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Characterization of an Antimicrobial Extract from *Elaeagnus angustifolia*

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Background: According to ethnobotanical data, *Elaeagnus angustifolia* fruit has wound healing activity, anti-inflammatory effect and antifebrile prosperities.

Objectives: This study was performed as to the best of our knowledge; there has been no scientific report on the characterization of antimicrobial effect of *E. angustifolia* extract.

Materials and Methods: An aqueous extract of *Elaeagnus angustifolia* was prepared and antimicrobial activity tests were performed on various target cultures. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of the extract was done using the broth dilution technique. To characterize the extract, shelf life, thermal and pH stability, effects of detergents such as Tween 80, Tween 20, Triton X100, toluene and enzymes on the antimicrobial activity of *Elaeagnus angustifolia* extract, were examined.

Results: The MIC values ranged from 7.5 to 0.1 mg/mL, showing maximum activity (1.62 mg/mL) against *E. coli*. Similarly, the MBC of the extract against *E. coli* was 1.62 mg/mL. Antimicrobial activity of the extract was relatively stable when kept in the refrigerator for 60 days. The antimicrobial activity of *Elaeagnus angustifolia* extract was absolutely stable at temperatures up to 700° C. After exposure of the *Elaeagnus angustifolia* extract to different pH solutions in the range of 4-10, almost 100% residual activity was found against *E. coli* at pH 4, 5, 6, and 7. Treatment of the extract with detergents, lipase and lysozyme eliminated its antimicrobial activity.

Conclusions: Our study gives an indication of the presence of promising antimicrobial compounds and provides basic information about the nature of the *Elaeagnus angustifolia* extract. Future studies should elucidate the components responsible for antimicrobial activity of these extracts against target cultures.

Keywords: Elaeagnus angustifolia; Antimicrobial Activity; Characterization

1. Background

For a long time, the therapeutic properties of various medicinal plants have been used to treat various human diseases and recently, there has been widespread interest in botanical medicines (1, 2). It has been estimated that between 60-90% of the population of developing countries use traditional and herbal medicines almost exclusively and consider them to be a normal part of primary healthcare (3). Although in most cases their efficacy and mechanisms of action have not been tested scientifically, these simple medicinal preparations often mediate beneficial responses due to their active chemical constituents (4). Moreover, the search for plants with antimicrobial activity has grown in importance during the recent years, due to a growing concern about the increase in the rate of infection caused by antibiotic-resistant microorganisms (1-5). Although the advances brought by technology has made life easier for people, many are still looking for better alternatives such as medicinal plants. Iran has a variety of plants of medicinal importance; among these plants the genus Elaeagnus (Araliaceae) consists of more than 80 species.

2. Objectives

Although, a few studies on the antimicrobial activity of *Elaeagnus spp* have been previously conducted (6, 7), yet, we could not find any information about the characterization or properties of *Elaeagnus spp*. Thus, in the present study, we report on the characterization of antimicrobial extracts of *Elaeagnus angustifolia.*

3. Materials and Methods

3.1. Extract Preparation

The fruits of *Elaeagnus angustifolia* were collected from mountains of Ardabil, Iran during October 2010 and were identified and approved by the Herbarium Department

of Pharmacognosy of Shahid Beheshti University of Medical Sciences (Tehran, Iran). The voucher specimen (No. 1057) is preserved in the herbarium of this department for reference. An aqueous extract was prepared by adding 2000 mL of distilled water to 100 g of fruit powder (without cores) and the resulting solution was boiled for 10 minutes. Next, the mixture was filtered and the solution was completely dehydrated for 8 to 10 hours in a water bath to provide a crude extract with 20% yield.

3.2. Antimicrobial Activity

Antimicrobial activity test was performed on various authentic pure cultures of human pathogenic bacteria maintained at the Microbiology Department. The following strains were used: *Staphylococcus aureus* (*S. aureus*), *Streptococcus pneumoniae* (*S. pneumoniae*), *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae (K. pneumoniae*) and *Candida albicans* (*C. albicans*). The bacteria and *Candida albicans* were sub-cultured on Mueller Hinton agar medium and Sabouraud Dextrose Agar (SDA) medium and stored at 4°C in the refrigerator to maintain stock culture.

3.3. Minimal Inhibitory Concentration and Minimal Bactericidal Concentration Determination

Minimal inhibitory concentration (MIC) of the antimicrobial extract from *Elaeagnus angustifolia* was found using the broth dilution technique. Seven test tubes containing 4 mL of sterile nutrient broth were prepared. For the assay, the plant extract was serially diluted at concentrations of 7.5, 3.25, 1.62, 0.81, 0.4, 0.2 and 0.1 mg/mL. To each of these test tubes, 0.2 mL of target culture was added. The tubes were incubated at 37 °C for 24 hours. The test tubes were examined for visible turbidity. Next, 100 μL from the above mentioned test tubes was transferred to and plated on Mueller Hinton agar plates. The end point of complete inhibition was defined as the minimum concentration of the test compound in the original tube, which fails to yield discernible growth when sub cultured. The tests were performed in duplicates for each bacteria. Similarly, the lowest dilution that yielded complete inhibition of growth was taken as the minimal bactericidal concentration (MBC). A similar experiment on SDB was carried out for *Candida albicans*.

3.4. Characterization of E. angustifolia Extract

3.4.1. Determination of Shelf Life

Elaeagnus angustifolia extract was kept refrigerated at two temperatures for 60 days and at different time intervals, 200 μL of the extract with the MBC value of 1.62 mg / mL was taken and residual antimicrobial activity was tested against *E. coli*. To determine the effect of temperature on the stability of *Elaeagnus angustifolia* extract, screw capped tubes containing the MBC value (1.62 mg / mL) of the extract were kept at 30, 40, 50, 60, 70, and 800°C for one hour in a water bath. The tubes were cooled to room temperature and the residual activity was determined against *E. coli*. To examine pH stability of *Elaeagnus angustifolia* extract, 200 μL aliquots of the extract were mixed with 200 μL of phosphate buffer of different pH (4-10), containing 1.62 mg/mL; the mixture was incubated for one hour and its residual activity was determined against *E. coli*.

3.4.2. Effect of Detergents on Activity of Elaeagnus angustifolia Extract

Susceptibility of *Elaeagnus angustifolia* extract to denaturation by various detergents (Tween 80, Tween 20, toluene, cetrimide and Triton X 100) was studied. Detergents were added to the extract at concentrations of 0.01 mg/mL or 0.1 mL/mL. The mixture (containing 1.62 mg/mL) was incubated at room temperature and then tested for residual activity against *E. coli*.

3.4.3. Effect of Enzymes on Antimicrobial Activity of Elaeagnus angustifolia Extract

The sensitivity of *Elaeagnus angustifolia* extract to enzymes, proteinase K (pH 8.5), trypsin (pH 8.1), lipase (pH 7) and lysozyme, (pH 6.1) was tested. All enzymes were dissolved in distilled water at concentration of 1 mg/mL. Next, 200 μL of the extract was mixed with 200 μL of enzyme, resulting a concentration of 1.62 mg/mL; the mixture was incubated at room temperature for three hours and then tested for its residual activity against *E. coli*.

4. Results

The MIC and MBC values of *Elaeagnus angustifolia* extract are given in Table 1. The MIC values ranged from 7.5 to 0.1 mg/mL, showing maximal activity (1.62 mg/mL) against *E. coli*. Similarly, the MBC of the extract against *E. coli* was 1.62 mg/mL. Since the antimicrobial activity of the extract was quite stable in the refrigerator for 60 days (data not shown), experiments were also conducted to see the effect of elevated temperature on stability of the extract. The antimicrobial activity of *Elaeagnus angustifolia* extract was absolutely stable at temperatures up to 700°C. Similarly, the effect of pH on stability of *Elaeagnus angustifolia* extract against *E. coli* was analyzed in terms of residual activity. After exposure of the *Elaeagnus angustifolia* extract to different pH solutions in the range of 4-10; almost 100% residual activity was detected against *E. coli* at pH 4, 5, 6, and 7. It is worth noting that the extract lost its activity at pH 8, 9 and 10 (Table 2).

The antimicrobial activity of the extract alone against *E. coli* was 1.62 mg/mL; while it lost activity when treated by Tween 80, Tween 20, Triton X100 and toluene. Upon treatment of the extract with proteinase K, trypsin, lipase and lysozyme; the extract lost its activity with lipase and lysozyme (Table 3).

Table 1. Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs) of *Elaeagnus angustifolia* Extract

Bacteria	MIC, mg/mL [a]	MBC, mg/mL
S. aureus	7.5 ± 1.2	7.5 ± 1.4
S. pnemonia	7.5 ± 1.5	7.5 ± 1.3
E. coli	1.62 ± 0.2	1.62 ± 0.3
K. pneumoniae	1.62 ± 0.4	1.62 ± 0.4
Candida krusii	7.5 ± 2.0	7.5 ± 1.6

[a] MIC; minimal inhibitory concentration, MBC; minimal bactericidal concentration

Table 2. Effect of Temperature and pH on Activity of *Elaeagnus angustifolia* Extract

Factor (pH)	Residual Activity *(Escherchia coli)*
3	-
4	-
5	-
6	-
7	-
8	+
9	+
10	+
Temperature, °C	
30	+
40	+
50	+
60	+
70	+
80	-

Table 3. Effect of Various Enzymes and Detergents on Antibacterial Activity of *Elaeagnus angustifolia* Extract

Factor	Residual Activity *(Escherchia coli)*
Enzyme	
Proteinase K	+
Trypsin	+
Lipase	-
Lysozyme	-
Detergent	
Tween 20	-
Tween 80	-
Cetrimide	+
Toluene	-
Triton X100	-

5. Discussion

Medicinal plants are an important source of potentially useful compounds/metabolites for the development of new antimicrobial agents (8). The first step towards development of new plant based antimicrobial agents is screening of medicinal plants for their in vitro antimicrobial activity. The present study was designed to obtain preliminary information about the antimicrobial activity of *Elaeagnus angustifolia* extract and furthermore to characterize the activity of the extract.

The MIC values of the extract against the target culture ranged from 7.5 mg/mL to 0.1 mg/mL. In particular, *Elaeagnus angustifolia* extract showed potent antimicrobial activity with MIC of 1.62 mg/mL against *E. coli*. Phytochemical studies on aqueous fruit extract of *Elaeagnus angustifolia* showed that it contains free fatty acids, flavonoids compounds, sitosterols, cardiac glycosides, terpenoids and tannins (9, 10) Therefore, since our extract lost its activity after treatment with lipase we can conclude that, it is possible perceptible that the nature of the extract could be a lipid compound. Furthermore, it can be hypothesized that the antimicrobial activities of *Elaeagnus angustifolia* extract depend on interactions between their lipid components and the net surface charge of microbe membranes (11). Our results from treatment of the extract with lipase and lysozyme could inactivate the antimicrobial effect of this extract. Many studies also revealed that glycosides are major groups of secondary metabolites present in the *Elaeagnus angustifolia* extract (12). Lysozyme is an enzyme that hydrolyzes glycosides (13). Thus, decreased antimicrobial activity of *Elaeagnus angustifolia* by lysozyme may be related to break down of polyphenolic glycosides (flavonoids). Our study on inactivation of the *Elaeagnus angustifolia* extract by detergents like Tween 80, Tween 20, Triton X100 and toluene showed that inactivation occurs because these detergents can interact with both proteins and lipids and this interaction is irreversible (14). It is therefore, tempting to propose the presence of a lipid compound which had a potent antimicrobial activity.

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Authors' Contributions

Enayatollah Kalantar, developed the idea and wrote the manuscript; Mohammad Hossein Dehghan, designed the chemical characterization of the extract; Jafar Soltani, helped with writing the manuscript; Morassa Farnad, did the antimicrobial testing; Mohammad Kamalnejad, performed the extraction; Shiva Hatami, prepared the bacterial media and helped in physical characterization of the extract; Mahboobeh Mehrabani, supervised the experiments and the literature search.

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