Original Article

Study The Biologic Effects Of Thymus Kotschyanus With Chlorhexidine 0.2% Mouthwash Solution On Some Mouth And Teeth Bacteria In Vitro Condition

Zahra Ataie[1,*], Mehrdad Ataie Kachoie[2,3,*], Mehrnoosh Tavakoli Dinani[1], Ebrahim Rahimi[4]

[1]Department of Periodontology, Dental Implants Research Center, Dental Research Iinstitute, School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran.
[2]Department of Medicinal Plants, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran.
[3]Medicinal Plants Processing Center, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran.
[4]Department of Food Hygiene and Public Health, Faculty of Veterinary Medicine, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran.

[*]Corresponding authors:
***Zahra Ataie**, Department of Periodontology, Dental Implants Research Center, Dental Research Iinstitute, School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran. Email address: zataiee@yahoo.com.
***Dr. Mehrdad Ataie Kachoie**, Associate Professor, Department of Medicinal Plants, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran. Medicinal Plants Processing Center, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran. Email address: Drataie@iaushk.ac.ir; Mehrdad.ataie@gmail.com.
[*]These two authors are equally recognized as the Corresponding authors.
DOI: 10.47750/pnr.2022.13.S09.1137

Abstract

Thymus kotschyanus is an essential oil with high antimicrobial effects. The present research was aimed to assess the antimicrobial effects of T. kotschyanus essential oil, chlorhexidine 0.2% mouthwash and antibiotic agents against some mouth and teeth bacteria in vitro condition. Aerial parts of the T. kotschyanus were prepared and used for essential oil extraction. Chemical components of T. kotschyanus essential oil were identified using the GC-MS. Antimicrobial effects were examined using the disk diffusion and MIC and MBC determination. Totally, 25 chemical compounents were identified in the T. kotschyanus essential oil. Carvacrol (52.01%), Sabinene hydrate (11.58%), 1-8 cineol (7.50%), Para saimene (6.89%) were the most commonly identified chemical compounents in the T. kotschyanus essential oil. In the disk diffusion, the diameter of the growth inhibition zones had ranges between 5.15±0.16 to 14.80±1.09 mm. The highest diameter of the growth inhibition zone was recorded for L. casei treated with T. kotschyanus (10 mg/ml), while the lowest was found for S. salvarius treated with T. kotschyanus (0.16 mg/ml). Statistically significant differences were obatined between some concentration of the T. kotschyanus and chlorhexidine, ampicillin, and metronidazol antibiotic agents ($P < 0.05$). The MIC values had ranges between 2.5 to 5 mg/ml. The MBC values hgd ranges between 5 to 10 mg/ml. The lowest MIC was identified for S. sanguis, S. salvarius, L. reutrei, L. acidophilus and L. casei treated by T. kotschyanus essentialk oil (2.5 mg/ml). The lowest MBC was identified for S. sanguis, S. salvarius, L. reutrei, L. acidophilus and L. casei treated by T. kotschyanus essentialk oil (5 mg/ml). According to the edible route of T. kotschyanus essential oil and its higher antimicrobial effects compatred to antibiotic agents and chlorhexidine mouthwash, formulation of mouthwash based on the T. kotschyanus essential oil is recommended to decrease the risk of oral infections, dental caries, and their subsequent complications.

Keywords: Thymus kotschyanus, Antimicrobial effects, Oral bacteria, Disk diffusion, MIC, MBC.

Introduction

In recent years, several innovation and development have established in dental sciences (1-5). In keeping with this, microbial community found in the oral cavity remain challenging in the cases of dental health (6, 7). There are about 500 types of microbes in the mouth, some of which are the cause of infectious oral disease, many are the cause of tooth decay. Tooth colonization by carious bacteria is one of the important risk factors in the development of dental diseases (8). A wide group of microorganisms have been identified in tooth decay, the most important of which are Streptococcus mutans, Lactobacillus acidophilus, proteolytic bacteria, anaerobic organisms such as Provetella species, Peptostreptococcus, Valionella, Fusobacterium, and Actinomyces viscosus, which play a role in the initiation and spread of tooth decay (9-12). Facultative anaerobes, especially S. mutans, is an important organism that is commonly found in human caries and participates in the progression of the lesion (13). Additionally, S. salvarius, and S. sanguis are other important genus of this family responsible for dental decay (14).

One of the ways to reduce the amount of oral microbes is to use disinfectant solutions called mouthwash. The use of mouthwashes effectively reduces the microbial population of the mouth and thus prevents gum diseases, tooth decay and accelerates the healing of oral wounds (15). Chlorhexidine is the most commonly used broad-spectrum antiseptic agent in oral hygiene. It is widely prescribed in different dental fields, especially in patients that cannot perform correctly mechanical biofilm control, due to physical/mental impairment, lack of motivation or xerostomia (16). Additionally, chlorhexidine has multiple applications in dentistry because of its substantivity. Chlorhexidine is adsorbed by the oral mucosa, oral proteins, and onto hydroxyapatite of the dental surface (17). It is mainly used in 0.2% concentration as mouthwash to decrease the microbial population of the oral cavity and mainly decreases the risk of dental decay (18). However, it has a chemical base and may have some bad effects on the human health (19, 20).

In recent years, using medicinal plants have developed in medical sciences (21). Herbal mouthwashes have better conditions than chlorhexidine, which is a common and available mouthwash, due to having natural ingredients in terms of compatibility with the body's physiology and less possibility of poisoning (22, 23). Therefore, it is recommended for people who cannot use chemical mouthwashes.

Iran is rich in ecological diversity and has a rich herbal flora which has not yet been well studied in terms of phytochemistry and bioactivity. Lamiaceae (formerly Labiatae) is one of the most important plant families in which Thymus with about 215 species is a significant genus (24). Thymus species are commonly used as a tonic, carminative, digestive, antitussive, and expectorant for the treatment of cold in Iranian traditional medicine. Recent studies have implied that these species have strong antibacterial activities (25). Thymus has several species including Thymus vulgaris, Thymus daenensis, Thymus schimperi, Thymus zygis and Thymus kotschyanus etc. (25). T. kotschyanus is a dicot herbal plant belonging to Lamiaaceae's family. The name of T. kotschyanus is gained from Greek "Thymos" meaning power and courage (26). In traditional medicine thymus species are used as antibacterial, antifungal, antiviral, anti-helminthic, antioxidative, antispasmodic, sedative and diaphoretic drugs (27). Carvacrol and Thymol are known as the major phenolic monoterpenes of thyme oil especially in T. kotschyanus oil showing anticancer and anti-depressant effects (28).

The present study was done to assess the biologic effects of T. kotschyanus with chlorhexidine 0.2% mouthmouthwash solution on some mouth and theet bacteria in vitro condition.

Materials and methods

Plant materials

Fully dried T. kotschyanus plant aerial parts were obtained from research farm in city of Shahrekord, Iran. The scientific name was confirmed by the Herbarium Center of the Islamic Azad University, Shahrekord Brnch, Shahrekord, Iran. In order to prepare the essential oil, the plant was crushed and grinded. Then, the essential oil was extracted by ethanol distillation using a Clevenger apparatus. Essential oil extraction was done for 3 hours from the time the liquid inside the balloon was boiled. After drying using sodium sulfate, the essential oil was passed through 0.45 µm microfilter, and stored in a dark glass container at 4 °C away from sunlight. The treatments that were prepared included 0.2% chlorhexidine and T. kotschyanus essential oil with specific concentrations.

GC-MS analysis

Analysis of essential oil composition using Gas chromatography mass spectrometry(GC-MS) Identification of essential oil components was done using retention indices by evaluating mass spectra of compounds and comparing them with standard mass spectra and valid references. For this purpose, the prepared essential oil was injected into the GC system, and the optimum column temperature was calculated for the complete separation of the essential oil's components. The essential oil was then injected into the mass chromatograph to study the mass spectra of the compounds. The GC-MS device Agilent 6890 with a HP-5MS capillary column (0.25 mm internal diameter, 30m length, film thickness0.25 µm) was used in this study. The column temperature program started with 70 °C heating for 2 min. Then, the temperature was increased to 220 °C at rate of 15 °C per minute, and continued to 300 °C for 2 min.

Antimicrobial evaluation

Pathogens include three types of S. salvarius, S. sanguis, and S. mutans, and the probiotics used include L. reutrei, L. acidophillus, and L. casei, which were obtained from the Collection Center of Industrial Microorganisms (Scientific and Industrial Research Organization, Tehran, Iran) along with the strain code. Each bacterium was cultured in the specific culture medium. In order to prepare a bacterial suspension from a fresh and young bacterial culture, several colonies were transferred to Mueller Hinton Broth culture medium (Merck, Germany) and to equalize the turbidity of the prepared microbial suspension according to 0.5 McFarland's standard, light absorption was set at a wavelength of 630 nm (29, 30). Eight different concentrations of essential oils (20, 10, 5, 2.5, 1.25, 0.63, 0.31, 0.16 µl/disk) were used, and the method of preparation of essential oil concentrations includes the final volume of each concentration of 20 µl, which from each concentration, the specified amount was taken with a sampler and dimethyl sulfoxide was added to reach the final volume, and the final volume of essential oil and solvent was 20 µl/disk. Dimethyl sulfoxide solvent was considered as a negative control in measuring the diameter of growth halo and evaluating Minimum Inhibitory Concentration (MIC) and Minimum Bacterial Concentration (MBC), and its possible effects were removed (31).

Disc diffusion method was used to determine the diameter of the halo of non-growth for the MIC and MBC and MBC of bacteria, in which a specific culture medium was prepared for each pathogen or probiotic and a thin layer of microbial suspension was cultured on the culture medium, then sterile 6 mm thick blank discs containing the required concentrations of essential oil were used on the culture media, then the media were kept in an incubator for 16-24 h at a temperature of 37 °C, and then the diameter of the halos of non-growth was measured with a caliper. To be sure, this experiment was repeated 3 times for each bacterial strain (32). A 0.2% chlorhexidine mouthwash was used to compare with the effects of essential oils by disking method. The MIC of essential oils was determined by the microbroth dilution method. For each treatment in the 96-well microplate, house number 1 to 8 corresponding to dilutions of 20 to 0.16 microliters of essential oils were considered. Then, it was placed in an incubator at a temperature of 37 °C for 16-24 h. Immediately after removing from the incubator, the absorbance of the microplate wells was read in the ELISA reader at a wavelength of 630 nm, and also, by visual inspection of the turbidity created in it, the lowest dilution of the test substance in which turbidity was observed in the well corresponding to that concentration It could not be considered as the minimum inhibitory concentration and the next well was considered as the minimum lethal concentration (29, 33).

Data analysis

This research was done factorially and based on completely random designs in 3 replications. The main factor includes T. kotschyanus essential oil, and the secondary factor includes different concentrations of the aforementioned T. kotschyanus essential oils, which have 8 levels including 20, 10, 5, 2.5, 1.25, 0.63, 0.31, 0.16 µl/disk. and also the concentration of chlorhexidine is 0.2% and antibiotics related to each bacteria, and the secondary factor includes the standard strains of selected microorganisms. Statistical analysis is done using Minitab version 16 statistical software and comparison of averages is done by Duncan's test at the statistical level of 1%. The data obtained from the experiment are entered into the Excel program for the above statistical analysis. P value < 0.05 was considered as significant level (34, 35).

Results

GC-MS analysis

Table 1 shows the GC-MS analysis of the chemical compounents of the T. kotschyanus essential oil. Totally, 25 chemical compounents were identified in the T. kotschyanus essential oil. Carvacrol (52.01%), Sabinene hydrate (11.58%), 1-8 cineol (7.50%), Para saimene (6.89%) were the most commonly identified chemical compounents in the T. kotschyanus essential oil.

Table 1. GC-MS analysis of the chemical compounents of the T. kotschyanus essential oil.

No	Chemical compounent	Inhibition idex	Frequency (%)
1	Sabinen	10.50	0.98
2	Alpha-pinene	10.75	1.69
3	Comphene	11.38	1.20
4	2 (10) pinene	12.69	0.50
5	Beta-mircene	13.59	1.01
6	Flandrene	14.08	0.99
7	3-Carene	14.32	0.009
8	2-carene	14.69	1.51
9	Para saimene	15.16	6.89
10	1-8 cineol	15.37	7.50
11	Sabinene hydrate	16.82	11.58
12	4 cerene	18.17	0.15
13	Beta terpineol	18.81	0.17
14	Comphore	20.81	1.49
15	Borneol	22.02	3.01
16	Paramentha 1-N, 4-L	23.56	0.91
17	Alpha terpineol	22.56	0.49
18	Iso thymol methyl ether	25.66	1.23
19	Carvacrol	28.80	52.01
20	Beta caryiophilen	33.22	1.86
21	Aromadandrene	34.02	0.24
22	Morolene	35.63	0.29
23	Cadinene	37.16	0.01
24	Cadina 3,9 di N	37.54	0.49
25	Caryiophilen oxide	39.91	0.18

Diameter oif the growth inhibition zone

Table 2 shows the diameter of the growth inhibition zone of bacterial strains against essential oils, antibiotics and Chlorhexidine 0.2%. the diameter of the growth inhibition zones had ranges between 5.15±0.16 to 14.80±1.09 mm. The highest diameter of the growth inhibition zone was recorded for L. casei treated with T. kotschyanus (10 mg/ml), while the lowest was found for S. salvarius treated with T. kotschyanus (0.16 mg/ml). Statistically significant differences were obatined between some concentration of the T. kotschyanus and chlorhexidine, ampicillin, and metronidazol antibiotic agents (P <0.05).

Table 2. Diameter of the growth inhibition zone of bacterial strains against essential oils, antibiotics and Chlorhexidine 0.2%.

Essential oil and antibiotics/concentrations		Diameter of the growth inhibition zone (mm)					
		S. mutans	S. sanguis	S. salvarius	L. reutrei	L. acidophilus	L. casei
Thymus kotschyanus	20	10.03±0.12 ^{b}	11.20±0.18 ^{c}	12.00±0.41 $_{c}$	11.93±0.55 $_{c}$	11.05±0.32 ^{c}	11.14±0.24 ^{c}
	10	12.84±1.11 ^{a}	14.43±1.00 $_{a}$	14.71±1.06 $_{a}$	14.56±1.12 $_{a}$	13.76±1.03 $_{a}$	14.80±1.09 $_{a}$
	5	11.79±0.81 ^{ab}	13.38±0.36 ^{b}	13.17±0.39 ^{b}	13.90±0.18 ^{b}	12.98±0.16 ^{ab}	13.89±0.52 ^{ab}
	2.5	9.93±0.23 ^{c}	10.99±0.38 ^{c}	11.87±0.68 $_{c}$	11.90±0.24 $_{cd}$	10.95±0.42 ^{c}	11.01±0.33 ^{c}
	1.25	7.75±0.51 ^{d}	8.86±0.63 ^{d}	9.81±0.37 $_{d}$	9.96±0.41 $_{e}$	9.02±0.20 ^{d}	8.93±0.53 ^{d}
	0.63	7.01±0.24 ^{e}	6.86±0.41 ^{e}	7.98±0.19 $_{e}$	7.80±0.26 $_{f}$	7.00±0.41 ^{e}	6.92±0.34 ^{e}
	0.31	6.12±0.20 ^{f}	6.03±0.17 ^{ef}	6.27±0.31 $_{f}$	6.92±0.28 $_{g}$	6.11±0.46 ^{f}	6.00±0.32 ^{f}
	0.16	5.52±0.35 ^{g}	5.49±0.28 ^{g}	5.15±0.16 $_{g}$	6.50±0.30 $_{h}$	5.49±0.25 ^{g}	5.58±0.34 ^{g}
Chlorhexidine 0.2%		11.18±0.48 $_{b}$	12.01±0.39 $_{b}$	12.55±0.23 $_{bc}$	13.03±0.44 ^{b}	12.07±0.26 ^{ab}	13.08±0.29 ^{b}
Ampicillin		12.93±1.14 ^{a}	13.45±0.22 $_{a}$	14.51±1.16 $_{a}$	13.15±0.61 ^{b}	12.19±0.33 ^{ab}	13.21±0.74 ^{b}
Metronidazole		11.25±0.51 $_{b}$	12.77±0.20 ^{ab}	12.98±0.69 $_{b}$	14.30±1.27 $_{a}$	13.58±0.42 $_{a}$	14.70±1.08 $_{a}$

*Dissimilar letters in each column show significant differences about P <0.05.

MIC and MBC determination

Table 3 shows the MIC and MBC concentrations of essential oil and chlorhexidine against tested bacteria. The MIC values had ranges between 2.5 to 5 mg/ml. The MBC values hgd ranges between 5 to 10 mg/ml. The lowest MIC was identified for S. sanguis, S. salvarius, L. reutrei, L. acidophilus and L. casei treated by T. kotschyanus essentialk oil (2.5 mg/ml). The lowest MBC was identified for S. sanguis, S. salvarius, L. reutrei, L. acidophilus and L. casei treated by T. kotschyanus essentialk oil (5 mg/ml).

Table 3. MIC and MBC concentrations of essential oil and chlorhexidine against tested bacteria.

Essential oil	MIC and MBC (mg/mL)											
	S. mutans		S. sanguis		S. salvarius		L. reutrei		L. acidophilus		L. casei	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Thymus kotschyanus	5	10	2.5	5	2.5	5	2.5	5	2.5	5	2.5	5
Chlorhexidine 0.2%	5	10	5	10	2.5	5	2.5	5	5	10	2.5	5

Discussion

In recent years, antibiotic-resistant bacteria have considered as important health threating factors globally (36-40). As a result, a big health threating issue will be occurred in future. The present survey was aimed to assess the antimicrobial effects of T. kotschyanus essential oil and chlorhexidine 0.2% on some probiootic and pathogenic bacteria of the oral cavity. Our findings showed that T. kotschyanus essential oil (10 mg/ml) harbored the highest antimicrobial effects against tested organisms, including S. mutans, S. sanguis, S. salvarius, L. reutrei, L. acidophilus, and L. casei. Additionally, T. kotschyanus essential oil harbored the lowest MIC and MBC values which showed its high potential antimicrobial effects even on low concentrations.

As it shows, carvacrol (52.01%), sabinene hydrate (11.58%), 1-8 cineol (7.50%), para saimene (6.89%) were the most commonly identified chemical compounents in the T. kotschyanus essential oil. All of these chemical compounents had high antimicrobial effects against diverse kinds of bacterial agents, including Staphylococcus aureus, Escherichia coli, and Candida albicans (41), Streptococcus species (42), and Bacillus subtilis, Enterobacter cloacae, E. coli O157:H7, Micrococcus flavus, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella enteritidis, S. epidermidis, S. typhimurium, and S. aureus (43). Additionally, high antimicrobial effects of T. kotschyanus essential oil was reported against Aspergillus niger and C. albicans (44), E. coli, S. aureus, Bacillus subtilis, Klebsiella pneumonia, and P. aeruginosa (45), multidrug resistance (MDR) Acinetobacter baumannii, P. aeruginosa, and S. aureus (46), and S. aureus and P. aeruginosa (47).

The most appropriate method for determining the bactericidal effect as well as a strong tool for obtaining information about the dynamic interaction between the anti-microbial agent and the microbial strain is the time-kill test. Also a time-dependent or a concentration-dependent antimicrobial effect is revealed by the time-kill test. Factors determining the activity of essential oils are composition, functional groups present in active components, and their synergistic interactions. The antimicrobial mechanism of action varies with the type of essential oil or the strain of the microorganism used. It is well known that in comparison to Gram-negative bacteria, Gram-positive bacteria are more susceptible to essential oils (48). This can be attributed to the fact that Gram-negative bacteria have an outer membrane which is rigid, rich in lipopolysaccharide (LPS) and more complex, thereby limiting the diffusion of hydrophobic compounds through it, while this extra complex membrane is absent in Gram-positive bacteria which instead are surrounded by a thick peptidoglycan wall not dense enough to resist small antimicrobial molecules, facilitating the access to the cell membrane (49). Moreover, Gram-positive bacteria may ease the infiltration of hydrophobic compounds of essential oils due to the lipophilic ends of lipoteichoic acid present in cell membrane (50).

An increase in bacterial resistance to antibiotics and the lack of new antibiotics introduced into the market resulted in a need to find alternative strategies so as to cope with infections resulting from drug-resistant bacteria (51, 52). Development of alternatives for antibiotics and the discovery or development of adjuvants are amongst the potential strategies proposed. In order to increase or restore antimicrobial efficacy against multi-drug-resistant bacteria, some efforts have been made. Addition of essential oils to antibiotics can induce a reduction in the antimicrobial MIC and the maximum effect has been observed with ampicillin and metronidazole as they are choices of oral infections.

Conclusions

In conclusion, we found that the T. kotschyanus essential oil, especially at 10 mg/ml concentration harbored the high antimicrobial effects against several types of bacteria in the oral cavity. Additionally, the antimicrobial effects of the T. kotschyanus essential oil was significantly highet than chlorhexidine 0.2% mouthwash and ampicillin, and metronidazole antibiotic agents. According to the edible route of T. kotschyanus essential oil, formulation of mouthwash based on the T. kotschyanus essential oil is recommended to decrease the risk of oral infections, dental caries, and their subsequent complications.

References

1. Maalekipour M, Safari M, Barekatain M, Fathi A. Effect of adhesive resin as a modeling liquid on elution of resin composite restorations. International Journal of Dentistry. 2021 Dec 28;2021.
2. Momeni-Moghaddam M, Hashemi C, Fathi A, Khamesipour F. Diagnostic accuracy, available treatment, and diagnostic methods of dental caries in practice: a meta-analysis. Beni-Suef University Journal of Basic and Applied Sciences. 2022 Dec;11(1):1-1.
3. Mortezagholi B, Movahed E, Fathi A, Soleimani M, Forutan Mirhosseini A, Zeini N, Khatami M, Naderifar M, Abedi Kiasari B, Zareanshahraki M. Plant-mediated synthesis of silver-doped zinc oxide nanoparticles and evaluation of their antimicrobial activity against bacteria cause tooth decay. Microscopy Research and Technique. 2022 Nov;85(11):3553-64.

4. Ebadian B, Fathi A, Khodadad S. Comparison of the effect of four different abutment screw torques on screw loosening in single implant-supported prosthesis after the application of mechanical loading. International Journal of Dentistry. 2021 Jul 19;2021.
5. Aminian A, Fathi A, Gerami MH, Arsan M, Forutan Mirhosseini A, Seyed Mohammad T. Nanoparticles to overcome bacterial resistance in orthopedic and dental implants. Nanomedicine Research Journal. 2022 Apr 1;7(2):107-23.
6. Khamisi N, Fathi A, Yari A. Antimicrobial resistance of Staphylococcus aureus isolated from dental plaques. Academic Journal of Health Sciencies: Medicina balear. 2022;37(1):136-40.
7. Fathi A, Salehi A. Antimicrobial resistance properties of Helicobacter pylori strains isolated from dental plaque and saliva samples. Academic Journal of Health Sciencies: Medicina balear. 2022;37(1):29-33.
8. Sedghi L, DiMassa V, Harrington A, Lynch SV, Kapila YL. The oral microbiome: Role of key organisms and complex networks in oral health and disease. Periodontology 2000. 2021 Oct;87(1):107-31.
9. Gendron R, Grenier D, Maheu-Robert LF. The oral cavity as a reservoir of bacterial pathogens for focal infections. Microbes and infection. 2000 Jul 1;2(8):897-906.
10. Aas JA, Paster BJ, Stokes LN, Olsen I, Dewhirst FE. Defining the normal bacterial flora of the oral cavity. Journal of clinical microbiology. 2005 Nov;43(11):5721-32.
11. Kaci G, Goudercourt D, Dennin V, Pot B, Doré J, Ehrlich SD, Renault P, Blottière HM, Daniel C, Delorme C. Anti-inflammatory properties of Streptococcus salivarius, a commensal bacterium of the oral cavity and digestive tract. Applied and environmental microbiology. 2014 Feb 1;80(3):928-34.
12. Badet C, Thebaud NB. Ecology of lactobacilli in the oral cavity: a review of literature. The open microbiology journal. 2008;2:38.
13. Nakano K, Ooshima T. Serotype classification of Streptococcus mutans and its detection outside the oral cavity. Future microbiology. 2009 Sep;4(7):891-902.
14. Willcox MD, Patrikakis M, Harty DW, Loo CY, Knox KW. Coaggregation of oral lactobacilli with streptococci from the oral cavity. Oral microbiology and immunology. 1993 Oct;8(5):319-21.
15. Ramamurthy JA, Mg V. Comparison of effect of hiora mouthwash versus chlorhexidine mouthwash in gingivitis patients: A clinical trial. Asian J Pharm Clin Res. 2018 Jul 7;11(7):84-.
16. Van der Weijden FA, Van der Sluijs E, Ciancio SG, Slot DE. Can chemical mouthwash agents achieve plaque/gingivitis control?. Dental Clinics. 2015 Oct 1;59(4):799-829.
17. Sajjan P, Laxminarayan N, Kar PP, Sajjanar M. Chlorhexidine as an antimicrobial agent in dentistry–a review. Oral Health Dent Manag. 2016 Apr;15(2):93-100.
18. Brookes ZL, Bescos R, Belfield LA, Ali K, Roberts A. Current uses of chlorhexidine for management of oral disease: a narrative review. Journal of Dentistry. 2020 Dec 1;103:103497.
19. Gagari E, Kabani S. Adverse effects of mouthwash use. A review. Oral surgery, oral medicine, oral pathology, oral radiology, and endodontics. 1995 Oct 1;80(4):432-9.
20. Wynder EL, Kabat G, Rosenberg S, Levenstein M. Oral cancer and mouthwash use. Journal of the National Cancer Institute. 1983 Feb 1;70(2):255-60.
21. Safarpoor Dehkordi F, Tirgir F, Valizadeh Y. Effects of Guajol® ointment synthesized from medicinal smoke condensate of jennet feces on burn wound healing on Wistar rat. Vet Res Forum. 2017 Summer;8(3):215-221.
22. Halberstein RA. Applications of medicinal plants in dentistry. European Journal of General Dentistry. 2012 Sep;1(03):123-4.
23. Mirzaei K, Fathi A, Asadinejad SM, Moghadam NC. Study the antimicrobial effects of Zataria multiflora-based mouthwash on the microbial community of dental plaques isolated from children: A candidate of novel plant-based mouthwash. Academic Journal of Health Sciencies: Medicina balear. 2022;37(3):58-63.
24. Prasanth Reddy V, Ravi Vital K, Varsha PV, Satyam S. Review on Thymus vulgaris traditional uses and pharmacological properties. Med Aromat Plants. 2014;3(164):2167-0412.
25. Hosseinzadeh S, Jafarikukhdan A, Hosseini A, Armand R. The application of medicinal plants in traditional and modern medicine: a review of Thymus vulgaris. International Journal of Clinical Medicine. 2015;6(09):635.
26. Zahra A, Abbas P, Reza E. Effect of nano-silver on stages of plant growth and yield and composition of essential oil of Thymus kotschyanus Boiss. & Hohen. African Journal of Agricultural Research. 2013 Mar 31;8(8):707-10.
27. Khoshsokhan F, Poormeidani A, Babalar M, Moghadam MF. Analysis of the essential oils of Thymus kotschyanus L.(10 populations) from Iran. Cercetari Agronomice in Moldova. 2014;47(2):49-59.
28. Rasooli I, Mirmostafa SA. Bacterial susceptibility to and chemical composition of essential oils from Thymus kotschyanus and Thymus persicus. Journal of agricultural and food chemistry. 2003 Apr 9;51(8):2200-5.
29. Shapiro S, Giertsen E, Guggenheim B. An in vitro oral biofilm model for comparing the efficacy of antimicrobial mouthrinses. Caries Res, 2002; 36(2): 93-100.
30. Haghighati F, Jafari S, Beitollahi J. Comparison of antimicrobial effects of ten Herbal extracts with chlorhexidine on three different oral pathogens: An in vitro study. Hakim Res J, 2003; 6(3): 71-6. (Persian).
31. Jalalvand, A. R., Zhaleh, M., Goorani, S., Zangeneh, M. M., Seydi, N., Zangeneh, A., & Moradi, R. Chemical characterization and antioxidant, cytotoxic, antibacterial, and antifungal properties of ethanolic extract of Allium Saralicum R.M. Fritsch leaves rich in linolenic acid, methyl ester. Journal of Photochemistry and Photobiology B: Biology, 2019; 192, 103–112. doi:10.1016/j.jphotobiol.2019.01.

32. Mozaffari B, Mansouri Sh, Rajabalian S, Alimardani A, Mohamadi M. In vitro study between anti-bacterialand cytotoxic effects of Chlorhexidine and Persica mouthrinses. Journal of Dental School Shahid Beheshti University of Medical Sciences, 2005; 23(3): 494-509.
33. Ranjbar R, Yadollahi Farsani F, Safarpoor Dehkordi F. Antimicrobial resistance and genotyping of vacA, cagA, and iceA alleles of the Helicobacter pylori strains isolated from traditional dairy products. Journal of Food Safety. 2019 Apr;39(2):e12594.
34. Dehkordi FS, Tavakoli-Far B, Jafariaskari S, Momtaz H, Esmaeilzadeh S, Ranjbar R, Rabiei M. Uropathogenic Escherichia coli in the high vaginal swab samples of fertile and infertile women: virulence factors, O-serogroups, and phenotyping and genotyping characterization of antibiotic resistance. New Microbes and New Infections. 2020 Nov 1;38:100824.
35. Mashak Z, Jafariaskari S, Alavi I, Sakhaei Shahreza M, Safarpoor Dehkordi F. Phenotypic and genotypic assessment of antibiotic resistance and genotyping of vacA, cagA, iceA, oipA, cagE, and babA2 alleles of Helicobacter pylori bacteria isolated from raw meat. Infection and drug resistance. 2020 Jan 29:257-72.
36. Dehkordi FS, Yahaghi E, Darian EK. Prevalence of antibiotic resistance in Escherichia coli isolated from poultry meat supply in Isfahan. Iran J Med Microbiol: Volume. 2014 Jul;8(2).
37. Safarpoor Dehkordi F, Gandomi H, Basti AA, Misaghi A, Rahimi E. Phenotypic and genotypic characterization of antibiotic resistance of methicillin-resistant Staphylococcus aureus isolated from hospital food. Antimicrobial Resistance & Infection Control. 2017 Dec;6(1):1-1.
38. Dehkordi FS, Yazdani F, Mozafari J, Valizadeh Y. Virulence factors, serogroups and antimicrobial resistance properties of Escherichia coli strains in fermented dairy products. BMC research notes. 2014 Dec;7(1):1-8.
39. Dehkordi FS, Barati S, Momtaz H, Ahari SN, Dehkordi SN. Comparison of shedding, and antibiotic resistance properties of Listeria monocytogenes isolated from milk, feces, urine, and vaginal secretion of bovine, ovine, caprine, buffalo, and camel species in Iran. Jundishapur Journal of Microbiology. 2013 May 31;6(3):284-94.
40. Dehkordi FS. Prevalence study of Coxiella burnetii in aborted ovine and caprine fetuses by evaluation of nested and real-time PCR assays. American Journal of Animal and Veterinary Sciences. 2011.
41. Candelaria-Dueñas S, Serrano-Parrales R, Ávila-Romero M, Meraz-Martínez S, Orozco-Martínez J, Ávila-Acevedo JG, García-Bores AM, Cespedes-Acuña CL, Peñalosa-Castro I, Hernandez-Delgado T. Evaluation of the Antimicrobial Activity of Some Components of the Essential Oils of Plants Used in the Traditional Medicine of the Tehuacán-Cuicatlán Valley, Puebla, México. Antibiotics (Basel). 2021 Mar 12;10(3):295.
42. Magi G, Marini E, Facinelli B. Antimicrobial activity of essential oils and carvacrol, and synergy of carvacrol and erythromycin, against clinical, erythromycin-resistant Group A Streptococci. Front Microbiol. 2015 Mar 3;6:165.
43. Soković M, Glamočlija J, Marin PD, Brkić D, Van Griensven LJ. Antibacterial effects of the essential oils of commonly consumed medicinal herbs using an in vitro model. Molecules. 2010 Oct 27;15(11):7532-46.
44. Mahboubi M, Heidarytabar R, Mahdizadeh E, Hosseini H. Antimicrobial activity and chemical composition of Thymus species and Zataria multiflora essential oils. Agriculture and natural resources. 2017 Oct 1;51(5):395-401.
45. Rasooli I, Mirmostafa SA. Bacterial susceptibility to and chemical composition of essential oils from Thymus kotschyanus and Thymus persicus. Journal of agricultural and food chemistry. 2003 Apr 9;51(8):2200-5.
46. Salehnia Sammak A, Anvari M, Matinizadeh M, Mirza M. Evaluation of Inoculation Pseudomonas fluorescens and Arbuscular Mycorrhizal Fungus on Growth, Morphological Characteristics and Essential Oil Percentage of Thymus kotschyanus. Journal of Medicinal plants and By-product. 2021 Feb 8.
47. Zhiani R, Dolatabadi S, Imani H, Moradi M, Emrani S. A Comparison of the Chemical Composition of Flowering Shoot Thymus kotschyanus and Thymus vulgaris by using GC-Mass and Antimicrobial Effects of the Bacteria Staphylococcus aureus and Pseudomonas aeruginosa. Journal of Essential Oil Bearing Plants. 2016 Oct 2;19(7):1639-47.
48. Chouhan S, Sharma K, Guleria S. Antimicrobial Activity of Some Essential Oils-Present Status and Future Perspectives. Medicines (Basel). 2017 Aug 8;4(3):58.
49. Galgano M, Capozza P, Pellegrini F, Cordisco M, Sposato A, Sblano S, Camero M, Lanave G, Fracchiolla G, Corrente M, Cirone F. Antimicrobial Activity of Essential Oils Evaluated In Vitro against Escherichia coli and Staphylococcus aureus. Antibiotics. 2022 Jul 20;11(7):979.
50. Man A, Santacroce L, Jacob R, Mare A, Man L. Antimicrobial Activity of Six Essential Oils Against a Group of Human Pathogens: A Comparative Study. Pathogens. 2019 Jan 28;8(1):15.
51. Alijani HQ, Fathi A, Amin HI, Lima Nobre MA, Akbarizadeh MR, Khatami M, Jalil AT, Naderifar M, Dehkordi FS, Shafiee A. Biosynthesis of core–shell α-Fe2O3@ Au nanotruffles and their biomedical applications. Biomass Conversion and Biorefinery. 2022 Nov 26:1-5.
52. Hasankhani A, Bahrami A, Mackie S, Maghsoodi S, Alawamleh HS, Sheybani N, Dehkordi FS, Rajabi F, Javanmard G, Khadem H, Barkema HW. In-depth systems biological evaluation of bovine alveolar macrophages suggests novel insights into molecular mechanisms underlying Mycobacterium bovis infection. Frontiers in Microbiology. 2022;13.