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Antioxidant, antimicrobial, and anticancer effects of the Russian olive, *Elaeagnus angustifolia* L. fruit extracts

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ABSTRACT

Elaeagnus angustifolia is a well-known medicinal plant in Iran with high pharmacological effects on human health. The present study was done to assess the antioxidant, antimicrobial, and anticancer effects of *E. angustifolia* L. fruit ethanolic and methanolic extracts. *E. angustifolia* was collected and identified. Extracts were prepared using methanol and ethanol solvents. Antioxidant effects were assessed using the DPPH assay. Total phenol and flavonoid contents were also examined. Disk diffusion, Minimum Inhibitory Concentration, and Minimum Bacterial concentration methods were used to assess extract antimicrobial effects. MTT assay was used to detect the anticancer effects. The concentrations at which the *E. angustifolia* methanolic and ethanolic extracts scavenged 50% of free radicals (IC_{50}) were 597.24 ± 10.09 and 605.33 ± 10.91 µg mL^{-1}, respectively. The total *E. angustifolia* ethanolic and methanolic extracts flavonoid contents were 15.25 ± 1.87 and 17.20 ± 1.30 mg QE/g, respectively. *E. angustifolia* ethanolic and methanolic extract's phenolic contents were 57.33 ± 2.14 and 60.13 ± 1.88 mg GAE g^{-1}, respectively. The methanolic extract exhibited higher antimicrobial effects, especially against *S. aureus* with low MIC and MBC levels. An elevation in the *E. angustifolia* extract concentration caused a significant decrease in cell viability. The first step of decrease in the cell viability in treatment with the *E. angustifolia* ethanolic and methanolic extracts was caused by the concentrations of 10 and 2.5 µg mL^{-1}, respectively. IC_{50} levels of *E. angustifolia* ethanolic and methanolic extracts were 15.97 and 8.23 µg mL^{-1}, respectively. Anticancer effects against the U87 cell line were performed within 24 h. These findings provided a better understanding of the widespread application of *E. angustifolia* as potential antioxidants, antibacterial and anticancer sources, and safe natural medicines.

Keywords: *Elaeagnus angustifolia*, Ethanolic extract, Methanolic extract. Antioxidant, Antimicrobial, Anticancer.
Article type: Research Article.

INTRODUCTION

Approximately, 25% of the world's therapeutic options have a botanical base, either synthesized from plant compounds or extracted straight from natural products (Silveira & Boylan 2023). Nowadays, oxidative stress and microbial pathogens are involved in a variety of diseases and disorders, ranging from cancer to infectious diseases and

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subsequent complications (Hajam *et al.* 2022). Therefore, the urgent need to produce various novel medicinal products based on natural plants is increasing day by day.

The Elaeagnaceae family is riparian tree growing close to rivers and contains 50 species (Belwal *et al.* 2022). *Elaeagnus angustifolia* also known as Russian olive, Oleander, and Russian silverberry, is a medicinal plant native to central Asia, Iran, Syria, and China, and exotically in some parts of Canada, the United States, and Spain (Gecer 2023; Nazir *et al.* 2020). *E. angustifolia* is traditionally applied as an antipyretic, analgesic, and diuretic agent (Tehranizadeh *et al.* 2016). It is full of flavonoids, phenolic, alkaloids, antimicrobial, anticancer, anti-inflammatory, and antioxidant compounds, as well as minerals and vitamins (Fakı *et al.* 2022). *E. angustifolia* fruits are mostly applied for the treatment of cough, cold, influenza, fever, jaundice, asthma, nausea, diarrhea, pain relief, rheumatoid arthritis, and bone fractions (Hamidpour *et al.* 2019). *E. angustifolia* extract antimicrobial effects were determined against *Staphylococcus aureus* and *Escherichia coli*. Additionally, the anticancer effects of *E. angustifolia* extract were assessed against diverse kinds of cancer cell lines (Zakaria *et al.* 2023).

In Iran, scarce investigations have been developed to assess the biological effects of *E. angustifolia* extract. As a result, a recent survey was conducted to evaluate its antimicrobial, antioxidant, and anticancer effects *in vitro* conditions.

MATERIALS AND METHODS

Chemical reagents, diagnostic kits, and devices

All chemical reagents, ethanol, methanol, Folin–Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution, NaOH, PBS, DMSO, DMEM medium, MTT assay kit, Mueller-Hinton Broth and agar media, and Aluminum chloride ($AlCl_3$) with analytical grade were purchased from the Sigma-Aldrich Company (St Louis, MO, USA). Whatman® cellulose filter paper No. 2 was also purchased from the Sigma-Aldrich company. For the centrifuge, the Shimadzu, Japan device was used. For spectrophotometric analysis, the Shimadzu, Japan device was also used. The magnetic shaker was purchased from CMVC, Germany. A laboratory mill (JXFM110 Hammer Mill, China) was also used. U87 cell line was purchased from the Pasteur Institute, Tehran, Iran. *E. angustifolia* fruit was collected from wild plants of Shahrekord, Iran (located at 32.3282° N, and 50.8769° E). *S. aureus* and *E. coli* were purchased from the Persian-type culture Collection (PTCC), Iran. Antibiotic disk was purchased from Oxoid, UK. Penicillin/streptomycin (Pen/Strep), kanamycin, and amphotericin B were purchased from GIBSO, USA. ELISA-reader (VIC, Australia) was applied in the MTT assay. mitomycin C (MMC: 10−7 M) antitumor agent was purchased from the Sigma-Aldrich, USA.

Fruits and extracts

E. angustifolia fruits were botanically and morphologically identified by an expert professor of the field of medicinal plants from the Islamic Azad University, Shahrekord Branch, Shahrekord, Iran. The voucher sample was preserved for reference in the herbarium of the General Directorate of Agricultural and Natural Resources of Shahrekord (Herbarium Number D-7138). A specimen sample was kept at the herbarium of the aforementioned organization, in a temperature-controlled place (22–25 °C and 30–40% relative humidity).

Collected fruits were washed and subjected to dehydration. Forced ventilation at room temperature (25 °C) was used up to a time that a constant weight was reached. After 10 days, the weight loss on drying was determined to be <10%, as recommended by the guidelines of European Pharmacopeia. A laboratory mill was used to grind the samples into powders. The powders were kept in sealed bags in the dark at 4 °C until further use.

A total of 30 g of *E. angustifolia* dried grind fruit samples were extracted in 100 mL ethanol and 100 mL methanol (99% purity separately) in a magnetic extraction shaker for 2 h then left at room temperature. The extraction mixture was decanted, filtered on paper, evaporated at 40 °C in the dark under vacuum, and immediately analyzed or stored at 4 °C. The final extraction concentrate was adjusted to 50 mg mL^{-1}. Extraction yields of samples were between 0.8 and 12 mg mL^{-1}.

E. angustifolia polyphenol content

Folin-Ciocalteu assay (Lozano-Puentes *et al.* 2023) was applied to determine *E. angustifolia* total polyphenols. A 750 µL of the *E. angustifolia* extracts and gallic acid were blended with 150 µL of Folin-Ciocalteu reagent. The mixture

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was then incubated at 22 ◦C for 5 min. Afterward, 150 µL of Na_2CO_3 (20%) was added into a mixture and achieved solution was incubated at 40 ℃ for half an hour. Solution absorbance was then checked at 750 nm. Total polyphenol was determined rendering the gallic acid curve. The following formula was applied to examine the *E. angustifolia* extract total polyphenols:

$$\text{Total polyphenol} = \frac{\text{Galic acid concentration (mg/mL)} \times E.angustifolia \text{ extract volume (mL)}}{E.angustifolia \text{ extract mass (g)}}$$

E. angustifolia antioxidant activity

E. angustifolia extracts at numerous concentrations were added to the 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution with a reaction mixture volume of 200 µL. Then, dark incubation was performed for 30 min at 37 ℃. Afterward, solution absorbance was assessed at 517 nm. Ascorbic acid was applied as a control. The following formula was applied to examine the DPPH radical scavenging activity of *E. angustifolia* extract (Choi & Choi 2022):

$$\text{Scavenging activity} = \frac{1-(E.angustifolia \text{ absorbance} - \text{blank absorbance})}{\text{Control absorbance}} \times 100$$

E. angustifolia flavonoid content

Aluminum chloride ($AlCl_3$) colorimetric assay was applied (Ashikaa *et al.* 2023). An aliquot of 400 µL of *E. angustifolia* extract and quercetin was added to $NaNO_2$ (5%, 30 mL) and mixed well for 5 min. Then, 30 µL of $AlCl_3$ (10%) was added to the achieved solution. The subsequent solution was incubated at 22 ℃ for 5 min. Afterward, NaOH (4%, 400 µL) was added to the previous solution. The subsequent solution was incubated at 22 ◦C for 15 min. Distilled water was applied to increase the mixture volume to 1 mL. After well mixing, solution absorbance was assessed at 510 nm. The total flavonoid was determined rendering the quercetin curve. The following formula was applied to examine the *E. angustifolia* extract total flavonoid:

$$\textit{E. angustifolia} \text{ total flavonoid} = \frac{\text{Quercetin concentration (mg/mL)} \times E.angustifolia \text{ extract volume (mL)}}{E.angustifolia \text{ extract mass (g)}}$$

E. angustifolia antimicrobial effects

The antimicrobial effects of *E. angustifolia* extracts were evaluated by disk diffusion and broth macro-dilution techniques. The *E. angustifolia* extracts were separately tested against *S. aureus* (PTCC 1276) and *E. coli* (PTCC 1112). The bacteria were cultured on Mueller-Hinton broth and further incubated for 24 h at 37 °C. Simple disk diffusion using the Bauer-Kirby method was employed for antimicrobial analysis. *S. aureus* and *E. coli* (0.5 McFarland concentration) were cultured superficially on the Mueller-Hinton agar contained antimicrobial disk (trimethoprim-sulfamethoxazole (30 mg/disk)) and the blank disks contained different concentrations (62.5, 125, 250, and 500 mg mL^{-1}) of *E. angustifolia* ethanolic and methanolic extract (1000 µL). Disks were placed on the surface of the media at a significant distance from each other. Mueller-Hinton agar media contained bacteria and disks were incubated for 24 h at 37 °C. The diameter of the bacterial growth inhibition zone surrounding each disk was measured and analyzed. Disks containing methanol and ethanol were used as positive controls (Shahreza & Soltani 2022).

The minimum inhibitory concentration (MIC) and minimal bactericidal concentration of *E. angustifolia* ethanolic and methanolic extracts were tested rendering the broth macro-dilution method. *S. aureus* and *E. coli* single colonies were inoculated to Mueller Hinton broth and incubated (24 h at 37 °C). Contents were then centrifuged (15 min at 5000 rpm) and formerly were suspended in another Mueller Hinton broth medium to adjust its turbidity at 106 CFU/mL (0.5 McFarland). The *E. angustifolia* extracts 62.5, 125, 250, and 500 mg mL^{-1} concentrations were prepared (using ethanol and methanol for each type of extract) and used in test tubes containing 4 mL of nutrient broth (sterile). Afterward, 0.5 mL of the obtained solution was added to 9.5 mL Mueller Hinton broth and incubated for 24 h at 37 °C. The inoculated medium comprising *S. aureus* and *E. coli* bacteria without *E. angustifolia* extract was applied as a positive control. The lowermost *E. angustifolia* extract concentration in which the bacterial growth is prevented was

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determined as MIC. For MBC, 100 µL of each tube without color change were sub-cultured in Mueller Hinton agar medium and incubated for 24 h at 37 °C. The lowest *E. angustifolia* extract concentrations which revealed bactericidal effects was considered as MBC (Moghtaderi *et al.* 2021).

E. angustifolia anticancer effects and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

U87 human glioblastoma cell line was applied and cultured in DMEM media containing fetal bovine serum (FSB, heat-treated), penicillin/streptomycin (Pen/Strep), kanamycin, and amphotericin B (about 20 µL of each). Formerly, they were incubated at 37 °C with a modified atmosphere (95% air and 5% CO_2; Veettil *et al.* 2022).

Effects of *E. angustifolia* ethanolic and methanolic extracts on the U87 cell lines viability were tested by the MTT. An average number of 5000 cells were cultured in a 96-well culture plate containing 100 µL of culture medium. Then, they were incubated for 48 h at 37 °C with 5% CO_2. Afterward, cells were treated with different extract concentrations (0, 2.5, 10, 20, and 40 µg mL^{-1}) and again incubated for 24 h at 37 °C with 5% CO_2. The previous solution was substituted with 20 µL MTT solution (0.5 mg mL^{-1}). Contents were incubated at 37 °C for 3.5 h. Moreover, formazan precipitation was solved in 200 µL DMSO and inoculated into wells. Solution absorbance was read at 490 nm using the ELISA reader. Cell viability was determined as the percentage of absorbance of *E. angustifolia* ethanolic and methanolic extracts-treated cultures compared with those of untreated control cultures. Cells containing mitomycin C (MMC: 10^{-7} M, Sigma-Aldrich ®) antitumor agent were applied as positive control. Cells without *E. angustifolia* ethanolic and methanolic extracts were applied as a negative control. Cell viability was measured using the following formula (Calderón-Montaño *et al.* 2021):

$$\text{Viability (\%)} = 100 \times \frac{\text{OD test}}{\text{OD control}}$$

Finally, the 50% inhibitory concentration (IC_{50}) of the extract was measured using the following linear regression formula:

$$Y = 1.904x + 80.41,\ y = 1.996x + 84.394$$

Data analysis

Collected data were assessed by analysis of variance (ANOVA) test. A post-hoc Scheffe multiple comparison test was also applied to compare the findings of diverse groups. The value of $p < 0.05$ was measured as a statistically significant level. All data were described as standard deviation (SD)

RESULTS

E. angustifolia radical scavenging, total phenol, and flavonoid contents

Table 1 shows the *E. angustifolia* ethanolic and methanolic extracts' radical scavenging effect as well as total polyphenol and flavonoid contents. The value at which the *E. angustifolia* methanolic and ethanolic extracts scavenged 50% of free radicals (IC_{50}) was 597.24 ± 10.09 and 605.33 ± 10.91 µg mL^{-1}, respectively. The total *E. angustifolia* ethanolic and methanolic extracts flavonoid contents were 15.25 ± 1.87 and 17.20 ± 1.30 mg QE g^{-1}, respectively. Finally, the total *E. angustifolia* ethanolic and methanolic extracts phenolic contents were 57.33 ± 2.14 and 60.13 ± 1.88 mg GAE g^{-1}, respectively.

E. angustifolia antimicrobial assessment through the disk diffusion

Table 2 shows the disk diffusion antimicrobial effects of *E. angustifolia* ethanolic and methanolic extracts. Examined extracts and antibiotics against bacteria exhibited the diameter of the growth inhibition zone ranged from 7.42 ± 0.39 mm (the lowest, *E. coli* treated with the *E. angustifolia* ethanolic extract) to 18.14 ± 0.63 mm (the highest, *S. aureus* treated with the trimethoprim-sulfamethoxazole). In a comparison of the growth inhibition zones, *E. angustifolia* methanolic extract exhibited significantly higher diameters than ethanolic ones ($p < 0.05$). Additionally, significant differences were revealed in the diameter of the growth inhibition zone between *S. aureus* and *E. coli* ($p < 0.05$).

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Table 1. *E. angustifolia* ethanolic and methanolic extracts radical scavenging, total polyphenol, and flavonoid contents.

Plant	DPPH IC_{50} (µg mL^{-1})	Total phenolics (mg GAE g^{-1})	Total flavonoids (mg QE g^{-1})
E. angustifolia ethanolic extract	605.33 ± 10.91	57.33 ± 2.14	15.25 ± 1.87
E. angustifolia methanolic extract	597.24 ± 10.09	60.13 ± 1.88	17.20 ± 1.30

Table 2. Disk diffusion antimicrobial effects of *E. angustifolia* ethanolic and methanolic extracts.

Extracts/Antibiotic agent	Disk diffusion diameter of the growth inhibition zone (mm)	
	S. aureus	***E. coli***
E. angustifolia ethanolic extract	10.33 ± 0.56^{Ca}	7.42 ± 0.39^{Cb}
E. angustifolia methanolic extract	13.95 ± 0.27^{Ba}	10.21 ± 0.19^{Bb}
Trimethoprim-sulfamethoxazole	18.14 ± 0.63^{Aa}	16.98 ± 0.42^{Aa}

Note: Different capital letters in each row: $p < 0.05$; Different small letters in each column: $p < 0.05$

E. angustifolia antimicrobial assessment through the MIC and MBC

Table 3 shows the MIC and MBC of *E. angustifolia* extracts against the tested bacteria. The lowest MIC and MBC levels were observed for *E. angustifolia* methanolic extract against *S. aureus* (62.5 and 125 mg mL^{-1}, respectively), while the highest for its ethanolic extract against *E. coli* (250 and 300 mg mL^{-1}, respectively).

Table 3. MIC and MBC of *E. angustifolia* extract against the tested bacteria.

Extract/Antibiotic agent	Inhibitory concentrations (mg mL^{-1})			
	S. aureus		***E. coli***	
	MIC	MBC	MIC	MBC
E. angustifolia ethanolic extract	125	250	250	500
E. angustifolia methanolic extract	62.5	125	125	250

E. angustifolia anticancer effect

Fig. 1 illustrates the cell viability of the cancer cell line based on the *E. angustifolia* ethanolic and methanolic extract concentrations. An elevation in the *E. angustifolia* extract concentration caused a significant drop in cell viability. The initial decrease in the cell viability of cancer lines treated with the ethanolic and methanolic extracts was caused by 10 and 2.5 µg mL^{-1}, respectively. At 40 µg mL^{-1} of ethanolic and methanolic extracts, cell viabilities were decreased to 25% and 7%, respectively. A significant difference was observed in cell viability between ethanolic and methanolic extracts at 10, 20, and 40 µg mL^{-1}.

Examination revealed that the IC_{50} levels of *E. angustifolia* ethanolic and methanolic extracts were 15.97 and 8.23 µg mL^{-1}, respectively. Anticancer effects of these extracts against the U87 cell line were performed within 24 h.

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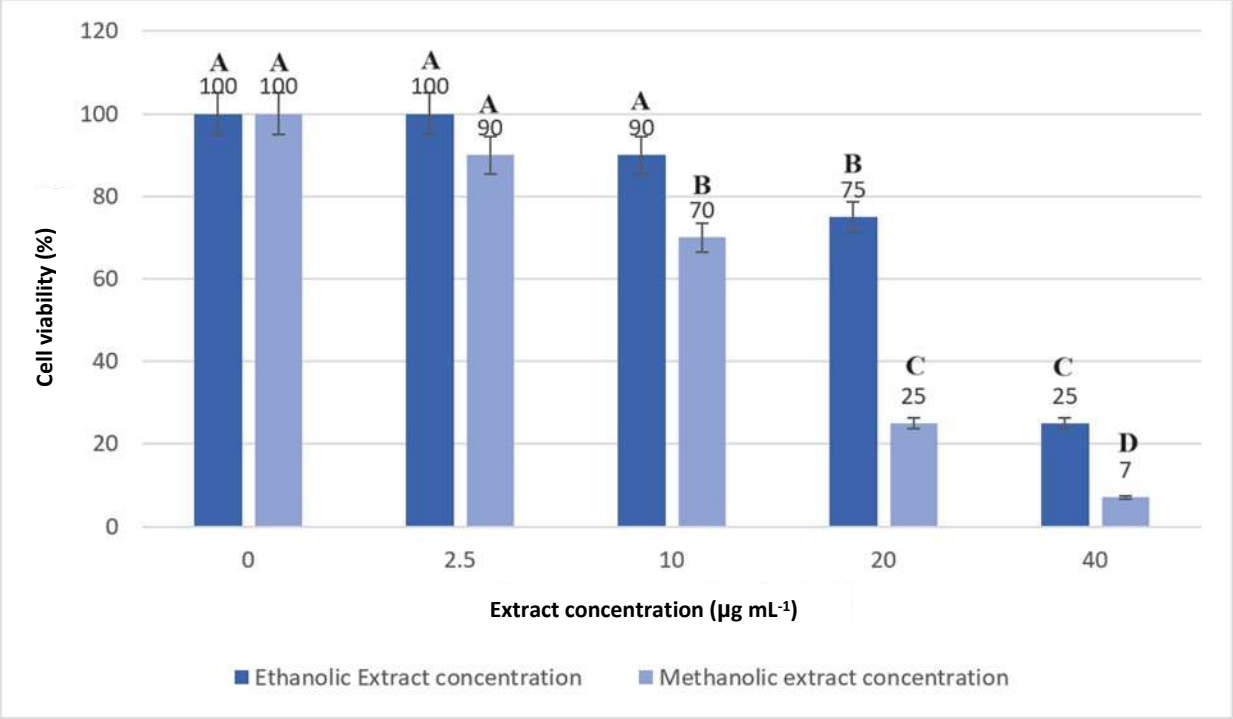

Fig. 1. Cell viability of cancer cell line based on the *E. angustifolia* ethanolic and methanolic extracts concentrations. Dissimilar letters showed significant differences (p <0.05).

DISCUSSION

Given the antioxidant agent's portion in lessening the complication of numerous diseases by oxidative stress neutralization, and based on their potential anticancer and antimicrobial effects, medicinal plant applications as sources of natural anticancer, antimicrobial and antioxidants can prevent oxidative stress and cancer (Ahmed *et al.* 2016). In keeping with the high antimicrobial effects, such medicinal plants may be beneficial for the fruitful microbial community of the gut (Pferschy-Wenzig *et al.* 2022). The present study showed that the *E. angustifolia* ethanolic and methanolic extracts possess substantial antioxidant, antimicrobial, and anticancer effects, similar to the studies conducted by Hamidpour *et al.* (2017), Piri Gharaghie & Hajimohammadi (2021), and Espinosa-Paredes *et al.* (2021). Findings showed that the concentration in which the *E. angustifolia* methanolic extract scavenged 50% of free radicals (IC_{50}) was lower (597.24 ± 10.09 µg mL^{-1}) than methanolic extract (605.33 ± 10.91 µg mL^{-1}). As a whole, the DPPH free radical inhibitory effect of extracts is dependent on diverse factors, including extraction solvent type and its polarity, methods of separation, active compounds purity, and the method of measurement (Baliyan *et al.* 2022). Previous studies conducted by Bisi *et al.* (2024) in France, Okmen & Turkcan (2014) in Turkey, and Sarvarian *et al.* (2022) in Iran also revealed that *E. angustifolia* extract possessed high antioxidant effects on DPPH radical scavenging assessment.

The total phenolic and flavonoid contents of the *E. angustifolia* methanolic extract were also higher than ethanolic extract. Plant phenolic components contain hydroxyl groups which facilitate radical scavenging effects (Nurzyńska-Wierdak 2023). These groups also may directly act as antioxidant agents (Nurzyńska-Wierdak 2023; Sun & Shahrajabian 2023). They directly exhibit inhibitory actions against carcinogenic and mutagenic agents (Sun & Shahrajabian 2023). The second plant-derived compounds with high antiradical features are flavones and flavonoids (Ivanišová 2023). Saboonchian *et al.* (2014) reported that the total phenolic and flavonoid contents were better extracted using ethanol and methanol solvents. They showed that *E. angustifolia* Fariman variant ethanolic and

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methanolic extracts had the uppermost phenolic (10.91 ± 0.18 and 10.28 ± 0.36 mg GAE/100 g FW, respectively) and flavonoid (5.80 ± 0.10 and 3.36 ± 0.05 mg QE/100 g FW, respectively) compounds.

Our findings also revealed that the *E. angustifolia* methanolic extract possesses higher antimicrobial effects. *E. angustifolia* methanolic extract showed a higher diameter of the growth inhibition zone against *S. aureus* (13.95 ± 0.27 mm) and *E. coli* (10.21 ± 0.19 mm) with lower MIC (62.5 and 125) and MBC (125 and 250) values, respectively. Findings revealed that both types of extract could restrict the growth of all tested strains. Additionally, both extracts were generally more effective on Gram-negative bacteria, compared to Gram-positive. Lower antimicrobial effects of extracts against Gram-negative bacteria are mainly because of the hard lipopolysaccharides wall of such bacteria which inhibit the extract penetration. The presence of polyphenols, triterpenoids, saponin, anthraquinones, glycosides, flavonoids, and steroids are the main reasons for the high antimicrobial effects of *E. angustifolia* methanolic and ethanolic extracts (Marvdashti *et al.* 2024). To date, the antimicrobial effects of *E. angustifolia* methanolic and ethanolic extracts have been reported against *E. coli*, *Candida albicans*, *Yersinia enterocolitica*, *Bacillus subtilis*, *S. aureus*, *Listeria monocytogenes*, *Enterococcus faecalis*, and *Salmonella typhimurium* (Okmen & Turkcan 2014).

Another part of this research revealed that the *E. angustifolia* methanolic extract exhibited higher anticancer effects than the ethanolic extract. Previously, *E. angustifolia* extracts anticancer effects were confirmed against HER2-positive breast cancer cells (Jabeen *et al.* 2020), Triple Negative Breast Cancer Cell Lines (TNBC; Fouzat *et al.* 2022), human breast cancer cell lines (MC-7; Naseri *et al.* 2023), and HELA human cervical cancer and PC3 human prostate cancer (Erdoğan *et al.* 2021). Flavonoids, phenols, and neo-clerodane diterpenoids contents of *E. angustifolia* play a substantial role in apoptosis promoting and cell-cycle progression, as well as angiogenesis and epithelial-mesenchymal transition (EMT) inhibiting, and as a consequence, possibly preventing cancer development. Furthermore, *E. angustifolia* extract compounds can regulate several cellular processes, including DNA repair, progression of cell cycle, apoptosis induction, and cell signaling cascades (Fouzat *et al.* 2022) which mainly act as an anticancer agent.

Put together, this research evaluated the antimicrobial, antioxidant, and anticancer effects of *E. angustifolia* ethanolic and methanolic extracts. The absence of *in vivo* examinations, including mice model test, lack of chromatographic-based examination of phytochemical compounds, and finally lack of antifungal and antiviral studies were the main limitations of this investigation.

CONCLUSION

In conclusion, we found that the *E. angustifolia* extracts, especially methanolic extract, exhibited a high DPPH-radical scavenging effect, high phenol and flavonoid contents, high antimicrobial effects against *S. aureus* and *E. coli* with low MIC and MBC levels, and high anticancer effects against U87 cell line. Given the edible route of *E. angustifolia*, the application of its methanolic extract as medicinal food by considerable antimicrobial, antioxidant, and anticancer effects should be further examined in animal models.

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Not applicable.

REFERENCES

Ahmed, SI, Hayat, MQ, Tahir, M, Mansoor, Q, Ismail, M, Keck, K & Bates, RB 2016, Pharmacologically active flavonoids from the anticancer, antioxidant and antimicrobial extracts of *Cassia angustifolia* Vahl. *BMC Complementary and Alternative Medicine*, 16: 1-9.

Ashikaa, BA, Zaruwa, MZ, Muhammad, BY, Bamidele, TO & Daikwo, MA 2023, Neuroprotective effect of *Stachytarpheta angustifolia* methanol extract against aluminum chloride ($AlCl_3$) induced Alzheimer's disease in albino rats. *Journal of Complementary and Alternative Medical Research*, 21:22-31.

Baliyan, S, Mukherjee, R, Priyadarshini, A, Vibhuti, A, Gupta, A, Pandey, RP & Chang, CM 2022, Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of *Ficus religiosa*. *Molecules*, 27: 1326.

Belwal, T, Bhatt, I & Devkota H 2022, Himalayan Fruits and Berries. Academic Press, 538 p.

Caspian J. Environ. Sci.
DOI:

Received:
Article type: Research

Bisi, H, Bonnard, M, Simon, L, Morille, M, Bégu, S & Parrot, I 2024, Antioxidant capacity of an ethanolic extract of *Elaeagnus x submacrophylla Servett*. leaves. *Heliyon*, 10: e28067.

Calderón-Montaño, JM, Martínez-Sánchez, SM, Jiménez-González, V, Burgos-Morón, E, Guillén-Mancina, E, Jiménez-Alonso, JJ, Díaz-Ortega, P, García, F, Aparicio, A & López-Lázaro, M 2021, Screening for selective anticancer activity of 65 extracts of plants collected in Western Andalusia, Spain. *Plants*, 10: 2193.

Choi IJ & Choi, IJ 2022, The antioxidant and anti-inflammatory activities of Echinacea angustifolia hot water extract. *Asian Journal of Beauty and Cosmetology*, 20: 373-381.

Erdoğan, Ö, Paşa, S & Cevik, O 2021, Green synthesis and characterization of anticancer effected silver nanoparticles with silverberry (*Elaeagnus angustifolia*) fruit aqueous extract. *International Journal of Pure and Applied Sciences*, 7: 391-400.

Espinosa-Paredes, DA, Cornejo-Garrido, J, Moreno-Eutimio, MA, Martínez-Rodríguez, OP, Jaramillo-Flores, ME & Ordaz-Pichardo, C 2021, *Echinacea angustifolia* DC extract induces apoptosis and cell cycle arrest and synergizes with paclitaxel in the MDA-MB-231 and MCF-7 human breast cancer cell lines. *Nutrition and Cancer*, 73: 2287-2305.

Fakı, R, Canbay, HS, Gürsoy, O & Yılmaz, Y 2022, Antioxidant activity, physico-chemical and fatty acid composition of oleaster (*Elaeagnus angustifolia* L.) varieties naturally grown in western Mediterranean region of Turkey. *Akademik Gıda*, 20: 329-335.

Fouzat, A, Hussein, OJ, Gupta, I, Al-Farsi, HF, Khalil, A & Al Moustafa, AE 2022, *Elaeagnus angustifolia* plant extract induces apoptosis via P53 and signal transducer and activator of transcription 3 signaling pathways in triple-negative breast cancer cells. *Frontiers in Nutrition*, 9: 871667.

Gecer, MK 2023, Diversity in important fruit traits of *Elaeagnus angustifolia* L. genotypes from Northeastern Turkey. *Erwerbs-Obstbau*, 65: 363-367.

Hajam, YA, Rani, R, Ganie, SY, Sheikh, TA, Javaid, D, Qadri, SS, Pramodh, S, Alsulimani, A, Alkhanani, MF, Harakeh, S & Hussain A 2022, Oxidative stress in human pathology and aging: molecular mechanisms and perspectives. *Cells*, 11: 552.

Hamidpour, R, Hamidpour, S & Doostmohamadi, P 2019, Chemistry, pharmacology and medicinal property of Russian olive (*Elaeagnus angustifolia* L.). *Cancer Science & Research*, 6: 1-7.

Hamidpour, R, Hamidpour, S, Hamidpour, M, Shahlari, M, Sohraby, M, Shahlari, N & Hamidpour, R 2017, Russian olive (*Elaeagnus angustifolia* L.): From a variety of traditional medicinal applications to its novel roles as active antioxidant, anti-inflammatory, anti-mutagenic and analgesic agent. *Journal of Traditional and Complementary Medicine*, 7: 24-9.

Ivanišová, E 2023, Herbs and Spices-New Advances. 2023 Mar 29. IntechOpen, Bratislava, Slovakia, 280 p.

Jabeen, A, Sharma, A, Gupta, I, Kheraldine, H, Vranic, S, Al Moustafa, AE & Al Farsi, HF 2020, *Elaeagnus angustifolia* plant extract inhibits epithelial-mesenchymal transition and induces apoptosis via HER2 inactivation and JNK pathway in HER2-positive breast cancer cells. *Molecules*, 25: 4240.

Lozano-Puentes, HS, Sánchez-Matiz, JJ, Ruiz-Sanchez, E, Costa, GM & Díaz-Ariza, LA 2023, *Guadua angustifolia* Kunth leaves as a source for bioactive phenolic compounds: Optimization of ultrasound-assisted extraction using response surface methodology and antioxidant activities. *Heliyon*, 9: e22445.

Marvdashti, LM, Mohammadbeigi, M, Arab, S, Ebrahimi, A, Rezaei, A & Abdolshahi, A 2024, *Elaeagnus angustifolia* L. whole fruit ethanolic extract: Phytochemical composition and antimicrobial effects. *Journal of Chemical Health Risks*, 14: 117.

Moghtaderi, M, Mirzaie, A, Zabet, N, Moammeri, A, Mansoori-Kermani, A, Akbarzadeh, I, Eshrati Yeganeh, F, Chitgarzadeh, A, Bagheri Kashtali, A & Ren, Q 2021, Enhanced antibacterial activity of Echinacea *angustifolia* extract against multidrug-resistant *Klebsiella pneumoniae* through niosome encapsulation. *Nanomaterials*, 11: 1573.

Naseri, Z, Piri, K, Naseri, M & Nuri, S 2023, Anticancer, antioxidant and antibacterial activities of extracts from *Scrophularia striata* and *Elaeagnus angustifolia*, growing in Ilam and Kurdistan provinces in Iran. *Plant Science Today*, 10: 152-165.

Caspian J. Environ. Sci.
DOI:

Received:
Article type: Research

Nazir, N, Zahoor, M & Nisar, M 2020, A review on traditional uses and pharmacological importance of genus *Elaeagnus* species. *The Botanical Review*, 86: 247-280.

Nurzyńska-Wierdak, R 2023, Phenolic compounds from new natural sources—Plant genotype and ontogenetic variation. *Molecules*, 28: 1731.

Okmen, G & Turkcan, O 2014, A study on antimicrobial, antioxidant and antimutagenic activities of *Elaeagnus angustifolia* L. leaves. *African Journal of Traditional, Complementary and Alternative Medicines*, 11: 116-120.

Pferschy-Wenzig, EM, Pausan, MR, Ardjomand-Woelkart, K, Röck, S, Ammar, RM, Kelber, O, Moissl-Eichinger, C & Bauer, R 2022, Medicinal plants and their impact on the gut microbiome in mental health: A systematic review. *Nutrients*, 14: 2111.

Piri Gharaghie, T & Hajimohammadi, S 2021, Comparison of anti-candida effects of aqueous, ethanolic extracts and essential oil of *E. angustifolia* with fluconazole on the growth of clinical strains of *Candida*. *New Cellular and Molecular Biotechnology Journal*, 11: 25-38.

Saboonchian, F, Jamei, R & Sarghein, SH 2014, Phenolic and flavonoid content of *Elaeagnus angustifolia* L. (leaf and flower). *Avicenna Journal of Phytomedicine*, 4: 231.

Sarvarian, M, Jafarpour, A, Awuchi, CG, Adeleye, AO & Okpala, CO 2022, Changes in physicochemical, free radical activity, total phenolic and sensory properties of orange (*Citrus sinensis* L.) juice fortified with different oleaster (*Elaeagnus angustifolia* L.) extracts. *Molecules*, 27: 1530.

Shahreza, MH & Soltani, A 2022, Genotyping and antibiotic resistance of methicillin-resistant *staphylococcus aureus* strains isolated from raw and frozen meat samples and assessment of the antimicrobial effects of *origanum vulgare* against MRSA isolates. *International Journal of Health Sciences*, 6: 4840-4852.

Silveira, D & Boylan, F 2023, Medicinal plants: advances in phytochemistry and ethnobotany. *Plants*, 12: 1682.

Sun, W & Shahrajabian, MH 2023, Therapeutic potential of phenolic compounds in medicinal plants—Natural health products for human health. *Molecules*, 28: 1845.

Tehranizadeh, ZA, Baratian, A & Hosseinzadeh, H 2016, Russian olive (*Elaeagnus angustifolia*) as an herbal healer. *BioImpacts*, 6: 155.

Veettil, SR, Sunil, EA, Mukunda, A, Mohan, A, John, S & Pynadath, MK 2022, Anticancer effect of *Piper betle* leaf extract on KB cell lines-an in vitro study. *Oral & Maxillofacial Pathology Journal*, 13.

Zakaria, ZZ, Ahen, Y, Ahmed, NH, Sokary, S, Bawadi, H & Al-Asmakh, M 2023, Exploring *Elaeagnus Angustifolia*: A Comprehensive Review of its Potential in Cancer Treatment and Management. *Preprints*, 2023110243.

Caspian J. Environ. Sci.
DOI:

Received:
Article type: Research