

# The Inhibitory Effect of Alcoholic Extraction of *Pimpinella anisum* against *Candida albicans* Isolated from Gingivitis

Toleen Khalied Ibrahim<sup>1</sup>, Widyan Alwan Khalaf<sup>1</sup> and Ali Ahmed Ali<sup>2\*</sup>

<sup>1</sup> Department of Field Crops- College of Agriculture-University of Samarra, Samarra, Iraq

<sup>2</sup> Department of Horticulture and Landscape Planning-College of Agriculture-University of Samarra, Samarra, Iraq

## Article Info

**Article Type**  
Original Article

Received: 05 October 2024  
Accepted: 07 November 2024  
© 2012 Iranian Society of Medicinal Plants.  
All rights reserved.

**\*Corresponding author**  
ali.a@uosamarra.edu.iq



## ABSTRACT

Anise, scientifically known as *Pimpinella anisum*, is a one-year herbaceous flowering plant commonly used as a spice, flavoring agent, and scent. Anise seeds have been extensively employed in traditional medicinal recipes throughout the Arab region, including Iraq. This study aimed to demonstrate the inhibitory effect of alcoholic extracts of *Pimpinella anisum* against *Candida albicans* isolated from patients with gingivitis. A cross-sectional study was conducted involving 120 samples from gingivitis patients aged 30 to 50 years, collected between September 2023 and July 2024. Samples were obtained using oral swabs from patients visiting a dental specialist center in Tikrit city and outpatient clinics, comprising both genders. Bacterial isolates were identified based on microscopic and macroscopic cultural characteristics, as well as biochemical tests. Additionally, a VITEK 2 compact system was employed for species-level identification. The results indicated that out of 120 samples, 80 tested positive for bacterial isolation while 40 tested negative. *Candida albicans* was isolated in 58.3% of the cases. The most common bacterial species causing infections were *S. mutans* (25%), followed by *Pseudomonas aeruginosa* (13%), *Staphylococcus aureus* (12%), and *Proteus mirabilis* and *Klebsiella pneumoniae* (8%). The least common bacterial species isolated, accounting for 3%, included *Staphylococcus epidermidis*, *Acinetobacter lowffii*, and *S. pneumoniae*. In conclusion, *Pimpinella anisum* exhibits an inhibitory effect against *Candida albicans* and may serve as an alternative medicine in dentistry, potentially acting as an adjunct to conventional therapy for oral candidiasis.

**Keywords:** *Pimpinella anisum*, *Candida albicans*, Gingivitis, Alcoholic extraction

## How to cite this paper

Ibrahim, T. K., Khalaf, W. A., Ali, A. A. The Inhibitory Effect of Alcoholic Extraction of *Pimpinella anisum* against *Candida albicans* Isolated from Gingivitis. Journal of Medicinal plants and By-Products, 2025; (1): 93-97. doi: 10.22034/jmpb.2024.367242.1778

## INTRODUCTION

For centuries, traditional medicinal herbs were used for nutrition and healing, playing an vital function in preserving human fitness and improving their life [1-3]. Aromatic herbs, like anise seeds, have a rich history of use in both traditional and modern medicine, including the pharmaceutical industry [4, 5]. Anise, scientifically referred to as *Pimpinella anisum*, is a type of herbaceous plant that completes its lifecycle within a 12 months. It is native to the Mediterranean place and is grown mainly for its fruits and seeds [6]. According to reports, the homeland of this species is Iran, India, and Turkey [7]. Anise seeds are used as a migraine reliever and possess carminative, aromatic, antiseptic, and diuretic properties in traditional medicine. Additionally, anise has strong antibacterial, antifungal, antioxidant, and antidiabetic characteristics [8]. Gingivitis and periodontitis are two conditions categorized under the broader term periodontal disease. This term encompasses a variety of conditions that impact the supporting tissues of the teeth [9]. Typically, one of the first indications of gingivitis is bleeding gums, which is a common symptom of the disorder [10]. If left untreated, gingivitis can progress to gingivitis, a condition

characterized by deterioration of the attachment of the gums and alveolar bone, eventually leading to tooth loss. Gingivitis can be treated with antibiotics [11]. In recent years, there has been a growing fascination with infections caused by the opportunistic fungi *Candida*. The significance of candidiasis has increased, partly due to the rise in HIV infections and the broader use of immunosuppressive therapy. Identifying the specific *Candida* strains responsible for infections is crucial, as different isolates of *Candida* species vary significantly in their ability to cause disease and their susceptibility to antifungal drugs [12]. The goal of this research was to study inhibitory effect of *Pimpinella anisum* against *Candida albicans* isolated from patients with gingivitis

## MATERIALS AND METHODS

### Fundamental Study Plant Samples

The *Pimpinella anisum* seeds were obtained from local markets. The seeds were washed with distilled water to remove dust and microorganisms. They were then placed in a controlled environment at a temperature of 22-26°C, with continuous stirring to ensure proper drying and prevent rotting. Once dry, the seeds were crushed, milled,

weighed, and stored in opaque glass containers. Each container was labeled with a piece of paper indicating the name of the plant and the specific plant part, and then stored in the laboratory until the extraction process was conducted.

### Preparation of Alcoholic Extraction

The alcoholic extraction method described in the study involved mixing 100 g of plant powder with 500 ml of 80% ethyl alcohol, resulting in a weight-to-volume ratio of 5:1. The mixture was heated to a boiling temperature of 100 °C, then removed from the heat source before adding the plant powder. It was subsequently placed in a vibrating incubator at 37 °C for 24 hours. After incubation, the mixture was filtered through multiple layers of medical gauze and transferred to glass dishes lined with thermal nylon to preserve the quantity of the extract. The extract was then dried in an electric oven at 38 °C for three days, confirmed to be dry, and stored in dark bottles with tight lids in the refrigerator at 4 °C until needed.

### Specimens Collection

A cross-sectional study analyzed 120 samples from patients aged 30 to 50 with gingivitis between September 2023 and July 2024. The samples were collected using oral swabs from patients of both genders attending a dental specialist center and outpatient clinics in Tikrit city. Bacterial isolates were identified based on their microscopic and macroscopic cultural characteristics, as well as biochemical tests. To ensure species-level identification, the VITKE2 compact system was utilized in addition to evaluating the biochemical characteristics of the isolates. Samples were collected after a dental examination conducted in the periodontal department.

### Gingival Crevicular Fluid (GCF) Specimens

After removing the supragingival calculus with a sickle scaler, the dental pockets were prepared for collecting gingival crevicular fluid (GCF). The teeth were isolated with cotton rolls to prevent contamination from saliva and blood. Following the scaling, pocket depth was evaluated using a periodontal probe. A sterile absorbent paper point measuring 30-45 mm was then used to collect the GCF sample. Two paper points were inserted into a periodontal pocket with a depth of 3-8 mm and left in place for 30 seconds. After this duration, the paper points were carefully removed with sterile tweezers and placed in a sterilized Eppendorf tube containing 5 ml of thioglycolate broth. This broth serves as a reducing and transportation medium for anaerobic bacteria. The bacteria were subsequently cultured on blood agar and incubated in a 5% CO<sub>2</sub> environment at a temperature of 35-37°C for up to 48 hours.

### Swap from Mouth

Please take a mouth swab to isolate aerobic bacteria, using transport media (Amies) to keep it moist until it reaches the college laboratory. The swab should be cultured

aerobically on Blood Agar, MacConkey Agar, Mannitol Salt Agar, and Sabouraud Dextrose Agar (SDA). Incubate the cultures at 37°C for 24 to 48 hours. Additionally, chocolate agar should also be used for incubation.

### Biofilms Formation Assay

To assess biofilm formation, we utilized pre-sterilized 96-well polystyrene microplates, following the modified method outlined in (12). Yeast was introduced into a tube containing 2 ml of YPD broth (yeast peptone dextrose broth) with 1% glucose using a loop, and the mixture was incubated at 37°C for 24 hours. After incubation, 200 µl of the resulting solution was transferred to each well of the microplate. The microplate was then sealed with lids and incubated at 37°C for another 24 hours. Following this incubation, the medium in the wells was extracted and rinsed twice with sterile phosphate buffer (PBS). The wells were then turned upside down to remove excess liquid and allowed to dry. A crystal violet solution (0.1 g/100 ml) was added to each well for staining, and the plates were incubated for 20 minutes. After incubation, the plates were rinsed twice with PBS, turned upside down to dry, and left to air dry. Next, a 200 µl volume of an acetone:ethanol mixture (20:80 v/v) was added to each well. After approximately 10 minutes, the absorbance results were measured at a wavelength of 450 nm using an ELISA reader. The tests were conducted in triplicate, and the means were calculated. The optical density (OD) of each strain was then compared with the average absorbance of the negative controls (OD<sub>nc</sub>), which represented the mean without any inoculation. Biofilm formation was classified as follows: no biofilm production (ODs = OD<sub>nc</sub>), poor biofilm production (OD<sub>nc</sub> < ODs ≤ 2OD<sub>nc</sub>), moderate biofilm production (OD<sub>nc</sub> ≤ ODs ≤ 4OD<sub>nc</sub>), and vigorous biofilm production (ODs > 4OD<sub>nc</sub>).

### Congo Red Agar Assay

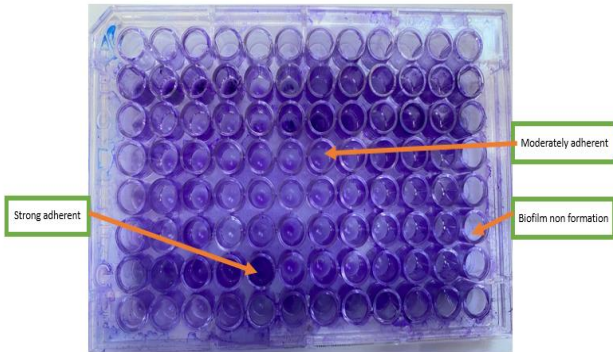
The medium was prepared using the method outlined by Freeman et al [13]. Brain-Heart infusion broth (37 g), sucrose (50 mg), agar (15 mg), congo red (0.8 mg), and distilled water (900 ml). All components, except for congo red, were dissolved in 900 ml of distilled water and sterilized by autoclaving at 121°C for 15 minutes. After sterilization, the mixture was cooled to 55°C. Meanwhile, congo red was prepared separately by dissolving 0.8 g in 100 ml of distilled water and sterilizing it by autoclaving at 121°C for 15 minutes. This solution was then added to the cooled medium before being dispensed into sterile Petri dishes.

### Statistical Analysis

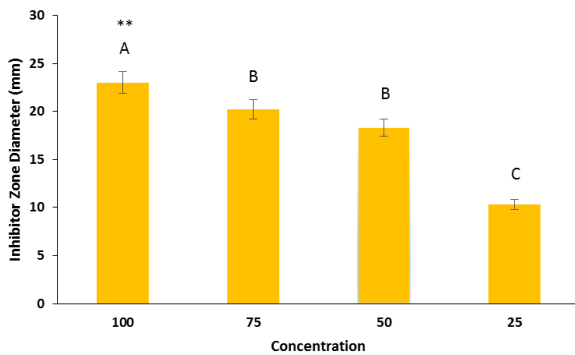
Statistical analysis was conducted using either Pearson's chi-square test or Fisher's exact test, depending on the characteristics of the contingency table and the qualitative variables involved. Results were presented as mean ± standard deviation and percentages, with a p-value of less than 0.05 considered statistically significant.

**RESULTS**

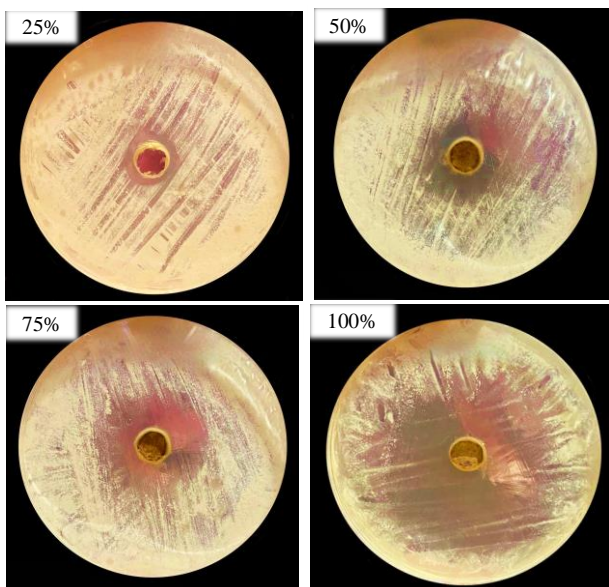
In the study the patients were distributed according to gender and age. In both Male and female, the most prevalent ages were 41-50 years (40%, 38.5%), respectively, while the less affected ages were (20-30) years with (27.3%, 27.7%), respectively, as showed in table 1.



**Fig 1** Biofilm formation by MTP



**Fig. 2** The inhibitory effect of *Pimpinella anisum* against *C. albicans* with different concentration



**Fig. 3** The inhibitory effect of alcoholic extraction of *Pimpinella anisum* against *C. albicans*

The results are presented in Table 2 and Figure 1 for bacterial and fungal isolation. The VITEK 2 compact system was used to accurately diagnose all bacterial isolates. Out of 100 samples, 80 samples showed positive isolation for bacteria and candida, while 40 samples showed negative bacterial isolation. The most commonly isolated species causing bacterial infections were *S. mutans* (25%), followed by *Pseudomonas aeruginosa* (13%), *Staphylococcus aureus* (12%), *Proteus mirabilis* and *Klebsiella pneumoniae* (8%). The least commonly isolated bacterial species was *Staph. epidermidis*, *Acinetobacter lowifii*, and *S. pneumonia*, each representing 3% of the isolates. Additionally, 43.75% of the isolates were positive for *candida albicans*.

in Table 3 and Figure 2 and 3, the results show that out of 35 *Candida albicans* samples, 19 (54.3%) displayed a strong biofilm when tested using the MTP method. Conversely, 16 (45.7%) samples showed a strong biofilm when tested using the CRA approach.

The results presented in Figure 2 and Figure 3 indicate that *C. albicans* exhibited the largest inhibitory zone diameter at a concentration of 100%, with a diameter of 23. At concentrations of 75%, 50%, and 25%, the inhibitory zone diameters were 20.19, 18.32, and 10.32, respectively.

**Table 1** the properties of the males and females were used in this research

Ages	MaleN (%)	Female N(%)
20-30	15(27.3%)	18(27.7%)
31-40	18(32.7%)	22(33.8%)
41-50	22(40%)	25(38.5%)
total	55 (100%)	65(100%)

**Table 2** Number and percentage of bacterial species isolated from gingivitis

Bacterial isolates	n	%
<i>Streptococcus mutans</i>	27	33.75
<i>Pseudomonas aeruginosa</i>	20	25
<i>Staphylococcus aureus</i>	11	13.75
<i>Proteus mirabilis</i> (12)	15	18.75
<i>Klebsiella pneumonia</i> (35)	12	15
<i>Staphylococcus epidermis</i>	6	7.5
<i>Acinetobacter lowifii</i>	3	3.75
<i>S. pneumonia</i>	12	15
<i>Candida albicans</i>	35	43.75
p-value	0.02	

In Table 2 and Figure 2 and 3, the results show that out of 35 *Candida albicans* samples, 19 (54.3%) displayed a strong biofilm when tested using the MTP method. Conversely, 16 (45.7%) samples showed a strong biofilm when tested using the CRA approach.

**Table 2** Biofilm formation by *Candida albicans*

Number of isolations	MTP			CRA		
	Negative	Moderate	Strong	Negative	Moderate	Strong
<i>Candida albicans</i> (35)	5(14.3%)	11(31.4%)	19(54.3%)	7(20%)	12(34.3%)	16(45.7%)

\*MTP: microtiter plate

\*CRA: Congo red agar

## DISCUSSION

The research findings indicate that a high percentage of *Candida albicans* was isolated from gingivitis patients, and 75% were isolated from the oral cavity of patients with periodontitis [14]. *Candida albicans* can act as a pathogenic microbe in patients with weakened immune systems, leading to the development of oral candidiasis and dental caries. This microorganism can cause each superficial and invasive infections, especially in people with weakened immune structures [15]. The transition of *Candida* spp. from an innocuous commensal organism to a pathogenic one is attributed to its virulent characteristics. These include its capacity to adhere to host tissue, invade, form biofilms, and secrete extracellular hydrolytic enzymes [16].

Studies show that for gingivitis, *S. mutans* is more common than other bacteria. According to references [17], the most frequently encountered vector species was *S. mutans* (18%), followed by *S. mitis* (13%) and *S. aureus* (12%). *Staphylococcus aureus*, *Klebsiella pneumonia*, and *Acinetobacter lowifii* accounted for 3.57%, 10.72%, and 7.14% of the isolates, respectively [18]. This finding contradicts the results of (18), which showed that *S. aureus* was the predominant bacteria. Oral streptococci species, such as *S. mutans* and *Streptococcus mitis*, significantly impact the development of plaque above the gum line and dental cavities [19].

An alcoholic extraction of *Pimpinella anisum* has shown an inhibitory effect against *Candida albicans* at concentrations of 100%, more than 75%, 50%, and 25%. Other studies suggest potent antifungal activity against various microorganisms, including *Candida albicans*, with inhibition zones ranging from 10 to 26 mm, compared to Clotrimazole using the agar diffusion method [20]. Basheer et al, mentioned that *Pimpinella anisum* contains a significant amount of anethole, which is an effective antifungal substance [21]. The inhibitory efficiency of the alcoholic extract of *Pimpinella anisum* on *C. albicans* yeast cells is due to the presence of multiple effective compounds within *Pimpinella anisum*, such as p-coumaric acid, catechin, oleuropein, gallic acid, caffeic acid, and others, which increase its effectiveness against various microorganisms [21]. Different mechanisms explain the effectiveness of the alcoholic extract of *Pimpinella anisum* in inhibiting *Candida albicans*, consisting of its potential to penetrate biofilms, exchange cell wall permeability, and adjust gene expression [22]. *Pimpinella anisum* is likewise characterized by using its antifungal and anti-biofilm results. The alcoholic extract of *Pimpinella anisum* has a greater inhibitory impact than the aqueous extract because of the natural compounds that dissolve higher in alcohol than in water [23]. Additionally, anise seeds are rich in chemical compounds that possess high-quality antioxidant, antibacterial, and anti-biofilm properties [24]. These properties are due to the high concentration of

primary ingredients, which possess harmful mechanisms that impact the entire bacterial cell. These mechanisms include disrupting cell walls, damaging membranes and proteins, and impairing the proton transfer system [25].

## Limitations of the Study

One limitation of our study was the small sample size of the participants. Future research should aim to include a larger number of individuals for more comprehensive investigation.

## CONCLUSION

This study explores the use of easily accessible medicinal plants, including *Pimpinella anisum*, in dentistry. These plants can serve as alternative treatments and supplements to traditional therapies, particularly in developing countries where economic resources and access to oral healthcare facilities are limited. The research was conducted in a laboratory setting outside of a living organism. The exact duration of contact between the extracts and *C. albicans* in vivo is uncertain. Further research is recommended to isolate the potentially active compounds of the plants.

## Authors' contribution

Conceptualization: Toleen Khalied Ibrahim

Data curation: Widyan Alwan Khalaf

Formal analysis: Ali Ahmed Ali

Funding acquisition: Toleen Khalied Ibrahim

Investigation: Toleen Khalied Ibrahim

Methodology: Widyan Alwan Khalaf

Project administration:), Ali Ahmed Ali

Resources: Toleen Khalied Ibrahim, Ali Ahmed Ali

Software: Ali Ahmed Ali

Supervision: Widyan Alwan Khalaf

Validation: Widyan Alwan Khalaf

Visualization: Toleen Khalied Ibrahim

Writing—original draft: Toleen Khalied Ibrahim, Widyan Alwan Khalaf

Writing—review & editing: Toleen Khalied Ibrahim, Ali Ahmed Ali

## Ethical Considerations

This study was conducted based on the ethical standards stipulated in the Declaration of Helsinki. The Ethics Committee of University of Samarra approved this study before taking the sample. The patient's informed written and verbal agreement was obtained, after the review and approval of the study protocol and subject's information by the local ethics committee according to the document number 1152. Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

## Conflicts of Interest

The authors declare that they have no competing interests.

**Funding/Support**

Non-Funding

**REFERENCES**

1. Ammar E.E., Aioub A.A., Elesawy A.E., Karkour A.M., Mouhamed M.S., Amer A.A., El-Shershaby N.A. Algae as Bio-fertilizers: Between current situation and future prospective. *Saudi Journal of Biological Sciences*. 2022; 29(5): 3083-3096.
2. Tabin S., Gupta R.C., kamili A.N., parray J.A. Medical and medicinal importance of *Rheum* spp. collected from different altitudes of the Kashmir Himalayan range. *Cell. Mol. Biomed. Rep.* 2022; 2(3): 187-201.
3. Fooladvand Z., Fazeli-nasab B. Antibacterial activities of *Stachys lavandulifolia* Vahl. extract against eight bacteria. *Journal of Herbal Drugs (An International Journal on Medicinal Herbs)*. 2014; 5(1): 13-18.
4. AbouZid S.F., Mohamed A.A. Survey on medicinal plants and spices used in Beni-Sueif, Upper Egypt. *Journal of Ethnobiology and Ethnomedicine*. 2011; 7: 1-6.
5. Ahani H., Attaran S. Therapeutic potential of Seabuckthorn (*Hippophae rhamnoides* L.) in medical sciences. *Cell. Mol. Biomed. Rep.* 2022; 2(1): 22-32.
6. Günay A., Gümüşçü A. The Effects of Different Fertilizer Applications on Yield and Quality of Anise (*Pimpinella anisum* L.). *International Journal of Agriculture Forestry and Life Sciences*. 2023; 7(1): 6-9.
7. Mohammed H.N. Broiler performance response to anise seed powder supplementation. *Iraqi Journal of Veterinary Sciences*. 2019; 33(1): 131-135.
8. Rodríguez-Hernández A.A., Flores-Soria F.G., Patiño-Rodríguez O., Escobedo-Moratilla A. Sanitary Registries and Popular Medicinal Plants Used in Medicines and Herbal Remedies in Mexico (2001–2020): A Review and Potential Perspectives. *Horticulturae*. 2022; 8(5): 377.
9. Jelavić S., Bajić Ž., Filipčić I.Š., Čulina I.J., Filipčić I., Aurer A. Periodontal status and the efficacy of the first-line treatment of major depressive disorder. *Clinical and experimental dental research*. 2022; 8(1): 366-373.
10. Umezudike K.A., Iwuala S.O., Ozoh O.B., Ayanbadejo P.O., Fasanmade O.A. Association between periodontal diseases and systemic illnesses: A survey among internal medicine residents in Nigeria. *The Saudi Dental Journal*. 2016; 28(1): 24-30.
11. Sanz M., D'Aiuto F., Deanfield J., Fernandez-Avilés F. European workshop in periodontal health and cardiovascular disease—scientific evidence on the association between periodontal and cardiovascular diseases: a review of the literature. *European Heart Journal Supplements*. 2010; 12(suppl\_B): B3-B12.
12. Byadarahally Raju S., Rajappa S. Isolation and identification of *Candida* from the oral cavity. *International Scholarly Research Notices*. 2011; 2011(1): 487921.
13. Freeman D., Falkiner F., Keane C. New method for detecting slime production by coagulase negative staphylococci. *Journal of Clinical Pathology*. 1989; 42(8): 872-874.
14. Jepsen K., Falk W., Brune F., Cosgarea R., Fimmers R., Bekeredjian-Ding I., Jepsen S. Prevalence and antibiotic susceptibility trends of selected enterobacteriaceae, enterococci, and *Candida albicans* in the subgingival microbiota of German periodontitis patients: a retrospective surveillance study. *Antibiotics*. 2022; 11(3): 385.
15. Nualmalang R., Thanomsridetchai N., Teethaisong Y., Sukphopetch P., Tangwattanachuleeporn M. Identification of pathogenic and opportunistic yeasts in pigeon excreta by MALDI-TOF mass spectrometry and their prevalence in Chon Buri province, Thailand. *International Journal of Environmental Research and Public Health*. 2023; 20(4): 3191.
16. Vila T., Sultan A.S., Montelongo-Jauregui D., Jabra-Rizk M.A. Oral candidiasis: A disease of opportunity. *Journal of Fungi*. 2020; 6(1): 15.
17. Jaseem R.M., Yasin L.Q. Isolation and identification of some predominant bacteria and assessment of TNF- $\alpha$  level in serum of patients with gingivitis. *Tikrit Journal of Pure Science*. 2023; 28(5): 1-11.
18. Periferakis A.-T., Periferakis A., Periferakis K., Caruntu A., Badarau I.A., Savulescu-Fiedler I., Scheau C., Caruntu C. Antimicrobial properties of capsaicin: available data and future research perspectives. *Nutrients*. 2023; 15(19): 4097.
19. Larsen T., Fiehn N.E. Dental biofilm infections—an update. *Apmis*. 2017; 125(4): 376-384.
20. Abd-Elhafeez E., Ramadan B.R., Abou-El-Hawa S.H., Rashwan M.R. Chemical Composition and Antimicrobial Activity of Anise and Fennel Essential Oils. *Assiut Journal of Agricultural Sciences*. 2023; 54(2): 127-140.
21. Basheer M., Abdelshakour A.K., Mohamed M.K., Elshobky A.M., Abdelmoneam A.S., Makeen F.E., Eissa Z.M., Hossny H.H., Elemam S.N., Mahfouz E.N. Mini review: Anise (*Pimpinella anisum* L.): phytochemical and Pharmacological activities. *ERU Research Journal*. 2023; 2(4): 723-733.
22. Dumitrescu E., Muselin F., Tîrziu E., Folescu M., Dumitrescu C.S., Orboi D.M., Cristina R.T. *Pimpinella anisum* L. essential oil a valuable antibacterial and antifungal alternative. *Plants*. 2023; 12(13): 2428.
23. Gülçm İ., Oktay M., Kireççi E., Küfrevioğlu Ö.İ. Screening of antioxidant and antimicrobial activities of anise (*Pimpinella anisum* L.) seed extracts. *Food chemistry*. 2003; 83(3): 371-382.
24. Kosalec I., Pepeljnjak S., Kuštrak D. Antifungal activity of fluid extract and essential oil from anise fruits (*Pimpinella anisum* L., Apiaceae). *Acta Pharmaceutica*. 2005; 55(4): 377-385.
25. Macwan S.R., Dabhi B.K., Aparnathi K., Prajapati J. Essential oils of herbs and spices: their antimicrobial activity and application in preservation of food. *International Journal of Current Microbiology and Applied Sciences*. 2016; 5(5): 885-901.