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RESEARCH ARTICLE

Comparison of Antifungal Activity of Thymus Vulgaris Essential Oil and Triple Antibiotic Paste Against Candida Albicans Isolated from Root Canal (In Vitro Study)

[version 1; peer review: 2 approved with reservations]

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Abstract

Background

The main objective of chemomechanical endodontic therapy is to reduce or eliminate microorganisms from the root canal system, and intracanal medications are used to accelerate the disinfection process.

The aim of this study

this study was to assess the antifungal activity of thymus vulgaris essential oil against *C. albicans* compared to that of triple antibiotic paste.

Materials and methods

C. albicans was isolated from patients who required endodontic treatment. Using the agar well diffusion method, the efficacy of Thymus vulgaris essential oil against *C. albicans* was determined at 25 mg/ml, 10 mg/ml, 8 mg/ml, 6 mg/ml, 4 mg/ml, and 2 mg/ml and compared to the triple antibiotic paste. The minimum inhibitory and fungicidal concentrations against *Candida albicans* were determined using the microbroth dilution method. The constituents were identified using a gas chromatography-mass spectrometer.

Results

Thymus vulgaris essential oil demonstrated antifungal activity against *C. albicans*, which appeared to increase as the extract concentration

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increased, and the difference between all concentrations and triple antibiotic paste was significant ($p \leq 0.01$). The MIC and MFC for *Thymus vulgaris* essential oil were 0.015 mg/ml and 0.031 mg/ml, respectively. 53 compounds were identified by (GC-MS) analysis, which represented more than 95% of the oil composition. The main component was thymol (35.48%).

Conclusion

Thymus vulgaris essential oil is effective against *C. albicans*, suggesting that it has the potential to be applied as an intracanal medication.

Keywords

Candida albicans, Essential oil, Regenerative endodontic, *Thymus Vulgaris*, Triple antibiotic paste

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Introduction

Revascularization, a new treatment method for managing an immature permanent tooth, was made available in endodontics.¹ The American Association of Endodontists approved the term “regenerative endodontics” in 2007.² Regenerative endodontic therapy (RET) aims to rebuild the pulp-dentine complex of immature necrotic permanent teeth that had been damaged by trauma, infection, or developmental abnormalities.³ A total of 65% of bacteria, 30% of fungi, and 5% of other organisms form heterogeneous microflora. Remarkably, these microbes can survive challenging root canal environments.⁴ *Candida albicans* is the most commonly identified root canal fungus and significantly contributes to the failure of root canal therapy.⁵ An important step in (RET) is the use of intracanal medications to clean the necrotic root canal. The most common intracanal medication used in endodontic regeneration is the triple antibiotic paste (TAP), which includes a combination of metronidazole, ciprofloxacin, and minocycline. It has been employed in more than half of published instances of endodontic regeneration.⁶ It has been reported that 40% of the teeth treated with TAP have discolored crowns.⁷ This large percentage indicated a significant clinical problem.⁸ Jain et al. (2014) reported that herbal remedies could be viable alternatives to traditional pharmaceuticals.⁹ *Thymus vulgaris* oil is composed of a blend of monoterpenes, which possess a variety of beneficial properties including antioxidative, antimicrobial, antispasmodic, and antibacterial effects.¹⁰ The purpose of this study was to assess the effectiveness of *Thymus Vulgaris* essential oil (TVEO) in comparison with TAP as a root canal medicament against *C. albicans*.

Methods

The Research Ethics Committee of the College of Dentistry, University of Baghdad approved this study (Reference number/839) at date 30/8/2023.

The raw material used during the experiment was acquired from a local market in Baghdad, Iraq, and was classified at the herbarium of the Department of Biology/College of Science/University of Baghdad, it consists from the aerial parts of *Thyme Vulgaris*. After washing and cleaning, the samples were dried. Drying was performed in a well-ventilated area at room temperature, without exposure to light. After drying, the leaves were collected and chopped in a household blender. The obtained powder was stored in an airtight container until extraction.⁴

Essential oil extract preparation

Dried thyme leaves (50 g) were hydrodistilled in a Clevenger-type apparatus for 3-4 h. The oils were maintained at 4 °C in sealed vials after drying over anhydrous sodium sulfate.¹¹

Patient selection and sample collection

Ten patients (their age ranged 25-45 years) who had pulpal necrosis, periapical lesions and needed endodontic treatment, were included in this study after signing an informed consent. All the selected teeth had one root. The teeth were symptomatic, and radiographic examination performed in the diagnostic department of a specialized dental clinic (Maysan) verified the diagnosis.

The selected teeth were isolated using a rubber dam. The working area was cleaned with 10% povidone-iodine solution. All coronal restorations and carious lesions were removed using a sterile carbide fissure bur, followed by field disinfection. Subsequently, an access opening to the root canal was created with a sterile diamond fissure bur, taking care not to go too deep inside the pulp chamber to preserve the microbiota. For the initial instrumentation, new and sterile K files of (15, 20) size were used, and a size #20 rotary file was used with no irrigants. Subsequently, normal saline was used to fill the canal, and the final file was agitated vertically to generate a microbiological suspension. After leaving the paper points inside the canal for one minute, they were transferred to the Brain Heart Infusion Broth to the laboratory for isolation and identification.¹²

Identification of *candida albicans*

- 1- Gram's stain: According to Gram's method of Naser and his colleagues 2023.¹³
- 2- colony morphology: Colonies of *C. albicans* were examined according to their morphological characteristics on Sabouraud dextrose agar plates as described by Webb *et al.* (1998).¹⁴
- 3- Germ tube formation: Human serum (0.5 ml of human serum was suspended in tubes containing a small inoculum from an isolated colony. The inoculated tubes were incubated for three hours at 37 °C. Subsequently, on a clean slide, a drop of the suspension was spread to search for germ tubes using a low-power light microscope.¹⁵

GC-MS analysis of TVEO

Thyme Vulgaris essential oil's chemical compounds analysis was analyzed using a gas chromatograph mass spectrometer (model 5973N, Agilent USA) equipped with a capillary column type HP-5ms (30 m length, 0.25 μ m thickness, 0.25 mm diameter) and helium, which was the carrier gas at a flow rate of 1 ml/min. The injector was operated at 280 °C. The oven temperature was programmed as follows: the starting temperature was 60 °C and held for 2 min. The temperature was increased at a rate of 10 °C/min to 300 °C and held for 5 min.¹⁶ This procedure was performed by the Ministry of Science and Technology, Department of Water and Environment/Environment Research Centre.

Test the sensitivity of *Candida albicans* to *Thymus Vulgaris* essential oil (TVEO) by Agar diffusion method

Dimethyl sulfoxide (DMSO) (LOBA Chemie, India) was used to dilute (TVEO) to various concentrations (25, 10, 8, 6, 4, and 2 mg/ml). The procedure involved the addition of 10% DMSO to the oil.

The Sabouraud dextrose agar (SDA) (OXOID, India) plates were inoculated with *Candida albicans* suspension that was produced as 0.5 McFarland using sterilized cotton swap. Six mm-diameter wells were created on the plate, and 100 μ l of TVEO at the previously determined concentrations were placed in each well. Metronidazole (Gulphar, U.A.E.), ciprofloxacin (Pharma International, Jordan), and minocycline (Vulga XR, Jordan) each of these powders was made separately by grinding it into a fine powder by using a clean mortar and pestle. To achieve the desired 1:1:1 proportion, the three antibiotic powders were individually weighed using a digital scale, and then, to make a paste, one gram of powder was combined with one milliliter of sterile water.¹⁴ 10% DMSO and TAP, on the other hand, served as the negative and positive controls, respectively. The plates were then kept in an incubator for approximately 24 h at 37°C, after which a ruler was used to measure in millimeters the fungal growth inhibition zones around the wells (involving six mm of each well).¹⁷

Determination of MIC and MBC of (TVEO)

Minimum inhibitory concentration was measured by broth microdilution. μ L each of the ten wells, numbered W1 to W10, received 100 μ L of SDA broth in a 96-well microtiter plate (NUNC™ Brand products), 100 μ L of TVEO at an 8 mg/ml concentration was added to W1, and W1 through W10 received a two-fold serial dilution. W11, which was the positive control, contained the inoculated media. In contrast, W12 contained uninoculated medium and acted as a negative control. 0.5 McFarland, a fungal suspension (20 μ l), was used to inoculate all wells and incubated overnight at 37 °C. Following incubation, 30 μ l of resazurin was added to each well (30 μ l/well) and incubated for 2–4 h¹⁸ for the observation of color changes. After completion of incubation, rows with no color change (blue resazurin color remained unchanged) were scored as MFC, whereas the last blue well in the row was recorded as the MIC and the next pink well was recorded as the sub-MIC. The MFC was detected by sub-culturing 100 μ l from the MIC well and the well before it on SDA plates. Results were obtained after 24 h of incubation at 37°C. After culturing, the concentration was considered to be MFC if it was equal to or higher than the MIC and there was no fungal growth on SDA.¹⁹

Statistical analysis

SPSS (Statistical Package for social Science) (version -22, Chicago, Illionis, USA) was used. Minimum, maximum, standard error, mean, and standard deviation, as well as the Levene test, Shapiro-Wilk test, and Tukey HSD with one-way analysis of variance (ANOVA) were used for data description, analysis, and presentation.

Level of significance when p value is less than 0.05.

Results

Chemical composition of the essential oil

The essential oil yield was 1%. Freshly isolated essential oil is a golden liquid with a hot-burning taste. The thyme essential oil was analyzed using GC-MS, and 53 components were identified, representing 95.95% of the total detected constituents. The major constituents of the oil are thymol (35.48%), o-cymene (13.82%), γ -terpinene (13.55%), carvacrol (4.04), α -terpinene (3.11%), caryophyllene (2.99%), linalool (2.57%), 3-Thujene (2%). Other ingredients were present in less than 2% of the samples (Table 1).

Table 1. GC-MS analysis of *Thymus vulgaris* essential oil.

No	Compounds	Area%	R.T
1	3-Thujene	2	12.900
2	2-Pinene	1.19	13.272
3	2,2-dimethyl-3-methylene-bicyclo	0.74	14.032

Table 1. *Continued*

No	Compounds	Area%	R.T
4	4(10)-Thujene	0.09	14.992
5	1 OCTEN 3 OL	1.37	15.369
6	3-Octanone	0.08	15.506
7	Beta.-Myrcene	1.82	15.689
8	3-Octanol	0.14	16.112
9	1-PHELLANDRENE	0.39	16.575
10	3-Carene	0.18	16.689
11	α -Terpinene	3.11	17.130
12	o-Cymene	13.82	17.724
13	2 -Oxabicyclo[2.2.2]octane	1.02	17.975
14	Beta. -Ocimene	0.14	18.404
15	γ -Terpinene	13.55	19.358
16	4-Thujanol	1.35	19.850
17	Linalool	2.57	21.199
18	Bicyclo[3.1.0]hexan-2-ol,2-methyl-5-(1-methylethyl)	0.31	23.554
19	Camphor	0.16	23.554
20	(+)-2-Bornanone	0.46	24.234
21	endo-Borneol	1.36	24.862
22	Terpinen-4-ol	1.39	25.171
23	3-Cyclohexene-1-methanol	0.27	26.463
24	8,9-Dehydrothymol	0.10	26.463
25	THYMYL METHYL ETHER	1.20	27.274
26	CARVACROL METHYL ETHER	0.91	27.726
27	2,6-Octadienal, 3,7-dimethyl	0.12	28.852
28	Geraniol	0.28	29.383
29	Thymol	35.48	31.161
30	Carvacrol	4.04	31.366
31	Phenol,5-methyl-2-(1-methylethyl)-acetate	0.11	32.787
32	Eugenol	0.11	33.184
33	Copaene	0.22	34.150
34	BETA. BOURBONENE	0.17	34.567
35	Caryophyllene	2.99	36.236
36	p-Cymene-2,5-diol	0.11	37.493
37	alpha.-Humulene	0.18	37.670
38	2,6-octadien-1-ol,3,7-dimethyl-,propanoate	0.23	37.808
39	gamma.-Muurolene	0.23	38.362
40	Germacrene	0.21	38.705
41	6.alpha.-Cadina-4,9-diene	0.13	39.088
42	alpha.-Muurolene	0.25	39.322
43	beta.-Bisabolene	0.10	39.591
44	delta.-Cadinene	0.86	40.117
45	trans-Calamenene	0.10	40.282
46	Spathulenol	0.12	42.900
47	Epicubenol	0.18	44.106

Table 1. Continued

No	Compounds	Area%	R.T
48	2-Naphthalenemethanol	0.25	44.449
49	tau.-Cadinol	0.58	45.112
50	alpha.-Cadinol	0.21	45.632
51	VULGAROL B	0.10	46.238
52	Cyclohexanol, 3-ethenyl-3-methyl	0.11	47.122
53	3-Methyl-4-isopropylphenol	0.12	58.360

Identification of *Candida albicans*

Under a microscope, the tested isolate showed gram-positive, oval-shaped, or budding yeast cells. *Candida* colonies on the SDA appeared smooth, creamy, white, and had a yeasty smell. The results of the germ tube test were positive, suggestive of *C. albicans*.

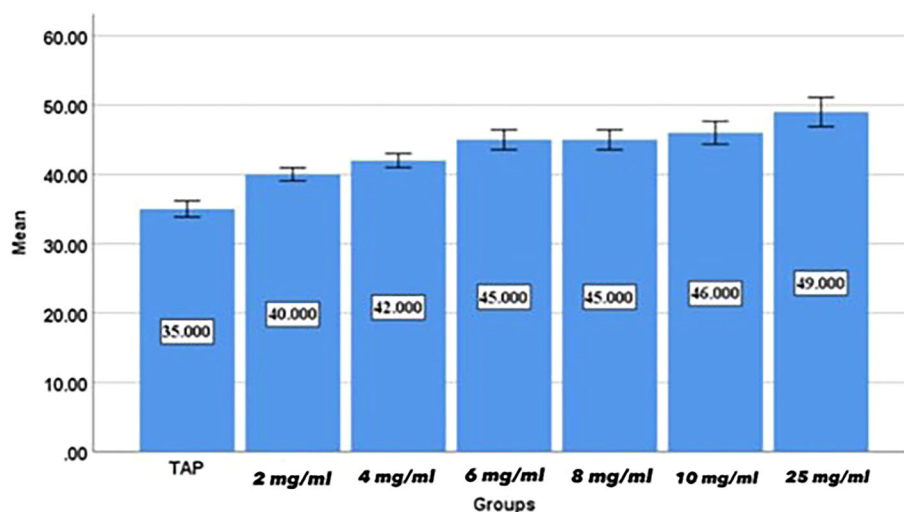
Antifungal activity of TVEO against *C. albicans*

TVEO was effective against *C. albicans* at all concentrations and regions around the wells with no fungal growth, termed growth inhibition zones. The lack of an inhibitory zone suggests that the fungi were resistant to the drug under evaluation. Triple antibiotic paste, in contrast to DMSO, demonstrated antifungal activity against *Candida albicans* and created an inhibitory zone.

Table 2. Comparison among the antifungal activity (inhibition zone diameter in mm) of *Thymus vulgaris* essential oil and triple antibiotic paste (control) against *Candida albicans*.

Groups	Minimum	Maximum	Mean	±SD	±SE	F	P value
TAP	32.000	37.000	35.000	1.633	0.516	52.031	0.000 Sig.
2 mg/ml	38.000	42.000	40.000	1.333	0.422		
4 mg/ml	40.000	44.000	42.000	1.414	0.447		
6 mg/ml	42.000	48.000	45.000	2.000	0.632		
8 mg/ml	42.000	48.000	45.000	2.000	0.632		
10 mg/ml	41.000	49.000	46.000	2.309	0.730		
25 mg/ml	45.000	55.000	49.000	2.944	0.931		

Levene test p value = 0.352 NS.

**Figure 1.** Activity of *Thymus vulgaris* essential oil and Triple Antibiotic Paste (control) against *Candida albicans*.

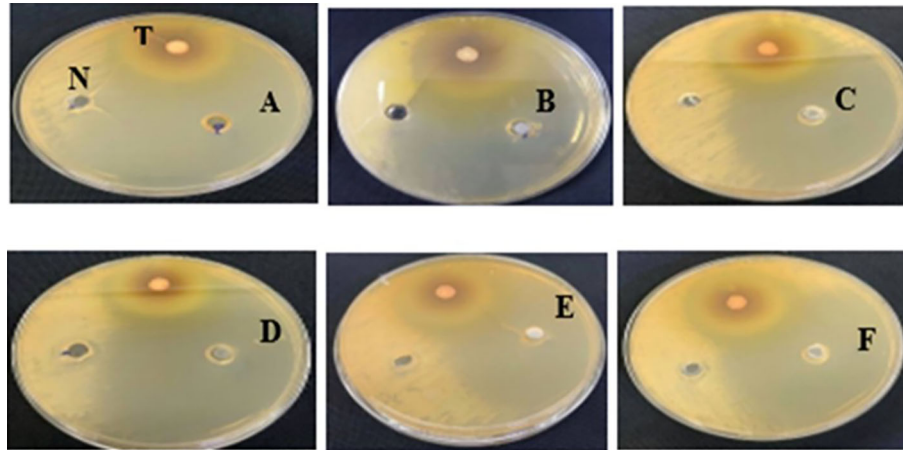


Figure 2. Inhibition zone of TVEO in different concentrations. A - 25 mg/ml, B - 10 mg/ml, C - 8 mg/ml, D - 6 mg/ml, E - 4 mg/ml, F - 2 mg/ml, T - triple antibiotic paste (positive control), N - dimethyl sulfoxide (negative control).

As TVEO concentration increased, the diameter of the growth-inhibitory zone also increased. The results of the ANOVA test showed that there was a highly significant difference between the concentrations of TVEO and TAP ($p \leq 0.01$) (Table 2, Figure 1, and Figure 2).

Since there was a significant difference between the extract concentrations and the control, each pair of TVEO concentrations was statistically compared using Tukey's HSD test, and the results revealed a significant difference between them ($p \leq 0.05$), while between 2 mg/ml and 4 mg/ml, 6 mg/ml and 8 mg/ml, and 8 mg/ml and 10 mg/ml was not significant ($p > 0.05$) (Table 3).

Table 3. Comparison among the antifungal activity (inhibition zone diameter in mm) of each two concentrations of Thymus Vulgaris essential oil against *Candida albicans* and triple antibiotic paste (control).

Groups		MD	P value
2 mg/ml	4 mg/ml	-2.000	0.301
	6 mg/ml	-5.000	0.000
	8 mg/ml	-5.000	0.000
	10 mg/ml	-6.000	0.000
	25 mg/ml	-9.000	0.000
	TAP	5.000	0.000
4 mg/ml	6 mg/ml	-3.000	0.023
	8 mg/ml	-3.000	0.023
	10 mg/ml	-4.000	0.001
	25 mg/ml	-7.000	0.000
	TAP	7.000	0.000
6 mg/ml	8 mg/ml	.000	0.999
	10 mg/ml	-1.000	0.923
	25 mg/ml	-4.000	0.001
	TAP	10.000	0.000
8 mg/ml	10 mg/ml	-1.000	0.923
	25 mg/ml	-4.000	0.001
	TAP	10.000	0.000
10 mg/ml	25 mg/ml	-3.000	0.023
	TAP	11.000	0.000
25 mg/ml	TAP	14.000	0.000

Determination of MIC and MFC of TVEO

MIC of TVEO was 0.015 mg/ml and MFC was 0.031 mg/ml.

Discussion

Different chemotypes can be identified in plants, and consequently, in the essential oils of *T. vulgaris*. Chemical composition analysis confirmed that the essential oil belongs to the thymol chemotype. These results were similar to those obtained by Al-Asmari et al.²⁰ and Micucci et al.,²¹ who studied the oil composition of thyme of the same species sample, in which thymol was the main component.

Many studies have documented the use of plant extracts in dentistry owing to their antimicrobial properties. The successful treatment of several oral diseases has been reported. Moreover, Herbs are better choices for disinfecting root canals because of their anti-inflammatory, antioxidant, sedative, and antibacterial properties.⁴

This study demonstrated the activity of TVEO against *C. albicans* in comparison with TAP, which had greater inhibition zones than TAP, and this activity increased as the concentration increased.

This result may be due to the EOs having an antifungal component such as phenol (especially thymol, which is the main component of the essential oil used), alcohols, aldehydes, ketones, ethers, and hydrocarbons, which have the potential to disrupt plasma membrane integrity and produce reversible changes in cellular management that would influence the chemical structure of the cells and their activity.²²

Our outcome is consistent with a previous study that found a direct relationship between concentration and antimicrobial activity.¹⁹ Mith et al. (2014) used the agar diffusion method to examine the antimicrobial effects of TVEO on several microorganisms and found that TVEO has antimicrobial properties that result in greater suppression of bacterial growth as TVEO concentration increases.²³ This result shows considerable promise for using TVEO as a replacement for TAP.

Conclusion

Compared with the synthetic antimicrobials that are currently available and applied for root canal therapy, TVEO showed noteworthy antifungal activity and could potentially be a potent herbal substitute.

Ethics and consent

The Research Ethics Committee of the College of Dentistry, University of Baghdad approved this study (Reference number/839) at date 30/8/2023.

Patients were included in this study after signing an informed consent.

Data availability

Underlying data

Zenodo: Raw data for [Comparison of Antifungal Activity of Thymus Vulgaris Essential Oil and Triple Antibiotic Paste Against Candida Albicans Isolated from Root Canal (in vitro study)] Doi: <https://zenodo.org/records/10917793>.²⁴

This project contains the following underlying data: Raw Data (F1000).xlsx (data available in excel format)

[Data available in word format: Under data.docx, Doi: <https://doi.org/10.5281/zenodo.10723658>]

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](#) (CC-BY 4.0).

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Marco Antonio Sánchez-Tito

Universidad Privada de Tacna, Tacna, Peru

Summary:

The full name of the species should be provided on its first mention: *Candida albicans*. For subsequent references, use *C. albicans*. Always use the correct spelling for species names according to taxonomy and binomial nomenclature; for example, it should be *Thymus vulgaris*. The term Gas Chromatography-Mass Spectrometry must be used instead of gas chromatography-mass spectrometer. Additionally, the conclusion should directly correspond to the research objective.

Introduction:

The authors could enhance the introduction by including more information about the potential applications of *T. vulgaris* based on other studies, particularly how it has been utilized in formulating experimental products for dentistry. References to consider include:

1. Marinković J., et. al. (2022) -Ref 1
2. Diouchi J., et. al. (2024)-Ref 2
3. Marinković J., et. al. (2020) -Ref 3

It is important to correct the spelling throughout the text: it should be *Thymus vulgaris* (*T. vulgaris*), not *Thymus vularis*.

Methods:

It is crucial to specify the calculation method for essential oil extraction yield. The authors mention that BHIB was used as the sample transport medium. However, the appropriate transport medium for isolating *C. albicans* is Sabouraud. In terms of identification of *C. albicans*, why wasn't a specific identification method utilized? The authors should clarify whether methods such as CHROMagar, a urease test, or a cycloheximide resistance test were employed, as these are commonly used for species identification in laboratories.

In the GC-MS section, the term "Thyme vulgaris" is incorrect; "Thyme" refers to the common name

in English and should not replace binomial nomenclature. It is also important to specify the library used for comparing the compounds during identification. In the sensitivity test, the authors did not indicate how repetitions were calculated for each concentration or the parameters used for this calculation. In the MIC and MBC section, the wording needs to be corrected to reflect "µL of each concentration in the ten wells."

In the statistical analysis section, it is essential to clarify that the one-way ANOVA test requires compliance with three main assumptions: independence of observations, normal distribution of the residuals, and homogeneity of the residuals. Concluding these last two assumptions based on the data alone can lead to misinterpretation. Authors should verify these assumptions to confirm the suitability of the ANOVA test and should indicate which multiple comparisons test was utilized.

Results:

From a methodological standpoint, it would have been ideal to first test the antifungal capacity of the essential oil and then prepare an experimental paste based on this concentration for comparison with the TAP.

Table 2 does not specify the statistical test used. It is misleading to mention Levene's test at the bottom of the table, as this only indicates homogeneity of variances. In fact, this table could also be used to present multiple comparisons using superscript letters, simplifying the presentation and avoiding the confusion caused by a lengthy Table 3.

Figure 1 unnecessarily reiterates information presented in the table.

Figure 2 displays Petri dishes with agar diffusion assays (wells) showing inhibition zones. However, the inhibition zones overlap, particularly evident in plate A between T and A. This overlap arises because the radial diffusion of the antimicrobials is too close together, making it impossible to delineate exact areas of inhibition for each agent. As a result, the zones become difficult to measure according to standard protocols, lacking a defined boundary between one inhibitor and another. According to CLSI (Clinical and Laboratory Standards Institute, M100) and EUCAST (European Committee on Antimicrobial Susceptibility Testing), inhibition zones should: - Be measured in millimeters, capturing the full diameter where there is no bacterial growth.

- If zones overlap, the experimental design is deemed inadequate; therefore, these measurements should not be reported as they can lead to interpretation errors.

In studies involving extracts or biomaterials (such as dental materials), the literature suggests that wells or discs should be placed at least 24 mm apart (center to center) to avoid overlap (Bauer et al., 1966; Balouiri et al., 2016, Journal of Pharmaceutical Analysis). While the presence of inhibition can be qualitatively reported ("+" or "inhibition present"), it should be noted that overlapping zones hinder quantitative measurement.

- 1. Bauer A., et. al. (1966) - Ref 9**
- 2. Balouiri M., et. al. (2016)- Ref 8**

Since the MIC and MFC determination method was detailed in the methods section, it would be beneficial to include a figure illustrating the details of the wells.

Discussion:

The results of the following studies could be discussed:

1. Sainudeen S., et. al. (2020)- Ref 4
2. Raoof M., et. al. (2023)- Ref 5
3. Agarwal L (2024) -Ref 6
4. Teja K., et. al. (2021)- Ref 7

Remember that pharmacognosy seeks to use the lowest effective concentrations for use as antimicrobial agents in experimental formulations.

This statement, "This result shows considerable promise for using TVEO as a replacement for TAP," is a bit premature. Remember that the evaluation of other properties (such as antioxidant activity, cytotoxicity, drug release, etc.) must be taken into consideration. Could the authors indicate the limitations of their study?

References

1. Marinković J, Nikolić B, Marković T, Petrović B, et al.: Essential Oils as Adjuvants in Endodontic Therapy: Myth Or Reality?. *Future Microbiology*. 2022; **17** (18): 1487-1499 [Publisher Full Text](#)
2. Diouchi J, Touré B, Ghoul S: Antibiofilm efficacy of plant extracts as root canal irrigants in endodontics: a systematic literature review. *Frontiers in Dental Medicine*. 2024; **5**. [Publisher Full Text](#)
3. Marinković J, Čulafić D, Nikolić B, Đukanović S, et al.: Antimicrobial potential of irrigants based on essential oils of *Cymbopogon martinii* and *Thymus zygis* towards in vitro multispecies biofilm cultured in ex vivo root canals. *Archives of Oral Biology*. 2020; **117**. [Publisher Full Text](#)
4. Sainudeen S, Nair V, Zarbah M, Abdulla A, et al.: Can herbal extracts serve as antibacterial root canal irrigating solutions? Antimicrobial efficacy of *Tylophora indica*, *Curcumin longa*, *Phyllanthus amarus*, and sodium hypochlorite on *Enterococcus faecalis* biofilms formed on tooth substrate: In vitro study. *Journal of Pharmacy And Bioallied Sciences*. 2020; **12** (5). [Publisher Full Text](#)
5. Raoof M, Zadeh M, Nejad Z, Sharififar F, et al.: Antimicrobial Efficacy of Different Herbal Extracts against Root Canal Pathogens - An in Vitro Study. *Indian Journal of Dental Research*. 2023; **34** (2): 204-208 [Publisher Full Text](#)
6. Agarwal L: Antimicrobial effect of herbal and conventional root canal endodontic irrigants against persistent pathogens. *Bioinformation*. 2023; **19** (13): 1312-1317 [Publisher Full Text](#)
7. Teja K, Janani K, Srivastava K, Shrivastava D, et al.: Comparison of Herbal Agents with Sodium Hypochlorite as Root Canal Irrigant: A Systematic Review of In Vitro Studies. *Evidence-Based Complementary and Alternative Medicine*. 2021; **2021**: 1-11 [Publisher Full Text](#)
8. Balouiri M, Sadiki M, Ibnsouda S: Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*. 2016; **6** (2): 71-79 [Publisher Full Text](#)
9. Bauer A, Kirby W, Sherris J, Turck M: Antibiotic Susceptibility Testing by a Standardized Single Disk Method. *American Journal of Clinical Pathology*. 1966; **45** (4_ts): 493-496 [Publisher Full Text](#)

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Biomaterials, Microbiology, Statistics, Orthodontics, Epidemiology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 10 June 2024

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ABSTRACT

Result: The MIC and MFC: abbreviations are not allowed in the abstract section of the manuscript, except after writing the whole word. Please mention the whole words first, followed by their abbreviations.

(GC-MS) analysis: what does this means? Please clarify.

MAIN MANUSCRIPT

Result:

As we compared the Antifungal Activity of Thymus Vulgaris Essential Oil and Triple Antibiotic Paste Against Candida Albicans Isolated from Root Canal, we should perform the paired samples t-test for comparison between these 2 variables. Also the Kaplan-Meier survival curve must be employed in the result section of the main manuscript to approve which variable is more convenient than the other.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: SURGERY

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

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