RESEARCH ARTICLE

In-vitro Activity of Essential Oils Extracted from *Thymus vulgaris* and *Origanum vulgare* against *Candida albicans*

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ABSTRACT

This study aimed to assess the antifungal efficacy of essential oils extracted from *Origanum vulgare* and *Thymus vulgaris* against *C. albicans* isolated from oral candidiasis patients *in-vitro*. Basra General Hospital was diagnosed based on the cultural and microbiological phenotypes and using Vitek2 Technique to the identification.

The study including a comparison of the inhibitory efficacy resulting from the use of EOs extracted from *O. vulgare* and *T. vulgaris* versus *C. albicans* was performed using the Agar well diffusion method of, used seven concentrations of each essential oil: (crude,1.5,1, 0.8,0.5, 0.2,0.1) mg/mL, as well as estimation of minimal inhibitory concentration (MIC) which is 0.2 mg/mL for *O. vulgare* and 0.1 mg/mL for *T. vulgaris*. The highest effect was to *O. vulgare* oil crude extract, which reached 3.26 cm, while the inhibtion zone of crude *T. vulgaris* oil was 2.53 cm which is highly significant ($p < 0.05$).

On the other side the study aimed to evaluate the activity of *T. vulgaris* oil store for one year comparing with freshly extracted oil found that the fresh oil has more activity reached to 2.53 cm and the activity of old oil reached to 1.51 cm was significant ($p < 0.05$), also using two typs of antifungal nystatin and fluconazole to compare with the activity of *O. vulgare* oil found that no effect of fluconazole and the activity of nystatin reach to 2.5 cm wich is less than the activity of *O. vulgare* oil wich reached to 3.26 cm comparing with the activity of antifungal highly significant p-value reached to 0.005, observed *in-vitro* suggests its administration may represent an alternative treatment for candidiasis. The antifungal sensitivity occurred to *C. albicans*, which record against fluconazole, voriconazole, caspofungin, micafungin, amphotericin B, and Flucytosine. also The study including investigation of the chemical compounds of (EOs) of *O. vulgare* using the chromatographic analysis(GC-MS) revealed that the main compounds present in the *O. vulgare* essential oil wich is the best activity againest *C.albicans*, revealed that the main compounds present in the essential oil were methyl-5-(1-methylethyl; 1-Pentacontanol; 2-Hexyl-1-octanol; sulfurous acid, ester; oxalic acid, 6-ethyloct-3-yl isohexyl ester 2-Hydroxy-p-cymene, and 2-Methyl-5-isopropylphenol.

Keywords: Antifungal activity, *Candida albicans*, *In-vitro*, *Origanum vulgare*, *Thymus vulgaris*.

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INTRODUCTION

Candida yeasts are the nearly common source of opportunistic infections worldwide, mostly affecting immunocompromised or hospitalized patients, as well as the elderly.[1] Candidiasis is a fungal infection caused by a variety of Candida species that can cause both superficial and systemic opportunistic infections all over the world.[2,3] *C. albicans* is the most common and important Candida species, which are considered important pathogens.[4] This yeast has been isolated from samples taken from various body locations, including blood, urine, lungs, and vaginal tissue.[5] The main species that causes candidemias*, C. albicans,* has specific properties that, when combined with local factors, can affect the spread of these infections' epidemiology.[6] When the causative pathogen is acquired from the hospital setting or a human source during a hospital stay, it is referred to as an exogenous source of candidemia.[7]

The majority of antifungals currently used to treat different clinical types of this disease have disadvantages that render them ineffective, necessitating the quest for safe and efficient antimycotic drugs or molecules.[8] Many people nowadays prefer medicinal plants to chemical products, so there is a clear need to identify new therapeutic agents that are both more effective and less harmful than chemical antifungal agents. *O. vulgare*, *S. hortensis*, *T. serpyllum* and *Thymus vulgaris* L. are examples of aromatic plants.[9] Many studies have been carried out in this field, demonstrating the importance of using plant extracts. Medicinal plants in various ways have been used. Researchers are looking at natural broad-spectrum alternatives, such as essential oils for candida, after broad-

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spectrum antibiotics and antifungals failed to stop the outbreak. According to a study out of Poland, both tea tree and rosemary essential oils are effective against candida which have the uncanny ability to change the morphology and metabolism of yeast enzymes.[10] Plants generate essential oils as a product of their secondary metabolism. These oils are derived from different parts of plants, such as leaves, and have a wide variety of pharmacological properties. Essential oils are used in animals and humans as antimicrobials, antioxidants, and anti-inflammatory agents.[11,12]

The study's aim was to look into the activities of *O. vulgare* and *T. vulgaries* (Eos) and study the activity of *T. vulgaries* (Eos) which storted for one-year aganest *C. albicans*, and compare these activity with two types of antifungals used fluconazole and Nystatine nowadays in order to evaluate its use as a therapy, also to analyze the chemical composition of Origanum vulgare essential oil by GC-MS.

MATERIALS AND METHODS

C. albicans was isolated from patients with oral candidiasis (thrush) at Basra General Hospital and classified to species level using standard morphological and physiological measures. Isolates were identified to the species level based on their ability to shape germ tubes and a range of macroscopic and microscopic characteristics on some culture media.[13-15]

Identification by Vitek2 of *C. albicans* and Antifungal Sensitivity

Identification and antifungal susceptibility tests (AST) were involved for *C. albicans* using Commercial kits vitek2 AST and ID YS01 obtained from BIOMERIEUX company (Biomerieux, USA). The procedure was carried out according to the manufacturer's instructions. And gram stain procedures are used according to gram stain kit procedures.

O. vulgare and T. vulgaries (Eos) Preparation

T. vulgaris and *O. vulgare* dry leaves were purchased from a market in Basrah and identified at the University of Basrah's College of Science herbarium Medicine, used for extraction after being cleaned and ground to a powder with an electric grinder In a soxhlet apparatus, 50 gm. of all individual samples using 250 mL of hexane at 40°C for three hours.[16] A rotary evaporator at 50°C for one hour was used to remove the separated oil from the solvent. The extracted oil was dried in a 40°C oven before being stored in sterile container in the refrigerator at 4°C. The procedure was repeated many times in order to obtain enough essential oil for further examination and study of the essential oil's biological activities.

Prepared *C. albicans* Suspension

A single isolating colony was cultured in SDA Petri dish for one day in suspension at a concentration of 310^6 cells/mL. *C. albicans* Colonies were suspended in test tubes containing normal saline and compared to test tubes containing MacFarland solution to reached 310^6 cells/mL; MacFarland standards was used according to the turbidity of suspensions to compare, were made by Barium Chloride 1% 0.2 mL with 1% sulfuric acid 9.8 mL.[17]

Well Diffusion Method Assay

The Agar well diffusion method was used to conduct in vitro inhibition testing and determine the minimum inhibitory concentration of plant extracts against *C. albicanis*. Using three replicates of SDA for each essential oil, 100 microliters of suspension was placed on the SDA and sprayed on the agar using a L shape sperader. Using a cork borer, make a hole in the agar and pour 0.1 microliter of oil into it. Control using the same steps as above, except in the hole placed distal water instead of oil, then the *T. vulgaris* and *O. vulgare* were explained, and the operation of old oil storied for one year and fresh extracted oil from thymus vulgaris was compared. samples were collected at sven concentrations and tested which are : (crude,1.5, 1, 0.8, 0.5, 0.2,0.1) mg/mL,[18], also to compared with nystatin and fluconazole antifungal.the emulsion were stabilized with 1% of Tween 80.[18]

GC/MS Analysis

The chemical composition of the most powerful oil was chemically analyzed at the GC laboratory at the College of Agriculture, University of Basrah, using gas chromatography (SHIMADZU GC MS-QP 2010). The inhibition zone diameter around each well was measured to determine the apility of each concentration of the different oil extracts.[19,20]

Toxicity Test of EOS Extracted from *T. vulgaris* and *O. vulgare*

To determine toxicity of EOS extracted from *T. vulgaris* and *O. vulgare*, 1 mL of human blood was drawn, which was used for the purpose of determining the toxicity of essential oils extracted from T. vulgaris and O. vulgare, 0.04 mg and 0.02 mg of oil extracted from T. vulgaris and O. vulgare, respectively. T. vulgaris and O. vulgare were dissolved in 0.8 mL of 0.85% regular saline, which had been prepared and sterilized, and 0.2 mL were applied to it in two separate test tubes containing anticoagulant derived from human blood. Also, 0.8 mL of physiological solution was taken alone, and 0.2 mL of human blood was added as a positive control, and 0.8 mL of tap water was taken, and 0.2 mL of human blood was added as a negative control, and the tubes were incubated at 37°C for 1 to 24 hours. The tubes were put in a centrifuge for 5 minutes at 1600 rpm an hour later, during which the results were obtained.[21]

Statistical Analysis

The results were statistically analyzed using the SPSS using the Krukal Wallis test and the least significant difference (LSD) using the absolute random design to compare the averages below the level Probability $p > 0.05$.[22]

RESULTS AND DISCUSSION

Vitek Technique used to Identification of *C. albicans* and Antifungal Susceptibility

Identification of *C. albicans* using the Vitek technique used to diagnose the candida isolate from oral candidiasis (thrush) from Basra General Hospital, the species found that was diagnosed

based on the cultural and microbiological phenotypes, and using the Vitek2 Technique to identify *C. albicanis* as shown in Figure 1.

Antifungal susceptibility to test the behavior of various antifungal types against *C. albicanis* using five distinct antifungal types: the isolate's susceptibility to fluconazole and voriconazole caspofungin, micafungin, and flucytosine was tested. As shown in Table 1, it was susceptible to all of the antifungal drugs listed above.

The effect of *Origanum vulgare* and *T. vulgaris* Essential Oil on *C. albicanis*

Several forms of antifungals have been discovered to treat fungal infections in humans, and a variety of antifungals are used as antiseptics, with fluconazole, nystatin suspension, and chlorhexidine among the most commonly used mouthwashes.[23] Several studies have been conducted to find natural antifungal products, aromatic plants, in particular, have a high concentration of biologically active compounds, primarily essential oil,[24,25] test the antifungal efficacy of *O. vulgare* Eos. in vitro against sixteen *Candida* species *O. vulgare* Eos. sensitivity was found in all isolates studied *in-vitro*.[2]

The findings shows that *O. vulgare* and *T. vulgaris* Eos. have antifungal activity against *C. albicanis* in general, with O. vulgare having more action than *T. vulgaris*, as measured by the inhibition region, which reached 3.26 cm and 2.53 cm for both of these essential oils, respectively non-significant $p > 0.05$.[26] It was discovered that *T. vulgaris* oil and *O. vulgare* essential oils have activity against *C. albicans*, also found that the activity of crude extract of essential oil in both plants gives a higher inhibition zone than other concentrations, suggesting that the active compound has a synergistic effect. The inhibition zone for *O. vulgare* essential oil as an antifungal was 3.26 cm, while the activity of crude extract of *T. vulgaris* oils was 2.53 cm, indicating high biological activity against *C. albicans*.[27] *T. vulgaris* essential oils were found to have inhibitory effects on cariogenic bacteria and decreased bacterial adhesion to dental surfaces.

Table 2 and Figure 3 indicate inoculums cultured on SDA media at seven different concentrations (crude,1.5, 1, 0.8, 0.5, 0.2, 0.1) mg/mL. some studies have been carried out to study natural antifungal compounds, emphasizing aromatic plants due to their high content of effective compounds, especially essential oil compounds.[28] Oregano essential oil has antifungal activity against *C. albicans*, with a MIC of 110 mg/L.[29] The result found that the MIC was 200 mg/mL for *T. Vulgaris* oil and 100 mg/mL for *O. vulgare* Eos. as appear in Table 3, indicating the studied essential oil has a positive fungicidal effect.

Each value in the table is the rate of three replicate
LSD = 0.3659

3.3 The effect of Fresh and Old *T. Vulgaris* Essential oil on *C. alpicanis*

Findings indicate discrepancies in susceptibility to *T. vulgaris* essential oil between fresh and oil stored for one year. The inhibition zone for fresh essential oil in crude concentration

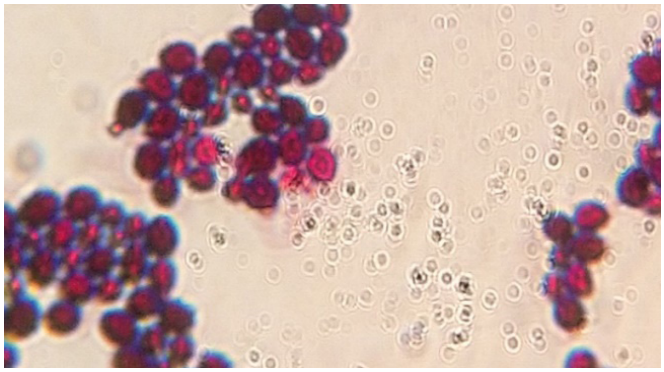

Figure 1: Candida albicanis G+ve staine 400 x

Table 1: Antifungal sensitivity patterns of *C. albicans* against antifungal by Vitek 2 Technique.

Susceptibility information	*Analysis time : 12.63 hours*	*Status : Final*
Antifungal	MIC (µg/ mL)	Interpretation
Fluconazole	<=1	S
Voriconazole	<=0.12	S
Caspofunigin	<=0.25	S
Micafungin	<=0.06	S
Flucytosine	<=1	S

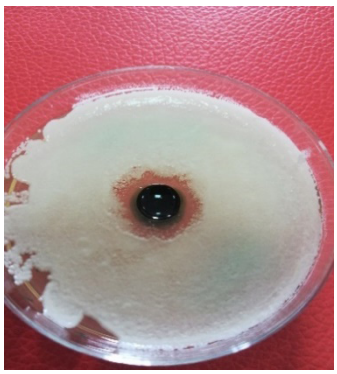

Figure 2: Origanum vulgare essential oil (crude)

Table 2: The effect of *O. vulgare* oil and *T. vulgaris* oil on *C. alpicanis*.

Concentration Mg / mL	*Rates of inhibition zone (cm)*		
	Type of extract		
	Thymus vulgaris	*Origanum vulgare*	*Average*
Crude	2.53	3.26	2.89
1.5	2.16	2.96	2.56
1	1.66	2.80	2.23
0.8	1.54	2.36	1.95
0.5	1.23	1.53	1.38
0.2	0.00	1.23	0.615
0.1	0.00	0.00	0.00
Total	1.82	2.598	2.209
0 control	0.00	0.00	0.00

Figure 3: Thymus vulgaris essential oil (crude)

Table 3: The effect of fresh and old thymus vulgaris essential oil on Candida alpicanis

Concentration mg/mL	*Rates of inhibition zone (cm)*	
	Fresh essential oil	*Old essential oil*
Crude	2.53	1.5
1.5	2.16	1.23
1	1.66	1.13
0.8	1.54	0.90
0.5	1.23	0.63
0.2	0.00	0.00
Total	1.82	1.08
control	0.00	0.00

Each value in the table is the rate of three replicates. LSD = 0.6056

was 2.53 cm, while the inhibition zone for old essential oil in crude oil crude concentration was 1.51 cm. Despite these differences, these differences were statistically significant, $p<0.05$. That means the activity of *T.vulgaris* essential oil is an effect with the store to a period reached to one year. as well as between different concentrations of essential oil as show in the following Table 3 that my be according to store conditions not suitable for storing that inhibit the activity of essential oil during storing period. The structure, consistency, and content of essential oil are all subject to change and are affected by various factors, including geographical and climatic conditions, as well as the conditions used for culture, drying, and storage, the harvesting season, and differences in oil extraction technology.[30,31] The activity of *O. vulgare* essential oil compared with old *T. vulgaris* essential oil was highly significant $p<0.05$, meaning the activity of *O. vulgare* essential oil highly effective reached to 3.26 cm.

The Activity of Antifungal Compartment with *O. vulgare* and *T. vulgaris* essential oil on *C. albicanis*

Compared to thyme essential oil and *O. vulgare* essential oil, the essential oil's antifungal effects against isolates of *C. albicans* were lower.[30,32,33] The *O. vulgare* essential oil

Figure 4: Nystatin tab (150 mg/ mL)

Figure 5: *O. vulgare* oil (1.5 mg/ mL)

Table 4: Comparison between the effect of Nystatin and Origanum essential oil

Type of antifungal	*Inhibition zone*
O. vulgare essential oil	3.26
fresh essential oil	2.53
old essential oil	1.5
Nystatine tab (150 mg)	2.5
Fluconazole cap(250 mg)	0.00
Control	0.00

Each value in the table is the rate of three replicates. LSD = 0.6286

appear high activity compartment with nystatin, as appeared in Figures 4 and 5, the inhibition zone which reached 3.26 cm and 2.5 cm for nystatin while fluconazole doesn't appear any inhibition as shown in Table 4, this result was highly significant $p<0.05$ thats may be according to the nystatin which works to inhibit the Ergosterol, which is the main structure in the yeast's cell membrane.

The Effective Compound in the Origanum vulgare Essential oil by using GC–MS

According to the high activity of *O. vulgare* oils, which were studied using GC–MS to determine the structure of these successful compounds in this research, 70 chemical

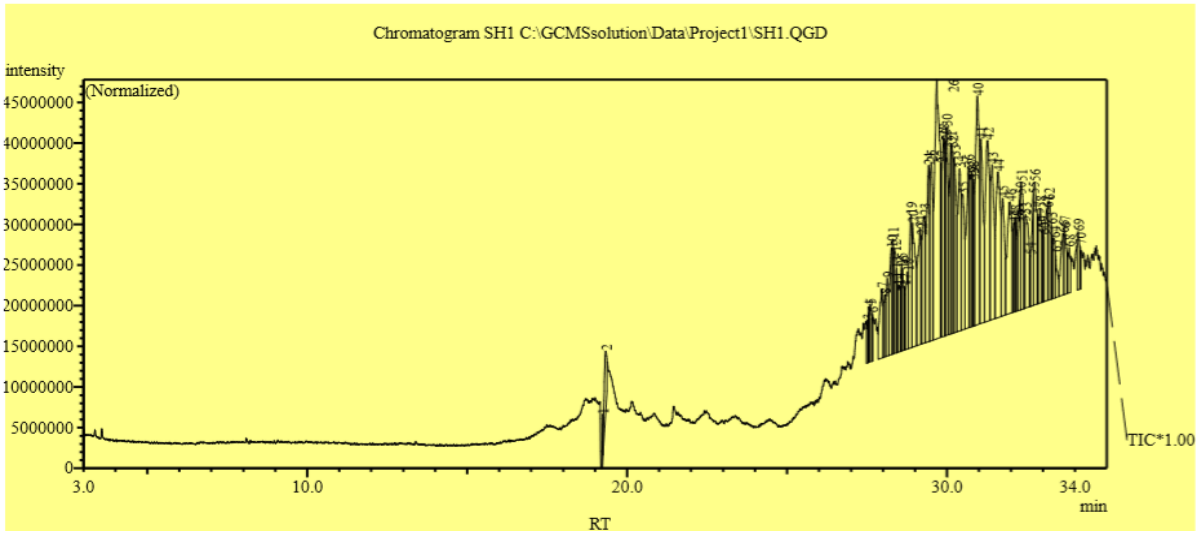

Figure 6: Gas Chromatography Mass Spectrometry chromatogram of the essential oil of *O.vulgare*

components of the examined *O. vulgare* oils were identified using GC-mass analysis. Moreover, this oil's components have been established., Tetracontane, 3,5,24-trimethyl-, Cyclododecanemethanol, Sulfurous acid, 2-ethylhexyl tridecyl ester. The remaining ingredients were used in smaller quantities., the main essential oil compounds in oregano oil are: Figure 6 appear these compounds according to GC –MS. The composition, consistency, geographic and climatic conditions, culture, drying, storage conditions, harvesting season, and oil extraction techniques all affect the composition and content of EOs. As a result, these differences can have an effect on the biological activity of essential oils. As a result, these differences can have an effect on the biological activity of essential oils.[30]

Toxicity Test of the Essential Oil Extracted from *T. vulgaris* and *O. vulgare*

According to the findings, the essential oils of *O. vulgare* and *T. vulgaris* are not toxic to human blood cells.[34]

CONCLUSION

The obtained results indicated that T.vulgaris and Origanum vulgare essential oils might be useful natural components within formulations used to prevent and treat candidiasis due to their high content of EOs responsible for their antifungal role. Furthermore, these essential oils can be used to replace a variety of conventional synthetic antifungal preparations.

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REFERENCES

1. Ghannoum MA, Jurevic RJ, Mukherjee PK, Cui F, Sikaroodi M, Naqvi A, et al. Characterization of the oral fungal microbiome (mycobiome) in healthy individuals. PLoS Pathog. 2010 Jan;6(1).
2. Cleff MB, Silva GM, Meinerz ARM, Madrid IM, Martins AA, Fonseca AO, Nascente PS, Meireles MCA, Mello JRB. (2007). Infecção cutânea em cão por Candida albicans. Rev. Vet. Zoot. 14 (2):164-168.
3. Lacaz CS, Porto E, Martins JEC, Vaccari EM, Melo NT (2002). *Tratado de Micologia Médica.* 9ed., Sarvier, São Paulo, SP.
4. Sullivan DJ, Moran GP, Pinjon E, et al. Comparison of the epidemiology, drug resistance mechanisms and virulence of *Candida dubliniensis* and *Candida albicans*. FEMS Yeast Res 2004;4:369-376.
5. Meis JF, Ruhnke M, De Pauw BE, Odds FC, Siegert W, Verweij PE. *Candida dubliniensis* candidemia in patients with chemotherapy-induced neutropenia and bone marrow transplantation. Emerg Infect Dis 1999;5:150-153.
6. Kojic EM, Darouiche RO. Candida infections of medical devices. Clin Microbiol Rev (2004) Apr;17(2):255-267
7. Brillowska-Dabrowska A, Schon T, Pannanusorn S, et al. A nosocomial outbreak of Candida parapsilosis in southern Sweden verified by genotyping. Scand J Infect Dis 2009;41(2):135-142.
8. Magill SS, Shields C, Sears CL, Choti M, Merz WG. (2006). Triazole Cross-Resistance among *Candida* spp.: Case Report, Occurrence among Bloodstream Isolates, and Implications for Antifungal Therapy. *J. Clin. Microbiol*., 44: 529-535.
9. Waller SB, Cleff MB, Serra EF, Silva AL, Gomes AD, de Mello JR, et al. Plants from Lamiaceae family as source of antifungal molecules in humane and veterinary medicine. Microb Pathog. 2017 Mar;104:232-237
10. Hina J, Shazia E, Sobia T, Farhana A. An overview on medicinal importance of *thymus vulgaris*. Journal of Asian Scientific Research. 2013.
11. Burt S. 2004. Essential oils: their antibacterial properties and potential applications in foods: a review. International Journal of Food Microbiology 94:223-253.
12. Lai PK, Roy J. (2004). Antimicrobial and chemopreventive properties of herbs and spices. Current Medicinal Chemistry 11, 1451-1460.
13. McGinnis, MR. (1980). Laborotary handbook of medical mycology. Academic press, New York, USA.

14. Buckley HR. (1989). Identification of yeast. In: Medical mycology: A practice approach. Evans, E. G. and Richaroach, M. D. (eds). I. RL. Press, Oxford University Press.
15. Lebboffe Michael (2014). Microbiology Laboratory Theory and Application (3rd ed.). Englewood, CO: Morton Puplishing Company. p.105.
16. Wallace S. Carrier, D. and Clausen, E. (2003). Extraction of nutraceuticals from milk thistle: part II. Extraction with organic Biochem Biotechnol. 891–903.
17. Andrews JM (2004). BSAC standardized disc susceptibility testing method (version 3). J. Anti microb. Chemoth.(53):713-728.
18. Zeinab L, Denisa M, Isabala N, Virginia V, and Denisa I. (2012). The Antimicrobial Activity Of Thymus vulgaris and Origanum Syriacum EOs ILS On Staphyalococcus Aureus, Streptococcus Pneumoniae And Cadida albicans. Farmacia 60:(6).
19. Sura MA (2015). Detection of Oral Candida Species and Investigation from Anti-Candida Activity of Origanum vulgare L Essential Oil. MiddleEast Journal of Scientific Research. 23(1):18-25.
20. Manuela-Anda R, Silvia,, DS, Enache-Soare CB, Diana P, Aurelian U, and Tatiana V (2010). Molecular biological techniquesused for the identification of Candida spp., Farmacia. 58(4):422–429.
21. Ahmed I, Mehmood, Z and Mohamma, F (1998). Screening of Indian Medicinal plants for their antimicrobial properties.J. Ethnopharm., 62:183-93.
22. Al-Rawi, Khasha Mahmoud and Khalafullah, Abdul Aziz Mohammed. (2000). Design and Analysis of Agricultural Experiments, University of Mosul, Dar Al Kutub Publishing.
23. Talebi S, Sabokbar A, Riazipour M, Saffari M. Comparison of the in vitro effect of chemical and herbal mouthwashes on Candida albicans Jundishapur J Microbiol. 2014 Dec;7(12): e12563.
24. Giordani R, Regli P, Kaloustian J, Mikai C, Abou L, Portugal H (2004). Antifungal effect of Various EOs against *Candida albicans*. Potentiation of Antifungal Action of Amphotericin B by Essential Oil from *Thymus vulgaris. Phytot. Res.,* 18:990-995.
25. Chavan PS, Tupe SG. Antifungal activity and mechanism of action of carvacrol and thymol against vineyard and wine spoilage yeasts. Food Control. 2014;46:11-120.
26. Baj, Tomasz, Biernasiuk Anna, Wróbel Rafał, Malm Anna, Chemical composition and *in vitro* activity of *Origanum vulgare* L., Satureja hortensis L., Thymus serpyllum L. and Thymus vulgaris L. EOs towards oral isolates of Candida albicans and Candida glabrata. Open Chem., 2020;18:108-118.
27. Oliveira,Maria AC de, Mariana R da C Vegian, Fernanda L Brighenti, Marcos J Salvador3 & Cristiane Y Koga-Ito. Antibiofilm effects of Thymus vulgaris and Hyptis spicigera essential oils on cariogenic bacteria, Future Microbiol. (2021) 16(4):241-255
28. Sarac N, Ugur A. The *in vitro* antimicrobial activities of the EOs of some Lamiaceae species from Turkey. J Med Food. 2009 Aug;12(4):902-907.
29. Vahedi G, Khosravi AR, Shokri H, Moosavi Z, Delirezh N, Sharifzadeh A, et al. Fungicidal effect of *Origanum vulgare* essential oil against Candida glabrata and its cytotoxicity against macrophages. J Herbmed Pharmacol. 2016;5(2): 78-84.
30. Soares IH, Loreto ÉS, Rossato L, Mario DN, Venturini TP, Baldissera F, et al. In vitro activity of EOs extracted from condiments against fluconazole-resistant and -sensitive Candida glabrata J Mycol Med. 2015 Sep; 25(3):2137.
31. Diba K, Ghabaie K, Heshmatian B, Sharbatkhori M. Antifungal activity of Satureja hortensis alcoholic extract against Aspergillus and Candida species. J Med Plants Res. 2013;7(30):2271-2274.
32. Adams A, Kumar S, Clauson J, Sahi S. Anti-yeast activities of Origanum oil against human pathogenic yeasts. Adv Biosci Biotechnol. 2011;2(2):103-107.
33. Çoşkun F, Sivri G, Demirci AŞ. Anti-Yeast effects of some commercial EOs against food-related yeasts. IOSR J Environ Sci Toxicol Food Technol. 2016;10(11):83-88.
34. Ahmed I, Mehmood Z,and Mohamma F. (1998). Screening of Indian Medicinal plants for their antimicrobial properties.J. Ethnopharm., 62:183-193.