IJIRR

International Journal of Information Research and Review
Vol. 05, Issue, 02, pp.5216-5220, February, 2018

RESEARCH ARTICLE

EFFECT OF EXTRACTION SOLVENT, EXTRACTION TIME AND EXTRACTION TEMPERATURE ON TOTAL POLYPHENOLS CONTENT EXTRACTED BY ULTRASONIC FROM SYRIAN FRESH OLIVE LEAVES

[1,*]Maya S Aljindy and [2]Prof. Faten Shouman

[1]Department of Analytical Chemistry, Faculty of Science, Tishreen University, Lattakia, Syria
[2]Chemistry Department, Faculty of Science, Tishreen University, Lattakia, Syria

ARTICLE INFO

Article History:

Received 05th November, 2017
Received in revised form
11th December, 2017
Accepted 15th January, 2018
Published online 28th February, 2018

Keywords:

Polyphenols,
Extraction, Ultrasonic,
Fresh Olive Leaves.

ABSTRACT

Total polyphenols were studied in olive leaves collected from Syria-Lattakia (Qurdaha area). Phenolic compounds were extracted from fresh olive leaves using two extraction methods, maceration and ultrasound assisted extraction. The effect of extraction solvent concentration was studied using the ethanol-water mixture at different percentages (60,70,80%), and the effect of extraction temperature was also studied at (20,30,40°C), in addition to the effect of extraction time on the total polyphenolic content. In the maceration extraction, the total polyphenolic content was studied in fresh olive leaves at different times (24,48,72 h), when the ultrasound extraction times were (10,20,30 min). The highest polyphenolic content was found in fresh olive leaves, which were carried out from the ultrasound extracted with ethanol/water (80%) at the temperature (40°C) and at the time of extraction (20 min). The total polyphenolic content extracted was estimated in milligram equivalent Gallic acid per gram of olive leaves (157.2539 mgGAE / gDM).

INTRODUCTION

Olive trees are known as trees of the subtropical region, which can survive and live for a few decades. The original homeland of olive trees is the Mediterranean region (Altemimi, Ammar, 2011). Syria is one of the countries that cultivate olive. The estimated cultured area is 684490 hectares with annual production of about 1000,000 tons of olive fruit (Tayoub *et al*., 2015). Olive tree belongs to the Oleaceae family which contains 20 to 29 genera according to the classification system (Yazici, 2012) Olive fruits are consumed as table olives and used for producing olive oil (Zam, Wissam, 2016). It is considered as the main source of dietary fat in Mediterranean countries and the most popular edible oil in the world (Xie, Pu-jun, 2015). However, full advantages of the leaves from the olive trees were not taken in the oil extraction process from olive fruit in most of oil mills. Olive leaves were burned or directly thrown away from the mills as environmentally detrimental waste products (Xie, Pu-jun *et al*., 2015), There is an intensive investigation of olive leaves because of their many positive and beneficial effects for human health. For instance, olive leaves can work as anti-inflammatory, anti-microbial, anti-diabetic, anti-atherosclerotic, and anti-carcinogenic agents (Altemimi, Ammar, 2014), antioxidant, antihypertensive, hypoglycemic, and hypocholesterolemic properties (Zam, Wissam *et al*., 2016). These activities are because theycontain bioactive compounds. Oleuropein, apigenin and vanillic acid are examples of polyphenols contained in olive leaves and their content changes depending on the cultivar, the region of growth and the harvesting time (Casazza, 2017). It is stated that there are approximately 40 phenolic compound when olive plants are considered as a whole as a tree, branch, root, and olive fruit. Among the phenolic compounds the concentrations of, especially, oleuropein, hydroxytyrosol, lutolein, rutin, transcinnamic acid and tyrosol are higher compared to other phenolic substances (Gamli, 2016). Phenols are sometimes called phenolics and are class of compounds consisting of a hydroxyl group - (OH) bonded directly to an aromatic hydrocarbon group. The simplest of the class is the phenol which is also called carbolic acid C6H5OH. Phenolic compounds are classified as simple phenols or polyphenols base on the number of phenol units in the molecules (Louis *et al*., 2017). It is known that, in the plant extraction field, conventional solvent extraction (CSE) was at a disadvantage of being time consuming, with a low extraction yield. Ultrasonication assisted extraction (UAE) could significantly improve the process due to oscillating and acoustic cavitation (Xie, Pu-jun, 2015). In this context, the use of ultrasound or sonication to break the cell membranes has the advantage of reducing considerably the extraction time and increasing the

Corresponding author: **Maya S Aljindy,**
Department of Analytical Chemistry - Faculty of Science - Tishreen University, Lattakia, Syria.

extract yield. The application of ultrasound disrupts the cell wall structure and accelerates diffusion through membranes; thus, the cell lyses and hence facilitates the release of cell contents (Falleh, Hanen *et al*., 2012). In this study, Olive tree (Oleaeuropaea) leaf native to the Mediterranean countries was selected as the potential antioxidant source rich in polyphenol. As an agricultural and industrial waste, olive leaves are a cheap, renewable and abundant source of polyphenols. Three important parameters, solvent concentration, extraction temperature, extraction time, were selected for optimizing the total polyphenolic content and Oleuropein concentration using Ultrasound assisted extraction (UAS) from the fresh olive leaves. Extractions with water and aqueous ethanol were also performed for comparison, because water and ethanol are the most common bio-solvents employed for polyphenol recovery.

MATERIALS AND METHODS

Sample Collection

Olive leaf samples were collected from Lattakia, Qardaha, Qubaisia, in October 2014, after which the fresh olive leaves were transferred to the laboratory, washed with tap water, placed in an electric mill before each extraction process to obtain olive leaf powder.

Chemicals

Ethanol, Gallic acid and Folin & Ciocalteu's phenol reagentwere obtained from Sigma-Aldrich company.

Polyphenols Extraction from Fresh Olive Leaves by Maceration

5 grams of fresh olive leaves were macerated in (100 ml) ethanol /water solvent (60-70-80%) for (24-48-72 h) at (20-30-40ºC) with constant stirring using the magnetic motor. The sample was then filtered through aWhatman No.1 filter (Whatman, UK) to separate coarse particles. The filtered extracts were then evaporated in rotary evaporator at room temperature under vacuum. The concentrated extracts were stored in a refrigerator at (4°C) until used.

Ultrasonic Assisted Extraction of Fresh Olive Leaves Polyphenols

5 grams of fresh olive leaves were placed in (100 ml) of the ethanol / water solvent (60-70-80%) for (10-20-30 min) at (20-30-40ºC) in a bath of ultrasound apparatus. The sample was then filtered through the WhatmanNo.1 filter (Whatman, UK) to separate coarse particles. The filtered extracts were then evaporated in rotary evaporator at room temperature under vacuum. The concentrated extracts were stored in a refrigerator at (4°C) until used.

Determination of Total Polyphenolic Content in Fresh Olive Leaves

Total phenolics were determined using Folin–Ciocalteu reagents. Fresh olive leave extract 1 ml were mixed with (0.5 ml) of Folin–Ciocalteu reagent (pre-diluted 10-fold with distilled water) and allowed to stand at room temperature for (5 min), and then (1.25 ml) of sodium bicarbonate (200 g/Kg) was added to the mixture. After standing for (90 min) at room temperature, absorbance was measured at (760 nm) (Chammem *et al*., 2015). Aqueous solutions of knowngallic acid concentrations in the range of (0.01-0.09 mg/ml) were used for calibration. Results were expressed as mg gallic acid equivalents (GAE)/ g sample (Casazza *et al*., 2017). For the gallic acid, the curve absorbance versus concentrations is described by the equation y = 1.333x +0.0014 (R^2 = 0,998). Allmeasurements were done in triplicate.

Statistical Analysis

Three samples of olive leaves extract of each treatment were independently analyzed in each sampling, and all of the determinations were carried out in triplicate. The results are expressed as means ± standard deviations.

RESULTS AND DISCUSSION

Effect of Extraction Solvent Concentration on Polyphenols Content

Effect of extraction solvent concentration (ethanol / water) on the total polyphenolic content was studied in fresh olive leaves by macerated5 grams of fresh olive leaves in three concentration of ETOH (60-70-80%) for (24 h) at (20°C). The results areshown in Table(1):

Table 1. Total Polyphenols content Extracted from Fresh Olive Leaves by Maceration with ETOH (60-70-80%) for (24h) at (20°C) expressed as (mgGAE/gFM)

ETOH Con.	TP (mgGAE/g FM± SD*)	LOD**	LOQ***
60%	114 ±0.75	1.68796	5.62655
70%	118 ±0.866	1.94909	6.49698
80%	121 ±1.145	2.57841	8.59469

* SD: standard deviation
** LOD: Limit of Detection
*** LOQ: Limit of Quantification

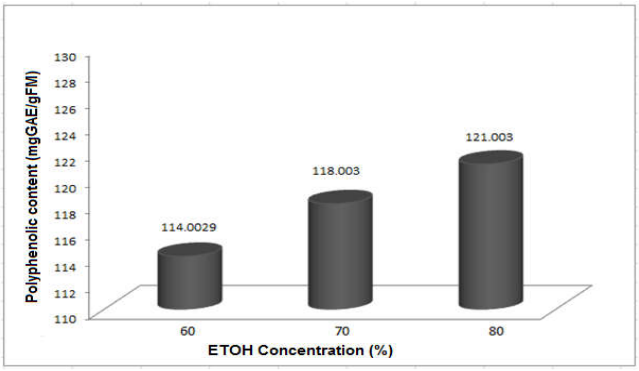

Fig. 1. Total Polyphenolic content Extracted from Fresh Olive Leaves by Maceration with ETOH (60-70-80%) for (24 h) at (20°C) expressed as (mgGAE/gFM)

The effect of extraction solvent concentration (ethanol / water) on the total polyphenols content in fresh olive leaves was also studied by placed the samples of fresh olive leaves in the ultrasonic device in three concentrations (60,70,80%) for (10min) at (20°C) .The results are shown in Table (2):Results in table (1) shows that total polyphenolic content which extracted from fresh olive leaves using different concentrations of ethanol/ water by maceration (60-70-805) for (24h) at

(20°C) ranged between (114.0029 - 121.003 mgGAE / gFM).And gave the highest amount of total polyphenols when using the concentration of solvent ethanol / water (80%) which amounted to (121.003 mgGAE / gFM).

Table 2. Total Polyphenols content Extracted from Fresh Olive Leaves by Ultrasonic with ETOH (60-70-80%) for (24h) at (20°C) expressed as (mgGAE/gFM)

ETOH Con.	TP (mgGAE/g FM± SD*)	LOD**	LOQ***
60%	127.25±0.433	0.974547	3.248489
70%	134±0.433	0.974547	3.248489
80%	140.75±0.866	1.949093	6.496977

* SD: standard deviation
** LOD: Limit of Detection
*** LOQ: Limit of Quantification

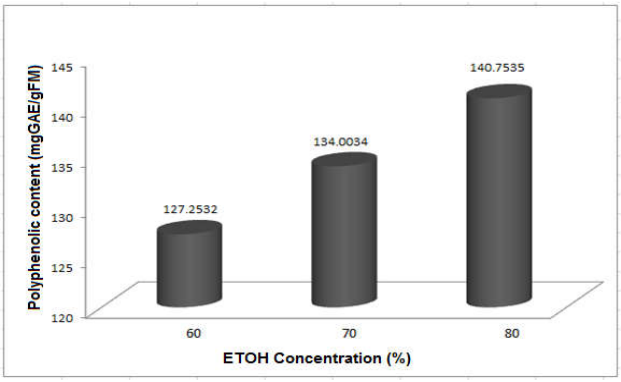

Fig. 2. Total Polyphenolic content Extracted from Fresh Olive Leaves using Ultrasonic with ETOH (60-70-80%) for (24 h) at (20°C) expressed as (mgGAE/gFM)

While the total polyphenols content show in table (2) which extracted from fresh olive leaves for (10 min) at(20°C) using ultrasonic ranged between (127.2532 - 140.7535 mgGAE / gFM).And the highest content of polyphenols extracted using Ultrasonic was obtained at ethanol/water concentration (80%), where the amount of polyphenols extracted was (140.7535 mgGAE / gFM). This is because the solvent polarity plays a major role in increasing solubility of phenolic compounds. The water mixtures of methanol, ethanol and acetone have been used in many studies to extract total polyphenols from olive leaves, Ethanol was found to be the best for being nontoxic (Dent, Maja *et al*., 2013).

Effect of Extraction Time on Polyphenols Content

The effect of extraction time on the total polyphenolic content by macerated 5 grams of fresh olive leaf in the solvent ethanol / water (80%) at a temperature (20°C) for different extraction times (24, 48,72h) was studied, and the results are shown in Table (3):

Table 3. Total Polyphenols content Extracted from Fresh Olive Leaves by Maceration with ETOH (80%) at (20°C) for (24-48-72h) expressed as (mgGAE/gFM)

زمن الاستخلاص (h)	TP (mgGAE/g FM± SD*)	LOD**	LOQ***
24	121 ±1.1457	2.578408	8.594693
48	119.75±0.433	0.974547	3.248489
72	97 ±0.433	0.974547	3.248489

* SD: standard deviation
** LOD: Limit of Detection
*** LOQ: Limit of Quantification

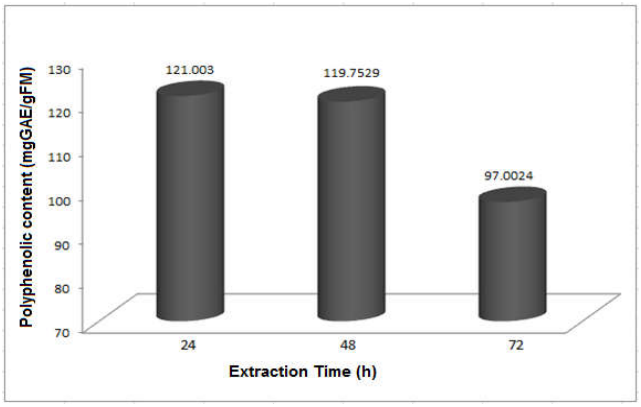

Fig. 3. Total Polyphenols content Extracted from Fresh Olive Leaves by Maceration with ETOH (80%) at (20°C) for (24-48-72h) expressed as (mgGAE/gFM)

The results in Table (3) show that the total polyphenolic content which extracted from fresh olive leaves by maceration method using ethanol / water (80%), extraction temperature (20°C) at different extraction times (24,48,72h) was (121.003 - 119.7529 - 97.0024 mgGAE / gFM), respectively. These results indicate that the total content of polyphenols extracted from fresh olive leaves was reduced by increasing the extraction time with the approximate total polyphenols content which extracted at extraction times (24,48h) and a clear decrease at (72h). Also the effect of extraction time on total polyphenolic content which extracted from fresh olive leaf by ultrasonic using ethanol / water solvent (80%) at (20°C) for different extraction times (10,20,30min) was studied. The results are shown in Table (4):

Table 4. Total Polyphenolic content Extracted from Fresh Olive Leaves byUltrasonicusing ETOH (80%)at (20°C) for (10-20-30 min), expressed as (mgGAE/gFM)

Extraction Time (min)	TP (mgGAE/g FM± SD*)	LOD**	LOQ***
10	140.75±0.866	1.949093	6.496977
20	143 ±0.433	0.974547	3.248489
30	116 ±0.433	0.974547	3.248489

* SD: standard deviation
** LOD: Limit of Detection
*** LOQ: Limit of Quantification

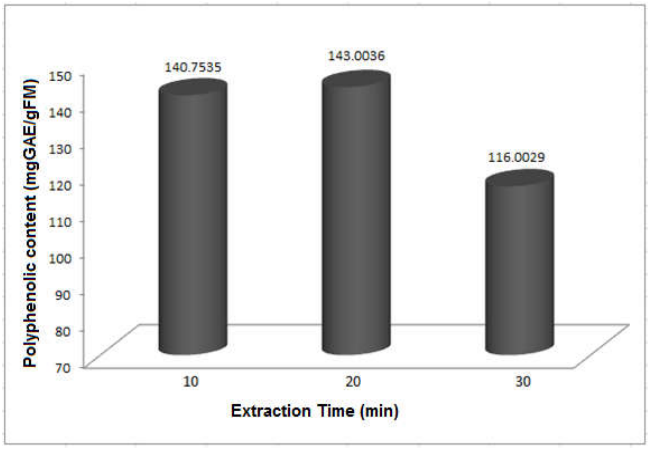

Fig.4. Total Polyphenolic content Extracted from Fresh Olive Leaves using Ultrasonic with ETOH (80%) at (20°C) for (10-20-30 min) expressed as (mgGAE/gFM)

The total polyphenols content shown in Table (4) which extracted by ultrasonic device from fresh olive leaves was obtained for (10,20,30min) and the same conditions as the solvent and the previous temperature was (140.7535 - 143.0036 - 116.0029 mgGAE / gFm).These results indicate that the total content of the extracted polyphenols increased with increasing extraction time up to (20 min) and then decreasing after prolongation time. The highest content of total polyphenols in the fresh olive leaves at extraction time (20 min) was (143.0036 mgGAE / gFM).Extraction time is an important variable in the extraction of phenolic compounds from plant materials by ultrasonic. The time of (20 min) allows cavity bubbles to reach and tear down more plant cells, increasing the total polyphenols content (Ilghami *et al.,* 2015). For the reduction of total polyphenols at the extraction time (72 h) in the maceration method and (30min) in the ultrasound method, Several studies have indicated that prolonged extraction lead to a decrease in the phenolic content of crude extracts due to the oxidation of these compounds by prolonging the exposure to environment factors such as light and oxygen (Morsy *et al.,* 2014),so the extraction time has a significant impact on the extraction yield, and increase extraction time does not increase the extraction yield. These circumstances could be well explained by Fick's second law of diffusion, which predicts that after a certain time, there will be a final equilibrium between the solute in the solid matrix (plant sample) and in the bulk solution (extraction solvent) (Benmeziane *et al*., 2014).

Effect of Extraction Temperature on Polyphenols Content

Effect of extraction Temperature on the total polyphenolic content was studied in fresh olive leaves by macerated 5 grams of fresh olive leaves in the solvent ETOH (80%) for (24 h) at three different temperatures (20-30-40°C). The results are shown in Table (5):

Table 5. Total Polyphenols content Extracted from Fresh Olive Leaves by Maceration with ETOH (80%) for (24h) at (20-30-40°C) expressed as (mgGAE/gFM)

Extraction Temp (°C)	TP (mgGAE/g FM± SD*)	LOD**	LOQ***
20	121 ±1.1457	2.578408	8.594693
30	128.75±0.433	0.974547	3.248489
40	152 ±1.1457	2.578408	8.594693

* SD: standard deviation
** LOD: Limit of Detection
*** LOQ: Limit of Quantification

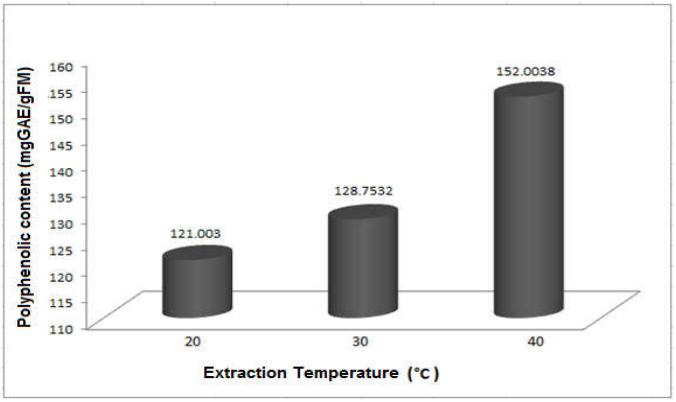

Fig. 5. Total Polyphenols content Extracted from Fresh Olive Leaves by Maceration with ETOH (80%) for (24h) at (20-30-40°C) expressed as (mgGAE/gFM)

The effect of extraction temperature on the total polyphenols content in fresh olive leaves was also studied by placed the samples of fresh olive leaves in the ultrasonic device at three temperatures (20-30-40°C) using ETOH (80%) for (10min) the results are shown in Table (6):

Table 6. Total Polyphenols content Extracted from Fresh Olive Leaves by Ultrasonic using ETOH (80%) for (24h) at (20-30-40°C) expressed as (mgGAE/gFM)

Extraction Temp (°C)	TP (mgGAE/g FM± SD*)	LOD**	LOQ***
20	143 ±0.433	0.974547	3.248489
30	151.25±0.433	0.974547	3.248489
40	157.25 ±1.5613	3.513778	11.71259

* SD: standard deviation
** LOD: Limit of Detection
*** LOQ: Limit of Quantification

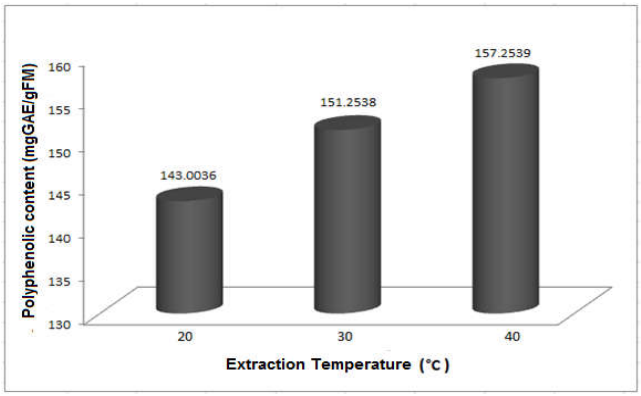

Fig. 6. Total Polyphenols content Extracted from Fresh Olive Leaves by Ultrasonic using ETOH (80%) for (24h) at (20-30-40°C) expressed as (mgGAE/gFM)

The results in Table (5) show that the total polyphenols content which extracted by maceration from fresh olive leaves using ethanol / water (80%) for (24h) at three different temperatures (20-30-40°C) were respectively (121.003 - 128.7532 – 152.003mgGAE/gFM). The total polyphenols content extracted by ultrasound device from fresh olive leaves using ethanol / water (80%), for (20 min) at temperatures (20-30-40°C) shown in Table (6) was (143.0036 - 151.2538 - 157.2539 mgGAE / gFM) respectively. We note the increase in the content of polyphenols extracted from fresh olive leaves when increasing the temperature up to (40 ° C). The highest amount of polyphenols in the fresh olive leaves was obtained from maceration (152.0038 mgGAE / gFM). And the highest content of polyphenols was extracted by ultrasound from Fresholives was (157.2539 mgGAE / gFM).This is because the use of high temperatures lead to a kinetic improvement, but it is limited by the fact that most of phenolic compounds are thermo labile (Mekinić, 2014), which is sensitive to high temperatures. Thus, heat treatments can improve the extraction kinetics, The previous reduces both the phenolic content and its stability and antioxidant capacity (Meneses, 2013; Ahmad-Qasem *et al*., 2013).

Conclusion

Ultrasonic Assisted extraction is one of the best ways to extract polyphenolic compounds from olive leaves compared to

maceration method in terms of saving time and economic cost. Many factors effect on the content of total polyphenols which extracted from fresh olive leaves such as solvent concentration, extraction temperature, extraction time.

REFERENCES

Ahmad-Qasem, Margarita; Canovas, Jaime; Barrajon-Catalan, Enrique; Micol, Vicente; Carcel, Juan Andres; García-Pérez, José Vicente. 2013. Kinetic and Compositional Study of Phenolic Extraction from Olive Leaves (var.Serrana) by Using Power Ultrasound. *Innovative Food Science and Emerging Technologies*, Vol. 17,120-129.

Altemimi, Ammar A. 2017. A Study of the Protective Properties of Iraqi Olive Leaves against Oxidation and *Pathogenic Bacteria in Food Applications. Antioxidants, 6 (34), 1-13.*

Benmeziane, F; Djamai, R; Cadot, Y; Seridi, R. 2014. Optimization of extraction parameters of phenolic compounds from Algerian fresh table grapes, (VitisVinifera). *International Food Research Journal*, 21 (3), 1061-1065.

Casazza, Alessandro; Aliakbarian, Bahar; Comotto, Mattia; Souza, Paula Monteiro; Perego, Patrizia. 2017. Olive Leaves Infuse and Decoct Production: Influence of Leaves Drying Conditions and Particle Size. *Chemical Engineering Transactions,* Vol. 57, 1807-1812.

Chammem, Nadia; Sifaoui, Ines; Mejri, Asma; Ben Slama, Mourad; Hamdi, Moktar; Abderabba, Manef. 2015. Optimization of Extraction of Phenolic and Antioxidant Contents from Olive Leaves Using Composition Central Design. G.J.B.B, 4 (2), 145-152.

Dent, Maja; Dragovic-Uzelac, Verica; Penic, Marija; Brncic, Mladen; Bosiljkov, Tomislav; Levaj, Branka. 2013. The Effect of Extraction Solvents, Temperature and Time on the Composition and Mass Fraction of Polyphenols in Dalmatian Wild Sage (*Salvia officinalis* L.) *Extracts. Food Technol. Biotechnol*, 51 (1), 84–91.

Falleh, Hanen; Ksouri, Riadh; Lucchessi, Marrie-Elizabeth; Abdelly, Chedly; Magné, Christian. 2012. Ultrasound-Assisted Extraction: Effect of Extraction Time and Solvent Power on the Levels of Polyphenols and Antioxidant Activity of Mesembryanthemumedule L. Aizoaceae Shoots. *Tropical Journal of Pharmaceutical Research*, 11 (2), 243-249.

Gamli, Ö.F. (2016). The Health Effects of Oleuropein, One of The Major Phenolic Compounds of Olives, OleaEuropaea L. *Ital. J. Food Sci*, Vol. 28, 178-189.

Ilghami, Amin; Ghanbarzadeh, Saeed; Hamishehkar, Hamed.2015. Optimization of the Ultrasonic-Assisted Extraction of Phenolic Compounds, Ferric Reducing Activity and Antioxidant Activity of the Beta vulgaris Using Response *Surface Methodology. Pharmaceutical Sciences*, 21 (1), 46-50.

Louis, H; Maitera, O.N; Boro, G; Barminas, J.T. 2017. Determination of Total Phenolic Content and Some Selected Metals in Extracts of Moringa Oleifera, Cassia Tora, Ocimum Gratissimum, Vernonia Baldwinii and Telfairia Occidentalis Plant Leaves. *World News of Natural Sciences*, Vol. 11, 11-18.

Mekinić, IvanaGeneralić; Gotovac, M; Skroza, Danijela; Ljubenkov, I; Burču, F; Katalinić, Višnja.(2014). Effect of the extraction solvent on the oleuropein content and antioxidant properties of olive leaf (cv. Oblica, Lastovka and Levantinka) extracts.*Croat. J. Food Sci. Technol,* 6 (1), 7-14.

Meneses, Nuno G.T; Martins, Silvia; Teixeira, Jose A; Mussatto, Solange I. 2013. Influence of extraction solvents on the recovery of antioxidant phenolic compounds from brewer's spent grains. Separation and Purification Technology, Vol. 108, 152‑158.

Morsy, Nashwa, F. S; Abdel-Aziz, M. E. 2014. Efficiency of olive (Oleaeuropaea L.) leaf extract as antioxidant and anticancer agents. *Journal of Agroalimentary Processes and Technologies*, 20 (1), 46-53.

Tayoub, Ghaleb;Sulaiman, Huda; Alorfi, Malik.2015. Oleuropein concentration in some Syrian olive oil mill waste water. *Journal of Natural Products*, Vol. 8, 33-38.

Xie, Pu-jun; Huang, Li-xin; Zhang, Cai-hong; You, Feng; Wang, Cheng-zhang; Zhou, Hao. 2015. Reduced-Pressure Boiling Extraction of Oleuropein Coupled with Ultrasonication from Olive Leaves (*Oleaeuropaea* L.). *Advances in Materials Science and Engineering*, Vol. 2015, 1-8.b

Xie, Pu-jun; Huang, Li-xin; Zhang, Cai-hong; You, Feng; Zhang, Yao-lei, 2015. Reduced pressure extraction of oleuropein from olive leaves (*Oleaeuropaea* L.) with ultrasound assistance. *Food and Bioproducts Processing,* Vol. 93, 29-38.a

Yazici, Işin; Karabey, Fatih; Akay, Şeref; Kaya, Ünal; Demiray, Hatice. 2012. The effect of different irrigation levels on the oleuropein contents of olive tree (Oleaeuropaea L. cv. Memecik) in the western coastal region of Turkey. *African Journal of Biotechnology*, 11 (90), 15664-15677.

Zam, Wissam; Ali, Ali; Rama, Hasan.2016. Optimization of Extraction Conditions for the Recovery of Phenolic Compounds and Antioxidants from Syrian Olive Leaves. *Journal of Pharmacognosy and Phytochemistry*, 5 (5), 390-394.

****