 µL of each of these extracts were mixed with 750 µL of the 1.10^{-4} M stable solution of the DPPH radical prepared in methanol, shaken vigorously in a vortex, and then incubated at room temperature for 50 minutes. Absorbances at 517 nm were measured using a Thermospectronic Heliosα brand UV-Vis spectrophotometer. Each sample was tested in duplicate and a tube containing only the sample solution and extract solvent of each concentration was used as a blank. Control tubes were prepared in triplicate and only DPPH· solution and its solvent (methanol) were placed in these tubes. After incubating for 50 minutes, their absorbances were measured at the same wavelength. The experiments were repeated using BHT as the reference compound. The means of the blanks were subtracted from the means of the absorbance values obtained. From these values, % scavenging (% I) values were calculated using Equation 2.

$$\%\ of\ DPPH\cdot\ Radical\ Scavenging\ Activity\ (I\%) = \frac{A_0 - A_1}{A_0} x100 \qquad (2)$$

where A_0 is the mean of the absorbance values of the control solutions (containing only DPPH· solutions), A_1 is the mean of absorbance values of sample tubes (containing extract and DPPH· solutions). The scavenging percentages were plotted against the concentrations of extract and reference compounds used. From these graphs, the concentration values (SC_{50}) of the extract and reference solutions, which reduced the I% values of the control tubes by half (50%), were calculated. Results are given in µg/mL.

The antioxidant activities of the extracts were also determined according to the cupric ion reducing antioxidant capacity (CUPRAC) method. For this purpose, the method of Apak et al. [14] was applied. After taking 1 mL of Cu-II solution, 1 mL of Neocuproin solution and 1 mL of NH_4Ac buffer solution (pH = 7) and mixing with X mL of antioxidant (or standard) solution, the total volume of the sample was adjusted to 4.1 mL by adding (1,1-X) ml of distilled water. After 1 hour of incubation, absorbance values were measured against the reagent blank at 450 nm using the same spectrophotometer mentioned above. The same procedures were repeated by preparing standard Trolox solutions with concentrations of 15.63, 31.25, 62.5, 125, 250, 500 µg/mL. From the values obtained, a calibration graph was prepared and CUPRAC values of the original extracts were calculated and given as g Trolox equivalent antioxidant capacity per 100 g of air-dried sample (g TEAC/100 g air-dried sample).

2.4. Statistical analysis

All experiments were performed in triplicate and the results are given as mean ± standard deviation (SD). Differences between the means were determined using statistical tests such as Kruskal Wallis, one-way analysis of variance (ANOVA). Tukey's Honestly Significant Differences (HSD) Post-Hoc test was used at a $p \leq 0.05$ significance level to explain the differences between the mean values. All statistical processes were performed using IBM SPSS Statistics for Windows (v. 20.0, IBM Corp., Armonk, NY, USA).

3. Results and discussions

3.1. Results of extraction yields

The yield values obtained from all three extraction techniques are given in Fig. 2 below in a comparative manner.

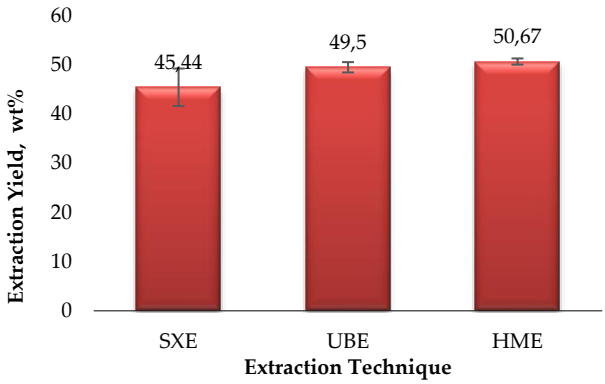

Figure 2. Extraction yield percentages obtained from the extraction techniques used (SXE: Soxhlet extraction, UBE: Ultrasonic bath extraction, HME: Hot methanol extraction)

As shown in Fig. 2, the highest extraction yield was obtained from HME. These values are 45.44 ± 3.81% for Soxhlet extraction, 49.50 ± 1.05% for ultrasound bath

extraction, and 50.67 ± 0.63% for hot methanol extraction, respectively. The Kruskal Wallis test, which is a nonparametric statistical test, was used instead of parametric one-way ANOVA since the data did not have in-group homogeneity ($p < 0.05$). Statistical treatment revealed that there were statistically significant differences between the extraction yields in terms of extraction techniques ($p < 0.05$). The yield of Soxhlet extractions was significantly lower than those of UBE and HME. The lower yield of the Soxhlet extraction compared to the other two techniques is thought to be due to the mass transfer problems between the solvent and the solid. Unless there is an external cause showing resistance, it is thought that the limiting step in the extraction of phenolic compounds from food samples is the diffusivities of the solutes from the solid to the solution [16]. Petrović et al. [17] showed that temperature and ultrasound positively affect mass transfer rate in slow extraction processes. Thus, considering the structure of the sample, it can be said that the transition of solutes from the matrix to the solution is more problematic in the Soxhlet technique compared to the other two techniques. Because the conditions in both UBE and HME techniques are harsher than in the SXE technique.

3.2. Results of TPC assay

The results obtained from the experiments performed using Folin Ciocalteu's Method are given in Fig. 3.

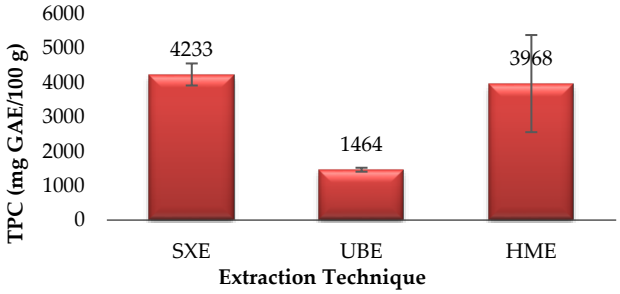

Figure 3. Total phenolic contents of the extracts (SXE: Soxhlet extraction, UBE: Ultrasonic bath extraction, HME: Hot methanol extraction)

The highest phenolic content in the experiments was obtained from SXE (4233 ± 321 mg GAE/100 g original sample) and HME (3968 ± 1412 mg GAE/100 g original sample) extracts, respectively ($p > 0.05$). The lowest TPC value was obtained in ultrasound-assisted extraction (1464 ± 57 mg GAE/100 g original sample). Of these three techniques, ultrasound-assisted extraction yielded significantly lower results than those of the other two techniques ($p < 0.05$). It is thought that this may be due to the fact that most of the compound groups that are extracted into the solution medium in ultrasound-assisted extraction do not have phenolic character. In the literature, many studies can be found showing that UBE is more advantageous than traditional extraction techniques in the extraction of phenolic compounds [18]. However, it should be noted that due to the complex nature of the sample structure and the diverse effects of phenolic compounds, there is no single and standard extraction method that can be applied to all herbal sample types at any time to extract their phenolic contents [19]. In addition, many variables such as device type (bath/probe), application power, frequency, temperature, solvent-solvent ratio, time, sample pretreatment should be considered, which affect the ultrasonic extraction process [20].

3.3. Results of antioxidant activity assays

3.3.1. Results of DPPH radical scavenging activity assay

Comparative SC_{50} values (mg/mL) obtained from DPPH radical scavenging antioxidant activity experiments are given in Fig. 4.

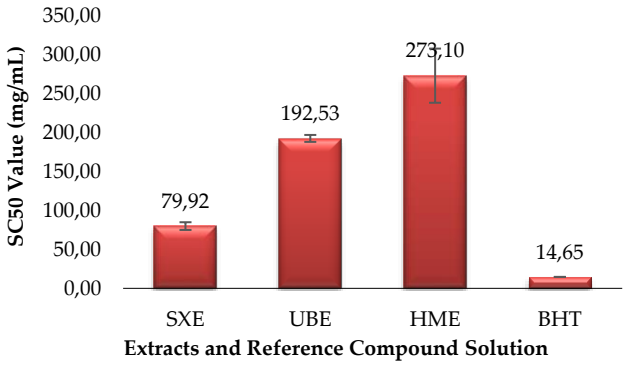

Figure 4. SC_{50} values of the extracts (SXE: Soxhlet extraction, UBE: Ultrasonic bath extraction, HME: Hot methanol extraction, BHT: Butylated hydroxytoluene) (Low value is better).

There were significant differences between the groups ($p < 0.05$) according to the nonparametric Kruskal-Wallis test performed because there was not any in-group homogeneity. In these experiments, the highest antioxidant activity was observed in Soxhlet extracts. The lowest values were obtained from hot methanol extracts. Values of ultrasonic bath extracts were between these two values (79.92 ± 4.96; 273.10 ± 34.79 and 192.53 ± 4.45 mg/mL, respectively). However, compared with BHT, all three extract types exhibited weaker antioxidant effects. For BHT, this value was obtained as 14.65 ± 0.08 mg/mL It is noteworthy that the antioxidant activity values of ultrasound-assisted extracts with lower phenolic content were higher than the antioxidant activity values of hot methanol extracts. This can be explained by the fact that in addition to the phenolic compounds, some other compound groups, which also have an antioxidant effect, pass into the solution in ultrasound-assisted extraction, or that some of the phenolic compounds that pass into the solution in hot methanol extraction do not show high antioxidant properties. Moreover, this reagent is not specific for

phenolic compounds, as it can be reduced with many non-phenolic compounds [21]. On the other hand, it can be said that some antioxidant compounds exposed to extreme conditions (high temperature and pressure) in the hot methanol extraction technique lose their antioxidant properties by breaking down or interacting with radical species formed under these conditions [22]. The DPPH radical scavenging antioxidant activity value measured in Soxhlet extracts of this plant is in agreement with the data in the literature [8].

3.3.2. Results of cupric ion reducing antioxidant capacity (CUPRAC) assay

The results obtained from the cupric ion reducing antioxidant capacity (CUPRAC) experiments are given in Fig. 5.

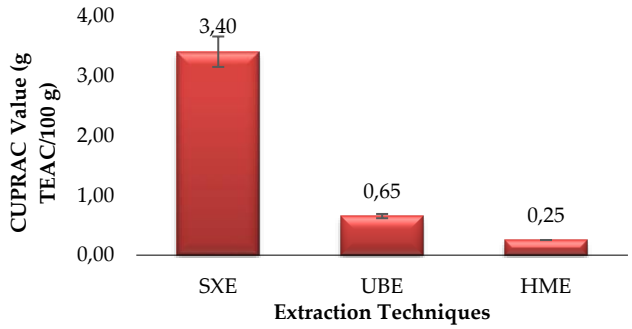

Figure 5. CUPRAC values of the extracts (SXE: Soxhlet extraction, UBE: Ultrasonic bath extraction, HME: Hot methanol extraction)

Although the non-parametric Kruskal-Wallis test showed that there was no significant difference between the CUPRAC values of the extracts obtained by three different methods ($p > 0.05$), it can be said that the Soxhlet technique revealed the best cupric ion reducing antioxidant capacity value (3.40 ± 0.25 g TEAC/100 g air-dried sample) according to the Mean Rank values. This value was followed by UBE (0.65 ± 0.04 g TEAC/100 g air-dried sample) and HME (0.25 ± 0.00 g TEAC/100 g air-dried sample), respectively. It can be easily seen that these results are in parallel with the results of the DPPH radical scavenging antioxidant activity method. Therefore, the comments made for the DPPH· method are also valid for this method.

In the literature, there are studies showing that high-pressure extraction processes are more successful than Soxhlet and ultrasound-assisted extraction processes in obtaining extracts with high total phenolic content [23, 24]. The results obtained in the present study also confirm these data. When it comes to the antioxidant properties of the extracts obtained, however, it is seen that different results are obtained. In one study, it was stated that the extracts obtained by high-pressure extraction showed higher antioxidant activity than the extracts obtained by ultrasound-assisted extraction technique [23]. However, in another study, it was reported that there was no difference between this technique and the other two techniques (Soxhlet and ultrasound-assisted extraction) compared with this technique [24]. A detailed literature search will reveal that quite different results are obtained for the extractions of similar compounds. It can be said that this variability is mainly related to the sample matrix [19], but besides this, the effect of some other variables such as extraction time, temperature, solvent type, and ratio, applied power should be considered [20].

It is an interesting research topic how the amount of phenolic content in a plant matrix affects the antioxidant activity of the extract obtained from that plant. While some researchers state that there is a strong linear correlation between them [25], others state that there is no such linear correlation or there is a more complex relationship than stated [24, 26]. Our study also revealed results that support this second group, that is, a positive linear correlation could not be established between TPCs and antioxidant activity values (both DPPH and CUPRAC).

4. Conclusions

In this study, dried fruits of the *D. Lotus* L. plant were extracted using three different extraction techniques such as Soxhlet, ultrasonic bath, and hot solvent (methanol) extraction, and their extraction yields and antioxidant activities were determined using two different methods (DPPH· and CUPRAC). Although the HME technique showed the highest extraction yield, it showed the same value as Soxhlet in terms of total phenolic content. UBE with the second-highest yield value revealed the lowest TPC value. In these respects, the HME technique seems to be competitive with the Soxhlet technique as it reaches a better extraction yield and the same TPC values with much less solvent consumption in a shorter time.

In terms of antioxidant activity values, the HME technique, which had the highest extraction efficiency and total phenolic content, revealed the lowest values. While the Soxhlet extraction technique showed the highest antioxidant activity values, interestingly the extract obtained from the UBE technique, which had the lowest TPC value, exhibited a higher antioxidant activity value than the extract obtained from the HME technique. This was attributed to the fact that there may be other components that can show antioxidant activity in the extract obtained from the UBE technique, apart from the phenolic components, or that the phenolic components in the extract obtained from the HME technique may be chemical species that do not have high antioxidant activity. However, more detailed analyzes are needed to

fully understand the reason for this phenomenon. Especially in UBE and HME techniques, it should be examined how the antioxidant activity values change depending on TPC values with the changing temperature and extraction times. However, it can still be said that the UBE technique has an advantage compared to the other two techniques due to its simplicity of application and the extraction of thermally sensitive compounds.

As a result, it can be said that the HME technique stands out with its advantages such as low solvent consumption and shorter extraction time in obtaining a phenolic-rich extract from the dried fruits of the *D. lotus* L plant, and the UBE technique is advantageous in terms of ease of application, shortened extraction time and protection of thermally sensitive compounds.

Acknowledgments

The authors gratefully thank Dr. Mustafa Kemal Altunoğlu for identifying the plant specimen and Prof. Dr. İsmail Çakmak for providing the ultrasonic bath system.

References

[1] D. Thompson, P. Moldéus, Cytotoxicity of butylated hydroxyanisole and butylated hydroxytoluene in isolated rat hepatocytes, Biochem Pharmacol, 37, 1988, 2201-2207.

[2] J.M. Luque-Rodriquez, M.D. Luque De Castro, P. Perez-Juan, Extraction of fatty acids from grape seed by superheated hexane, Talanta, 68(1), 2005, 126-30.

[3] R.P.F.F. da Silva, T.A.P. Rocha-Santosa, A.C. Duarte, Supercritical fluid extraction of bioactive compounds, TrAc-Trend Anal Chem, 76, 2016, 40-51.

[4] K. Kumar, S. Srivastav, V.S. Sharanagat, Ultrasound assisted extraction (UAE) of bioactive compounds from fruit and vegetable processing by-products: A review, Ultrason Sonochem, 70, 2020, 105325.

[5] A.P. Da Fonseca Machado, J.L. Pasquel-Reátegui, G.F. Barbero, J. Martínez, Pressurized liquid extraction of bioactive compounds from blackberry (*Rubus fruticosus* L.) residues: a comparison with conventional methods, Food Res Int, 77(3), 2015, 675-83.

[6] S. B. Bagade, M. Patil, Recent Advances in Microwave Assisted Extraction of Bioactive Compounds from Complex Herbal Samples: A Review, Crit Rev Anal Chem, 51(2), 2021, 138-49.

[7] T. Baytop, Türkiye'de Bitkiler ile Tedavi: Geçmişte ve Bugün, 1999, Turkey, Nobel Tıp Kitabevleri.

[8] M.R. Loizzo, A. Said, R. Tundis, U.W. Hawas, K. Rashed, F. Menichini, N.G. Frega, F. Menichini, Antioxidant and antiproliferative activity of *Diospyros lotus* L. extract and isolated compounds, Plant Food Hum Nutr, 64(4), 2009, 264-270.

[9] S.M. Nabavi, M.A. Ebrahimzadeh, S.F. Nabavi, M. Fazelian, B. Eslami, In vitro Antioxidant and Free Radical Scavenging Activity of Diospyros lotus and Pyrus boissieriana growing in Iran, Pharmacogn Mag, 5(18), 2009, 122-126.

[10] H. Gao, N. Cheng, J. Zhou, B. Wang, J. Deng, W. Cao, Antioxidant activities and phenolic compounds of date plum persimmon (Diospyros lotus L.) fruits, J Food Sci Technol, 51(5), 2014, 950-956.

[11] F.A. Ayaz, A. Kadioglu, Fatty acid compositional changes in developing persimmon (*Diospyros lotus* L.) fruit, New Zeal J Crop Hort, 1999, 27(3), 257-261.

[12] K. Slinkard, V.L. Singleton, Total phenol analysis: automation and comparison with manual methods, Am J Enol Viticult, 1977, 28, 49-55.

[13] M. Cuendet, K. Hostettmann, O. Potterat, W. Dyatmiko, Iridoid glucosides with free radical scavenging properties from fagraea blumei, Helv Chim Acta, 1997, 80(4), 1144-1152.

[14] R. Apak, K. Güçlü, M. Özyürek, S.E. Karademir, Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method, J Agric Food Chem, 2004, 52, 7970-7981.

[15] F. Akdeniz, M. Gündoğdu, Direct and alkali medium liquefaction of *Laurocerasus officinalis* Roem, Energ Convers Manage, 2007, 48, 189-192.

[16] D.D. Gertenbach, Solid–Liquid Extraction Technologies for Manufacturing Nutraceuticals from Botanicals, In: Functional Foods: Biochemical and Processing Aspects, Editors: J. Shi, G. Mazza, M. Le Maguer, 2001, USA, CRC Press Inc.

[17] S.S. Petrović, J. Ivanović, S. Milovanović, I. Žižović, Comparative analyses of the diffusion coefficient from thyme for different extraction processes, J Serb Chem Soc, 2012, 77(6), 799–813.

[18] A. Dobrinčić, M. Repajić, I.E. Garofulić, L. Tuđen, V. Dragović-Uzelac and B. Levaj, Comparison of different extraction methods for the recovery of olive leaves polyphenols, Processes, 2020, 8(9), 1008.

[19] J.O. Chaves, M.C. de Souza, L.C. da Silva, D. Lachos-Perez, P.C. Torres-Mayanga, A.P.F. Machado, T. Forster-Carneiro, M. Vázquez-Espinosa, A.V. González-de-Peredo, G.F. Barbero, M.A. Rostagno, Extraction of flavonoids from natural sources using modern techniques, Front Chem, 2020, 8, 507887.

[20] N. Medina-Torres, T. Ayora-Talavera, H. Espinosa-Andrews, A. Sánchez-Contreras, N. Pacheco, Ultrasound assisted extraction for the recovery of phenolic compounds from vegetable sources, Agronomy, 2017, 7(3), 47.

[21] L.K. MacDonald-Wicks, L.G. Wood, M.L. Garg, Methodology for the determination of biological antioxidant capacity in vitro: a review, J Sci Food Agric, 2006, 86(13), 2046-2056.

[22] M.M. Küçük, S. Ağırtaş, Liquefaction of *Prangmites australis* by supercritical gas extraction, Bioresource Technol, 1999, 69(2), 141-143.

[23] X. Li, X. Meng, H. Tan, Q. Ren, D. Li and B. Li, Ultra-high pressure extraction of anthocyanins from *Lonicera caerulea* and its antioxidant activity compared with ultrasound-assisted extraction, Int J Agric Biol, 2018, 20, 2257-2264.

[24] R. Arbianti, T. S. Utami, A. Kurmana, A. Sinaga, Comparison of antioxidant activity and total phenolic content of *Dillenia indica* leaves extracts obtained using various techniques, 14th Regional Symposium on Chemical Engineering, Dec 4-5, Yogyakarta, Indonesia, 2007, ISBN 978-979-16978-0-4.

[25] S. Aryal, M. K. Baniya, K. Danekhu, P. Kunwar, R. Gurung, N. Koirala, Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from Western Nepal, Plants, 2019, 8(4), 96.

[26] A. M. Chahardeh, D. Ibrahim, S. F. Sulaiman, Antioxidant activity and total phenolic content of some medicinal plants in urticaceae family, J Appl Biol Sci, 2009, 3(2) 27-31.