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Study the antimicrobial activities of Russian olive (*Elaeagnus angustifolia* L. fruit)-based mouthwash on bacteria isolated from dental caries; A preliminary survey

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ABSTRACT:
Background: *Elaeagnus angustifolia* also known as Russian olive is an important medicinal fruit with high antimicrobial and antioxidant effects. In the present survey, the antimicrobial effect of *E. angustifolia* fruit-based mouthwash was tested against bacteria isolated from dental caries. The cotton swab was used to sample the dental caries areas in the oral cavity. **Methods**: Samples were cultured and isolated bacteria were subjected to antimicrobial assessment. *E. angustifolia* mouthwash was produced with a routine laboratory formula using the *E. angustifolia* ethanolic extract. Then, its antimicrobial effects were assessed using disk diffusion and Minimum Inhibitory Concentration. **Results**: *S. mutans* (25%), *E. cloacea* (15%), and *S. aureus* (20%) were isolated from dental caries samples. *E. angustifolia* mouthwash (1%) harbored the highest antimicrobial effects against *S. mujtans* (15.61±0.94 mm), and *S. aureus* (12.01±1.10 mm), while showed the lowest against *E. cloacea* (9.73±0.27 mm). Findings showed that the lowest MIC levels were obtained for *S. mutans* (2 mg/ml). The highest MIC level was found for *E. cloacea* (8 mg/ml). **Conclusion**: *E. angustifolia* mouthwash can be effectively used as a novel mouthwash with high antimicrobial effects against the pathogens responsible for dental caries. However, additional clinical trials should be done to distinguish other aspects of the *E. angustifolia* mouthwash in the oral cavity.

Keywords: E. angustifolia mouthwash, Dental caries, Antimicrobial effects.

INTRODUCTION:

Oleaster, also known as Russian olive or Wild olive (*Elaeagnus angustifolia* L.) belongs to the Araliaceae (Elaeagnacea) family and is native to some parts of North America, Europe, and Asia (1). It contains phenolic, flavonoids, antimicrobial, alkaloids, anti-inflammatory, anticancer, and antioxidant compounds and minerals and vitamins (2). *E. angustifolia* fruits are mostly applied for the treatment of colds, coughs, fever, influenza, asthma, jaundice, diarrhea, nausea, rheumatoid arthritis, pain relief, and bone fractions (3). *E. angustifolia* extract antimicrobial effects were determined against *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*). Additionally, the anticancer effects of *E. angustifolia* extract were assessed against diverse kinds of cancer cell lines (4). The edible nature of *E. angustifolia* will make it safe and easy to use as an edible antimicrobial agent for the treatment of dental caries and plaques.

Totally, scarce studies have been developed to assess the antimicrobial effects of *E. angustifolia* extract against dental and oral microorganisms. As a result, a present

survey was conducted to evaluate the antimicrobial activities of *E. angustifolia* fruit-based mouthwash on bacteria isolated from dental caries.

METHODS:

Fruits and extracts:

E. angustifolia fruits were purchased from groceries. Samples were botanically and morphologically identified by an expert professor. Collected fruits were washed and subjected to dehydration. Forced ventilation at room temperature (25 ℃) and shed was used up to a time that a constant weight was reached. A laboratory mill was used to grind the samples. A total of 30 g of *E. angustifolia* dried grind fruit samples were extracted in 100 mL ethanol 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 stored at 4 °C.

Mouthwash formulation:

As much as 100 ml of mouthwash was produced for each formulation with *E. angustifolia* extract as the active substance. The formulations of *E. angustifolia* mouthwash were done according to Table 1. Propylene glycol was included in the *E. angustifolia* extract and placed in a glass beaker. It was then raised to 60 °C, stirred with a magnetic stirrer at 300 rpm, and Tween 80, and sorbitol and aquadest were added. Benzoic acid and sodium benzoate were dissolved in aquadest and added to the solution and stirred with a magnetic stirrer until homogeneous. Subsequently, 100 ml of the sorbitol and aquadest and was stirred until the solution became clear, and *E. angustifolia* was added. Organoleptic, acidity, stability, weight mass, viscosity, irritation, and contact time of *E. angustifolia* mouthwash were assessed using the method described previously (5).

Table 1. Formulation of *E. angustifolia* mouthwash.

Components	Frequency (%)
E. angustifolia ethanolic extract	1
Propylene glycol	25
Tween 80	5
Oleum menthe piperitae	0.25
Benzoate acid	0.1
Sodium benzoate	1
Sorbitol 70%	15
Aquadest	100

Isolation of bacteria from the dental plaque samples:

The dental caries samples were identified in the oral cavity of patients who were referred for routine check-ups. For sampling, cotton sterile swabs were soaked with normal saline and after full contact with the dental caries surface, it was transferred to the laboratory using separate tubes. Cotton swabs were cultured into a sterile tube containing 5% sheep blood agar, chocolate agar, and a selective medium and transported to the microbiology laboratory. All media were incubated at 37 °C and 42 °C for 24 to 48 h. Different biochemical tests were performed after Gram staining and microscopy to identify bacterial strains. The basic biochemical tests to identify bacterial strains include the Starch Test, Simon Citrate, Oxidase, Catalase, Voges Proskauer, Urease, Indole, Methyl Red, and Coagulase Test. Analytical Profile Index (API 20E) (BioMeriouxVitek, Inc., MO, USA) system was used to identify bacteria (6).

Antibacterial effects of mouthwash against isolated bacteria:

The simple disk diffusion method was used to assess the antimicrobial effects of synthesized mouthwash. For this purpose, isolated bacteria were cultured on Muller Hinton agar media. A total of 1000 µl of 1% *E. angustifolia* mouthwash was poured into the blank disk and located at the surface of each culture media. For comparison, tetracycline (30 µg/disk) and gentamicin (10 µg/disk) (Himedia, India) antibiotic disks were accompanied. All guidelines were performed according to the Clinical and Laboratory Standard Institute (CLSI) (7-9). The Minimum Inhibitory Concentration (MIC) of synthetic *E. angustifolia* mouthwash was also assessed. For this purpose, 1, 2, and 4 mg/ml concentrations of mouthwash were prepared and the MIC value was determined using the previously described method (10).

***Statistical analysis**:*
Microsoft Office Excel and SPSS software and chi-square and analysis of variance (ANOVA) tests were used. $P < 0.05$ was considered a significant level (11-15).

RESULTS:
Table 1 indicates the bacterial distribution among the dental caries samples. *S. mutans* (25%), *E. cloacea* (15%), and *S. aureus* (20%) were isolated from dental caries samples. A statistically significant difference was obtained between the distribution of different bacteria ($P < 0.05$) (Figure 1).

Table 2. Bacterial distribution among the dental caries.

Samples	N. collected	Bacterial distribution (%)		
		S. mutans	*S. aureus*	*E. cloacea*
Dental caries	20	5 (25)	4 (20)	3 (15)

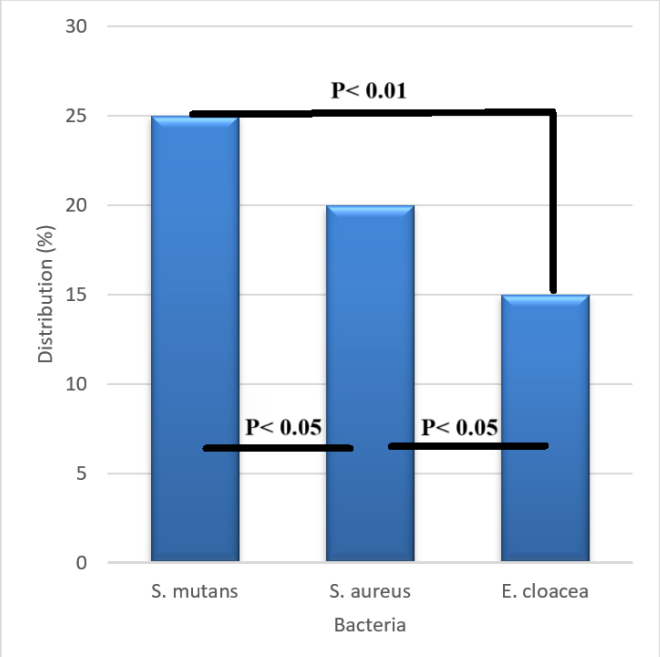

Figure 1. Statistical differences between the frequency of isolated bacteria.

Table 3 indicates the diameter of the bacterial growth inhibition zone against synthetic mouthwash and antimicrobial agents. *E. angustifolia* mouthwash (1%) harbored the highest antimicrobial effects against *S. mujtans* (15.61±0.94 mm), and *S. aureus* (12.01±1.10 mm), while showed the lowest against *E. cloacea* (9.73±0.27 mm). Statistically significant differences were obtained between the diameter of the growth inhibition zone of bacteria treated with different antimicrobial agents ($P < 0.05$).

Table 3. The diameter of the growth inhibition zone of bacteria against synthetic mouthwash and antimicrobial agents.

Antimicrobial agents	Growth inhibition zone (mm)		
	S. mutans	*S. aureus*	*E. cloacea*
E. angustifolia mouthwash (1%)	15.61±0.94 [a]	12.25±1.00 [a*]	9.73±0.27 [a]
Tetracycline	6.03±0.28 [c]	6.27±0.19 [b]	8.96±0.41 [a]
Gentamicin	10.64±0.13 [b]	6.33±0.15 [b]	10.09±0.28 [a]

[*]**Dissimilar small letters in columns show significant statistical differences ($P < 0.05$).**

Table 4 indicates the *E. angustifolia* mouthwash MIC values. Findings showed that the lowest MIC levels were obtained for *S. mutans* (2 mg/ml). The highest MIC level was found for *E. cloacea* (8 mg/ml).

Table 4. *E. angustifolia* mouthwash MIC values.

Treatment	MIC (mg/ml)		
	S. mutans	*S. aureus*	*E. cloacea*
E. angustifolia mouthwash	2	4	8

DISCUSSION:

This study showed that *E. angustifolia* mouthwash can effectively prevent the growth and proliferation of *S. mutans*, *S. aureus*, and *s* isolated from dental caries cases. Compared to antimicrobial agents, *E. angustifolia* mouthwash had better antimicrobial effects on tested bacteria.

E. angustifolia mouthwash produced by the ethanolic extract constrains the growth of all tested strains. Additionally, the *E. angustifolia* mouthwash was generally more effective on Gram-positive bacteria, compared to Gram-negative. 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 is the main reason for the high antimicrobial effects of *E. angustifolia* ethanolic extract (16).

Up to now, the antimicrobial effects of *E. angustifolia* ethanolic extract have been reported against *Candida albicans, E. coli, Bacillus subtilis, Yersinia enterocolitica, Listeria monocytogenes, S. aureus, Salmonella typhimurium* and *Enterococcus faecalis* (17).

Through recent years, owing to the increased number of diseases caused by antibiotic-resistant bacteria, the standing of using better replacements including medicinal plants with their natural antimicrobial effects has grown meaningfully (18). As *E. angustifolia* mouthwash had lower antimicrobial effects and a higher rate of MIC against *E. cloacea*, it has been recommended to use it in combination with another antimicrobial natural plant to decrease also the risk of this microorganism in the cases of dental caries. However, *E. angustifolia* mouthwash had the best antimicrobial effects against the most important pathogen in the case of dental caries, which means *S. mutans*.

As this is a preliminary survey, it may be limited to the absence of clinical trials on human models, lack of other pathogenic agents to assess the antimicrobial effects of *E. angustifolia* mouthwash, lack of *E. angustifolia* mouthwash concentrations to find the best one, and finally lack of the comparison of the findings with chlorhexidine mouthwash.

CONCLUSIONS:

In conclusion, the antimicrobial effects of *E. angustifolia* mouthwash were tested and were effective on the microbial pathogens isolated from the cases of dental caries. To be honest, additional trilas, especially on human beings should be performed to assess other aspects of the application of *E. angustifolia* mouthwash on cases of dental caries. However, its application is an oral mouthwash can be effective in preventing dental caries occurrence.

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