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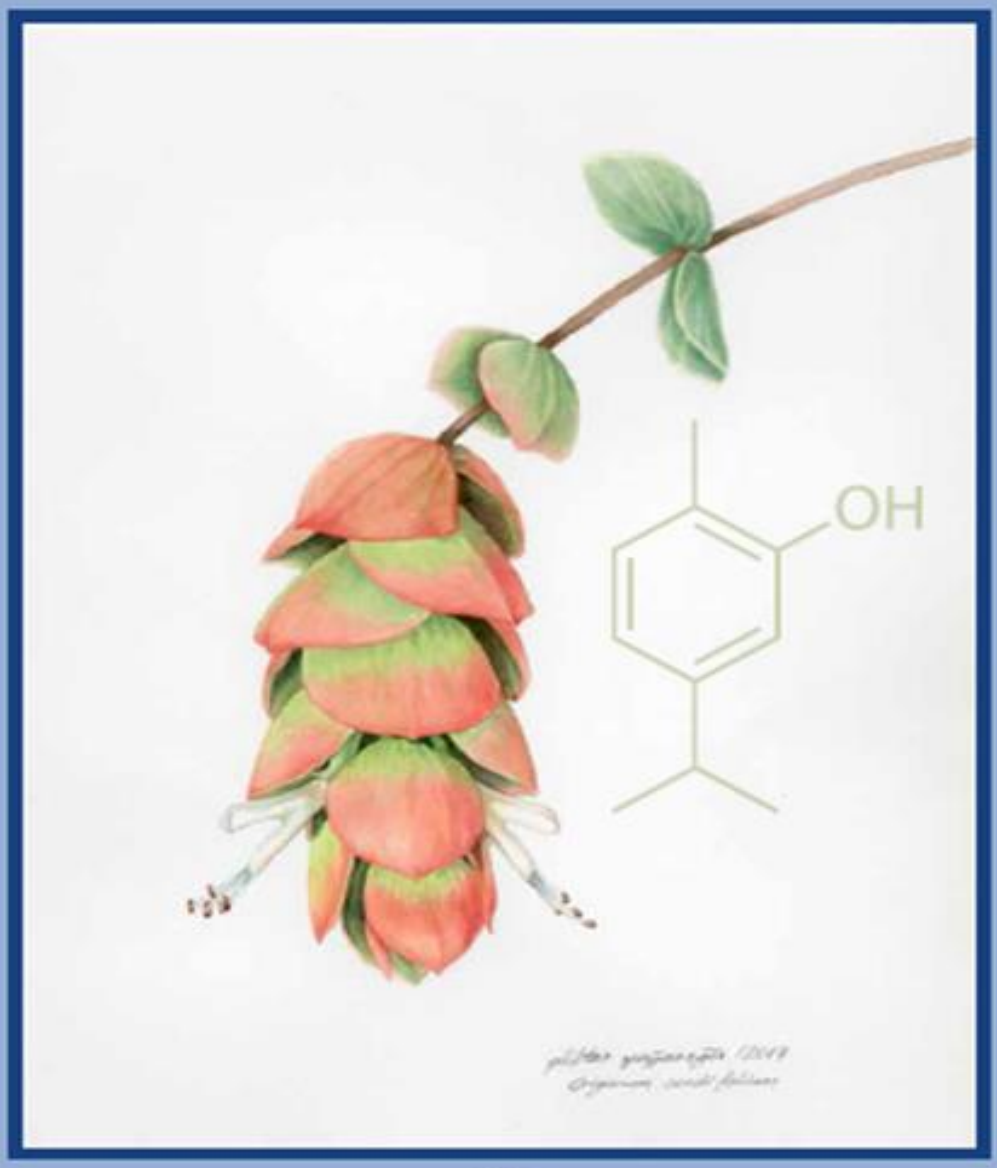

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Authors (11 pt.)

Neriman Özhatay & Çağın Korkmazer *

Eastern Mediterranean University, Faculty of Pharmacy Famagusta, North Cyprus

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Oleuropein amounts of olive leaves from different regions of Northern Cyprus

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Abstract

Olive (*Olea europea* L.) trees are widely grown in Mediterranean region and leaves are used as a traditional medicine. Oleuropein is the main active compound for the olive leaves. Olive leaves have potentially beneficial effects on certain health conditions, including antimicrobial and antioxidant and other biological activities. These beneficial effects may be due to the antioxidant components of olive leaves, especially oleuropein. Oleuropein containing extracts are used in many supplements for different pharmacological activities. In this study, oleuropein amounts of olive leaves from seven different localities of North Cyprus were investigated by HPLC. Olive leaves were extracted by methanol according to European Pharmacopoeia's method. Oleuropein amounts of the extracts were calculated by reversed phase HPLC with PDA. Buyukkonuk and Guzelyurt samples contain the highest amount of oleuropein, followed by Tatlisu, Dipkarpaz, Lapta, Zeytinlik and Lefke.

Keywords

HPLC, North Cyprus, *Olea europea*, oleuropein.

Article History

Submitted: 30 October 2019 Accepted: 18 November 2019 Published Online: 30 December 2019

Article Info

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Research Article:

Volume: 2 Issue: 2 December 2019 Pages: 68-74

INTRODUCTION

Medicinal plants play a vital role in healthcare and have been widely used as main treatment for approximately all ailments in ancient times (Anza *et al.*, 2017). Chemical drug discovery was a revolution in the era of medicine. However, increase in the rater of drug resistance and presence of undesirable side effects led scientists to investigate safer alternatives to synthetic medications. Therefore, the prevalence of phytochemicals such as phenolic compounds, flavonoids and tannins, to which the likehood of resistance development is much lower, has been increasing (Qin and Hou 2017).

The Mediterranean diet is a modern nutritional concept that is useful to enhance life quality. The idea was based on the premise that animal fats were predisposing for heart diseases whereas unsaturated plant fats or oils promoted good health. By pressing (squeezing) or centrifuging the crushed fruit and the seed, olive oil can be obtained. All oils extracted from olives are sorted as Virgin Olive Oils and the highest quality Virgin Olive Oils are called Extra Virgin Olive Oil. This class contains high amounts of monounsaturated fatty acid (60 to 80% oleic acid), medium amounts of polyunsaturated fatty acid (4 to 20%) and small amounts of polyphenols, tocopherols, sterols and many aromatic compounds.

O. europaea subsp. *europaea* L. has also many other auxiliary uses. The olive fruits and leaves falling from mature trees provide animals a source for food. The remnants from the process like the pomace are used as plant fertilizer, compost, and fuel. In many countries, olive trees are planted extensively as decoration or for shade. Because the tree timber is durable, it is used for furniture, as kitchen equipment and ornamental items. It can be used as a windbreak for the lighter fruit or plants or flowers that may be affected by wind and pulled out of soil like orchid family flowers. Olives have a pretty famous and special history and are symbolic. The branch of olive was and still a symbol of grace, victory, peace and blessing. The leafy branches of the olive tree were ritually offered to gods and significant figures as immolation of purity and benediction. They were also offered to winners of competitions or victors of wars. Nowadays, olives have still been used in religious rituals and curative sessions. Olive trees are importantly mentioned in Romanian and Greek culture as well as in Holy books as in Torah, Bible and the Holy Quran (Kapellakis *et al.*, 2008; Talhaoui *et al.*, 2015).

The aim of this study is to investigate oleuropein amounts within the different region in Northern Cyprus. Leaf samples were collected from 7 different places in Northern Cyprus (Tatlisu, Buyukkonuk, Dipkarpaz, Zeytinlik, Lapta, Guzelyurt and Lefke) and oleuropein amounts were calculated using reverse phase HPLC.

MATERIALS AND METHODS

Plant materials

Olive leaves were collected from 7 different regions (Tatlisu, Buyukkonuk, Dipkarpaz, Zeytinlik, Lapta, Guzelyurt and Lefke) in North Cyprus during the first week of March 2018 which are in order. 500 g of olive leaves were gathered from every area and the leaves were detached from the stems. The leaves were left to dry at room temperature for one week. Then leaves were crushed and kept in dry storage until extraction.

Preparation of the extracts

The extraction was carried out with dried and powdered leaves according to the European Pharmacopoeia (2014). 1 g of olive leaves extracted with 50 ml methanol was sonicated at 60 ºC for 30 minutes. The mixture was left to stand and cool down. Afterwards, it was filtered and washed with methanol till 100 ml. The extract was stored in the fridge. Then the extract was diluted with distilled water 1/10 dilution by adding 100 µl of extract to 900 µl water. All samples were extracted twice at same conditions.

Oleuropein analysis by HPLC

Reversed phase HPLC method was performed for oleuropein analysis using an isocratic elution. Oleuropein was eluted from C18 column (150 x 0.46 mm, 5 □m) using methanol: water: acetic acid (70:30:1) as a mobile phase. Flow rate was 1 mL/min and injection volume was 20 µL. Oleuropein was detected by PDA detector at 240 and 280 nm and the retention time of oleuropein was 5.4 minutes. Oleuropein standards were prepared at 1 mg/mL concentration for stock solution and then five dilutions (0.15, 0.1, 0.08, 0.06 and 0.04 mg/ml) were prepared for calibration curve. All standards and extracts were injected 3 times and mean values and standard deviations were calculated.

RESULTS AND DISCUSSION

Olive leaves were collected from seven different locations from North Cyprus (Table 1). Oleuropein was identified as major compound in the leaf extract of *Olea europea* by authentic standard oleuropein. The UV spectrum is given in Figure 1 and the calibration curves were shown in Figure 2 for both wavelengths 240 and 280 nm.

Table 1: Oleuropein amounts in *Olea europaea* from different localities of North Cyprus.

Sample Name	Locality	Amount of oleuropein (%, in plant) 240 nm	Amount of oleuropein (%, in plant) 280 nm
Sample 1	Tatlisu	**0.079 ± 0.003***	**0.080 ± 0.004**
Sample 2	Buyukkonuk	**0.088 ± 0.006**	**0.087 ± 0.006**
Sample 3	Dipkarpaz	0.072 ± 0.006	0.072 ± 0.007
Sample 4	Zeytinlik	0.057 ± 0.007	0.057 ± 0.007
Sample 5	Lapta	0.065 ± 0.003	0.065 ± 0.004
Sample 6	Guzelyurt	**0.086 ± 0.004**	**0.087 ± 0.005**
Sample 7	Lefke	0.046 ± 0.004	0.046 ± 0.004

*mean ± SD (n = 6)

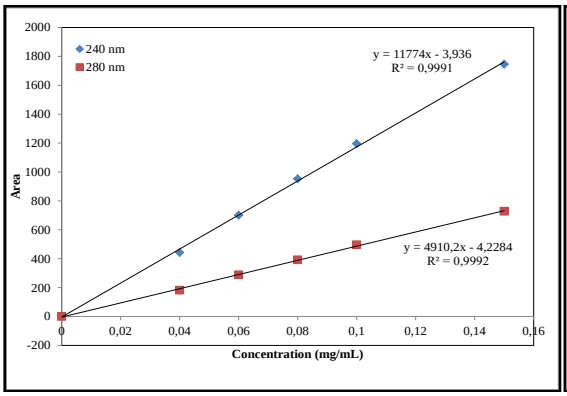

Figure 1: UV spectrum of *oleuropein*.

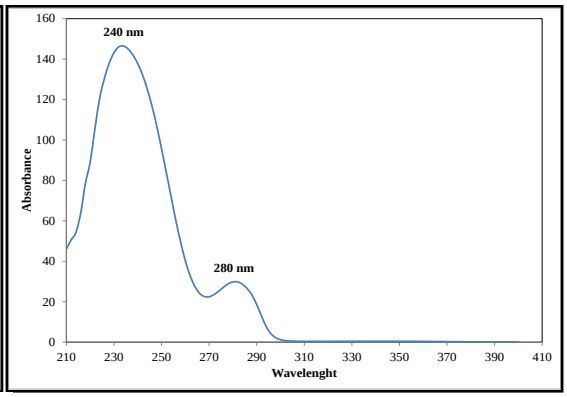

Figure 2: Calibration curves for oleuropein at 240 nm and 280 nm.

All samples were injected 3 times and at mean values and standard deviations are given in Table 1. The HPLC chromatograms of standard oleuropein and all extracts are given in Figure 3 and 4, respectively.

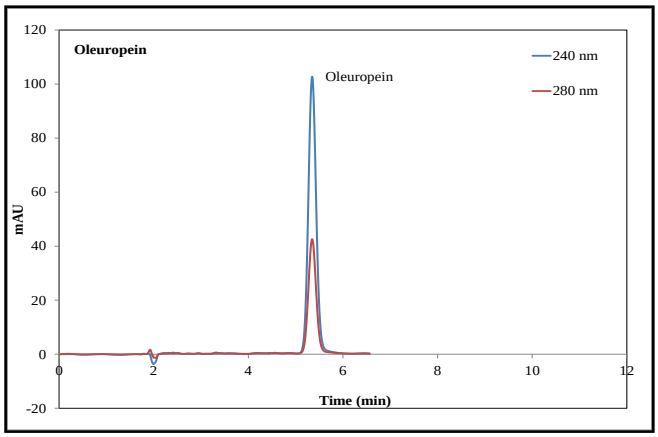

Figure 3: HPLC chromatogram of oleuropein.

After HPLC analysis of oleuropein within the extracts, areas of oleuropein were collected at 240 and 280 nm. The 240 nm is registered in European Pharmacopoeia and 280 nm in the specific Λmax from UV spectrum of oleuropein. The results for both wavelengths are given in Table 1.

Our results were accurate and show slight differences among the different localities. According to the results, Buyukkonuk, Guzelyurt and Tatlisu samples contained the highest amount of oleuropein but Lefke sample had less. The order of amounts of oleuropein within the extracts was as Buyukkonuk > Guzelyurt > Tatlisu > Dipkarpaz > Lapta > Zeytinlik > Lefke.

In Spain, olive leaves were analysed and different oleuropein derivatives and glycosides were detected between 0.028-0.329 % in dry mass (Talhaoui *et al.*, 2016). Oleuropein amount within the *O. europea* was found in wide range (0.03-2.16 %) according to the location of the raw materials (Vinha *et al.*, 2005).

According to the literatures (Talhaoui *et al.*, 2016; Hayes *et al.*, 2011; Czerwińska *et al.*, 2016), the results were correlated with the other Mediterranean countries.

In conclusion, oleuropein is considered as one of the most crucial secoterpenic compounds that have many biological benefits. Olive leaves were gathered from different locations in North Cyprus, and they were dried and then extracted with methanol, and injected to HPLC for qualification and quantification of the oleuropein within the samples. According to the results, oleuropein was found to be major phenolic compound within all olive leaves collected from different locations from North Cyprus. The order of the amount of oleuropein was found as Buyukkonuk > Guzelyurt > Tatlisu > Dipkarpaz > Lapta > Zeytinlik > Lefke.

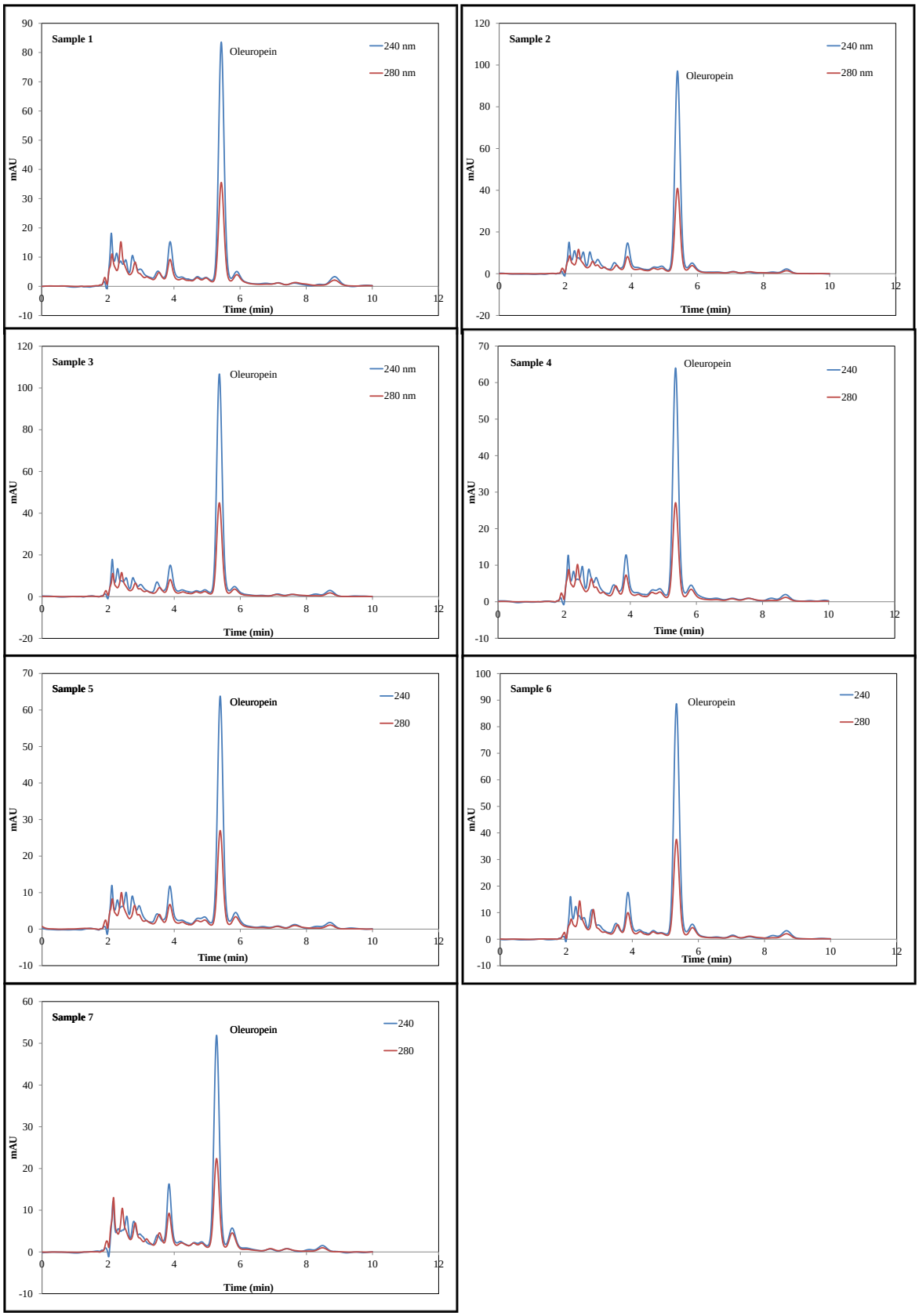

Figure 4: HPLC chromatogram of all samples.

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Research Article EMUJPharmSci ISSN 2651-3587 https://dergipark.org.tr/emujpharmsci

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Forced degradation studies of new formulation containing naltrexone

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Abstract

Naltrexone is one of the classical opioid antagonists. In substantially lower than standard doses, they apply different pharmacodynamics. A daily dose of 1 to 5 mg is considered as low-dose naltrexone (LDN). Clinical reports of LDN have demonstrated its possible benefits in diseases such as fibromyalgia, Crohn's disease, multiple sclerosis, complex-regional pain syndrome, Hailey-Hailey disease, and cancer.

The aim of the present study was to establish the inherent stability of naltrexone and stability indicating assay method for simultaneous determination of naltrexone after being subjected to acidic and basic stress.

A forced degradation study of naltrexone in its tablet form was conducted under the acidic and basic conditions in order to develop a rapid and sensitive stability indicating UV-Visible method for the quantification of naltrexone. Quantification was achieved by UV detection at 286.60 nm, on the basis of peak area. Naltrexone was found to be unstable and degraded in the acidic and basic buffer up to 3 hours.

Keywords

Forced degradation, low dose naltrexone, naltrexone, stress condition.

Article History

Submitted: 15 October 2019 Accepted: 22 October 2019 Published Online: 30 December 2019

Article Info

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Research Article:

Volume: 2 Issue: 2 December 2019 Pages: 75-83

INTRODUCTION

Addiction is one of the well-known chronic brain diseases (Volkow, 2004). One of the common sources of addiction is opioids, which have been known to cause both medical and social problems. There are currently over half a million people addicted to opioids receiving treatment in Europe and America according to the European Drug Report.

The sixth most common cause of premature death and illness in high-income countries is alcohol (Lee *et al.,* 2016). The term Alcohol Use Disorder (AUD) is used to describe the progression of the addiction and varies in terms of intensity, frequency, and symptoms.

While the most effective treatment for the AUD is abstinence, the majority of patients with alcohol addiction are not able to avoid drinking, thus making it necessary to develop effective alternative therapies.

Nowadays, naltrexone is one of the more common drugs used in the treatment of alcohol and opiate dependence (Modesto-Lowe, 2002). Naltrexone is an oral opioid antagonist which is more closely related to the μ-receptor relative to its affinity for the the δ- and κ-receptors. It is used in the treatment of chronic substance abuse (Brown, 2001).

Naltrexone has been found to effectively block and even reverse the effects of opioids with lower affinity reversible agonists like heroin and methadone and is also used to treat instances of alcohol dependence.

Naltrexone hydrochloride (NLX) is divided into low dose naltrexone (LDN) and full dose naltrexone. In many countries, access to full dose naltrexone is possible by the prescription of either 25 mg or 50 mg oral tablets. 50 mg tablet effectively blocks the effects of heroin for up to 24 hours. Conversely, LDN is a reversible competitive antagonist available in 1.75 mg and 4.5 mg doses that works by temporarily blocking the opioid receptors in the brain and subsequently increasing the production of endorphins through a positive feedback mechanism. The increase in production raises the level of endorphins as well as the level of encephalin in the body.

LDN is an opioid antagonist that blocks the reception of both exogenous opiates and endogenous opioids (endorphins). However, its administration in only small doses results in relatively short lived endorphin-block lasting somewhere between 3-4 hours. In consequence, endorphin deficit in the body causes the hypothalamus to signal for an increase in endorphin production. This is known as “the rebound effect”.

Chemical stability of pharmaceutical molecules is a matter of great concern as it affects the safety and efficacy of the drug product. The degree to which a drug maintains its characteristics and properties at the time it was manufactured over the period of storage and use within specific limits is known as drug stability. Overall drug stability is typically divided into different specific kinds which are physical, chemical, therapeutic, microbiological, and toxicological.

Drug stability is further divided into two categories: pre-market stability and commercial stability. Pre-market stability is determined using clinical trials in which the drug is subjected to various conditions as a way to evaluate its safety and efficacy during both the clinical trial and filing period. On the other hand, commercial stability refers to the monitoring of the long-term stability of post-approval batches of the drug. The evaluation of drug stability is usually done by testing the drug product or substance using a stability-indicating method to determine the retest period (in the case of pre-market stability) and shelf life (in the case of commercial stability).

Forced degradation studies are an integral step in designing a stability program for drug products and substances that comply with regulatory standards. Such studies involve forcefully degrading the drug product/substance using conditions that are severe and regularly accelerated, thus resulting in degradation products best suited for the study of molecular stability. As such, forced degradation is an essential stability testing that use more stringent conditions than those found in speeding up testing (Klick, 2005). Forced degradation studies are useful for the development and regulation of drug products as they aid the identification of potential degradation products. Moreover, they provide data for prediction of the degradation pathway, the efficacy of stability-indicating methods, conditions not suitable for the stability of the drug, the selection of storage conditions and packaging material(s) (Alsante, 2007). There are a number of similarities between forced degradation and preformulation degradation studies, both of which are useful in supporting the development of a stability-indicating method. International Conference on Harmonization (ICH) guidelines state that the purpose of stress testing is to determine the potential degradation of drug products as part of the effort to determine the molecule's intrinsic stability and validate its stability-indicating pathway. ICH guidelines contain many stipulations regarding the management of forced degradation, although these fail to outline the process of stress testing through a detailed practical approach. It is widely accepted that stress tests can be useful for the secondary analysis of drug products

before they are entered into the registration dossier, despite the fact that they are not considered essential in formal stability testing studies. Stress testing is an integral part of the preformulation assessment of the stability of a drug candidate in which stress is intentionally applied to induce degradation by adding other reactants (eg., acids, bases, and peroxides), exposing materials to compressive or sheer forces (eg., solid-state physical stability), increasing humidity when relevant (eg., solid-state chemical and physical stability), raising temperature, subjecting the test materials to various pH conditions or intense ultraviolet (UV) and visible light (eg., photo stability). By making the drug more susceptible to degradation, forced degradation provides a valuable guide for the selection of storage conditions. Furthermore, it also helps to determine the degradation pathway of the drug, distinguish between drug-related and non-drug related degradation in the formulation, identify the degradation product structure, and evaluate the intrinsic stability of the drug. Forced degradation studies are also useful for generating degradants in a short span of time (typically a few weeks). The samples they generate are useful for the development of a stability-indicating method to be utilized in later analyses of samples generated by studies on accelerated and long-term stability.

The aim of the study was to establish the inherent stability and stability indicating assay method for simultaneous determination of naltrexone after being subjected to stress conditions, such as acidic and basic stress.

MATERIALS AND METHODS

The materials used in this study include: Naltrexone HCl reference standard, distilled water, sodium hydroxide (NaOH), hydrochloric acid (HCl). Naltrexone HCl reference standard was obtained from United States Pharmacopeia (USP) (Lot: 120449). NaOH and HCl were purchased from Merck.

Preparation of standard solution

Standard solution was prepared by dissolving approximately 6.0 mg of NLX reference standard in 25 mL of distilled water to get a concentration of 0.24 mg/mL.

Preparation of sample stock solution of capsules containing 3 mg NLX (stock-1)

Contents of 4 capsules were unlocked and weighted accurately. The content was dispersed in distilled water using a 25 mL

volumetric flask. The dispersion was kept in the ultrasonic bath (Selecta Ultrasound H-D) for 30 minutes at 25 ^{0}C. The volume was completed to 25 mL by using distilled water and then filtered through a quantitative cellulose filter (Millipore Millex- HN, Nylon 0.45 µm). Finally, the sample was placed in a UV-Vis spectrophotometer. All absorbance measurements were obtained in a quartz cuvette (1 cm optical path length), from 200-700 nm even though the wavelength of naltrexone was 280.65 nm.

Preparation of sample solution of capsules containing 3 mg NLX (sample-1)

Sample solution was prepared using sample stock solution (stock-1). 2.5 mL stock-1 solution was taken into 5 mL volumetric flask and diluted to 5 mL with distilled water to obtain a concentration of 0.24 mg/mL.

Preparation of sample stock solution of capsules containing 4.5 mg NLX (stock-2)

Contents of 3 capsules were unlocked and weighted accurately. The content was dispersed in distilled water using a 25 mL volumetric flask. The dispersion was kept in the ultrasonic bath (Selecta Ultrasound H-D) for 30 minutes at 25 ^{0}C. The volume was completed to 25 mL by using distilled water and then filtered through a quantitative cellulose filter (Millipore Millex- HN, Nylon 0.45 µm). Finally, the sample was placed in a UV-Vis spectrophotometer. All absorbance measurements were obtained in a quartz cuvette (1 cm optical path length), from 200-700 nm even though the wavelength of naltrexone was stipulated to about 280.65 nm.

Preparation of sample solution of capsules containing 4.5 mg NLX (sample-2)

Sample solution was prepared using sample stock solution (stock-2). 2.5 mL stock-2 solution was taken into 5 mL volumetric flask and diluted to 5 mL with distilled water to obtain a concentration of 0.24 mg/mL.

The absorbances of the standard, sample-1 and sample-2 solutions were measured by UV-spectrophotometer. The assay amounts of these solutions were calculated according to the UV absorbance results.

Force Degradation Study

Forced degradation of NLX solutions was carried out under acid/base hydrolysis. 0.1 N HCl and 0.1 N NaOH solutions were prepared for the force degradation method.

Preparation of sample solution of capsules containing 3 mg and 4.5 mg NLX via acid induced hydrolysis (sample-3 and sample-4)

For the preparation of capsule solutions containing 3 mg and 4.5 mg NLX, 2.5 mL of the sample stock of each NLX (stock-1 and stock-2) were taken into 5 mL volumetric flask separately, and 0.5 mL

acid was added. The solutions were stirred for 3 hours on the magnetic stirrer by protecting from light. After 3 hours, the solutions were neutralized with 0.5 mL base. The volume was completed to 5 mL by using distilled water and then filtered through a quantitative cellulose filter (Millipore Millex- HN, Nylon 0.45 µm). Finally, the absorbance was measured by UV-Vis spectrophotometer.

Preparation of sample solution of capsules containing 3 mg and 4.5 mg NLX via base induced hydrolysis (sample-5 and sample-6)

For the preparation of capsule solutions containing 3 mg and 4.5 mg NLX, 2.5 mL of the sample stock of each NLX (stock-1 and stock-2) were taken into 5 mL volumetric flask separately, and 0.5 mL base was added. The solutions were stirred for 3 hours on the magnetic stirrer by protecting from light. After 3 hours, the solutions were neutralized with 0.5 mL acid. The volume was completed to 5 mL by using distilled water and then filtered through a quantitative cellulose filter (Millipore Millex- HN, Nylon 0.45 µm). Finally, the absorbance was measured by UV-Vis spectrophotometer.

The absorbances of the sample-3, sample-4, sample-5 and sample-6 solutions were measured by UV-spectrophotometer. The assay amounts of these solutions were calculated according to the UV absorbance results.

RESULTS AND DISCUSSION

The UV absorbance values of NLX standard, capsules containing 3 mg and 4.5 mg NLX are shown in Table 1. The naltrexone peak appeared at 280.65 nm. The aborbance values of the standard, capsules containing 3 mg and 4.5 mg NLX solutions were 0.85139, 0.83068 and 0.83173, respectively.

Table 1. The UV absorbance results of NLX standard and drugs containing 3 mg and 4.5 mg NLX.

Type of the solution	Concentration (mg/mL)	Wavelength (nm)	Absorbance
Standard of naltrexone	0.24	280.65	0.85139
Capsule containing 3 mg naltrexone	0.23	280.65	0.83068
Capsule containing 4.5 mg naltrexone	0.23	280.65	0.83173

The UV absorbances of the sample-3, sample-4, sample-5 and sample-6 solutions are shown in Table 2. The assay amounts of the solutions calculated according to the UV absorbance results are given in Table-3.

Table 2. The absorbances of the solutions 3 mg and 4.5 mg naltrexone with acid and base.

Type of the solution	Wavelength (nm)	Absorbance
Capsule containing 3 mg naltrexone with acid (sample-3)	280.65	0.70930
Capsule containing 3 mg naltrexone with base (sample-4)	280.65	0.75815
Capsule containing 4.5 mg naltrexone with acid (sample-5)	280.65	0.72241
Capsule containing 4.5 mg naltrexone with base (sample-6)	280.65	0.73349

Table 3. Assay analysis before and after forced degradation.

Type of the solution	Assay (%)
Capsule containing 3 mg naltrexone	101.81
Capsule containing 4.5 mg naltrexone	101.93
Capsule containing 3 mg naltrexone with acid	86.89
Capsule containing 3 mg naltrexone with base	92.92
Capsule containing 4.5 mg naltrexone with acid	88.54
Capsule containing 4.5 mg naltrexone with base	89.90

It is obviously clear that the treatment of NLX drugs with acid or base decreases the assay amounts of the NLX capsules. The degraded amounts are shown in Table 4.

Table 4. Summary of forced degradation study of naltrexone drug.

Stress condition	Time (h)	Assay for the capsule containing 3 mg NLX (%)	Assay for the capsule containing 4.5 mg NLX (%)	Remark
Acid hydrolysis	3	86.89	88.54	Degradation observed
Base hydrolysis	3	92.92	89.90	Degradation observed

UV spectra of the standard and all samples of naltrexone are given in Figure 1.

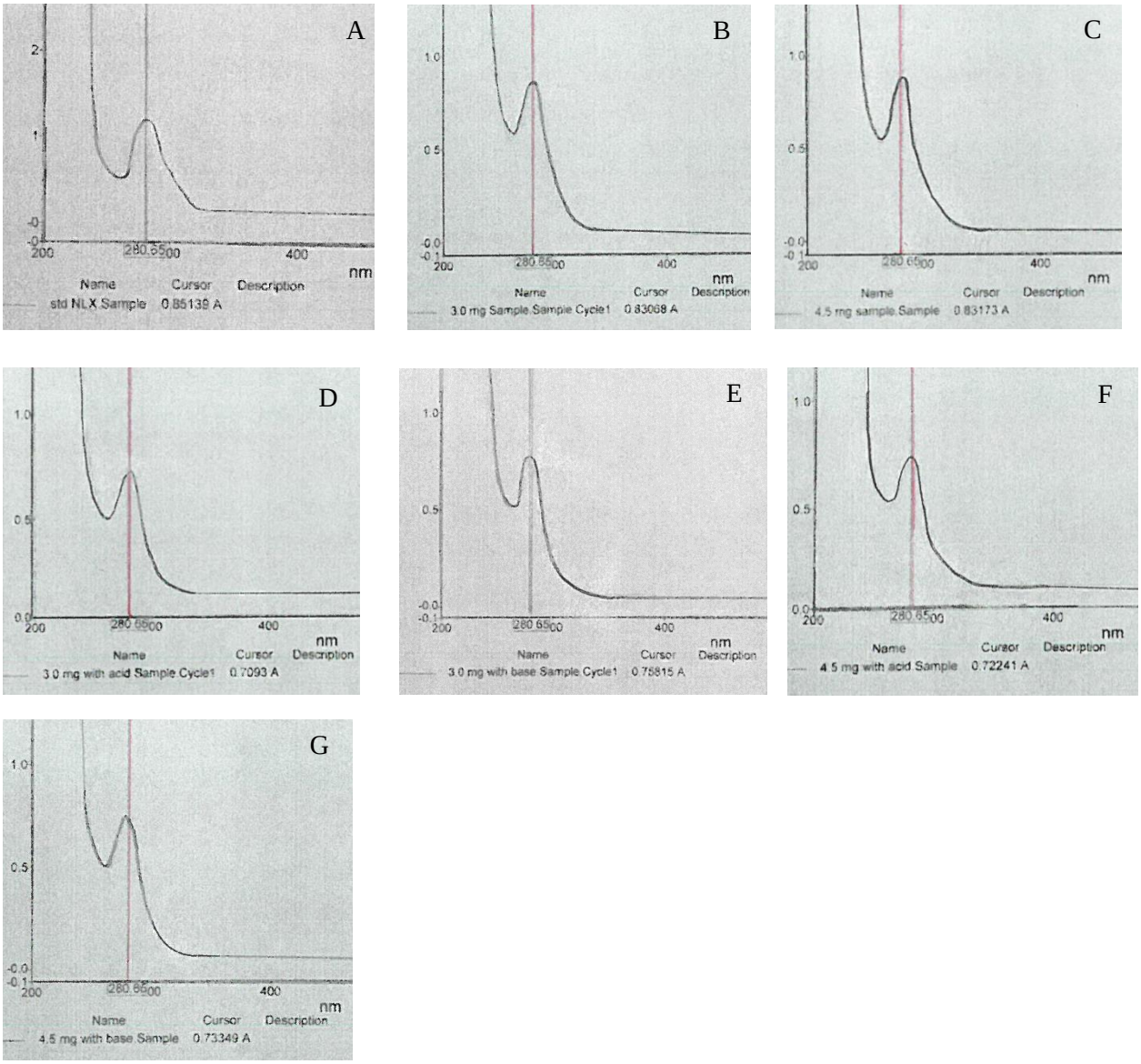

Figure 1. The UV spectra of NLX standard and NLX samples. **A)** The peak of standard of naltrexone **B)** The peak of capsule containing 3 mg naltrexone **C)** The peak of capsule containing 4.5 mg naltrexone **D)** The peak of capsule containing 3 mg naltrexone with acid **E)** The peak of capsule containing 3 mg naltrexone with base **F)** The peak of capsule containing 4.5 mg naltrexone with acid **G)** The peak of capsule containing 4.5 mg naltrexone with base.

Forced degradation studies can help to understand the degradation pathways. Moreover, it determines the active ingredients in the drug and it helps to clarify the structure of the degradants. The information gained from the stability analysis can be helpful for improving the formulation, manufacturing, and storage of the drug. In order to have adequate time to get more information about the stability of the molecule, it is better to start degradation studies earlier in the drug development process.

The aim of this experiment was to compare the assay analysis before and after acidic and basic forced degradation reaction. As it was shown, degradation was observed for naltrexone sample during acidic and basic stress conditions. Naltrexone was degraded in acidic and basic buffer. For acidic degradation of 3 mg naltrexone, the amount decreased from 101.81% to 86.89% and for 4.5 mg naltrexone, the amount decreased from 101.93% to 88.54%. On the other hand, for basic degradation of 3 mg naltrexone, the amount decreased from 101.81% to 92.92% and for 4.5 mg naltrexone, the amount decreased from 101.93% to 89.90%.

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Research Article EMUJPharmSci ISSN 2651-3587 https://dergipark.org.tr/emujpharmsci

EMU Journal of Pharmaceutical Sciences

EMUJPharmSci

Healthy life-style patterns of pharmacists in Turkey

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Abstract

Healthy Life-Style Behavior is defined as all the behaviors one engages in to maintain health, including health responsibility, nutrition, exercise, spiritual development, interpersonal relationships, stress management, and protection from disease. Therefore, the aim of this study was to evaluate the life-styles of pharmacists in Turkey.

This is a cross-sectional study, with a universe of 24,925 pharmacists in Turkey. While calculating the sample size, expected prevalence was predicted as 50% (often unknown), standard deviation as 5%, confidence interval as 95%, and design effect as 1.0. For the sample size, a randomization table was used with the pharmacists list, and 10% were selected as spare. In total, the life-styles of 398 pharmacists were evaluated, using the "Healthy Life Style Behavior of The Pharmacists in Turkey Questionnaire" and the "Healthy Life-Style Behavior Scale."

The highest scores on the Healthy Life-Style Behavior Scale were on the spirituality subgroup (27.57 $\pm$ 3.69). This group consists of interpersonal relations (26.29 $\pm$ 3.61), nutrition (24.58 $\pm$ 4.39), physical activity (20.34 $\pm$ 5.23), health responsibility (19.44 $\pm$ 3.86), and stress management (19.43 $\pm$ 3.22).

New interventional methods, awareness policies, and strategies are required for pharmacists.

Keywords

Awareness policies, behavior, Healthy Life-Style, pharmacists.

Article History

Submitted: 21 June 2019 Accepted: 02 December 2019 Published Online: 30 December 2019

Article Info

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Research Article:
Volume: 2 Issue: 2 December 2019 Pages: 84-94

INTRODUCTION

Health is defined by the World Health Organization (WHO) as "a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity." (WHO, 2019). According to WHO, the causes of 70-80% of deaths in developed countries and 40-50% of deaths in less-developed countries are diseases causes by unhealthy life-styles. Because of this, health services offered to patients should include protection from disease, sustainability, and health improvement (Dickey and Janick, 2001; Yalçınkaya *et al.*, 2007).

A healthy life-style is defined as maintaining self-control over all behaviors that may affect one's health and organizing one's daily activities by selecting the appropriate behavioral pattern for one's health status (Yalçınkaya *et al.*, 2007; Zaybak and Fadıloğlu, 2004). Healthy life-style behavior (HLSB) is defined as all the behaviors one engages in to maintain health, including health responsibility, nutrition, exercise, spiritual development, interpersonal relationships, stress management, and protection from disease (Özkan and Yılmaz, 2008). Healthy life-style is an important factor in protection from disease and improving health. For instance, according to the "National Disease Burden Study" and "Global Health Risks Report" that were conducted in Turkey, the fundamental risk factors in preventing chronic disease are preventable, controllable, and changeable life-styles (WHO, 2009; T.C. Sağlık Bakanlığı, 2006).

Healthcare professionals have important roles and responsibilities in improving and maintaining healthy life-style behaviors. Healthcare professionals can model healthy life-styles and influence the individuals they provide with health education (Ecevit *et al.*, 2003). One particular type of healthcare professional that the public frequently comes into contact with is the pharmacist. According to the data in the "2016 Turkish Pharmacists Association Database," 25,453 pharmacists who own a pharmacy in 2015 and 24,928 pharmacists who own a pharmacy in 2016 (TEB, 2016).

In the "Regulations about Pharmacists and Pharmacies" that were published in Turkey by the Official Gazette in 2014, a

"pharmacist" is defined as a professional authority for conducting pharmacy activities, and a "pharmacy" is defined as an institution that is responsible for: preparing pharmaceuticals from natural and synthetic medicinal substances that are used for protection from diseases; diagnosis and treatment of diseases; presentation of medicine to the patient; analyzing medicine, continuation of pharmacological effect, and surveillance for safety, effectiveness, and cost; providing standardization and quality control for medicine; informing patients about problems regarding the use of medicine and health services; and conducting facilities in accordance with the report of subsequent health problems (Resmi Gazete, 2014). This study aimed to evaluate the healthy life-style patterns of pharmacists, who serve as consultants and are more easily accessible to the public than other healthcare professionals. The findings can serve as a reference for new interventional studies about pharmacists. To our knowledge, this is the first study to investigate the HLSBs of pharmacists in Turkey.

MATERIALS AND METHODS

This study is cross-sectional, with a universe of 24,928 pharmacists in Turkey. While calculating sample size, expected prevalence was predicted as 50% (often unknown), standard deviation as 5%, confidence interval as 95%, and design effect as 1.0. Sample size was calculated as 379, using a randomization table with the pharmacists list; 10% were selected as spare.

This study was conducted in May-August 2017 and all participants were working in their pharmacy stores.

In Turkey, all private pharmacists are members of the union, so the Association has a list of e-mail addresses and telephone numbers, which was utilized for this study. Then, informed consent forms were obtained from all of the participants. The survey was conducted by telephone.

In total, the life-styles of 398 pharmacists were evaluated, using the "Healthy Life-style Behavior of The Pharmacists in Turkey Questionnaire (which is include demographic

informations)" and the "Healthy Life-style Behavior Scale" (HLSBS-II).

The HLSBS-II was developed by Walker, Sechrist, and Pender in 1987 to measure health improvement behaviors in relation to individuals' healthy life-styles. This scale consists of 52 items in six subgroups (Table 1) (Esin, 1999):

- **Health Responsibility**, which determines the level of contribution to and responsibility for one's health.
- **Physical Activity**, which shows the level of physical activity, an essential factor of healthy life.
- **Nutrition**, which determines changes in an individual's selection and regulation of meals and food.
- **Spirituality**, which determines life goals, self-development ability, self-awareness, and self-satisfaction.
- **Interpersonal Relations**, which determines the level of communication and sustainability with one's inner social circle.
- **Stress Management**, which determines the level of recognition of stress sources and stress control mechanisms.

Table 1: Healthy Life-Style Behavior Scale subgroups.

Subgroups	Question Numbers in Scale	Lowest Possible Score	Highest Possible Score
Health responsibility	3, 9, 15, 21, 27, 33, 39, 45, 51	9	36
Physical activity	4, 10, 16, 22, 28, 34, 40, 46	8	32
Nutrition	2, 8, 14, 20, 26, 32, 38, 44, 48	9	36
Spirituality	6, 12, 18, 24, 30, 36, 42, 46, 50,52	9	36
Interpersonal Relations	1, 7, 13, 19, 25, 31, 37, 43, 49	9	36
Stress management	5, 11, 17, 23, 29, 35, 41, 47	8	32
Total		52	208

Each subgroup can be scored independently. The total score on all scales yields the healthy life-style behavior score. While the scale originally consisted of 48 items, currently it consists of 52 items after the addition of 4 items by Walker, Sechrist, and Pender in 1996. The only difference in the new 52-item scale is the number of items.

All items on the HLSBS-II are positive. It uses a 4-point Likert scale, with 1 for

"Never," 2 for "Sometimes," 3 for "Often," and 4 for "Regularly." The lowest possible score is 52 and the highest is 208. The validity and reliability of the HLSBS-II were verified by Esin in 1997, and the Cronbach Alpha internal consistency coefficient was found to be 0.91 (Esin, 1999). The present study's data was evaluated with the SPSS 21.0 statistics package. The variables were analyzed by visual (histogram and probability graphs) and analytical methods (Kolmogorov-Smirnov/Shapiro-wilk), and descriptive statistics were presented as mean (±) standard deviation, frequency distribution, and percentage. Our study protocol was approved by the Gazi University institutional review board, and all participants provided informed consent in the format required by the board.

RESULTS

The average age of the 398 pharmacists who participated in the study was 43.3 ± 8.7 and the median age was 46 (minimum: 26; maximum: 67). The ages of 9% of participants were between 18 and 29, the ages of 22.6% of participants were between 30 and 39, the ages of 48.2% of participants were between 40 and 49, the ages of 19.1% of participants were between 50 and 64, and the ages of 1.1% of participants were over 65 years of age. 58.5% of participants were women and 41.2% were men, with the majority of them being over 40 years of age. The average weight (kg) of participants was 73.40 $\pm$ 10.06 and the median weight was 72 (minimum: 52; maximum: 97). The average body mass index (BMI) (kg/m^2) was 25.91 $\pm$ 2.87 and the median BMI was 25.91 (minimum: 19; maximum: 34).

A total of 86.4% of participants did not use alcohol and 13.6% did. While 29.9% of participants did not smoke cigarettes, 12.3% did, and 57.8% of them had smoked cigarettes but had stopped.

A total of 75.6% of participants did not have any chronic diseases (Table 2). The most common disease was thyroid disease (67.4%) and the most common type of medicine used was anti-hypertensive (26.3%).

Table 2: Number of participants' for the presence of chronic diseases diagnosed by physicians**'.**

	Number	**(%)***
Presence of chronic disease diagnosed by physicians' (n=398)		
No	303	75,6
Yes	95	24,4
Presence of chronic diseases (n=95)**		
Thyroid	64	67,4
Hypertension	16	16,8
Diabet	10	10,5
Chronic lung diseases	7	7,4
Cardiovascular system diseases	4	4,2
Other***	2	2,1

*Column percentage
**More than one answer was given to the question. Percentages are calculated from the given answers.
***Among the other, the most common is menier vertigo.

According to participants' answers on the HLSBS-II (Table 3), the spirituality (27.57 ± 3.69) subgroup had the highest score. The other scores were interpersonal relations (26.29 ± 3.61), nutrition (24.58 ± 4.39), physical activity (20.34 ± 5.23), and health responsibility (19.44 ± 3.86). Stress management (19.43 ± 3.22) was the lowest, ranked at the bottom. The median subgroup score was 139 (minimum: 76; maximum: 196). Spirituality, interpersonal relations, and nutrition subgroups had the highest median scores, and the average of the other four subgroups was similar.

Table 3: Participants' total scores and item averages for subgroups on the Healthy Life-Style Behavior Scale.

HLSB-II Subgroups	**Average Total Subscale Score**	**Median (minimum–maximum)**
Health responsibility (n = 398)	19.44 ± 3.86	20 (10–28)
Physical activity (n = 398)	20.34 ± 5.23	21 (8–32)
Nutrition (n = 398)	24.58 ± 4.39	25 (10–36)
Spirituality (n = 398)	27.57 ± 3.69	27 (18–36)
Interpersonal relations (n = 398)	26.29 ± 3.61	26 (19–36)
Stress management (n = 398)	19.43 ± 3.22	20 (11–28)
Total (n = 398)	137.65 ± 24.00	139 (76–196)

According to the distribution of score averages, several interesting facts emerge:

- Item 4, "I follow a regular exercise program," had the lowest score (2.35 ± 0.81).
- Item 46, "I feel that I have a relationship with a divine power," had the highest score (3.63 ± 0.50).
- There are no items whose average score was below 2 (meaning "never" or "sometimes").
- The items whose average score was above 3 (meaning "often" or "regularly") are presented in Table 4.

Table 4: Propositions on the Healthy Life-Style Behavior Scale with a median participant score above 3.

Item No	Proposition	Median (minimum-maximum)
49	When I need advice and guidance from others, I receive it.	3.04 ± 0.72
24	It is easy for me to show interest, love, and closeness to others.	3.05 ± 0.68
17	I look to the future with hope.	3.05 ± 0.64
18	I spend time with my best friends.	3.06 ± 0.67
47	I resolve conflicts with communication and compromise.	3,08 ± 0,56
7	I appreciate the success of other people.	3.10 ± 0.61
30	I embrace the people I love.	3.10 ± 0.65
23	I feel adequate and at peace with myself.	3.14 ± 0.69
12	I believe that my life has a purpose.	3.16 ± 0.67
13	I maintain meaningful, satisfactory relationships with people.	3.17 ± 0.63
50	I'm open to new experiences and situations.	3.18 ± 0.68
48	I eat breakfast regularly.	3.21 ± 0.84
46	I feel that I have a relationship with a divine power.	3.63 ± 0.50

"When I need advice and guidance from others, I receive it" is the only Health Responsibility subgroup proposition with an average score of 3 or above. None of the Physical Activity subgroup propositions had an average score of above 3. A score of 3 or higher was rarely given to Physical Activity propositions. "I eat breakfast regularly" was the only Nutrition subgroup proposition with an average score above 3, and an answer of 3 or higher was rarely given to these propositions. In the Spirituality subgroup, "I feel that I have a relationship with a divine power" had the highest average score of any proposition.

All other propositions with an average score of 3 or higher belong to the Interpersonal Relations subscale.

DISCUSSION

The present study measured the HLSBs of pharmacists throughout Turkey. Social-demographic properties were evaluated using questions about health protection behaviors and habits on the HLSB-II scale (52 items) questionnaire form. To our knowledge, this is the first study to investigate the HLSBs of pharmacists in Turkey.

According to WHO, 60% of all deaths are linked to chronic disease, and this number is expected to increase to 75% by 2020 (WHO, 2019). In the case of Turkey, seven of the top ten diseases that cause death are chronic diseases (T.C. Sağlık Bakanlığı, 2013).

In this study, 24.4% of participants had a chronic disease. Hypertension frequency was the most common at 16.8%, which is lower than average in Turkey. According to the conclusions of the Turkish Hypertension Prevalence Study-2012 (Türk Hipertansiyon ve Böbrek Hastalıkları Derneği, 2012) and the Türkiye Diyabet Prevelans Çalışması-II (TURDEP-II) study (Satman *et al*., 2013), hypertension frequency was 30% in Turkey. In our research, the smoking frequency was 12.3%, which is quite low. The overall frequency in Turkey was reported as 31% in the "Global Adult Tobacco Investigation Turkey Report-2010" of the Ministry of Health, 33% in "Attitudes and Behavior Investigation on Tobacco, Alcohol and Substance Use in General Population-2011" and 30% in "Health Statistics Annual-2011" (T.C. Sağlık Bakanlığı, 2011; 2012; Türkiye Uyuşturucu ve Uyuşturucu Bağımlılığı Merkezi, 2012; TÜİK, 2012). The low frequency in pharmacists may create a positive effect on societal behavior, because healthcare providers are role models of HLSBs. Healthcare professionals should be more conscious about living a healthy life-style and applying those principles to daily life. When healthcare professionals give advice to the other people about living a healthy life-style, firstly they need to maintain these principles in their own lives so that their advice can impact other people's lives as well as provide motivation and courage for people to apply the advice to their own lives. In Turkey, as well as in many other countries, when a person buys medicine, they often disclose information that they do not even tell their doctors; this is exactly why we give pharmacy students

lessons about communication and consultancy.

In our sample, the frequency of alcohol use was 13.6%. According to the 2011 Turkish Ministry of Health report "The Frequency of Turkey Chronic Diseases and Risk Factors Study," overall alcohol consumption frequency in Turkey is 13% (TÜİK, 2012), which is similar to our result.

The HLSB-II answers of our participants indicated that the reason for the low level of physical activity was a combination of intensive work, difficult living conditions, a lack of fitness habits, and a lack of adequate public fitness facilities.

One of the reason for having a chronic disease may be inadequate implementation of HLSBs. The scores of these healthcare providers, who can serve as role models, on HLSB issues are low, and they should work hard to adjust it. Healthcare providers should act more consciously about HLSB issues and implement these behaviors.

Future studies should take the data obtained in this study into account, and investigate how HLSBs can be integrated into the daily life of pharmacists. This is necessary to ensure that courses about health protection and improvement can be added to the curriculum of pharmaceutical study, then discuss concrete ways to put these things into practice in daily life and identify the issues that pharmacists tend to do a poor job with (that is, healthy life-style, coping with stress, nutrition, exercise, etc.). This training is necessary for pharmacists to develop HLSBs. Moreover, the effect of the spirituality subgroup of the HLSB-II on HLSBs, which was the subgroup with the highest median score, should be investigated with a conclusion-interference study with the purpose of increasing this score. Studies should also be conducted on how to improve socio-economic conditions in order to create healthier life-styles for pharmacists. These methods may include the creation of places where health providers can do physical activities during leisure time at their offices, providing more balanced and healthier nutrition at the office, providing a stress-free workplace environment, education about not smoking and the benefits of physical training programs, providing easier-to-utilize health services, and carrying out more health screening programs.

In conclusion, new interventional methods, awareness policies, and strategies are required for pharmacists.

These can be designed by conducting qualitative and quantitative studies that investigate the factors of pharmacists' adoption of healthy life-style attitudes and behaviors.

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Research Article EMUJPharmSci ISSN 2651-3587 https://dergipark.org.tr/emujpharmsci

EMU Journal of Pharmaceutical Sciences

EMUJPharmSci

Plant biodiversity and unique yew stands of Istranca (Yıldız) mountains in European Turkey

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Abstract

Population of *Taxus baccata* in the western point of Turkey has been identified in cooperation with Central Anatolia Forestry Research Institute and Bulgarian Academy of Science (BAS) during the project entitled "Conservation and Sustainable use of Biodiversity in Istranca (Yıldız) Mountain – Challenges and Opportunities for Promotion and Implementation of the Transboundary Biosphere Reserve Concept (37534303 TUR)". *Taxus baccata* hot spot contains *Cylamen coum* var. *coum*; an endangered species according to the Bern Convention appendix I list, on European scale. The most important characteristics of this population is the height of the trees. They are also the secondary tree population in the rainforest, which are completely grown under shade conditions.

Keywords

Conservation, European Turkey, *Taxus*, *Taxus baccata*, yew

Article History

Submitted: 2 December 2019 Accepted: 30 December 2019 Published Online: 30 December 2019

Article Info

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Research Article:

Volume: 2 Issue: 2 December 2019 Pages: 95-101

INTRODUCTION

Taxus baccata L. (yew) is a small, evergreen, dioecious and coniferous tree which can grow up to 20 meters in height. The bark of the tree is reddish-brown. Leaves are alternate, needleless and narrow with flattened margins. The ripe fruit consists of a seed enclosed by a red fleshy cup-shaped structure, named as aril (Figure 1). All parts of the plants, especially the seeds, except the red arils are extremely poisonous. The red arils contain taxol modified diterpenes which, interestingly, have a broad spectrum of activity (Trease and Evans 1983).

Figure 1: An illustration of *Taxus baccata* (Hand drawn by Gülten Yeğenağa).

Taxus baccata is distributed naturally to western, central and southern Europe, northwest Africa, northern Iran and southwest Asia (Tutin *et al*., 1964-1980). In Turkey, it is mainly spread out along North Anatolia. It is often planted out as an ornament in parks and gardens with several different varieties.

***Taxus baccata* in European Turkey**

European Turkey (Thrace) occupies a small part of Turkey, and has a surface area of 23.500 km². It is situated in the north side of the Sea of Marmara, which connects the Black Sea and Aegean Sea via the straits of Bosphorus and Dardanelles that separate Europe from Asia. In comparison with Turkey's general topography, Thrace generally has low elevations. Davis (1965-1985), Webb (1966), Tutin *et al*., (1964-1980) and Turill (1924) published the principal references that dealt with the flora of European Turkey. Apart from these main references, several recent papers, published by Baytop & Byfield (1997), Özhatay (1975), Özhatay *et al*., (2003), Seçmen and Leblebici (1991, 1997), Yarcı (1997, 1999),

Yıldız (2009) and several check-lists, such as "Flowering plant & Fern of European Turkey" and "Check-list of additional taxa to the supplement flora of Turkey: Checklist III, IV, V, VI, VII, VIII and IX (Özhatay and Kültür 2006; Özhatay *et a*l., 2009, 2011, 2013, 2015, 2017, 2019)" have been published.

The distribution of *Taxus baccata* forest in European Turkey has been determined during two different projects:

Project I: 'Conservation and Sustainable use of Biodiversity in Istranca (Yıldız) Mountain 2007'. *Taxus baccata* forest was declared as Gene Conservation Forest (Mehtap & Karadağ 2008).

Project II: The plant diversity survey for the 'Yıldız Mountains Biosphere Project' was conducted between May to October 2009. The project area was located in the North-western corner of European part of Turkey and covered about 1300 km². The overall purpose of the Yıldız Mountain Biosphere Project was to assist landscape scale conservation of biodiversity of Yıldız Mountains in a long-term plan.

Floristically, flowering plants and ferns (vascular plants) were the focus of the field survey (Akalın *et al*., 2013). A total of 1364 plant taxa (1273 species) have been recorded from the project area. Within the study area, 15 endemic taxa and 55 rare species have been recorded, including *Allium rumelicum* Koçyiğit & Ozhatay (Koçyiğit and Özhatay, 2010) and *Allium urusakiorum* Ozhatay & Seregin (Kocyigit *et al*., 2016) introduced as new species to science.

Eleven floristic hot spots were assessed, identifying the İğneada and Kasatura areas as high-importance centres of plant diversity. Their coastline and sand dune habitats support the highest diversity of rare and endemic species. Figure 2 shows the boundry of the project area in North-western corner of European Turkey with bold black lines.

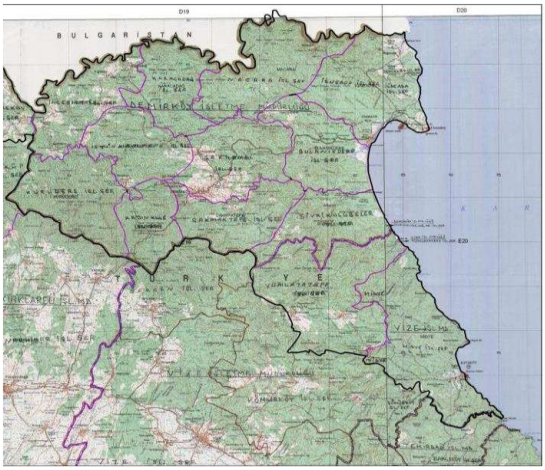

Figure 2: Boundry of the project area in North-western corner of European Turkey.

MATERIALS AND METHODS

The materials of *Taxus baccata* were collected and examined during the surveys of two different projects:

Project I: Conservation and Sustainable use of Biodiversity in Istranca (Yıldız) Mountain – Challenges and Opportunities for Promotion and Implementation of the Transboundary Biosphere Reserve Concept (37534303 TUR) (2008) project was carried out in cooperation with Central Anatolia Forestry Research Institute and Bulgarian Academy of Science (BAS). The field survey was performed using smallest area estimation method (Poore 1955), in a specific area of the forest which has a rich population of *Fagus orientalis*. During the field studies of the population of *Taxus baccata*, approximately 50 individuals were counted. The area was determined as Gene Protection Forest.

Project II: Yıldız Mountains Biosphere Project supported by EuropeAid/125289/D/SER/TR. The plant diversity survey for the 'Yıldız Mountains Biosphere Project' was conducted during May-October 2009. Flowering plants and ferns (vascular plants) were the focus of the field survey. The specimens, that were collected and examined, were kept within the herbaria via ISTE (Herbarium of the University Istanbul, Faculty of Pharmacy) and ANKO (Herbarium of Ankara Forestry Research Institute).

RESULTS

Short description of *Taxus baccata* within the protected area of European Turkey is shown in Table 1.

Table 1: Short description of Taxus baccata within the protected area of European Turkey.

Protected area: A1 (E) Kirklareli/Demirköy - Mahya Mountain (Northwest)
Size: 121,5 ha
Characteristic vegetation and habitat: Beech forests and *Taxus baccata* (yew) forests
Number of plant species: 52 (taxa)
Number of endemic species: None
Conservation status: ÖBA NO 4 - Strandzha Mountains
Gene Conservation Forest: Yew (*Taxus baccata*)

Flora and vegetation of the area

The area, containing approximately 50 individuals of *Taxus baccata* trees, is under widespread *Fagus orientalis* (Oriental beech) forests and in between dense *Rhododendron ponticum* ssp. *ponticum* (*Rhododendoron* with pink flower) bushes.

This habitat is utterly dense and receives less sunlight. *Fagus orientalis* (oriental beech) trees cover this area with a dense forest vegetation. *Acer trautvetteri* (maple), *Sambucus nigra* (elderberry) and *Laurocerasus officinalis* (cherry laurel) are found in the spaces near forest roads. There are few individuals of *Ilex colchica* (restharrow) and *Daphne pontica* within a part of the forest under low sunlight.

Cylamen coum var. *coum* and *Anemone nemorosa* are endangered species according to the national scale of Bern Convention Appendix I List.

Taxus baccata hot spot includes 2 endangered habitats as stated by Bern Convention Appendix IV List. These are 41.1E122 Strandzha Mountains (Istranca) *Rhododendron* – Oriental beech forests and 42.A7 Western palearctic yew forest communities. The list of the species which occur in the *Taxus baccata* hot spot:

Acer pseudoplatanus L.
Acer trautvetteri Medw.
Anemone nemorosa L.
Anthemis cretica L.subsp. tenuiloba (DC.) Grierson
Asplenium adianthum – nigrum L.
Aphodeline lutea (L.) Reichb.
Bellis perennis L.
Brassica nigra (L.) Koch
Calamintha grandiflora (L.) Moench
Campanula rapunculus L.var. lambertiana (A. DC.) Boiss.
Cardamine bulbifera (L.) Crantz. (Foto 1)
Ceterach officinarum DC.
Cornus mas L.
Cyclamen coum Miller var. coum
Daphne pontica L.
Digitalis viridiflora Lindley
Dorycnium graecum (L.) Ser.
Epilobium angustifolium L.
Fagus orientalis Lipsky
Fagus sylvatica L.
Fragaria vesca L.
Galium rotundifolium L.
Genista carinalis Gris.
Gentiana asclepiadea L.
Geranium robertianum L.
Hedera helix L.
Hypericum androsaemum L.
Hypericum bithynicum Boiss.
Ilex colchica Poj.
Laurocerasus officinalis Roemer
Ligustrum vulgare L.
Limodorum abortivum (L.) Swartz
Lysimachia punctata L.
Petasites hybridus (L.) Gaertner
Polygonatum hirtum (Bosc ex Poiret) Pursh
Polypodium vulgare L. subsp. vulgare
Primula vulgaris Huds subsp. vulgaris
Prunus spinosa L. subsp. dasyphylla (Schur) Domin
Pteridium aquilinum (L.) Kuhn.
Rhododendron ponticum L. subsp. ponticum
Ruscus hypoglossum L.

DISCUSSION

Taxus baccata (yew) communities in *Taxus baccata* hot spot are declared as Gene Conservation Forests in 2010 (OGM Or-Tohum, 2010). Since then, the area had gained conservation status. Since the area was located in Kurudere Forest Sub-District Directorate, forestry activities had continued until 2007. However, there was no logging during the breeding period on the authority of management policy for a decade before 2007. Furthermore, by the same management policy, road maintenance had not been done and the access into the hot spot area was very difficult. Hence, the area was kept free from human disturbance and pollution.

Taxus baccata hot spot contains *Cylamen coum* var. *coum*; an endangered species according to Bern Convention appendix I list, on European scale. Additionally, on national scale, the endangered species *Acer pseudoplatanus*, *Anemone nemorosa*, and *Digitalis viridiflora* are present in the area. Besides the two endangered habitats, Strandzha Mountains *Rhododendron*–Oriental beech forests and Western palearctic yew forest communities, the area is surrounded by *Rhododendron ponticum* ssp. *ponticum* community within dense oriental beech (*Fagus orientalis*) forest. This habitat is utterly dense and receives low sunlight. *Acer trautvetteri* (maple), *Sambucus nigra* (elderberry) and *Laurocerasus officinalis* (cherry laurel) are found in the spaces near forest roads. The area has only one gene conservation region. Silviculture activities continue in the area. There was no logging in the last decade. Silviculture activities should be planned and monitored in a sustainable manner and the changes, which might result from biotic and abiotic factors, should be observed and recorded.

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Review EMUJPharmSci ISSN 2651-3587 https://dergipark.org.tr/emujpharmsci

EMU Journal of Pharmaceutical Sciences

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The metabolites of ellagitannin metabolism urolithins display various biological activities

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Abstract

Dietary consumption to various nuts, berries, and particularly pomegranate is an important source of ellagitannins. These molecules are particularly subject to gastrointestinal metabolism producing urolithins as their metabolites. Urolithins (i.e., hydroxylated benzo[c]chromen-6-one analogues) have a greater absorption than the ellagitannins thus; greater bioavailability is of great significance. Therefore, the biological activities obtained through the use of ellagitannin rich foods are mainly attributed to urolithins. These compounds possess a good peripheral distribution. In addition, some of their further metabolites can penetrate to the central nervous system which, is of a topic of interest for CNS related pathologies. This review has aimed to introduce the structure and metabolism related formation of different urolithins concomitant to their biological activities discovered so far in the literature.

Keywords

Antioxidant, anticancer, antimalarial, anti-inflammatory, cholinesterases, ellagitannins, metabolism, urolithins.

Article History

Submitted: 25 October 2019 Accepted: 2 December 2019 Published Online: 30 December 2019

Article Info

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Research Article:

Volume: 2 Issue: 2 December 2019 Pages: 102-110

INTRODUCTION

Ellagitannins are present in a large number of dietary sources (Clifford *et al*., 2000; Abe *et al*., 2010). Nuts, berries, and pomegranate are rich sources of ellagitannins (Garcia-Villalba *et al*., 2015). So far, numerous studies have been conducted to investigate nutraceutical potential and biological actions of ellagitannin rich foods (Okuda *et al*., 1989; Lipińska *et al*., 2014). Some of these studies have mainly exploited different extracts of related plants, particularly the fruits.

Ellagitannin is a macromolecule condensed with glucose units and upon metabolism releases ellagic acid (Quideau *et al*., 1996; Seeram *et al*., 2006). Regarding the nature of the chemical composition of ellagitannin, it is quite difficult to attribute the resulting biological actions for such a big molecule (González-Barrio *et al*., 2010). Indeed, metabolism studies have shown that ellagitannins are subject to gastrointestinal system metabolism yielding out the disintegration of sugar units to produce ellagic acid (Seeram *et al*., 2004). It is known that ellagic acid has almost no absorption potential (Lei *et al*., 2003). In other words, it has negligible bioavailability. From this perspective, it is very difficult to relate the biological actions of ellagitannin rich food to ellagitannins and ellagic acid.

So far, numerous studies have been conducted for the investigation of the metabolism of ellagitannins and ellagic acid in various living things including mammalian and non-mammalian species. These studies point out an ellagitannin initiated metabolism cascade that leads to the microbiota dependent formation of urolithins in the gastrointestinal tract (Tomas-Barberan *et al*., 2014; Landete, 2011; Selma *et al*., 2014; García-Villalba *et al*., 2013).

Urolithins are hydroxylated benzo[c]chromen-6-one derivatives (Figure 1). Regarding the metabolism pathway, poly-hydroxylated urolithins are produced first, and then they are further subject to produce less hydroxylated metabolites ending up with the main compounds such as urolithin A (i.e., 3,8-dihydroxy-6H-benzo[c]chromen-6-one) and urolithin B (i.e., 3-hydroxy-6H-benzo[c]chromen-6-one) (Giménez-Bastida *et al*., 2012; Zhao *et al*., 2018). This cascade, although it may vary on the amount and the type depending on the metabolism differences among living things, is consistent including in humankind (Bialonska *et al*., 2010). Since urolithin A and B are the major

metabolites found in systemic circulation, they are considered as biomarkers of ellagitannins (Cerdá *et al*., 2005; Tomas-Barberan *et al*., 2018).

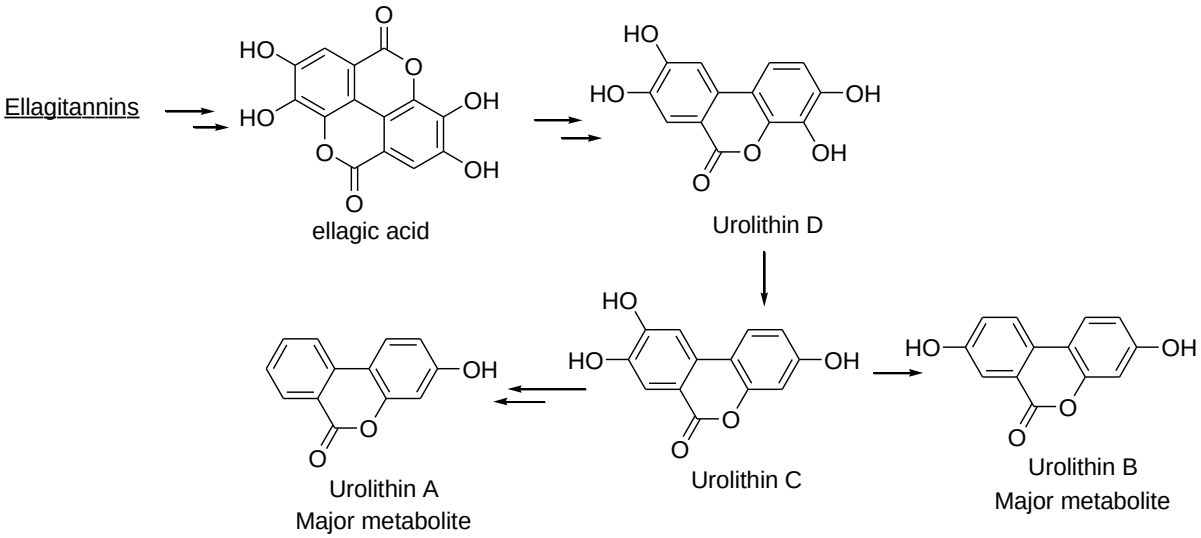


Figure 1: The formation of major urolithins through gastrointestinal tract metabolism reactions.

Metabolism studies have also pointed out that urolithin also tends to undergo further metabolism reactions, particularly phase II conjugation reactions (González-Sarrías *et al* 2014; Piwowarski *et al*., 2017; Pfundstein *et al*., 2014; González-Sarrías *et al*., 2017) (Figure 2).

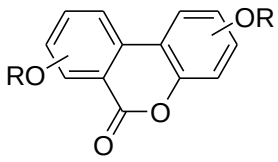


Figure 2: The phase II conjugates of urolithins.

The glucuronide and the sulfate metabolite formation through the hydroxyl groups are very common. Thus, it is not surprising to find urolithin in feces and urine. In addition, catechol-O-methyl transferase (COMT) catalyzed reactions are also observed and subsequently, methyl ether metabolites are observed within the central nervous system (Espín *et al*., 2013; Sala *et al*., 2015).

Regarding these basic features, it has been of interest for the identification of biological activities of urolithins and their metabolites for the last two decades. Therefore, the aim of this review is to focus on the key concepts of urolithins, with an emphasis on their reported biological effects. Some of these beneficial effects include antioxidant, anti-

inflammatory, anticancer, and antimicrobial effects.

Antioxidant activity

Poly-hydroxylated phenols, also considered as natural phenols found abundant in nature, are known to act as antioxidant compounds. They have been shown to be involved particularly in the prevention of certain diseases, mainly including metabolism disorders and central nervous system diseases (Scalbert *et al*., 2005; Manach *et al*., 2004). Although their mechanism of action is not proven, metal chelation and radical scavenging activities have been linked to their antioxidant activity (Hadi *et al*., 2007; Eghbaliferiz and Iranshahi, 2016). Recent findings have also pointed the significance of sulfate and glucuronide conjugates, even having function (Heleno *et al*., 2015). Since, urolithins are also hydroxylated phenolic compounds, they have been shown to act as antioxidants in various antioxidant assay systems (Bialonska *et al*., 2009; Kallio *et al*., 2013). In an earlier study by Cerdá *et al.* (2004), when urolithins were compared to ellagitannins they were reported as poorer antioxidants. It is noteworthy to mention that, radical scavenging activities have been linked to the number of hydroxyl groups. Therefore, the major urolithins (i.e., urolithin A and B) that contain only one and two hydroxyl groups, respectively are poorer antioxidants when compared to a greater number of hydroxyl groups present in urolithin C and D (Bialonska *et al*., 2009). As implied previously, the last studies on urolithins indicated the possible physiological roles of glucuronide and sulfate conjugates of urolithins.

Anti-inflammatory activity

Several studies have been conducted to evaluate the anti-inflammatory effects of urolithins on the gastrointestinal system upon the use of pomegranate juice or extract (Larrosa *et al*., 2010; Espín *et al*., 2013). Although there is no mechanistic study indicating the role of urolithins on some important inflammatory cascades, including the arachidonic acid pathway derived formation of prostaglandins, there are only a few research studies which examined the level of some inflammatory responses upon the use of urolithins. It is important to note that urolithin A has been found to be an inhibitor on the activation of nuclear factor kappa b and mitogen activated protein kinase (González-Sarrías *et al*., 2010). Moreover, it was also shown that both urolithin A and B have the potential to down-regulate the expressions of inflammation markers; COX-2 and prostaglandin E synthase (Larrosa *et al*., 2010; González-Sarrías *et al*., 2010).

Inflammation of the blood vessel wall plays a role in the development of atherosclerosis. Urolithin A glucuronide

conjugate was found to down-regulate chemokine ligand 2 and plasminogen activator inhibitor 1 thereby, inhibiting monocyte adhesion to endothelial cells (Giménez-Bastida *et al*., 2012).

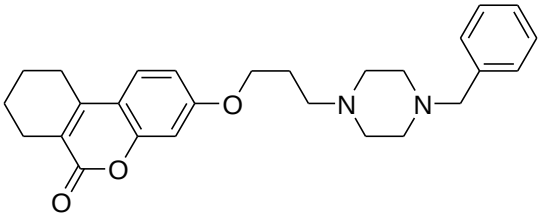
Anti-cancer activity

Anticancer activities of urolithins have been tested in various assay systems. Particularly, they were found to inhibit cancer-cell proliferation in colon, kidney, prostate, liver, breast, and bladder cancer cell lines (Tomás-Barberán *et al*., 2017). The mechanism is mainly associated with the blockage of cell cycle and the induction of apoptosis. However, it is noteworthy to state that these studies do not cover each urolithin and urolithin metabolite produced through metabolism. Furthermore, the dose used in these studies is also a topic of debate regarding the actual concentrations of urolithins found upon ellagitannin exposure.

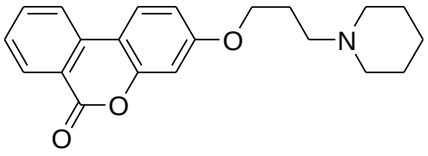
Cholinesterase inhibitory activity

Emerging role of polyphenols in neurodegenerative disorders, particularly in Alzheimer's Disease, has also been studied using pomegranate juice. In this concept, it is already known that phenolic compounds (such as ellagitannins) generally have poor potential to penetrate through blood-brain barrier. Therefore, their basic effect within the central nervous system is a question that remains to be clarified. Using in silico computational methods, Ahmed *et al.* (2014) have reported that urolithins, particularly methylated urolithin A and B, have shown possible penetration. From this point of view, methyl ether derivatives or methyl ether urolithin derived novel metabolite formation in central nervous system might be responsible for the effects within the central nervous system (Ahmed *et al.*, 2014; Yuan *et al*., 2015).

Our previous findings with urolithin B have indicated the low potential of this compound to inhibit cholinesterase enzymes A and B (i.e., around 50 µM IC_{50}) (Gulcan *et al*., 2014). The compound was shown to be more selective for acetylcholinesterase (Norouzbahari *et al*., 2018, Gulcan *et al*., 2014). Urolithins are more successful cholinesterase inhibitors, possessing low IC_{50}s. Some of these examples are shown in Figure 3.



0,9 and 3,1μM IC_{50}s, respectively for acetylcholinesterase and butyrylcholinesterase (Gulcan, HO et al 2014)



2,1 and 11,5μM IC_{50}s, respectively for acetylcholinesterase and butyrylcholinesterase (Gulcan, HO et al 2018)

Figure 3: Representative cholinesterase inhibitors derived from urolithins.

Antimalarial activity

As folk medicine, dried *Punica granatum* rinds (i.e., a source of ellagitannins and punicalagins) have long been used to treat malaria (Dell'Agli *et al.*, 2010). Recent studies have indicated that urolithins act on MMP-9 which is a proteolytic enzyme that degrades matrix proteins and associated in the pathogenesis of malaria. MMP-9 was shown to be up-regulated in haemozoin (malarial pigment) or trophozoite-fed human monocytes (Prato *et al.*, 2008). Urolithin A and B inhibited the release and expression of MMP-9, pointing out the significance of urolithins as active constituents in the traditional treatment of malaria (Dell'Agli *et al.*, 2010).

CONCLUSION

It is apparent that the research on urolithins is relatively new and more data is required to explain their preventive and protective potential in disease states, particularly at the molecular level. Many xenobiotics have the potential to act on the inhibition or activation of many proteins. From this perspective, trying to explain some of the activities of urolithins through the induction or inhibition of the expression of related protein synthesis cascades does not thoroughly display the certain mechanistic background. On the other hand, some research studies have used high concentrations of urolithins either in *in vivo* or *in vitro* experiments which are practically impossible to be reached with regular ellagitannin rich food exposure. From the medicinal chemistry perspective, urolithins are also important scaffolds to be utilized in drug design studies. Our focus continues to make

research on the design of novel urolithin derivatives with diverse biological actions, particularly focusing on the treatment of Alzheimer's Disease.

ACKNOWLEDGEMENT

The authors declare no conflict of interest.

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