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Chemical composition, antibacterial, and antifungal effects of *Citrus medica* (citron), *Pimpinella anisum* (anise), and *Artemisia dracunculus* (tarragon) on oral pathogens: An *in vitro* study

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ABSTRACT

Background: Dental caries are caused by acidic by-products from bacterial fermentation of dietary carbohydrates and can lead to oral complications. Oral candidiasis is another disease affecting quality of life, especially in diabetic and immunocompromised patients. Interest in using Persian medicine to manage oral diseases has been growing recently. Persian medicine texts highlight medicinal plants such as *Artemisia dracunculus*, *Citrus medica*, and *Pimpinella anisum* for oral health benefits. The present research explores the antimicrobial effects of these plants against microorganisms causing caries and oral candidiasis.

Materials and Methods: This is an *in vitro* study, aimed to evaluate the chemical composition, and antimicrobial effects of *C. medica*, *P. anisum*, and *A. dracunculus* on oral pathogens. Based on criteria such as accessibility, recognizability, and novelty, leaves of *A. dracunculus,* peels of C. *medica,* and seeds of *P. anisum* were chosen. Their chemical compositions were analyzed after procuring the plant samples and preparing their essential oils (EOs). Subsequently, the diameters of the inhibition zones and their minimum inhibitory concentration (MIC) and minimum bactericidal concentration/minimum fungicidal concentration values were measured.

Results: The major chemical components of *P. anisum*'s EO (PAEO), *A. dracunculus'* EO (ADEO), and *C. medica*'s EO (CMEO) were estragole (75.77%), anethole (89.03%), and limonene (92.31%), respectively. All pathogens were susceptible to all EOs except *Streptococcus salivarius,* which was resistant to CMEO and had the highest MIC. Except for this EO, all the other EOs showed inhibition zones with diameters ranging from 6 to 30 mm, ADEO being the most effective. In MICs, *Lactobacillus acidophilus* was the most sensitive microorganism tested with MIC. In contrast, the most resistant microorganism was *S. salivarius*.

Conclusion: The attained results demonstrated that the examined plants possess notable antimicrobial properties against oral pathogens.

Key Words: Anti-infective agents, dental caries, medicinal plants, oral candidiasis, volatile oils

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INTRODUCTION

Dental caries is mainly caused by acidic by-products produced through bacterial fermentation of dietary carbohydrates.[1] This oral condition can significantly influence patients' quality of life and general health by causing complications such as pain, gingival bleeding, abscesses, oral malodor, decreased productivity, nutritional impairment, tooth loss, and subsequently compromising the available space in the dental arch. Moreover, treating dental caries is costly.[2] Although many studies have shown *Streptococcus mutans* as the primary etiological factor in dental caries, other bacteria such as *Lactobacillus acidophilus, Streptococcus sanguinis*, and *Streptococcus salivarius* also play a role in caries initiation.[3]

Oral candidiasis, an opportunistic infection of the mouth by *Candida* yeast, is another oral condition that significantly affects the quality of life. Diabetic and immunocompetent patients frequently suffer from this disease. Even though it is often undiagnosed, it can be easily prevented with good oral hygiene. Many studies indicate the role of candidiasis in the etiopathogenesis of premalignant oral diseases and its association with a higher risk of malignant transformation. *Candida albicans* cause most cases of candidiasis, whereas other *Candida* species may also be responsible for this infection.[4,5]

Chlorhexidine (CHX) and nystatin are effective antimicrobial agents that are frequently used in dentistry. CHX is effective against dental microbial biofilms, plaque, gingivitis, and candidiasis. Despite its frequent uses, it can lead to side effects such as dry mouth, altered taste sensations, and lingual discoloration. In addition, 0.12% CHX mouthwash has been linked to increased calculus formation despite having antiplaque properties. Nystatin, mainly used for oral candidiasis treatment, has side effects such as delayed hypersensitivity reactions and cross-reactivity with other macrolides, as well as a notable bitterness. Therefore, it can lead to increased use of sweeteners in the drug solution and raise the risk of dental caries.[6,7]

Persian medicine is among the world's medical systems, with a rich history spanning thousands of years. In this system, maintaining health and preventing disease are very important and are given priority, whereas the treatment of the diseases is next essential.[8,9] Persian medical books have mentioned various medicinal plants used as gum and dental tonic and for treating bad breath and oral ulcers.[10,11] Among the mentioned plants in these texts, many therapeutic effects on oral and dental diseases have been attributed to *Artemisia dracunculus*, *Citrus medica*, and *Pimpinella anisum*, such as gum tonic, mouth freshening, and dental pain-relieving effects.[11,12] In the present study, these three aromatic plants were selected after reviewing Persian medicine texts. In the next step, their essential oils (EOs) were prepared, and their antimicrobial effects were investigated on some of the common bacteria causing dental caries (i.e., *L. acidophilus, S. sanguinis*, *S. salivarius,* and *S. mutans*) and the leading cause of candidiasis, *C. albicans*.

MATERIALS AND METHODS

Study design

This *in vitro* study evaluated the chemical composition, and antibacterial and antifungal effects of *C. medica*, *P. anisum*, and *A. dracunculus* on oral pathogens. The current study was approved by the Ethics Committee of Zanjan University of Medical Sciences with ethics code IR.ZUMS.BLC.1401.012.

Plants selection

In this study, an initial list of efficacious herbal remedies for oral and dental conditions was prepared by examining Iranian medical texts. Based on criteria such as accessibility, recognizability (alignment of the ancient name mentioned in the texts with the plant that has been scientifically named), and novelty, three plant materials were chosen for investigation: leaves of *A. dracunculus*, peels of *C. medica*, and seeds of *P. anisum*.

Materials

Plant samples were purchased from a local market, and their scientific names were confirmed by the Department of Pharmacognosy, the School of Pharmacy, ** University of Medical Sciences and received herbarium codes (*P. anisum*: 4020, *A. dracunculus*: 4118 and *C. medica*: 5138). Sabouraud dextrose broth/agar, DeMan, Rogosa, Sharpe (MRS) broth/agar, and brain–heart infusion (BHI) broth/agar media were purchased from Merck company (Darmstadt, Germany). Iranian Scientific and Industrial Research Organization supplied the following bacterial and fungal strains: *C. albicans* 5027 (ATCC10231), *S. salivarius* subsp. *Salivarius* PTCC1448 (CIP 53.158), *S. sanguinis*

PTCC1449 (CIP 55.128), *L. acidophilus* PTCC1643, and *S. mutans* PTCC1683.

Preparation of essential oils

After drying at room temperature and in a shaded place, the plant materials were finely ground and transferred into round-bottom flasks. Two-thirds of each flask volume was filled with distilled water. Subsequently, the flasks were heated for 3 h using the Clevenger apparatus. The obtained EOs were then separated, dried by adding anhydrous sodium sulfate, and kept at 4°C until it was used for the assay.

Analysis of the essential oils using gas chromatography-mass spectrometry

The chemical composition of the EOs was analyzed using an Agilent 7890A/5975C Gas chromatography/mass spectrometry (GC/MS) system equipped with a 30 m × 0.25 mm × 0.25 m semipolar HP-5 ms capillary column (Agilent Technologies). EO samples diluted 1:10 in hexane (1.0 µL) were injected in a split mode (50:1). The injection port temperature was set at 250°C. Helium was used as the carrier gas with a flow rate of 1 mL/min. Injector and auxiliary temperatures were set at 260°C and 280°C, respectively. The temperature program started at 60°C for 4 min, increased to 100°C at a rate of 3°C/min, and held isothermally at 100°C for 2 min. Then, the temperature increased to 260°C at a rate of 4°C/min and finally kept constant at 260°C for 5 min. The constituents of the EOs were identified by comparing their mass spectra with data from the Wiley Database Library and the National Institute of Standards and Technology mass spectral library. The retention index of each separated component was also calculated using n-alkane (C9-C23) standards, as shown in Adams (2017)[13] and used to confirm identification.

Preparation of microbial suspension

Streptococcus species and *L. acidophilus* were grown in BHI broth/agar and MRS broth/agar at 37°C for 24–48 h, respectively. *C. albicans* was also cultured on sabouraud dextrose broth/agar at 35°C ± 2°C for 18–48 h. The antimicrobial activity of EOs was evaluated by preparing a microbial suspension into sterile PBS equivalent to 0.5 McFarland with a concentration of 1.5×10^8 CFU/mL.

Determining the antimicrobial activity of essential oils using agar well diffusion method

After preparing a microbial suspension equivalent to the 0.5 McFarland standard, it was cultured as lawn onto the surface of culture media using a sterile swab. Subsequently, wells were punched into the culture medium surface using a sterile Pasteur pipette, and 50 µL of EOs were inoculated into each well. After 24 h of incubation at 37°C, the diameter of the growth inhibition zone was measured in millimeters using a ruler. CHX 0.12% and DMSO were used as positive and negative controls, respectively.

Determining the minimum inhibitory concentration and minimum bactericidal concentration/minimum fungicidal concentration using broth microdilution method

The broth microdilution method was used to determine the minimum inhibitory concentration (MIC) of EOs using a 96-well microplate. For this purpose, 150 µL of culture media was added to each well. Next, 150 µL of the EOs were added to the first well. After thoroughly mixing the EOs and the culture medium, 150 µL of this mixture was transferred to the adjacent well (well 2). This process was serially continued until well 9. Wells 11 and 12 were used as negative and positive controls, respectively. Except for well 11, 15 µL of microbial suspension containing 1.5×10^8 CFU/mL were added to each well. Finally, the microplate was incubated at 37°C for 24 h. MIC was calculated as the lowest concentration of EOs that inhibited the visible growth of microorganisms. Furthermore, wells without visible growth of bacteria were selected, and 50 µL of them streaked on agar medium. The culture media was incubated at 37°C for 24 h. The plate without bacterial growth with the lowest EOs concentration was considered for the minimum bactericidal concentration (MBC). The plate devoid of fungal growth and possessing the minimal EOs concentration was considered for the minimum fungicidal concentration (MFC).

RESULTS

Chemical composition of essential oils

The mean yield of *C. medica*, *P. anisum,* and *A. dracunculus* EOs s after three hydrodistillation extractions was 1.04 g oil/100 g dried peels, 0.3 g oil/100 g dried seeds, and 0.22 g oil/100 g dried leaves, respectively.

The chemical compositions of the EOs are summarized in Tables 1-3. The results showed that the chemical content of *P. anisum*'s EO (PAEO) and *A. dracunculus*'s EO (ADEO) are almost close to each other. In contrast, *C. medica*'s EO (CMEO) has a different chemical content. In ADEO, the

Table 1: Chemical composition of *Artemisia dracunculu's* essential oil

Name	RI	Area (%)
Methyl chavicol (Estragole)	1187	75.77
(Z)-beta-Ocimene	1038	6.24
(E)-beta-ocimene	1049	4.27
Limonene	1030	3.42
Spathulenol	1569	2.07
Methyl eugenol	1384	1.80
Alpha-pinene	937	1.70
Bicyclogermacrene	1497	0.92
Germacrene D	1481	0.67
(E)-Caryophyllene	1422	0.65
Isobornyl acetate	1277	0.43
Alpha-Phellandrene	1005	0.33
Carvone	1222	0.32
Caryophyllene oxide	1574	0.31
Beta-Pinene	979	0.20
(E,E)-alpha-Farnesene	1513	0.19
O-Cymene	1021	0.18
(E)-beta-Ionone	1473	0.17
6,10,14-trimethyl-2-Pentadecanone	1850	0.17
Sabinene	975	0.09
Total identified compounds		99.9
Total identified hydrocarbons		18.67
Total identified oxygenated compounds		80.87
Total identified monoterpenes		17.18
Total identified sesquiterpene		4.81
Total identified phenylpropanoids		77.57

RI: Retention index

Table 2: Chemical composition of *Pimpinella anisum's* essential oil

Name	RI	Area (%)
(E)-Anethole	1275	89.03
Carvone	1222	5.00
Limonene	1030	2.26
Methyl chavicol (Estragole)	1185	1.86
(E)-Pseudoisoeugenyl 2-methylbutyrate	1830	0.53
Gamma-himachalene	1478	0.46
Cis-Dihydrocarvone	1178	0.20
Germacrene D	1481	0.18
Zingiberene	1499	0.17
Alpha-phellandrene	1006	0.11
(Z)-Anethole	1239	0.11
Alpha-himachalene	1450	0.10
Total identified compounds		99.96
Total identified hydrocarbons		3.28
Total identified oxygenated compounds		96.73
Total identified monoterpenes		7.57
Total identified sesquiterpenes		0.91
Total identified phenylpropanoids		91.53

RI: Retention index

predominant compounds included oxygenated compounds (80.8%) and phenylpropanoids (77.5%). Similarly, in PAEO, the primary compounds were also found to be oxygenated compounds (96.73%) and phenylpropanoids (91.53%). CMEO was entirely composed of hydrocarbon compounds that mainly belong to monoterpenes (94.05%). The major components of ADEO included estragole (75.77%), (Z)-beta-ocimene (6.24%), and (E)-beta-ocimene (4.27%). The major components of PAEO were anethole (89.03%), carvone (5.00%), and limonene (2.26%). Limonene (92.31%), beta-bisabolene (2.12%), and alpha-trans-bergamotene (2.09%) were the major components of CMEO.

Antimicrobial activity

The antimicrobial properties of CMEO, PAEO, and ADEO against five different strains of oral pathogens were evaluated using the agar well diffusion and broth microdilution methods. The results are summarized in Table 4. All pathogens were susceptible to all EOs except *S. salivarius*, which was resistant to CMEO. According to the results, no inhibition zone was observed after evaluating the effect of CMEO on *S. salivarius*. Except for this strain, the EOs showed inhibition zones with diameters ranging from 6 to 30 mm; the results demonstrated that the most effective EO, compared to CHX and nystatin, was ADEO (inhibition zones ranging from 7 to 30 mm). PAEO also showed relatively suitable results as compared to the two standard drugs. Conversely, the least effective one was CMEO, with inhibition zones ranging from 0 to 15 mm. Among the oral pathogens, *C. albicans* demonstrated the highest sensitivity to EOs, whereas *S. salivarius* was the most resistant.

The outcomes regarding MIC and MBC/MFC of EOs have been briefly outlined in Table 5. All EOs had an inhibitory effect on microbial isolates. *L. acidophilus* was the most sensitive microorganism tested with MIC of 1.82 mg/mL for PAEO, 1.8 mg/mL for ADEO, and 1.56 mg/mL for CMEO. In contrast, the most resistant microorganism was *S. salivarius*, with MIC of 233.25 mg/mL for PAEO, 113.75 mg/mL for ADEO, and 400 mg/mL for CMEO. PAEO induced a strong bactericidal effect on *S. mutans, S. sanguinis,* and *L. acidophilus* in the same concentration of MIC (1.82 mg/mL). Nevertheless, it had no bactericidal/fungicidal effect on *C. albicans* and *S. salivarius*. ADEO exerted a strong bactericidal effect on *S. mutans* and *S. salivarius* in the same concentration of MIC (1.8 mg/mL). Meanwhile, it had no bactericidal/fungicidal effect on *C. albicans* and

L. acidophilus. CMEO had no bactericidal/fungicidal effect on *S. sanguinis*. In comparison, it induced the bactericidal effect on *L. acidophilus* and *S. salivarius* in the same concentration of MIC.

DISCUSSION

Lately, the use of herbal medicine in the treatment of various diseases has been on the rise. Recent studies have shown the significant role of plant extracts and isolated compounds as natural antibacterial agents in oral care products. In various fields (e.g., dentistry), EOs have found their application and are an area of interest for researchers. EOs are complex mixtures of compounds with low-molecular weight (e.g., monoterpenes, sesquiterpenes, and phenylpropanoids) with hydrocarbon structures or with functional groups, often aldehyde, alcohol, ester, and ketone. These plant extracts have significant antimicrobial effects, which are generally the result of the interaction of their compounds with the cell membrane of the microorganism. Their lipophilic nature and low-molecular weight allow them to penetrate the cell wall, interact with membrane lipids, and cause irreversible damage to it. Disruption of the electron transport chain, absorption of nutrients, synthesis of protein and nucleic acid, coagulation of cell content, and inhibition of enzymes necessary for energy metabolism are the consequences of this damage, ultimately leading to cell death. The bioactivity of the EO depends on its constituent compounds, ratio, and structural configuration.[14,15] For example, Anthole, as the predominant substance in PAEO, was identified in this study as the major compound in PAEO that induces cell death due to its lipophilic nature and ability to traverse the cell membrane, interfering with cellular membranes and proteins.[16] The major substance in

Table 3: Chemical composition of *Citrus medica's* essential oil

Name	RI	Area%
N-Octane	-	0.63
Alpha-pinene	937	0.53
(Z)-beta-farnesene	1422	0.83
Myrcene	995	1.21
Alpha-trans-bergamotene	1443	2.09
Beta-bisabolene	1514	2.12
Limonene	1030	92.31
Total identified compounds		99.73
Total identified hydrocarbons		99.73
Total identified oxygenated compounds		0.00
Total identified monoterpenes		94.05
Total identified sesquiterpenes		5.04

RI: Retention index

Table 4: Antimicrobial activities of *Pimpinella anisum's* essential oil, *Artemisia dracunculu's* essential oil, and *Citrus medica's* essential oil against selected microorganisms using agar well diffusion method (mm)

Microorganisms	*Candida albicans* (PTCC 5027)	*Streptococcus salivarius* (PTCC 1448)	*Streptococcus sanguinis* (PTCC 1449)	*Streptococcus mutans* (PTCC 1683)	*Lactobacillus acidophilus* (PTCC 1643)
PAEO	23	14	15	14	12
ADEO	30	7	16	16	19
CMEO	12	0	15	6	14
CHX	ND	16	17	16	23
Nystatin	30	ND	ND	ND	ND

ND: Not determined; PAEO: *Pimpinella anisum's* essential oil; ADEO: *Artemisia dracunculu's* essential oil; CMEO: *Citrus medica's* essential oil; CHX: Chlorhexidine

Table 5: Minimum inhibitory concentration and minimum bactericidal concentration/minimum fungicidal concentration values (mg/mL) of *Pimpinella anisum's* essential oil, *Artemisia dracunculu's* essential oil, and *Citrus medica's* essential oil on selected microorganisms determined by broth microdilution method

Microorganisms	*C. albicans* (PTCC 5027)		*Streptococcus salivarius* (PTCC 1448)		*Streptococcus sanguinis* (PTCC 1449)		*Streptococcus mutans* (PTCC 1683)		*Lactobacillus acidophilus* (PTCC 1643)	
	MFC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC
PAEO	-	29.16	-	233.25	1.82	1.82	1.82	1.82	1.82	1.82
ADEO	-	14.22	113.75	113.75	56.88	28.44	1.8	1.8	-	1.8
CMEO	400	200	400	400	-	200	400	200	1.56	1.56
CHX	ND	ND	>0.0047	>0.0047	>0.0047	>0.0047	>0.0047	>0.0047	>0.0047	>0.0047
Nystatin	>0.13	>0.13	ND	ND	ND	ND	ND	ND	ND	ND

-: No bactericidal/fungicidal effect; ND: Not determined; MBC: Minimum bactericidal concentration; MIC: Minimum inhibitory concentration; MFC: Minimum fungicidal concentration; PAEO: *Pimpinella anisum's* essential oil; ADEO: Artemisia dracunculu's essential oil; CMEO: *Citrus medica's* essential oil; CHX: Chlorhexidine

CMEO (i.e., limonene) similarly disrupts membrane integrity, causes cellular leakage, and ultimately, cell death. It also inhibits DNA transcription and translation.[17] Given the noted properties of the EOs from the studied plants, they appear to be suitable candidates for use in dental materials and oral hygiene products such as mouthwash, toothpaste, dental floss, and more. Moreover, previous studies have demonstrated that the plants under investigation have shown no toxic effects, even at high doses, and their use in living specimens has not caused any adverse effects on the kidneys or liver.[18-20] This, coupled with their antimicrobial properties, highlights the strong potential of these plants for use as a mouthwash.

In the present article, we investigated the antibacterial and antifungal effects of CMEO, PAEO, and ADEO against microorganisms such as *S. mutans*, *S. sanguinis*, *S. salivarius*, *L. acidophilus*, and *C. albicans*.

The results of the agar well diffusion method showed that all microorganisms were sensitive to the prepared EOs, except for *S. salivarius*, indicating their resistance to CMEO. Overall, CMEO exhibited less potency on studied Candida and the bacterial strains. In addition, it showed weaker inhibitory and cidal effects against *C. albicans*, S. *salivarius*, *S. sanguinis*, and *S. mutans*. The results of both methods indicate that the ADEO and PAEO had stronger effects.

The GC/MS analysis results revealed that PAEO and ADEO possess high levels of oxygenated compounds, but the compounds identified in CMEO were not oxygenated. Various studies have demonstrated that EOs rich in oxygenated compounds exhibit greater antibacterial properties.[21] Moreover, previous studies have considered the lower antimicrobial effects of hydrocarbon derivatives to be related to their limited diffusion in the medium due to their lower solubility.[22] In many studies on the antifungal effects of EOs, the predominant effects of EOs rich in oxygenated compounds were also reported. Meanwhile, hydrocarbon compounds have shown a limited role in the antifungal effects of EOs.[23] The results of the mentioned studies can explain the stronger antibacterial and anticandida effects of PAEO and ADEO than those of CMEO. Although the antimicrobial effect of CMEO on the *Candida* and *Streptococcus* strains was weak, according to Table 5, it had a potent effect on *L. acidophilus*. Tang *et al*. showed the antibacterial activity of CMEO and D-limonene (its major compound) against *Escherichia coli* K99 and *L. acidophilus*. They indicated that CMEO inhibits the growth of *E. coli* K99 and *L acidophilus* by disrupting bacterial cell membrane permeability and surface hydrophobicity, causing intracellular macromolecule formation and preventing cell membrane formation.[24] In general, *L. acidophilus* showed the most sensitivity and *S. salivarius* indicated the least sensitivity to the EOs among the studied bacteria. PAEO and ADEO showed notable anticandida effects and can be the objective of further studies as anticandida agents.

Several studies have been conducted on the plants whose EOs were examined in the present study. In most of these studies, the antimicrobial effects of extracts other than EOs have been investigated.[25-27] To the best of our knowledge, the antimicrobial effects of these three EOs on oral pathogens have not been investigated so far. Arabestani *et al*. investigated the impact of *A. dracunculus* on oral pathogens, preparing n-hexane, ethyl acetate, methanol, and water extracts and assessing their antibacterial activity against *S. mutans* and *S. sobrinus* through the agar well diffusion and microdilution methods. Methanolic extracts exhibited significant superior activity against both bacteria.[28] In another study, Sadeghi-Nejad *et al*. used *A. dracunculus*, *Satureja khuzestanica*, and *Myrtus communis* aqueous extracts to prepare a polyherbal toothpaste formulation. The results showed their significant effects against *S. mutans*, *L. casei*, *S. sanguinis*, *S. salivarius*, and *C. albicans*.[29]

Lavaee *et al*. (2023) measured the antimicrobial properties of ethanolic and methanolic extracts of *P. anisum* and *Oregano vulgare* on *S. sanguinis*, *S. mutans*, and *S. salivarius* obtained from dental caries. *P. anisum* was an effective antibacterial agent against selected bacteria and showed the most antibacterial activity, especially against *S. mutans*.[30] Awad *et al*. measured the antimicrobial effect of *P. anisum* extract as a mouth rinse in 6–12-year-old children against *S. mutans* and lactobacilli. The children were randomly divided into three groups (A, B, and C) of 20 each. Group A received 1.6 g/40 mL *P. anisum* solution mouth rinse three times/day for 1 min for 1 week, Group B received 10 g/40 mL *P. anisum* solution mouth rinse three times/day for 1 min for 1 week, and Group C received only 40 mL of distilled water three times/day for 1 min for 1 week. Group B showed a significant decrease in bacterial counts, indicating the efficacy of the stronger *P. anisum* extract.[31] Dumitrescu *et al*. investigated the structure and antimicrobial

properties of PAEO. The antibacterial and antifungal effects were analyzed against specific strains, such as *C. albicans*, using the broth microdilution method, and GC/MS was used for structure identification. These authors identified a total of 13 compounds, of which transanethole was in the highest proportion (72.49%), followed by limonene (10.01%), anisole (5%), and α-pinene (3.26%). This finding bears some resemblance to the results of the GC/MS of our study. The existing disparities may be attributed to the differences in plants' growth conditions in various regions. Excellent antifungal activity against *C. albicans* was also ascertained.[25] In another study, Chaudhry and Tariq investigated the effects of the aqueous extract of *P. anisum* on 176 bacterial isolates, including *Staphylococcus aureus, S. salivarius,* and *S. sanguinis*. The aqueous extract did not affect *S. mutans* and *S. sanguinis*; however, it exhibited an inhibition zone with a diameter of 14 mm against *S. salivarius*.[32]

Jayaram *et al*. evaluated the chemical composition and anthelmintic, antimicrobial, antioxidant, and anticancer activities, of chloroform extract of fruit peel of *C. medica*. Chloroform extract of *C. medica* exhibited good antibacterial activities with inhibition zones ranging between 8 and 11 mm, however, no significant inhibition zones were seen in antifungal assays. Unfortunately, none of the microorganisms examined in this study bore any resemblance to those in our research. Their research showed that in addition to its strong antimicrobial properties, this extract also has promising anthelmintic, antioxidant, and anticancer properties. Furthermore, key phytochemicals were identified n-hexadecanoic acid, which was in complete contradiction to our study.[33] Krumina *et al*. (2015) assessed the impact of 10 plant extracts, 6 juices, and propolis, individually and in combinations, on the *in vitro* growth of oral pathogens *S. mutans* and *C. albicans*. Using agar-well diffusion and broth dilution methods revealed that all tested 70% ethanolic extracts inhibited the growth of both pathogens, with cloves, cinnamon, propolis, lavender, and sage showing the highest inhibitory activity. Nevertheless, juices, including *C. medica*'s juice demonstrated limited effectiveness. Combinations of extracts showed diverse effects, with some combinations exhibiting synergistic actions while others displayed antagonistic interactions, particularly against *C. albicans*.[34] Sah *et al*. investigated the antimicrobial properties of fruit juice and ethanolic extracts from various parts of *C. medica* against a range of bacteria, fungi, and yeast of clinical origin. *C. albicans* proved resistant to all plant samples. Notably, the fruit juice and pulp extracts exhibited broad-spectrum antimicrobial activity with MIC and MBC. The root extract demonstrated particularly high potency against *S. aureus*. Conversely, leaf and peel extracts showed comparatively weaker antimicrobial activity.[35]

All reviewed studies indicate that the examined plants possess significant antimicrobial effects. None of the discussed studies have addressed the EO effects on the plants we investigated. Besides, since they have employed different methodologies in the preparation of the plant product or selected different microorganisms, making direct comparison of the results with our study is challenging. This result shows a gap in research on their impact on oral pathogens. Investigating this area could help develop strategies for oral hygiene and health. Bridging this gap could lead to natural and effective methods of combating oral infections. This field of research is an opportunity for researchers to investigate and discover more about the potential impacts of these plants on oral health.

CONCLUSION

The attained results have demonstrated that the examined plants possess notable antimicrobial properties against oral pathogens. This result suggests their potential as promising candidates for future investigations as therapeutic agents in oral diseases.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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