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# RESEARCH ON THE CAROTENOID CONTENT (BETA-CAROTENE AND LYCOPENE) IN THE FRUITS OF *ELAEAGNUS ANGUSTIFOLIA* L.

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#### Abstract

Elaeagnus angustifolia L., is a shrub species widely used for therapeutic purposes. This plant's edible fruits are a rich natural source of bioactive compounds, particularly carotenoids, whith antioxidant, provitaminic, and protective effects against chronic degenerative diseases.

The present study aimed to evaluate the beta-carotene and lycopene content in aqueous extracts from dried fruits of E. angustifolia L., as influenced by extraction temperature (ambient, 50 °C and 80 °C). Following thermal treatment and purification via solid-phase extraction (SPE), carotenoids were quantitatively determined using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), employing atmospheric pressure chemical ionization (APCI) for detection.

The results revealed a significant increase in the carotenoid concentration correlated with extraction temperature, with peak values of 105.8 µg/g beta-carotene and 190.2 µg/g lycopene in the extract obtained at 80 °C. That moderate heat treatment boosts the release of oil-loving pigments from the plant material, even in water-based systems and without the use of organic solvents. This study outlines an efficient, environmentally friendly and reproducible extraction method. Elaeagnus angustifolia L. fruits possess a high potential as a raw material for the creation of functional food products, antioxidant dietary supplements, and standardized phytopharmaceutical extracts.

Keywords: aqueous extraction, beta-carotene, carotenoids, Elaeagnus angustifolia, lycopene, LC-MS/MS.

#### 1. INTRODUCTION

Elaeagnus angustifolia L., commonly known as silver olive or Russian olive, is a species native to Southeast Asia, yet it has adapted to numerous regions with varied climates, ranging from arid steppes to temperate areas of Europe, Asia, and North America. The plant is valued for its drought resistance,

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tolerance to poor or saline soils, and its ability to colonize degraded land, playing a significant role in land stabilization and the rehabilitation of areas affected by pollution. It is frequently used in urban green spaces due to its ornamental appearance and capacity to monitor pollution although it may become invasive.

The species is distinguished by a unique morphology, which includes an irregular stem, exfoliating bark, lanceolate silver-green leaves, fragrant campanulate flowers, and edible, sweet, floury false fruits with a yellow-orange interior that are rich in bioactive compounds.

The size (length, width, and thickness) of the fruits may vary depending on their moisture content. The literature reports average dimensions of 18.11 mm (length), 12.56 mm (width), and 12.2 mm (thickness) at a moisture content of 5.75% d.b.; or a length of 20.72 mm, width of 15.73 mm, and thickness of 14.69 mm at a moisture content of 14.43% (Zare et al., 2012).

The fruits represent a valuable natural source of carotenoids such as beta-carotene and lycopene, both of which exhibit significant antioxidant potential (Ozen et al., 2017).

The chemical composition of the plant encompasses flavonoids, phenolic acids, terpenoids, glycosides, vitamins (C, E, B1), minerals and sugars, these being dispersed throughout various parts of the plant (Patel, 2015).

Recent studies (Sevindik et al., 2025) have focused on the phytochemical profile and scientific validation of traditional uses, emphasizing its role in pharmacology and nutrition. In traditional medicine, E. angustifolia has been used for its anti-inflammatory, antiseptic and analgesic properties, particularly in the treatment of respiratory, digestive and chronic conditions (Tehranizadeh et al., 2016).

The fruits of Elaeagnus angustifolia constitute a rich natural source of bioactive compounds with remarkable therapeutic and nutritional potential (Hassanzadeh & Hassanpour, 2018). Modern phytochemical studies have highlighted a complex composition comprising carotenoids, flavonoids, phenolic acids, sugars, essential fatty acids, phytosterols, vitamins and other substances of high biological value, which explains the increasing scientific interest in their valorization (Farzaei et al., 2015; Panasenko et al., 2021).

Among the major compounds of interest, carotenoids stand out, particularly beta-carotene and lycopene, which give the fruits their characteristic yellow-orange hue (Zglińska et al., 2021). These lipophilic pigments are well-known for their powerful antioxidant properties, which help to neutralise free radicals and protect cells from oxidative stress (Hamidpour et al., 2017). As such, the consumption of these fruits may be associated with a reduced risk of chronic degenerative diseases, including cardiovascular conditions and neoplasms.

The fruits also contain a high percentage of flavonoids, specifically quercetin, kaempferol and isorhamnetin, which are found in glycosylated forms, and have documented anti-inflammatory, antioxidant and vasoprotective properties (Kumar & Pandey, 2013). Phenolic acids, with 4hydroxybenzoic acid and caffeic acid being the most abundant, also contribute significantly to the total antioxidant capacity of fruit extracts (Tehranizadeh et al., 2016).

Regarding the sugar content, chemical analysis revealed the predominant presence of fructose (approximately 27%) and glucose (approximately 22%), which are responsible for the fruit's characteristic sweet taste (Ayaz, 1999). These carbohydrates vary in concentration depending on the stage of fruit ripening.

The fruits also contain a diverse spectrum of fatty acids, especially linoleic acid (omega-6), present in high quantities in the seeds, and palmitic acid, both involved in maintaining cellular health.

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Phytosterols, notably  $\beta$ -sitosterol, contribute to reducing cholesterol absorption and exhibit anti-inflammatory and hypoglycemic effects (Marvdashti et al., 2024).

Additionally, the composition is enriched with fat-soluble vitamins (notably vitamin E – tocopherol, and provitamin carotenoids such as beta-carotene), as well as water-soluble vitamins such as vitamin C and thiamine (B1), alongside essential minerals, such as potassium, magnesium, calcium, iron and manganese, distributed across different parts of the plant (fruit, leaves, bark) (Nirmala et al., 2022; Kharlyngdoh et al., 2025).

The presence of condensed tannins and saponins contributes to the fruit's anti-inflammatory and antimicrobial potential (Sabouri et al., 2021). Furthermore,  $\beta$ -carbonyl alkaloids with potential pharmacological activity, such as elaeagnin, have been identified in the root and bark.

This rich phytochemical composition positions *Elaeagnus angustifolia* as a species of high therapeutic and nutritional value, justifying an in-depth investigation of its carotenoid content and the evaluation of its potential as a functional ingredient in the food and pharmaceutical industries. The findings of such research may support the scientific basis for the use of the fruits in the development of natural supplements with antioxidant and preventive action.

# 2. MATERIALS AND METHODS

The research examined the dried fruits of *Elaeagnus angustifolia* L., which were collected at full physiological maturity, air-dried naturally, and ground into a fine powder to facilitate efficient extraction of bioactive compounds. The average mass of the plant material used for each extraction was 19.80 g.

To determine the content of beta-carotene and lycopene, three aqueous extracts were prepared using the Ultrasound-Assisted Extraction (UAE) method, an ecological and efficient technique that involves the use of ultrasound to enhance the extraction process of bioactive compounds.

Three types of extracts were prepared, corresponding to different temperatures: ambient temperature (24-hour maceration), 50°C and 80°C (1-hour extraction) using 100 ml of distilled water. Each sample was subjected to ultrasound treatment using a thermally controlled ultrasonic bath, which favored the diffusion of carotenoid compounds from the plant matrix into the aqueous solvent. After extraction, the samples were centrifuged and the supernatant was collected for subsequent steps.

Due to the aqueous nature of the extracts and the fact that the mobile phases used in the liquid chromatography were non-aqueous, purification and concentration were required via solid-phase extraction (SPE). This step was performed using C18-S cartridges (Perkin Elmer). The 12 mL sample was applied to the cartridge, and the extracted compounds were eluted with 5 mL of a 30:70 (v/v) methanol:acetonitrile mixture, leading to a concentration factor of  $2.4\times$ .

Quantitative determination of beta-carotene and lycopene was performed by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) using a 5500 AbSCIEX system equipped with an APCI (Atmospheric Pressure Chemical Ionization) detector. Separation was performed on a Perkin Elmer Cyano column (150 mm  $\times$  4.6 mm, 5  $\mu$ m) under isocratic elution conditions, with mobile phases being 100% methanol (A) and 100% acetonitrile (B), mixed in a 30:70 (v/v) ratio, at a flow rate of 0.5 mL/min.

For beta-carotene, the transitions  $537.4 \rightarrow 519.1$  Da and  $537.4 \rightarrow 445.4$  Da were monitored with an injection volume of  $10 \mu L$ . The retention time was 3.52 minutes, and the calibration curve, established over a concentration range of 1-25 micrograms per millilitre, adhered to the equation  $y = 1.5 \times 10^4 \text{ x} + 2.46 \times 10^4$ , with a correlation coefficient of  $R^2 = 0.9978$ .

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For lycopene, the monitored transitions were  $537.3 \rightarrow 519.1$  Da and  $537.3 \rightarrow 281.0$  Da, with an injection volume of  $20 \,\mu\text{L}$ . Two isomers were detected, of which only isomer 1 (retention time 3.53 minutes) was quantified, based on a reference standard at a concentration of  $140 \,\mu\text{g/mL}$ .

The conversion of concentrations expressed in  $\mu g/mL$  (obtained instrumentally) to  $\mu g/g$  of dried fruit was performed taking into account the total extract volume (100 mL), the final volume of the purified extract (5 mL), the concentration factor, and the mass of the sample used (19.80 g). The calculation was performed using the following formula:

$$Conc_{\frac{ug}{g}fruct} = Conc_{\frac{ug}{mL}} * \frac{V_{baloncotatfinal}}{V_{probasupusaSPE}} * V_{extractinitial} * \frac{1}{m_{fructuscat}}$$

Details regarding the instrumental method for carotenoid extraction are presented in Table 1.

Table 1. Details of the instrumental method for carotenoid extraction

| Transition ID | Q1<br>(Da) | Q3 (Da) | Declustering<br>Potential<br>(DP, volti) | Entrance<br>potential<br>(EP, volti) | Collision<br>Energy<br>(CE, volti) | Collision Cell<br>Exit Potential<br>(CXP, volti) |
|---------------|------------|---------|--|--------------------------------------|------------------------------------|--|
| Beta-Caroten  | 537.4      | 519.1   | 15                                       | 5                                    | 12                                 | 16   |
| Beta-Caroten1 | 537.4      | 445.4   | 15                                       | 10                                   | 25                                 | 10   |
| Lycopene      | 537.3      | 519.1   | 10                                       | 15                                   | 11                                 | 10   |
| Lycopene1     | 537.3      | 281.0   | 10                                       | 15                                   | 29                                 | 14   |

#### 3. RESULTS AND DISCUSSIONS

Following the determination of the carotenoid content (beta-carotene and lycopene) in *Elaeagnus angustifolia* L. fruits, using the LC-MS/MS method after extraction of the compounds from dried plant material with distilled water at three different temperatures—ambient temperature (cold extract), 50 °C, and 80 °C—the following results were obtained:

# Determination of the Beta-Carotene Content in Aqueous Extracts of Russian Olive

The quantitative data regarding the beta-carotene content in aqueous extracts of *E. angustifolia* fruits are presented in Table 2. The results show a progressive increase in beta-carotene concentration with increasing extraction temperature.

Table 2. Beta-carotene content as a function of extraction temperature

| Sample name      | Concentration (µg/mL) | SPE sample<br>volume (mL) | Final volumetric flask volume (mL) | Concentration (µg/g dried fruit) |
|------------------|-----------------------|---------------------------|------------------------------------|----------------------------------|
| Cold extract     | 33.6                  | 12                        | 5                                  | 70.7                             |
| Extract at 50 °C | 36.8                  | 12                        | 5                                  | 77.4                             |
| Extract at 80 °C | 50.3                  | 12                        | 5                                  | 105.8                            |

The beta-carotene concentration values in dried fruit were obtained starting from the instrumentally measured concentration ( $\mu g/mL$ ) by applying the calculation formula presented in the methodology section. This formula accounts for all key parameters: extraction volume, SPE-applied volume, final analyzed volume, and sample mass, allowing for comparable and reproducible results.

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The chromatogram of the beta-carotene standard (Figure 1) clearly shows that a retention time of 3.52 minutes corresponds to a distinct and reproducible separation of beta-carotene in a 30:70 (v/v) methanol:acetonitrile solvent mixture. The monitored transitions  $-537.4 \rightarrow 519.1$  Da and  $537.4 \rightarrow 445.4$  Da, correspond to the primary ions resulting from APCI fragmentation, thereby verifying the compound's identity, and the symmetrical shape of the chromatographic peak indicates efficient separation and correctly calibrated instrumentation. The correlation coefficient R<sup>2</sup> close to 1 (0.9978) (Figure 2) ensures that the experimental data fit the mathematical model very well, allowing for accurate quantification even at low concentrations.

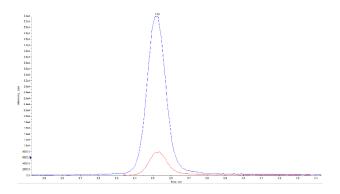


Figure 1. Chromatogram of the beta-carotene standard (25 µg/mL) in a methanol:acetonitrile solvent mixture (30:70, v/v)

Figure 2. Calibration curve for beta-carotene, within the range of 1–25  $\mu$ g/mL, with curve equation  $y = 1.5 \times 10^4 x + 2.46 \times 10^4$ ,  $R^2 = 0.9978$ 

The results obtained for beta-carotene from *Elaeagnus angustifolia* L. fruits are precise and support the foundation for rigorous quantitative analysis. Although aqueous extracts are theoretically less efficient for carotenoids, they become highly relevant when combined with solid-phase extraction (SPE) and LC-MS/MS analysis, supporting their potential use in phytopharmaceutical applications. The three extract samples—cold extract, 50 °C, and 80 °C—were purified by solid-phase extraction (SPE) using C18-S cartridges (Perkin Elmer), which ensured selectivity for hydrophobic compounds such as beta-carotene. The chromatograms obtained for the three types of extracts are presented in Figures 3, 4, and 5.

The cold extract (Figure 3) presented a well-defined chromatographic peak, but with a relatively low intensity compared to the other extracts. This result indicates a lower yield of beta-carotene extraction in the absence of thermal treatment, which can be explained by the reduced solubility of carotenoids in water at low temperatures. The absorption peak intensity in the 50 °C extract was significantly higher (Figure 4), reflecting a more efficient release of beta-carotene under conditions where heat contributed to the breakdown of carotenoid–protein complexes and the permeabilisation of cell walls, thus facilitating extraction. The 80 °C extract showed the most intense signal among the three samples, with a clear and symmetrical peak (Figure 5), indicating the maximum extraction yield at this temperature. No peak degradation or major impurities were observed, suggesting that beta-carotene remained stable under these extraction conditions, supported by SPE and gentle elution.

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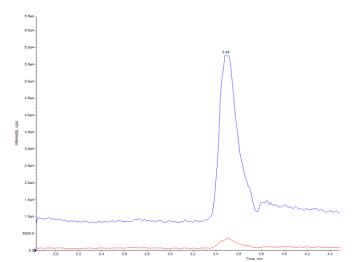


Figure 3. Chromatogram of beta-carotene in the cold extract, purified with SPE using C18-S cartridge (Perkin Elmer), concentration factor 2.4×

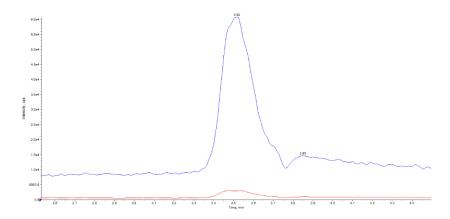


Figure 4. Chromatogram of beta-carotene in the 50 °C extract, purified with SPE using C18-S cartridge (Perkin Elmer), concentration factor 2.4×

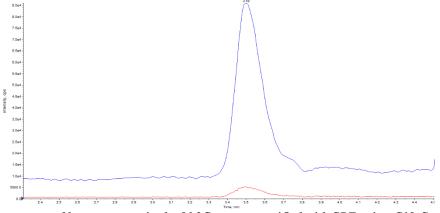


Figure 5. Chromatogram of beta-carotene in the 80 °C extract, purified with SPE using C18-S cartridge (Perkin Elmer), concentration factor 2.4×

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The chromatograms confirm the trend observed in the quantitative data: higher temperature favors beta-carotene extraction, with the 80 °C extract exhibiting the highest concentration,  $105.8 \,\mu\text{g/g}$  of dried fruit.

# Determination of the Lycopene Content in Aqueous Extracts of Russian Olive

The quantitative data on the lycopene content in the aqueous extracts of *E. angustifolia* fruits are summarized in Table 3. As with beta-carotene, the lycopene content was significantly influenced by the extraction temperature.

Table 3. Lycopene content as a function of extraction temperature

| Sample Name      | Concentration (µg/mL) | SPE Sample<br>Volume (mL) | Final Volumetric<br>Flask Volume (mL) | Concentration (µg/g<br>dried fruit) |
|------------------|-----------------------|---------------------------|---------------------------------------|-------------------------------------|
| Cold extract     | 64.3                  | 12                        | 5                                     | 135.3                               |
| Extract at 50 °C | 78.2                  | 12                        | 5                                     | 164.6                               |
| Extract at 80 °C | 90.4                  | 12                        | 5                                     | 190.2                               |

In the chromatogram of the lycopene standard (140  $\mu$ g/mL) (Figure 6), two isomers are evident: isomer 1 (retention time ~3.52 min) and isomer 2 (retention time ~3.97 min). The monitored MS transitions (537.3  $\rightarrow$  519.1 Da and 537.3  $\rightarrow$  281.0 Da) confirmed the identification of lycopene and its cis-trans isomers.

Based on this 140  $\mu$ g/mL reference standard, only isomer 1 of lycopene (retention time 3.53 min) was identified in the samples and was used for quantification as follows:

In the non-heated samples (cold extract), only isomer 1 at 3.53 min displayed both monitored transitions, indicating that the original structure of lycopene was preserved. The signal intensity reflected a concentration of 135.3  $\mu$ g/g dried fruit (Figure 7). The chromatogram of the extract at 50 °C shows that the signal corresponding to isomer 1 is more intense, indicating a higher concentration of 164.6  $\mu$ g/g (Figure 8). This increase suggests a more efficient extraction because of thermal permeabilisation of plant cells. The highest signal intensity for isomer 1 and a concentration of 190.2  $\mu$ g/g observed in the chromatogram of the extract at 80 °C (Figure 9) confirm the optimal extraction efficiency at this temperature. The chromatogram does not show signs of degradation (no major impurities are detected), indicating the thermal stability of lycopene under the tested conditions.

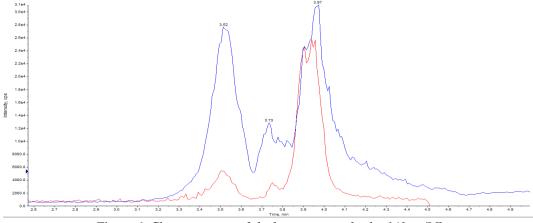


Figure 6. Chromatogram of the lycopene standard – 140 µg/Ml

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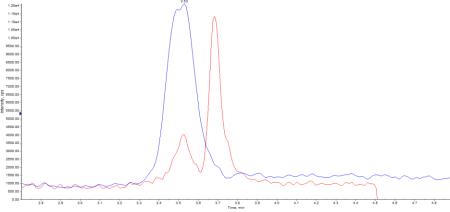


Figure 7. Chromatogram of the cold extract

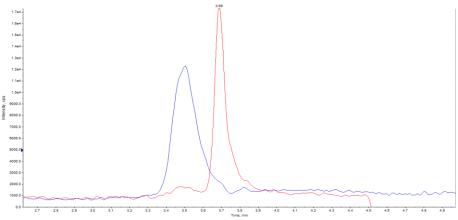


Figure 8. Chromatogram of the extract at 50 °C

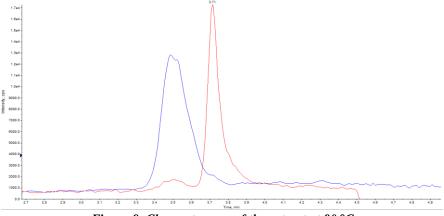


Figure 9. Chromatogram of the extract at 80 °C

The maximum value obtained, 190.2 µg/g of dried fruit, corresponding to the extract processed at 80 °C, confirms that lycopene, although more prone to oxidation, can be extracted more efficiently under moderate to elevated thermal conditions.

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### 4. DISCUSSION

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The significant differences among the extracts obtained at different temperatures indicate that the extraction temperature plays a crucial role in the recovery of hydrophobic carotenoids, even from an aqueous me and Beta-carotene and lycopene, although poorly soluble in water, can still be extracted to a certain extent due to the capacity of hot water to penetrate the plant matrix and release pigments associated with cellular proteins or lipids. The choice of water as an extraction solvent aligns with the concept of green solvent, due to its toxicological safety, availability, biodegradability and ecological character, which makes the entire proposed extraction method fit within the principles of green chemistry (Clark & Tavener, 2007).

The direct correlation between the chromatographic peak intensity and temperature indicates a higher extraction yield of beta-carotene at elevated temperatures, as also supported by Khoo et al. (2011). Furthermore, the efficiency of the purification method using solid-phase extraction (SPE) allowed for the concentration of carotenoids and the removal of water-soluble impurities, thereby increasing the detection sensitivity through LC-MS/MS analysis.

Shen et al. (2009) demonstrated that solid-phase extraction (SPE) can concentrate the extracted betacarotene by up to fourfold, confirming the specific efficiency of this method in purifying lipophilic compounds.

In the LC-MS/MS chromatograms obtained for the aqueous extracts of *Elaeagnus angustifolia* L., only the all-trans isomer (Isomer 1) of lycopene was identified, with no indication of cis-isomer formation, even at the extraction temperature of 80 °C. This result is consistent with the findings of Colle et al. (2010), who demonstrated that in tomato/oil emulsions heated at 80–140 °C, an isomeric equilibrium is established in which the all-trans form remains predominant. After prolonged heating, the conversion reached a temperature-dependent equilibrium. That study revealed that thermal treatments can increase the bioaccessibility of lycopene without significantly compromising its structure, similar to the increase observed at 80 °C in our study. Likewise, the review by Shi and Le Maguer (2000) indicated that under moderate heat treatments, lycopene isomerization remains limited, maintaining the trans form as predominant.

The method applied in the present study—aqueous extraction followed by SPE-C18 purification with sequential elution—is also supported by Naviglio et al. (2008), where the same steps led to the recovery of all-trans lycopene with a purity of  $\geq 98\%$ , demonstrating the high efficiency of this method for lipophilic compounds.

The effects of extraction conditions (solvent-to-sample ratio, temperature, and extraction time) on the yield and active compound content of methanolic extracts of *Elaeagnus angustifolia* (peel, pulp, and seeds) obtained by different methods were investigated by Sardarodiyan et al. (2016), who also indicated that the influence of temperature on the extraction yield was more significant than that of the other factors (p < 0.05). In the abovementioned experiment, the optimal extraction conditions were: solvent-to-sample ratio, 31.79 mL/g; temperature, 52.87 °C; and extraction time, 25.44 min (Sardarodiyan et al., 2016).

The results obtained are comparable to, or even superior to, those reported in the scientific literature for extracts obtained using organic solvents, which supports the feasibility of using aqueous extracts in the development of phytopharmaceutical or functional food products. Sabir et al. (2007) comparatively tested the antibacterial activity of aqueous and organic solvent extracts (chloroform, methanol, acetone) from fresh fruits of Elaeagnus umbellata, and the results revealed strong inhibition of Escherichia coli and Staphylococcus aureus growth in the case of the aqueous extract, while the chloroform and methanol extracts exhibited low activity against these bacteria.

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The effects of E. angustifolia on inflammation and pain have been extensively studied, with several clinical trials documented in the scientific literature (Hosseinzadeh et al., 1999; Ahmadiani et al., 2000; Karimi et al., 2010; Farahbakhsh et al., 2011) investigating aqueous extracts from Russian olive fruit for this purpose. Although the extracts were administered in very different doses (20 mg/kg, 50 mg/kg, 250-500-700 mg/kg, 1-1.5 g/kg), all studies reported efficacy comparable to that of nonsteroidal anti-inflammatory drugs (NSAIDs) such as Diclofenac, Indomethacin, Sodium salicylate, and Imipramine. Specifically, low doses of Russian olive fruit extract (20-40 mg/kg) were effective only in suppressing chronic pain (Ahmadiani et al., 2000), whereas higher doses (130–450 mg/kg) were shown to be effective in managing both chronic and acute pain (Karimi et al., 2010).

Preparations from oleaster fruits are widely used across Europe and Central Asia for wound healing in patients with peptic ulcers. Gürbüz et al. (2003) revealed that the carotenoid fraction of the fruit oil exhibited a protective effect against gastrointestinal ulcers.

The fruits of another, lesser-known and less-utilized species of the *Elaeagnus* genus, *E. latifolia*, were evaluated for their bioactive compound content and antioxidant activity using extracts obtained through various methods (shaking, percolation, Soxhlet extraction, ultrasound-assisted ethanol extraction, and hydrodistillation). Among these, the highest carotenoid extraction yield was obtained via Soxhlet extraction for 120 minutes (176.6 mg β-carotene equivalents/kg dry matter) (Magnusson et al., 2022).

These findings underscore the high nutritional value of *Elaeagnus angustifolia* L. fruits, demonstrating their potential as a significant natural source of carotenoids (Carradori et al., 2020), with promising applications in the food, cosmetic, and pharmaceutical industries. Beta-carotene, a precursor of vitamin A, plays a key role in maintaining visual health, supporting immune function, and protecting the skin, while lycopene is regarded as one of the most effective natural antioxidants, with demonstrated activity in reducing the risk of cardiovascular disease (Kohlmeier et al., 1997) and certain cancers, particularly prostate and colon cancer (Kapała et al., 2022).

Our results confirm the presence of substantial amounts of lycopene in the fruits of E. angustifolia, which is consistent with findings for other species within the *Elaeagnus* genus as reported in the literature (Patel, 2015; Abdalla, 2019; Nazir et al., 2020). For example, the lycopene content in E. umbellata fruit is reported to be 17 times higher than that of tomatoes (Lycopersicum esculentum), according to Fordham et al. (2001), with the two fruits being comparable in terms of other carotenoids (β-carotene, phytoene, and phytofluene).

#### 4. CONCLUSIONS

This study convincingly demonstrated the *Elaeagnus angustifolia* L. fruit's capacity to provide significant amounts of the biologically valuable carotenoids beta-carotene and lycopene. In this context, the experimental determinations revealed that the analyzed fruits contained high concentrations of both carotenoids, with the maximum values - 105.8 µg/g for beta-carotene and 190.2 µg/g for lycopene being obtained following extraction at 80 °C. These findings clearly highlight the favorable impact of the elevated temperature on the efficiency of the extraction process. This observation is particularly noteworthy given the use of an aqueous extraction medium, considering the well-documented poor water solubility of carotenoids.

The findings suggest that using a controlled temperature during the extraction process facilitates the effective release of pigments from the plant matrix, primarily due to the thermal breakdown of cell walls and the separation of carotenoid-protein or carotenoid-lipid complexes. As such, the study

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supports the development of a green, efficient, and safe extraction method, with potential for industrial scale-up to produce carotenoid-rich extracts.

The application of SPE purification, followed by LC-MS/MS analysis with APCI detection, allowed the acquisition of a clear chromatographic profile and high-fidelity quantification, comparable to methods based on organic solvents. Using water exclusively as an extraction solvent provides substantial benefits in terms of sustainability and safety, offering promising prospects for the creation of functional foods and natural dietary supplements. Clean chromatograms devoid of significant cisisomers, coupled with the high concentrations measured, validate the practicability of this approach for producing standardised extracts from *Elaeagnus angustifolia*.

From a biological and nutritional viewpoint, the carotenoids identified contribute to the distinctive coloration of the fruits, while also performing beneficial physiological functions, including protection against oxidative stress, enhancement of the immune system, and a decrease in the risk of cardiovascular diseases and tumours. In addition, complementary phytocompounds such as flavonoids, phenolic acids, and liposoluble vitamins further enhance the therapeutic value of the species.

In summary, *Elaeagnus angustifolia* L. has been confirmed as a valuable and promising plant source of natural carotenoids, with potential uses in the development of dietary supplements, natural pharmaceutical products, and cosmetics that exhibit antioxidant and anti-aging properties. The data obtained confirm the potential of incorporating these fruits into the production line of antioxidant and preventative products, and justify the need for further research to validate the bioactivity of the isolated extracts in vitro and in vivo.

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