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Studying The Effects of (Thyme, Sage, And Marjoram) Essential Oils Against Biofilm Produce by *Candida albicans* Isolated From Urinary Tract And Vaginal Infections

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Abstract

Background: The urinary and reproductive tracts are among the most vulnerable organs in the human body to microbial infections due to their mucous membranes and the fact that they are a moist area, making them a reservoir for fungi in particular. They may be opportunistic, infecting those with weak immunity and those suffering from chronic diseases. These yeasts may acquire resistance to antifungal treatments. Therefore, the global trend has become towards discovering therapeutic alternatives to limit their growth and reduce side effects. **Objective**: The study aimed to test the effectiveness of plant oils (sage oil, thyme oil, and marjoram oil) in reducing the growth of fungi resistant to manufactured antifungals and in inhibiting the growth of biofilms, and to consider them as alternative treatments to manufactured treatment. **Methods**: The isolates were collected from the Maternity and Child Hospital in Maysan and identified by chromogenic agar to confirm them. After that, the antifungal test was performed, and the efficiency of the vegetable oils was tested through the disc diffusion method, followed by the biofilm inhibition test.**Results**: The results for marjoram oil and sage oil were notable, with inhibition diameters of 19.72 mm and 11.67 mm, respectively. In contrast, thyme oil showed no results. Regarding the biofilm test, marjoram oil achieved the highest percentage at 24.69%. Sage oil followed, which had the highest inhibition of *C. albicans* at 14.25%. Then, it was found that thyme oil had the highest inhibition of *C. albicans* at 12.44%. **Conclusion**: Marjoram and sage oils have shown effective results against *Candida albicans* isolates from both urine and vagina, indicating their potential as antifungal agents. Further clinical trials are recommended, along with their application in topical or oral forms. Thyme oil, despite its efficacy, did not affect biofilms, This is attributed to the oil's sensitivity to heat and light, which may alter its chemical and therapeutic properties.

Keywords : Antifungal , essential oils, Antifungal sensitive test , biofilm, *Candida albicans*

1. Introduction

Yeasts, in particular, play two distinct roles in the human body. First, they help maintain the natural balance of the microbiome. However, if this balance is disrupted, diseases may arise (Wilson, 2019). Candida fungi, specifically *Candida albicans*, are responsible for candidiasis infections and play a prominent and important role in them. Especially in individuals with weakened immune systems, whether they are elderly, have autoimmune diseases, or have taken antibiotics or corticosteroids for extended periods, these yeasts can lead to inflammations of the mucous layer of the mouth, vagina, digestive tract. In severe cases, they can even enter the bloodstream (Mayer et al., 2013). *Candida albicans* possesses many virulence factors that are important to it and play a key role in causing infections. These factors help it adhere to the host and cause infection. It also has a symbiotic relationship with the pharynx, digestive tract, female reproductive organs, and urinary tract. Its ability to adhere to the host is due to the presence of mucous membranes. The spores take on multiple forms, including filamentous and yeast-like, which is their most important characteristic and one of the virulence factors that helps them cause infection. In addition to their varied forms, they have an important characteristic: the production of hydrolytic enzymes (phospholipase and protease) and the formation of biofilms that give them the ability to adhere to surfaces (Lal et al.,2021).
Recent studies are focusing on producing treatments with fewer or no side effects, as well as reducing microbial resistance (Abdula et al., 2024; Mohammed et al.,2025; Rasool et al., 2025), Plant based products are rich in natural compounds that are effective and antimicrobial. (Seira et al., 2018; Al-Siraj et al., 2024). Many plants possess properties and characteristics rich in phenolic and terpenoid compounds, including sage oil, thyme oil, and marjoram oil (Madras et al., 2016). The surge in the trend towards alternative treatments occurred due to the increasing resistance of fungi to antifungals, including commonly used antifungals such as fluconazole and amphotericin B. Thujone and cineole compounds, which are abundant in sage oil, make it unique in its inhibitory effect on Candida growth because it possesses a property that disrupts fungal cell membranes and reduces their ability to adhere to host cells (Abo-Altemen et al.2019; Naji et al.,2025) . A study by Badiee (2012) has shown Most plant oils have active antimicrobial compounds, and their concentration varies from plant to plant. For example, studies indicate that the compounds in sage oil have a future in terms of their use for topical treatment. As for thyme oil, it contains thymol and carvacrol, which are very effective and can disrupt the plasma membranes of fungi and

affect their enzymes. (Bachir et al., 2022). The journal Frontiers in Microbiology stated that some oils can be used in medicinal cosmetics, including thyme oil, due to its antimicrobial properties, even in small doses (Alshaikh and Perveen, 2021). Some plant oils possess aromatic properties due to their content of sabinene, terpenes, and linalool, which give them antimicrobial properties. Marjoram oil, for example, inhibits biofilm growth (Manohar, 2001). Natural products were chosen because they are active and effective against Candida, as well as being safe and having few side effects, if any. They have become a global trend as an alternative or supportive treatment (Mohammed, 2018). The research aims to study the effect of three types of plant oils (sage, thyme and marjoram) and to determine their activity in reducing the growth of mites and inhibiting their biofilm. It examines their active properties, as demonstrated by previous scientific studies on their chemical characteristics and mechanisms of action (Cid-Chevecich et al., 2022). In addition, the research aims to evaluate its role in prevention and treatment, and to determine the possibility of its use in future medical treatments and its consideration as part of natural or supportive treatment protocols.

2. Materials and Methods

collection of samples

50 isolates of 63 specimens were obtained from the Children's and Maternity Hospital in Maysan City from both urine and vaginal sources at various intervals, and the women's ages were between 22 and 37 years old.

Wet Mount

A drop of 10% potassium hydroxide solution was placed on the slide, then the sample to be examined was added, and the slide was covered to prevent the formation of bubbles. It was left for a few minutes and then examined under a microscope using 10x and 40x magnification lenses.

Preparation of Sabouraud Dextrose Agar

Use this medium to isolate, grow and reproduce fungi, yeasts, pathogenic and non-pathogenic and prepare according to as stated in (Sabouraud, R. 1892).

Preparation of Mueller Hinton medium

Use this medium to test the sensitivity of microbes to antibiotics and prepare according as reported in Microbiologyinfo.com. (2022).

Preparation of the center of CHROMagar Candida

Use this medium to diagnose yeasts and determine the type, and prepare according to as stated in Bio-Rad Laboratories. (2022).

McFarland's experiment

McFarland's experiment (McFarland Standard) was used to determine the concentration of candida in microbial suspension, which is a very important step in testing the effectiveness of essential oils or antibiotics, especially in the experiments of "disk propagation" or "dicks" on dishes containing agar. The McFarland scale is used to calibrate the number of yeasts in the suspension (usually the standard 0.5 is used), which approximately corresponds to (Knoll et al.,2022) :

• 1.5 × 10^8 CFU/mL (colony-forming units per milliliter).

Prepared a pure culture of yeasts (from a culture for 18-24 hours), and then suspended part of the culture in a sterile saline solution or nutritious broth. Then the turbidity of the suspension was compared to the McFarland 0.5 standard using a reference tube (by sight or with a spectrometer). If it is more turbid, it is diluted. If it is less, more cells are added.

Antifungal and Essential Oil

When testing Candida, it's critical to measure the dimensions of the antifungal agents' effects on the Candida and the resistance of the *Candida* to these agents in order to measure the effectiveness and activity of the agents. For this reason, use concentrations of Itraconazole 10µg and Flucanozole 25 µg,Clortrimazole 10 µg. Types of aromatic oils of commercial origin (Turkish) and in 100% concentration were used, mainly thyme oil, marjoram oil, and sage oil.

The method of distribution of tablets (discs) in the experiment of testing essential oils:

After processing the bacterial sample based on the McFarland standard: Prepared the agar: Poured the Müller-Hinton agar medium into a petri dish, then inoculated the fungal surface with a sterile swab, dipping it in the fungal suspension (titrated with McFarland). Then wiped the entire surface of the agar with it in a criss-cross manner to ensure an even distribution (Geraldi et al.,2022). After that, the discs were distributed. Sterile paper tablets (approximately 6 mm in diameter) were used. Followed by adding a specified amount of essential oil to them (usually 10-20 microliters) and then placing the tablets on the surface of the agar using sterile forceps. Several tablets with different oils or in different concentrations can be placed on the same plate. Incubated dishes were stewed at 37°C for 18-24 hours, and the results were measured by the diameter of the inhibition zones (the growth-free zone around the disc). The larger the diameter, the more effective the oil is against fungi (Ermenlivera et al.,2021; Hamza et al.,2025).

Tissue culture plate method to detection the Biofilm formation by Candida isolates

Biofilm inhibition was assessed using a microplate biofilm inhibition assay. 100 µL of Candida strains at a 0.5 McFarland concentration (1 × 10^6 CFU/ml) was added to the wells of a 96-well microplate. Then, 100 µL of essential oil was added to all wells, bringing the total volume to 200 µL per well. The essential oils were then dissolved in 50% dimethyl sulfoxide (DMSO). The wells were incubated for 48 hours at 37°C. To create a positive well for comparison, an essential oil-free suspension was used. The suspension was then removed, the wells were allowed to dry, and the wells were washed three times with phosphate-buffered saline after 48 hours of incubation. 200 µL of 0.1% crystal violet dye was added to a 96-well plate, which was placed at room temperature and incubated for 30 minutes. The violet dye was then removed, and the wells were washed three separate times with the same phosphate-buffered saline solution. The plate was then left to air dry at room temperature for 30 minutes. The crystal violet was then dissolved in 95% ethanol, and its absorbance at 550 nm was measured using a SPECTROstar Nano microplate reader (BMG Labtech). The biofilm inhibition percentage was determined using the equation (Norouzi et al., 2021; Karpiński et al., 2023:

$$\% \text{ Inhibition} = 100 - \frac{\text{OD 570 sample})}{\text{OD 570 control)}} \times 100$$

Statistical Analysis:

The Statistical Packages of Social Sciences-SPSS (2019) program was used to detect the effect of difference groups in study parameters. T-test and Least significant difference-LSD was used to significant compare between means in this study.

Results

***Candida albicans* Isolation**

After collecting 50 samples, Candida was seen in urine samples after microscopic examination and culture media. 50 samples out of 63 were obtained. The results of the samples collected from different places at different times.

Cultural Characteristics

Candida albicans on Sabouraud dextrose agar appeared as small, creamy, or white colonies with a smooth, shiny, and yeast-like appearance. They also appeared in a different color when using differential chromogenic agar; they appeared in bright green, Fig 1,2.

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Fig (1) SAD agar

Fig (2)Chromogenic agar

Microscopic Appearance

Digital microscopy use of the wet mount method can be used to identify the small, oval, spherical, single-celled organisms known as Candida, Fig (3).

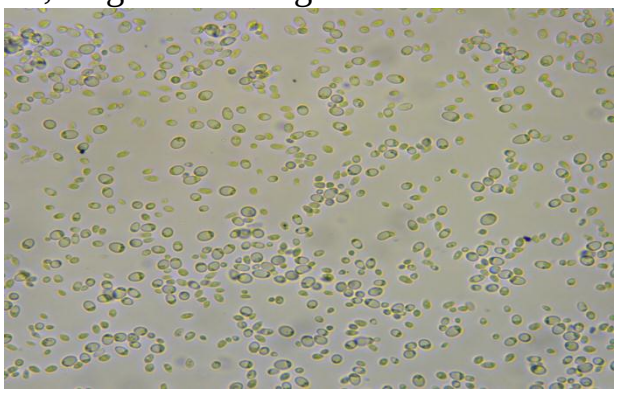

Fig (3) *Candida albicans* under microscope

The effect of Essential oils on *Candida albicans*

The study tested sensitivity to essential oils through the utilization of the modified disk diffusion method. The widths of the zones of inhibition were measured in millimeters for each essential oil disk using a ruler. The determination of the susceptibility of essential oils (sensitivity, intermediate, and resistance) was conducted based on the methods described in earlier studies. Marjoram oil 100% had the highest effect of sensitivity on *C. albicans* for urine and vaginal isolates, 18.95 and 19.57 mm, respectively, and then sage oil was 10.89 and 11.67 mm, respectively, and no significant difference was observed compared to itraconazole. As for fluconazole and clotrimazole, they were the best. The results are shown in Table 1, and Figure

4,5. As observed, there is no inhibitory effect of thyme oil on *Candida albicans*, which indicates that it has 100% resistance to it.

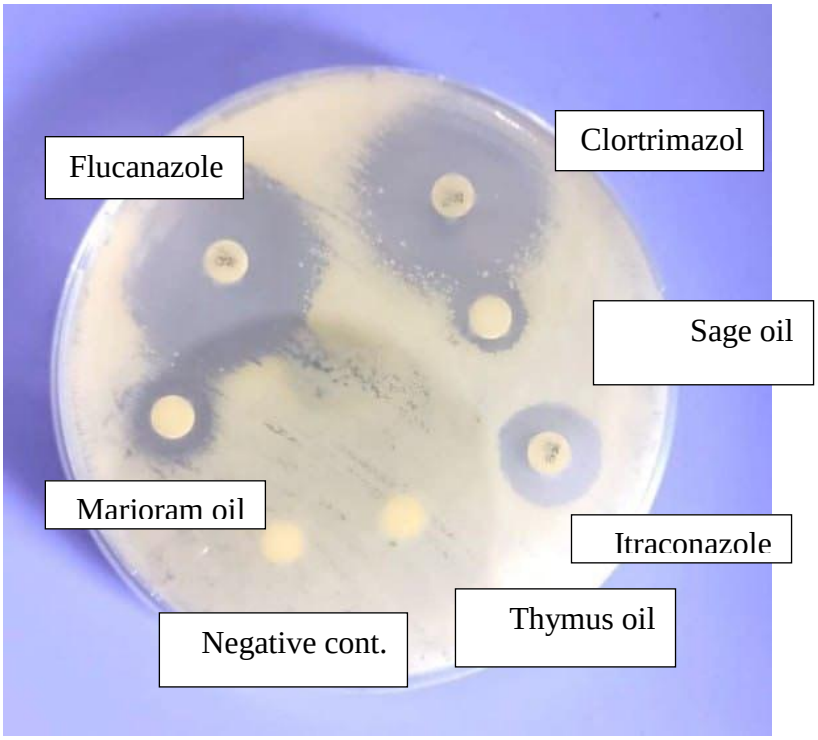

Fig (4) Antifungal Test

Table 1: Comparison between difference groups in Inhibition of Urine and Vaginal *C. albicans* isolates

Group	Means ±SD		L.S.D.	P-value
	Urine isolates	Vaginal isolates		
Marjoram oil	18.95 ±3.61 b	19.72 ±2.32 ab	1.904 NS	0.4207
Sage oil	10.89 ±1.75 c	11.67 ±1.26 c	0.943 NS	0.1048
Thymus oil	0.00 ±0.00 d	0.00 ±0.00 d	0.00 NS	0
Clotrimazole	24.93 ±2.86 a	23.67 ±4.53 a	2.101 NS	0.2242
Fluconazole	27.47 ±2.25 a	23.83 ±6.74 a	2.607 *	0.0073
Itraconazole	16.64 ±1.85 b	16.97 ±1.87 b	1.102 NS	0.5477
DMSL/ Negative control	0.00 ±0.00 d	0.00 ±0.00 d	0.00 NS	0
L.S.D.	5.026 **	6.362 **	---	
P-value	0.0001	0.0001		
* (P≤0.05), ** (P≤0.01), NS: Non-Significant.				

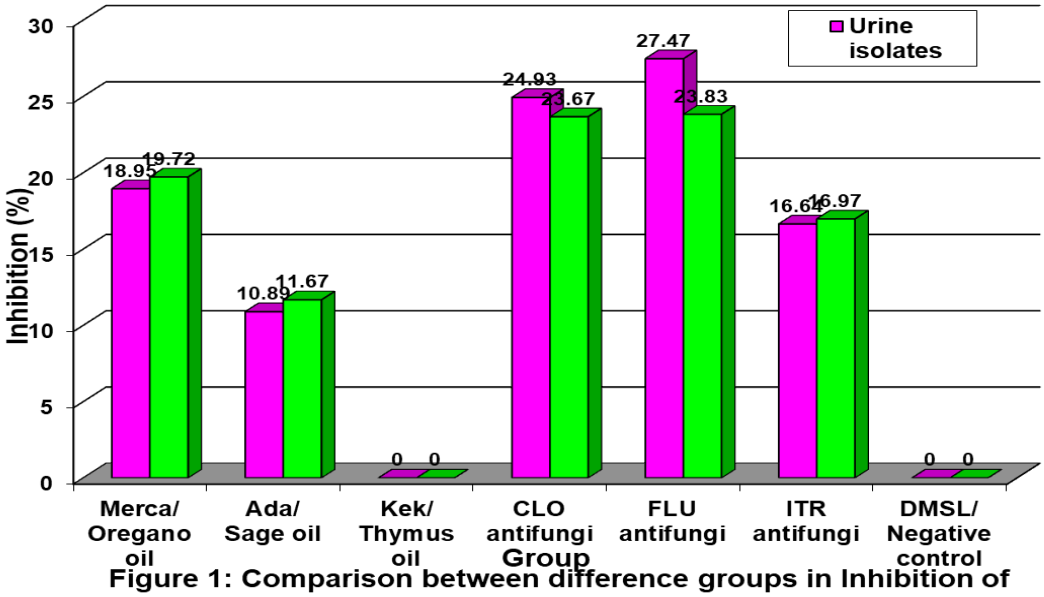

Figure 1: Comparison between difference groups in Inhibition of Urine and Vaginal *C. albicans* isolates

The results indicate that there is no notable difference between essential oils and antifungals, but there are significant differences between the two types of Candida vaginalis and urinary tract. However, the differences between the averages were minor and may not be of obvious biological or clinical significance, and it is likely that the statistical effect is due to the large sample size and low variability without being reflected on the real effectiveness in the practical context, so it recommends more studies to assess the real biological impact of these differences

Tissue culture plate method to detection the Biofilm formation by Candida isolates

The microtiter biofilm inhibition assay method was utilized to study the biofilm test and the inhibitory impact of essential oils. The results indicated that Marjoram oil had the highest inhibition of biofilm *C. albicans* for urine and vaginal isolates, 24.69% and 23.17%, respectively. And then sage oil followed, which had the highest inhibition of 14.25% and 11.67%. Then, thyme oil had an inhibition of biofilm of 12.44% and 10.22%. The results are shown in Table 2 and Figure 6,7.

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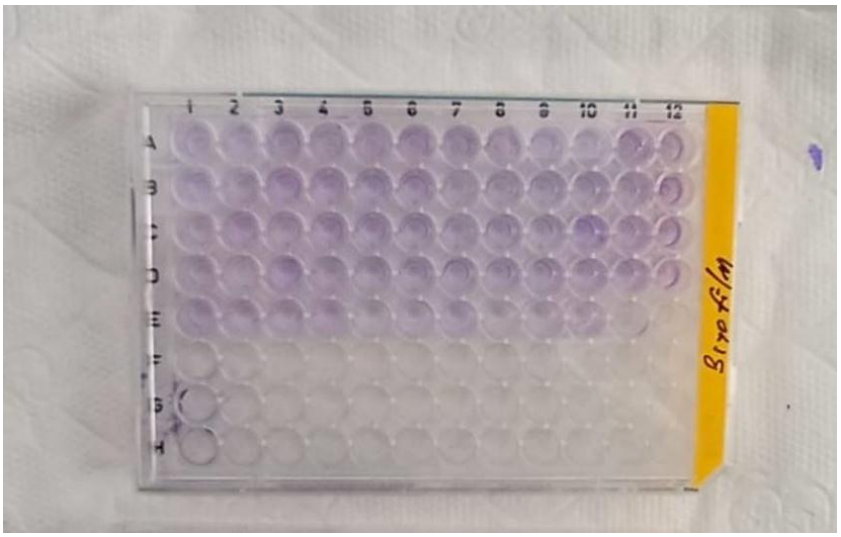

Fig (6) Detection the Biofilm formation by Candida isolates

Table 2: Comparison between difference groups in Biofilm results of Urine and Vaginal *C. albicans* isolates

Group	Means ±SD		L.S.D.	P-value
	Urine isolates	Vaginal isolates		
Marjoram oil	24.69 ±11.36 a	23.17 ±9.55 a	6.397 NS	0.6334
Sage oil	14.25 ±8.07 b	11.67 ±5.07 b	4.239 NS	0.2264
Thymus oil	12.44 ±9.72 b	10.22 ±8.94 b	23.04 NS	0.4305
L.S.D.	9.028 *	8.619 *	---	
P-value	0.03372	0.0458		
* (P≤0.05), NS: Non-Significant.				

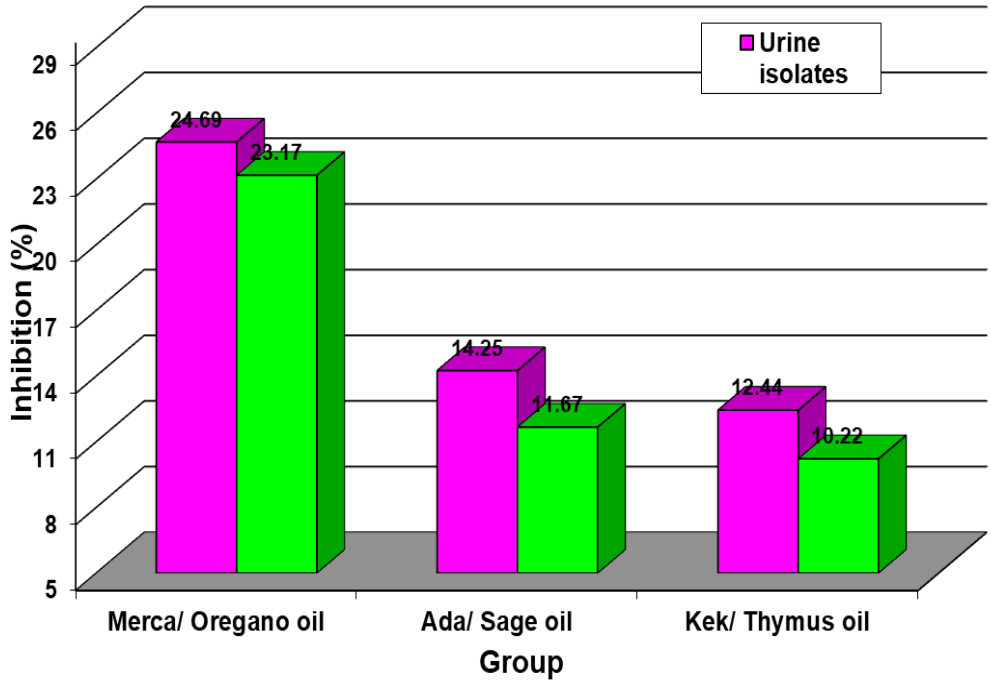

Figure 2: Comparison between difference groups in Biofilm results of Urine and Vaginal *C. albicans* isolates

The results indicate that there are very significant differences between the essential oils used, but between Candida vaginalis and the urinary tract, there are no obvious significant differences.

Discussion

The objective of this study was to evaluate the impact of essential oils on *Candida albicans* and to minimize the reliance on antibiotics due to their adverse effects. The modified disc diffusion method was used to identify fungi sensitive to certain antibiotics. The results indicated that marjoram oil was effective against Candida albicans, exhibiting greater efficacy than itraconazole. However, the results obtained from isolates using sage and thyme oils did not match those of previous studies. This discrepancy can be explained by the study by Pluhar et al. (2016) The reason for this difference is that the active compounds in the oils are affected by temperature, humidity, light, weather conditions, and human and animal interference, thus weakening their effectiveness against Candida. The thyme oil used in previous studies is of European origin, while the one used in my current study is not of European origin, which makes it different in terms of chemical composition and polyphenol production. The results are consistent with those of Sheikh and Pervin (2021). Plant oils affect the effectiveness of fungal biofilms and thus inhibit them, with marjoram oil being the most effective in

inhibiting them, followed by sage oil. then followed that Thymus oil had the highest inhibition. Many studies have demonstrated the significant impact of essential oils on biofilm, as mentioned by Proskovcova et al. (2021) and Fernandes et al. (2023). Additionally, the extent of biofilm formation is contingent upon the specific location and nature of the colonized surface, as described by Marak and Dhanashree (2018). The anti-biofilm activity is likely related to carvacrol and thymol, which are the primary compounds of essential oils. This evidence correlates with the study by Hajibabaei et al. (2023). All results align with the current study, although variations in inhibition rates exist.

The results align with the findings reported by Karpinski et al. (2023). Of the three oils used, sage oil and marjoram oil stood out for their effectiveness and proved to be potent antifungal agents, which aligns with our study.

Conclusion

Based on the results obtained, sage and marjoram oils exhibit promising antifungal activity against Candida albicans, whether applied topically or orally. They are considered good natural alternatives with high activity levels of active compounds. Although thyme oil was not effective against Candida, further research is needed on different concentrations of the oils, comparing commercial oils with those used in treatment, as well as examining oil storage, packaging, transportation, origin, and geographical location.

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دراسة تأثيرات الزيوت العطرية (الزعتر والمريمية والمردقوش) ضد الغشاء الحيوي الذي تنتجه المبيضات البيضاء المعزولة من التهابات المسالك البولية والمهبلية

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مستخلص البحث:
خلفية: تُعدّ المبيضة البيضاء سببًا رئيسيًا للعدوى الفطرية الشائعة، وترتبط ارتباطًا وثيقًا بعدوى المسالك البولية والتناسلية. ويُعدّ الأفراد ذوو المناعة الضعيفة أكثر عرضةً للإصابة بها. ولمعالجة مقاومة مضادات الفطريات، يجري استكشاف طرق علاجية وبدائل متنوعة، مثل استخدام الزيوت العطرية ذات الخصائص المضادة للميكروبات والفطريات. **الهدف**: تقيم هذه الدراسة الفعالية المضادة للفطريات للزيوت الأساسية (زيت المريمية وزيت الزعتر وزيت البردقوش) ضد المبيضات البيضاء المعزولة من عينات البول والمهبل ، ومقارنة نشاطها بمضادات الفطريات التقليدية مثل فلوكونازول وكلوتريمازول وإيتراكونازول. **الطرق**: تم الحصول على العزلات السريرية للبيض من عينات البول والمهبل للمرضى في مستشفى الأطفال والولادة في ميسان وتم تحديدها لاحقا باستخدام أجار كروموجينيك. تم تقييم مضادات الفطريات وفعالية الزيوت الأساسية عن طريق فحوصات انتشار القرص ومقايسات تثبيط الأغشية الحيوية الدقيقة ، بالإضافة إلى قياس تثبيط الأغشية الحيوية. **النتائج**: زيت البردقوش يثبت نشاط مضاد للفطريات كبير ، مع منطقة تثبيط من 19.72 ملم. أظهر زيت المريمية آثار فطريات جيدة من 11.67 ملم ، وزيت الزعتر لم تظهر النتائج. أشار اختبار البيوفيلم إلى أن زيت البردقوش كان لديه أعلى تثبيط للبيض بنسبة 24.69٪. تبع ذلك زيت المريمية ، والذي كان لديه أعلى تثبيط للبيض بنسبة 14.25٪. ثم وجد أن زيت الزعتر يحتوي على أعلى نسبة تثبيط للبيض بنسبة 12.44٪. **الخلاصة**:قد تحل زيوت البردقوش والمريمية محل العوامل المضادة للفطريات ضد عزلات البول البيضاء. هناك حاجة إلى التجارب السريرية لتحديد تطبيقاتها العلاجية الجلدية أو الفموية. يؤثر الزعتر على الأغشية الحيوية ، ولكن ليس كمضاد للفطريات. نوصي بحماية الزيوت التجارية والعلاجية من الحرارة والضوء ، والتي يمكن أن تغير خصائصها الكيميائية والعلاجية.

الكلمات الرئيسية: مضاد للفطريات ، الزيوت الأساسية ، اختبار حساسية مضاد للفطريات ، بيوفيلم ، المبيضات البيض