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BREEDING PERSPECTIVES OF OLEASTER (*ELAEAGNUS ANGUSTIFOLIA* L.): POLLEN PHENOLIC COMPOSITION AND ANTIOXIDANT PROPERTIES

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SUMMARY

The Russian olive (*Elaeagnus angustifolia* L.) is a commonly known resilient shrub indigenous to Central and Western Asia and Southern Europe. This important species possesses numerous ecological and economic significances. It also serves as a vital nutritional resource for bees within the Nakhchivan Autonomous Republic. The presented study aimed to examine the phenolic composition and antioxidant properties of pollen grains derived from *E. angustifolia*. The total phenolic content (TPC) and total flavonoid content (TFC) reached quantification at 26.117 mg GAE g^{-1} DW and 0.449 mg QUE g^{-1} DW, respectively, in pollen grains, employing the Folin-Ciocalteu and DPPH (2,2-Diphenyl-1-picrylhydrazyl) assays. The antioxidant capacity, as assessed through the FRAP (ferric reducing antioxidant power) method, showed the value of 485.491 µmol Fe (II) g^{-1}. Additionally, the DPPH assay resulted in the SC50 value (0.059 mg/ml). Utilizing reversed-phase high-performance liquid chromatography (RP-HPLC-PDA), six major phenolic compounds were identified, including ellagic and gallic acids. These findings underscore the potential applications of *E. angustifolia* pollens in food supplements and therapeutic contexts, thereby accentuating its rich biochemical profile along with ecological and medicinal importance.

Keywords: Russian olive (*E. angustifolia* L.), pollen grains, biochemical profile, phenolic compounds, antioxidant properties, ecological and medicinal importance, RP-HPLC

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Key findings: The Russian olive (*E. angustifolia* L.) biochemical composition revealed six major phenolic compounds, including ellagic and gallic acids. These findings underscore the potential applications of *E. angustifolia* pollens in food supplements and therapeutic contexts.

INTRODUCTION

Elaeagnus angustifolia L., commonly referred to as the Russian olive, is a tall shrub that belongs to the genus *Elaeagnus* L. within the family Elaeagnaceae Adans. The said species is predominantly native to Central and Western Asia and Southern Europe and widespread in regions such as Russia, the Caucasus, Turkey, and Iran. The genus *Elaeagnus* L. comprises over 90 recognized species globally (Saboonchian *et al.,* 2014).

In Azerbaijan, two wild species documented were *E. caspica* and *E. angustifolia*, along with two cultivated species, *E. pungens* and *E. orientalis* (Askerov, 2006). However, in the Nakhchivan Autonomous Republic, one wild species, *E. angustifolia*, and two cultivated species, *E. pungens* and *E. orientalis,* exist (Talibov and Ibrahimov, 2008). *E. angustifolia* is a deciduous shrub or small tree, typically reaching a height of three to seven meters. It has narrow, lanceolate leaves that are silvery-gray due to dense, scaly trichomes on both surfaces, giving them a distinctive powdery appearance. The leaves have an alternate arrangement and are about 4–9 cm long and 1–2.5 cm wide.

The bark is brown to gray and becomes fissured with age, while young branches often have coverings of silver or brown scales. The plant produces fragrant, yellow, star-shaped, bisexual flowers, usually in clusters of one to three, located in the leaf axils. These flowers lack petals but have a four-lobed calyx and are highly attractive to pollinators, particularly honeybees, which serve as the primary agents of pollination.

Flowering occurs from late March to early April, depending on climatic conditions. The fruit is an elliptical, drupe-like achene, 1 to 1.5 cm long, initially silver but maturing to red-brown. The fleshy outer layer is edible and sweet, while the seed inside is hard and stony. *E. angustifolia* is a well-adapted plant to arid and semi-arid climates, exhibiting high drought resistance due to its deep root system and xerophytic leaf traits (Figure 1) (Askerov, 2006).

The species *E. angustifolia's* leaves, flowers, and fruits possess substantial economic value and are beneficial in alternative and conventional medicines and foods (Shilin and Yulin, 2006). Historically, employing the fruits and flowers in folk medicine has succeeded in treating various ailments, including nausea, cough, asthma, fever, and jaundice (Ahmadiani *et al*., 1998; Gürbüz *et al*., 2003; Hamidpour *et al*., 2017; Emaminia *et al*., 2020). Particularly in Chinese Uyghur medicine, the use of flowers has served for the treatment of thoracalgia and asthma (Xie *et al*., 2020). Contemporary scientific research has indicated that the plant exhibits a wide range of pharmacological effects.

For instance, fruit extracts of *E. angustifolia* have been practically applicable to alleviate pain and inflammation in patients suffering from rheumatoid arthritis. Meanwhile, fruit flour acts as an analgesic, and flower extracts have demonstrated efficacy in managing nervous and cardiovascular diseases (Mohammed *et al*., 2006; Dehghan *et al*., 2016). Components of the species *E. angustifolia* are notably rich in flavonoids, coumarins, cardiac glycosides, phytosterols, phenolic acids, amino acids, terpenoids, vitamins, and minerals (Aksoy and Sahin, 1999; Ayaz and Bertoft, 2001; Boudraa *et al.,* 2010; Cansev *et al.,* 2011; Okmen and Turkcan, 2014; Incilay, 2014; Yıldırım *et al.,* 2015; Fakı *et al.,* 2022).

Phenolic compounds are recognized plant metabolites known for their considerable free radical scavenging properties, attributed to the presence of hydroxyl groups. Therefore, the incorporation of plants into human diets is often proposed to enhance antioxidant defense. These phenolic substances are synthesized primarily in the leaves, flowers, fruits,

Figure 1. *E. angustifolia.*

and seeds of the plant. Based on the above discussion, the prospective study aimed to evaluate the phenolic compounds and antioxidant properties of the species *Elaeagnus angustifolia* pollen grains, which also serve as a vital nutritional resource for bees in the Nakhchivan Autonomous Republic.

MATERIALS AND METHODS

Samples and extraction

Samples collected of *E. angustifolia* came from Ashabi Kahf, District Julfa, Nakhchivan Autonomous Republic. The stamens entailed systematic separation from the flowers and subsequent drying in a shaded and well-ventilated environment. For the extraction process, 1 g of the dried pollen was combined with 50 ml of 98% methanol and sustained agitation on a magnetic stirrer for a duration of 24 hours. The resultant mixture underwent filtration through Whatman No. 4 and No. 1 filter papers. The final extract was preserved in a deep freezer at -18 °C.

Total phenolic content (TPC)

The total phenolic content (TPC) evaluation of the pollen extract utilized the Folin-Ciocalteu method (Singleton *et al*., 1999). A solution preparation combined 20 µl of pollen extract, 400 µl of Folin-Ciocalteu reagent, and 680 µl of distilled water before incubating for 3–4 minutes. Subsequently, the addition of 400 µl of a 7.5% Na_2CO_3 solution allowed the mixture to stand at room temperature for two hours. The absorbance at 760 nm was measured using a Thermo Scientific Evolution™ 201 UV–Vis spectrophotometer. Expressing the TPC was in milligrams of gallic acid equivalents (GAE) per gram of dry pollen.

Total flavonoid content (TFC)

The total flavonoid content (TFC) assessment utilized the colorimetric assay developed by Fukumoto and Mazza (2000). In this procedure, 50 µl of a 10% $Al(NO_3)_3$ solution and 50 µl of a 1.0 M NH_4CH_3COO solution were combined with 25 µl of the pollen extract. The mixture continued to incubate at room temperature for 45 minutes, with the absorbance later measured at 415 nm. Quercetin standards, as employed, express TFC in milligrams of quercetin (QUE) per gram of dried weight (DW) of the sample (Fukumoto and Mazza, 2000).

Total antioxidant capacity

The total antioxidant properties of the pollen extract were evaluated through the ferric reducing antioxidant power (FRAP) method, which involves measuring the reduction of Fe^{3+} to Fe^{2+} (Benzie and Strain, 1996; Pulido *et al*., 2000). The FRAP reagent preparation comprised mixing 2.5 ml of 10 mM TPTZ, 2.5 ml of 20 mM $FeCl_3$, and 25 ml of a 300 mM acetate buffer at pH 3.6. Following a 4-minute incubation, the recorded absorbance was at 595 nm, with $FeSO_4 \cdot 7H_2O$ serving as a reference. The study reported FRAP results as micromoles of $FeSO_4 \cdot 7H_2O$ per gram of dry weight.

DPPH radical scavenging activity

The antioxidant capacity of the extract bore further scrutiny by assessing its ability to neutralize DPPH (2,2-Diphenyl-1-picrylhydrazyl) free radicals (Molyneux, 2004). A solution containing 750 µl of the pollen extract was combined with 750 µl of a DPPH solution.

This mixture was kept in the dark at room temperature for 45 minutes, after which the absorbance was measured at 517 nm, and the results were expressed as SC_{50}.

Determination of phenolic compounds

Phenolic compounds entailed analysis utilizing a Shimadzu Corporation LC 20AT liquid chromatography (HPLC) system, equipped with a photodiode array (PDA) detector and a C18 column (250 mm × 4.6 mm, 5 µm, GL Sciences). The conduct of elution used a mobile phase A (10% acetonitrile in water) and a mobile phase B (2% acetic acid in water). The flow rate setting was at 1 ml/min, with an injection volume of 20 µl at a column temperature of 30 °C. The established detection wavelengths were at 250, 280, 320, and 360 nm, with all samples filtered through a membrane with a pore size of 0.45 µm before analysis (Kolaylı *et al*. 2024). Identification of phenolic components was successful using 25 standard phenolic compounds.

Standard phenolics

The simultaneous analysis of 25 phenolic standards continued. This included the acids—gallic and protocatechuic, p-OH benzoic, m-OH benzoic, chlorogenic, caffeic, syringic, p-coumaric, and ferulic. Likewise, the analysis occurred for epicatechin, rutin, myricetin, quercetin, apigenin, resveratrol, daidzein, t-cinnamic acid, hesperetin, luteolin, rhamnetin, chrysin, pinocembrin, CAPE, curcumin, and ellagic acid.

All experiments performed had three independent biological replicates. For each replicate, pollen samples were collected from separately gathered *E. angustifolia* individuals under the same environmental conditions. Statistical analyses engaged the SPSS version 20.0 software (SPSS Inc., Chicago, IL, USA). One-way ANOVA and Tukey's test helped compare the values obtained for TPC, TFC, FRAP, and DPPH parameters across different pollen samples.

RESULTS AND DISCUSSION

In pollen samples of the plant species *Elaeagnus angustifolia*, the content of polyphenols, flavonoids, and antioxidant properties has been studied (Table 1). The DPPH SC_{50} value of 0.059 mg/mL demonstrates a highly effective ability to scavenge free radicals at a low concentration. Complementarily, the FRAP result of 485.491 µmol Fe^{2+} g^{-1} indicates a strong capacity to reduce oxidized molecules, suggesting that the extract possesses considerable efficacy in both radical scavenging and oxidant reduction. These values were significantly higher than values reported in previous studies for mixed bee pollen (DPPH: 70.81 µmol TE/g), implying the superior antioxidant potential of the species *E. angustifolia* pollen (Çobanoğlu, 2024). Similarly, Cansev *et al*. (2011) declared lower FRAP values for *E. angustifolia* fruit extracts, which further highlights the remarkable efficacy of pollen grains in antioxidant performance.

In the pollen extract, the total phenolic content was markedly higher, recorded at 26.117 mg GAE g^{-1} DW, in contrast to the flavonoid content (0.449 mg QUE g^{-1} DW). One can infer that phenolic compounds are the principal contributors to the observed antioxidant capacity. This trend aligns with the findings of Saboonchian *et al*. (2014), who reported higher phenolic content with modest flavonoid content in flowers. Cansev *et al*. (2011) observed varied values for phenolic accumulation in fruits based on the solvent; however, they still fall short of pollen's phenolic richness. These findings emphasize the role of phenolics as dominant bioactive molecules in the antioxidant mechanism.

The presence of narrow confidence intervals and low standard deviations across all measured parameters reinforces the precision and reproducibility of the results. Such robust

Table 1. Pollen extract of the *Elaeagnus angustifolia* flower.

Pollen grains extract	Total phenolic mg (GAE/g)	Total flavonoid (mg QUE/g)	FRAP (μmol $Fe_2SO_4.7H_2O$/g)	DPPH SC_{50} (mg/ml)
Mean±Std	26.117 ± 0.101	0.449 ± 0.012	485.491 ± 2.125	0.059 ± 0.011
Intervals	26.218 – 26.016	0.461 - 0.43	487.616 – 483.366	0.07 - 0.048

data can support the potential for consistent performance of the extract in practical applications. Bendaikha *et al*.'s (2014) findings also emphasized the need for reproducibility when assessing plant-based antioxidants and highlighted how environmental stability in sampling increases data reliability—a condition mirrored in the consistent measurements of our study.

The highest antioxidant capacity of the pollen extract was confirmed by its phenolic compounds, which makes it an excellent candidate for various applications, including nutraceutical and therapeutic uses. A similar potential was evident in Faramarz *et al*. (2015) in their study based on *E. angustifolia* fruits; however, the concentration of ellagic and phenolic acids in pollen grains suggested even higher pharmacological values. Tepe and Doyuk (2024) also highlighted the importance of phenolic acids in combating oxidative stress, reinforcing the targeted use of the present extract.

The results revealed compelling evidence regarding the antioxidant properties of the species *E. angustifolia* pollens, primarily attributed to its highest phenolic content. Although the flavonoid content was relatively low, the identified flavonoids, chrysin and pinocembrin, exhibited significant pharmacological relevance, particularly for neuroprotective and anti-inflammatory effects. Research conducted by Javaid *et al*. (2021) and Kaur *et al*. (2015) also supported the therapeutic implication of ellagic acid, especially in neurological contexts. These past findings align with the presented results and underscore the broader health-promoting potential of the *E. angustifolia* pollen extracts.

In the *E. angustifolia* pollen extract, 25 phenolic compounds were analyzed received analysis, resulting in the detection of six phenolic compounds (Table 2). However, some were visible, and various others were labeled as 'ND' (not detected), suggesting either species-specific variations or environmental influences on phytochemical profiles. These variations were consistent with the conclusions of Bendaikha *et al*. (2014) and Faramarz *et al*. (2015), who also observed tissue- and environment-specific differences in the compounds found across the different plant parts.

Ellagic acid is the predominant phenolic acid identified in the pollen grain extract, with a concentration of 1047.967 μg/g. This finding was in sharp contrast to the phenolic profiles of fruits and flowers, where ellagic acid was absent or detected at much lower levels. Tepe and Doyuk's (2024) findings enunciated that ferulic and vanillic acids emerged to be dominant in fruits, while Dilek and Doyuk (2020) did not report ellagic acid at all in floral tissues. The contrary findings support the hypothesis of pollen being a unique reservoir for ellagic acid accumulation.

Additional noteworthy phenolic acids include gallic acid and p-coumaric acid, which have common associations with antimicrobial and anti-inflammatory activities. Although chlorogenic acid and caffeic acid were absent, their absence also gained validation by past findings in flower samples, further supporting a tissue-specific phytochemical distribution (Saboonchian *et al*., 2014).

Identifying a limited diversity of flavonoids was successful, with chrysin and pinocembrin being the most noteworthy. In contrast, the flavonoids quercetin, rutin, and kaempferol, typically found in other parts of plants, were notably absent. Faramarz *et al*. (2015) illustrated abundant flavonoid compounds in the fruits and may not entail synthesis in pollen grains. Although detecting chrysin and pinocembrin occurred in relatively modest quantities in the analyzed samples,

Table 2. Analysis of the phenolic content in *E. angustifolia* pollen.

Phenolic acids	Content (µg/g)
Gallic Acid	95.975
Protocatechuic Acid	ND
Chlorogenic Acid	ND
p-OH Benzoic Acid	ND
t-Cinnamic Acid	ND
Caffeic Acid	ND
Syringic Acid	ND
m-ON Benzoic Acid	ND
p-Coumaric Acid	23.747
Ellagic Acid	1047.967
Ferulic Acid	43.855
Flavonoids	
Resveratrol	ND
Daidzein	ND
Luteolin	ND
Quercetin	ND
Epicatechin	ND
Apigenin	ND
Hesperidin	ND
Rhamnetin	ND
Chrysin	9.833
Pinocembrin	10.420
CAPE	ND
Curcumin	ND
Rutin	ND
Myricetin	ND
Resveratrol	ND
Daidzein	ND

*ND = Not detected

their presence is nonetheless significant due to their well-documented pharmacological properties. Both compounds are flavonoids known for their antioxidant, anti-inflammatory, and neuroprotective activities. Previous studies have highlighted their ability to modulate neuronal signaling pathways, reduce oxidative stress, and inhibit neuroinflammation, thereby offering potential therapeutic benefits in the prevention and management of neurodegenerative disorders such as Alzheimer's and Parkinson's diseases. Their detection in *E. angustifolia* pollen, despite low concentrations, reinforces the biofunctional value of this plant matrix and suggests it may serve as a supplementary source of neuroprotective agents in nutraceutical or pharmaceutical applications. Further investigation into the bioavailability and synergistic interactions of these compounds with other phenolics present in the pollen could provide profound insights into their therapeutic potential (Kaur *et al*., 2015; Javaid *et al*., 2021).

It is plausible that pollen with a high content of phenolic compounds and strong antioxidant properties may contribute to enhanced fertilization potential, thereby positively influencing reproductive success and overall plant productivity. Phenolic compounds in pollen appear to play a protective role in pollen viability and germination by mitigating oxidative damage and stabilizing cellular structures during pollen tube growth. Antioxidants can maintain pollen function under environmental stress conditions, which is crucial for successful fertilization (Sharma *et al*., 2012). Phenolic content and antioxidant activity may vary among different populations. These characteristics can help in the selection of genotypes with superior qualities in breeding programs.

CONCLUSIONS

The pollen grains of *E. angustifolia* L. (Russian olive) exhibit a distinct and potent phenolic composition, characterized by a high concentration of ellagic acid and significant antioxidant capacity. In contrast to the plant's fruits and flowers, which possess a broader yet less concentrated phenolic spectrum, the pollen provides a more focused profile of bioactive compounds. This concentrated antioxidant potential underscores its promising applications in nutraceutical and therapeutic contexts, particularly for mitigating oxidative stress and supporting neuroprotective mechanisms. Although flavonoid content was relatively low, the detection of pharmacologically active compounds, such as chrysin and pinocembrin, further enhances the therapeutic value of the pollen, warranting advanced investigation into its functional properties and potential health benefits.

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