Comparing the Antifungal Effect of Hanse Dent Endomix with Ledermix and Metapaste on Candida Albicans: An In Vitro Study

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Abstract

Aim of study: Candida albicans is one of the microorganisms that causes the failure of endodontic treatment, as it is stubborn to removal and resistant to many procedures in the canal system, so intracanal medicaments are used during the endodontic treatment to reduce its presence in the canal system, thus controlling one of the causes of endodontic treatment failure and relieving periapical lesions, so this study done to evaluate the effectiveness of three medicaments, which are Metapaste®, Ledermix® and Hanse Dent Endomix® against Candida albicans grown in pure cultures in Petri dishes within a special medium for its growth. Materials and Methods: Three Petri dishes A, B, and C were taken and divided into four parts: 1,2,3,4, each dish containing PDA culture medium. The medicaments were dissolved and impregnated on 6 mm diameter filter paper (Whatman paper) discs and then a suspension of Candida albicans was spread on the PDA medium in Petri dishes, Then the discs impregnated with medicaments were transferred to the dishes and placed over the fungal suspension. It was waited 24 hours of incubation at 37°C for the growth of Candida albicans, and then the results were taken. After clarifying the effectiveness of Hanse Dent Endomix® medicament and its effect on inhibiting the growth of Candida albicans, the medicament was extended to 6 diluted concentrations in glass tubes and impregnated these concentrations on 6 mm diameter Whatman discs. The effect of these concentrations on Candida albicans suspension in the PDA medium was studied to determine the minimum inhibitory concentration (MIC) of Hanse Dent Endomix®, within three groups of the experiment, namely, A, B, and C. Each experiment included two Petri dishes divided into eight sections. The first dish from each experiment was divided into 1,2,3,4. And the second dish was divided into 5,6,7. 8, The results were taken after 24 hours of incubation at 37°C. Results: Hanse Dent Endomix® medicament showed effectiveness in inhibiting the growth of Candida albicans, while Metapaste® and Ledermix® medicaments did not show any effectiveness in inhibiting the growth of Candida albicans fungi, the MIC of Hanse Dent Endomix® after 24 hours is 9µg\ml. Conclusion: This study demonstrated the efficacy of Hanse Dent Endomix®, Metapaste® and Ledermix® in inhibiting the growth of Candida albicans, and determined the minimum inhibitory concentration of Hanse Dent Endomix® after 24 hours.

Keywords: Candida albicans; Metapaste; Ledermix; Hanse Dent Endomix.

1. Introduction

- Microorganisms are the main cause of the development of pulpitis and periapical inflammation. Periapical lesions have been shown to heal at a higher rate in teeth with no root canal system infection [1], The primary goal of endodontic treatment is to eliminate microorganisms from the affected root canal system. Chemical-mechanical disinfection removes the majority of microorganisms; however, it is difficult to completely eradicate microorganisms due to anatomical complexity and limitations in accessing the canal system by tools and irrigation. [2] Therefore, the need for intracanal medicaments increases especially in cases where the infection is resistant to regular treatment and the outcome of endodontic treatment is at risk. Many microbiological studies on persistent periapical inflammation have shown that Candida albicans is the most common fungus. [3]
- C. albicans is a very adaptable microorganism with the ability to survive in diverse and distinct anatomical sites. Micromorphological and physiological properties of C. albicans are rapidly modified in response to different growth conditions and environmental changes. Accordingly, pathogenicity may be increased after adaptation to the environment and

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phenotypic switching.[4,5,6]

- Several intracanal medicaments, such as Metapaste and Ledermix, have been tested against a wide range of microorganisms and have shown highly variable results [7,8], For instance, Paul and colleagues In their laboratory study evaluated the efficacy of Ledermix against Candida albicans suspension and they found that Ledermix is effective in inhibiting its growth, while Chu and colleagues found in their clinical study that Ledermix is not effective in eliminating Candida albicans.[9, 10]
- Metapaste mainly contains calcium hydroxide, which is the most common medicament. The effect of calcium hydroxide has been studied recently on Candida albicans, in some studies, no significant effect has been found, [11] other studies have found it effective in inhibiting the growth of Candida albicans [12]
- As for the Hanse Dent Endomix medicament [Patent No.6075, On 20\Dec\ 2016 by Prof Dr. MSc. Aziz Abdullah], it mainly contains tea tree oil (T.T.O), which has anti-bacterial and anti-fungal properties [13, 14], It also contains chamomile extract, which is also known for its anti-bacterial and anti-fungal properties. [15,16], It also contains nystatin and cortisone in different proportions.

2. Material and Methods

This study was divided into two experiments, the first experiment used the method of (*Antibiotic resistance test*) to find out the actual effect of each of the Three medicaments which is Ledermix®, Metapaste® and Hanse Dent Endomix® on *Candida albicans*, second experiment included determining the Minimum Inhibitory Concentration (MIC) of the effective medicaments according to the (filter *paper disk agar diffusion*) Method.

the first experiment (Antibiotic resistance test)[22]

C. albicans was suspended in 20 ml of Peptone Water; the suspension was adjusted to match the turbidity equivalent to 0.5 McFarland standards. (Figure NO.1)

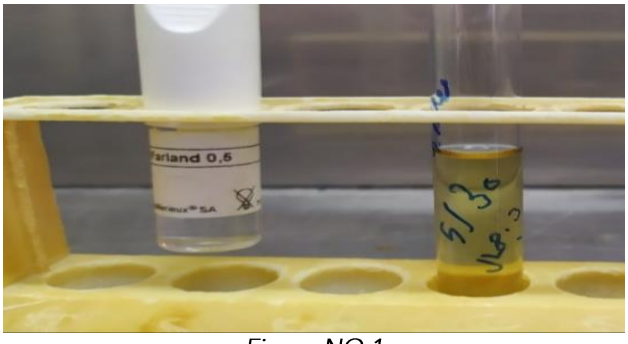

Figure NO.1

Medicaments were extended in a 1: 1 ratio and impregnated on Whatman discs with 6mm diameter in two sterile Petri dishes each one divided into two sections, the sum of the numbering of the sections is 1, 2, 3, 4.

Section 1 contains discs impregnated with Hans Dent Endomix medicament, Section 2 contains discs impregnated with Ledermix medicament and Section 3 contains discs impregnated with Metapaste medicament, and Section 4 discs impregnated with sterile distilled water. (Figure NO.2)

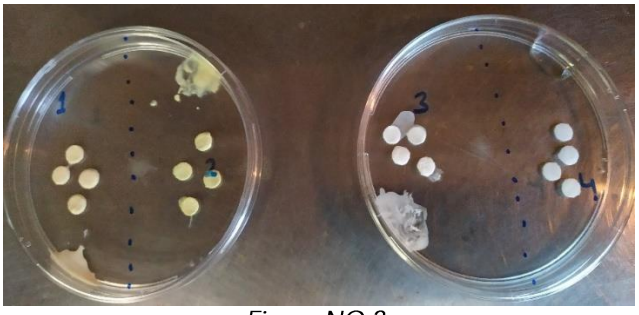

Figure NO.2

Three Petri dishes containing PDA medium were taken and named A, B, and C each dish was divided into four parts 1, 2, 3, and 4 as follows:

A: 1,2,3,4

B: 1,2,3,4 C: 1,2,3,4. (Figure NO.3)

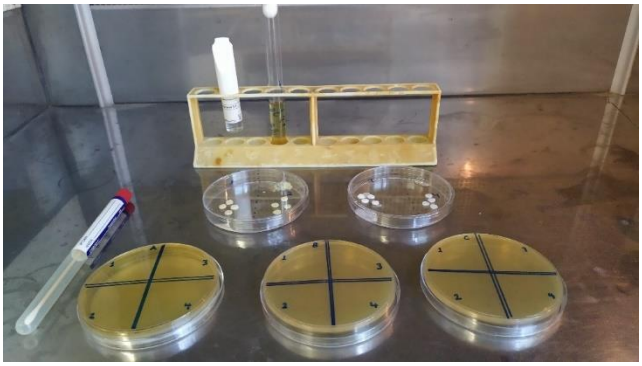

Figure NO.3

Using a sterile cotton swab, the *Candida albicans* suspension was transferred to dishes and spread over the entire area of the dish containing PDA medium. Then the discs were transferred to the dishes according to the corresponding numbers. discs impregnated with Hanse Dent Endomix are placed in part 1, discs impregnated with Ledermix are placed in part 2 and discs impregnated with Metapaste are placed in part 3 and discs impregnated with distilled water (control) are placed in part numbered 4.

The dishes A, B and C were placed in the Refrigerator for two hours to ensure a good spread of the medicaments from the impregnated discs and then transferred to the incubator at 37° temperature for 24 hours.

The second experiment: determining the (MIC) of Hanse Dent Endomix medicament using (*filter paper disk agar diffusion*) method.[22]

Prepare the concentration tubes:

Six sterile glass tubes were obtained and numbered 1, 2, 3,4,5,6 and filled with sterile Peptone Water in a specific amount using the micropipette (Figure

NO.4).

Table NO.1						
Tube No.	1	2	3	4	5	6
Amount of peptone water	1ml	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml

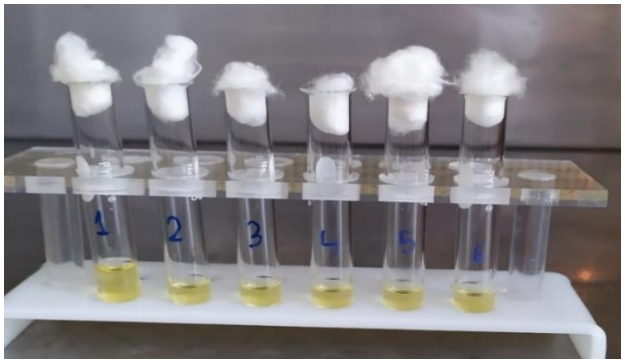

Figure NO.4

Extending Hanse Dent Endomix medicament within the tubes and adjusting the concentrations:

36 µg of Hans Dent Endomix medicament were dissolved in tube No.1 to adjust concentration **36µg\ml.**
Using a micropipette, an amount of 0.5 ml was taken from tube No.1 and added to Tube No.2; the concentration became **18µg/ml.**
The process was repeated by taking 0.5 ml from each tube and adding it to the next tube to divide the concentration in half as shown in the Table NO.2: **(Figure NO.5)**

Table NO.2						
Tube No.	1	2	3	4	5	6
Concentration in µg\ml	36	18	9	4.5	2.25	1.125

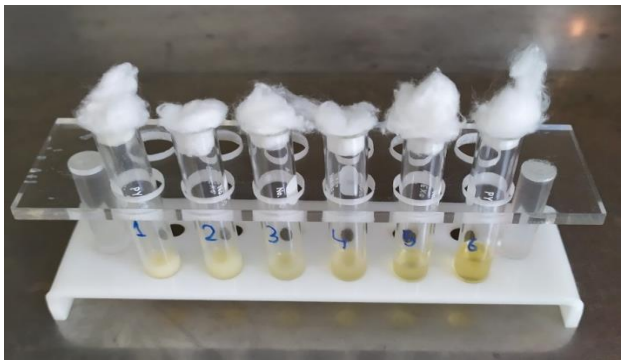

Figure NO.5

impregnate diluted concentrations into whatman filter paper discs in sterile Petri dishes:

Four sterilized Petri dishes were taken and each dish was divided into two parts to obtain eight parts 1_2_3_4_5_6_7_8. (**Figure NO.6**)
In parts 1 to 6, Whatman discs were impregnated from the tubes of diluted concentrations according to tube number (part 1 contains discs impregnated from tube No.1,…..etc.), in part 7 Whatman discs were impregnated with sterile Peptone water only (sterility control), in part 8 Whatman discs were impregnated with Hanse dent Endomix medicament with a dilution of 1:1(effectiveness control).

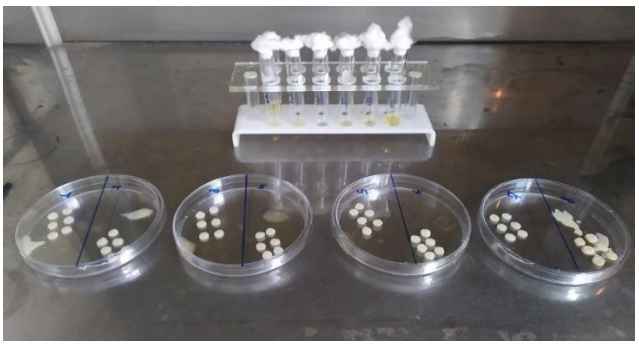

Figure NO.6

Prepare Petri dishes containing PDA medium:

Six Petri dishes containing PDA medium were prepared and divided into three groups, group A, group B, and group C. Each group contains two Petri dishes, each dish divided into four sections. Each group is numbered from 1 to 8 as follows: (**Figure NO.7**)
Group A: the first dish numbered 1,2,3,4; second dish numbered 5,6,7,8.
Group B: the first dish numbered 1,2,3,4; second dish numbered 5,6,7,8.
Group C: the first dish numbered 1,2,3,4; second dish numbered 5,6,7,8.

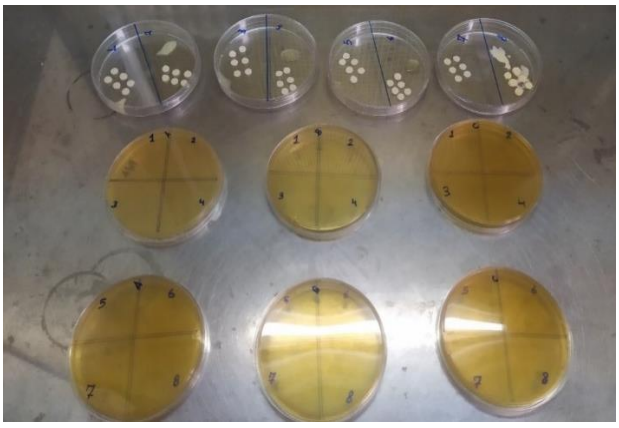

Figure NO.7

Using a sterile cotton swab, the *Candida albicans* suspension was transferred to dishes and spread over the entire area of the dish.

Transfer the discs to Petri dishes :

Whatman discs impregnated with Hanse Dent Endomix medicament concentrates were transferred to the prepared Petri dishes according to the number sequence in each group separately (the discs impregnated in part 1 were transferred to section No.1 of the Petri dish, and the discs impregnated in part 2 were transferred to section No.2 of the dish Petrie, etc.)
The dishes group A, B and C were placed in the refrigerator for two hours to ensure a good spread of the medicament from the impregnated discs and

then transferred to the incubator at 37° temperature for 24 hours.

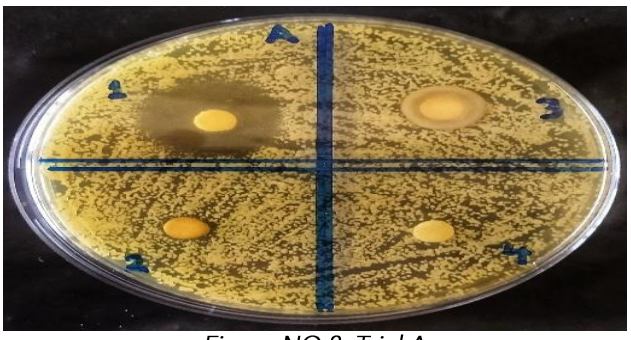

Figure NO.8: Trial A

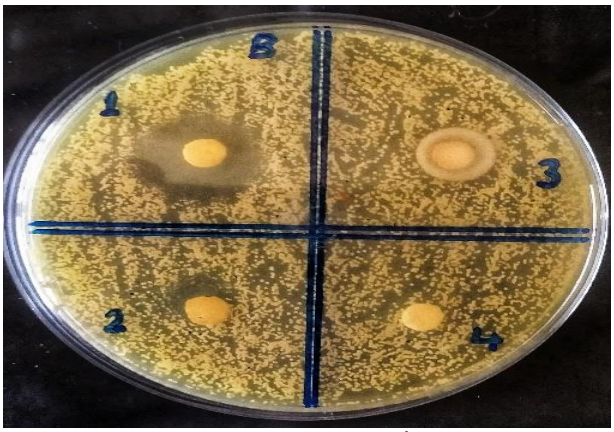

Figure NO.9: Trial B

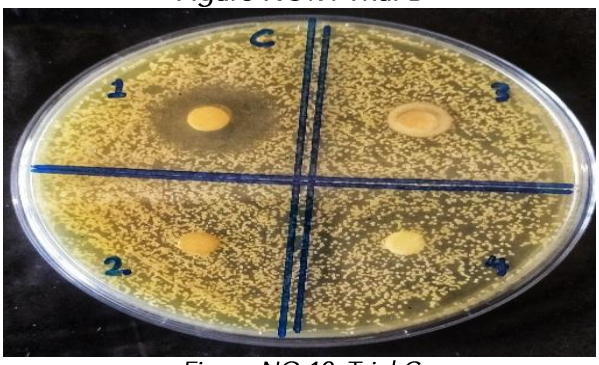

Figure NO.10: Trial C

3. Result

Result of the first experiment (Antibiotic resistance test)

The results were taken after 24 hours: they were as follows:

Disk No. 1 has a zone of inhibition around it in each of the three trials A, B and C.

Disc No. 2 has no zone of inhibition in each of the three trials A, B and C.

Disc No. 3 has no zone of inhibition in each of the three trials A, B and C.

Disc No. 4 (the control) has no zone of inhibition in each of the three trials A, B and C.

Table NO.3

Materials	Effectiveness Against C.albicans		
	A	B	C
Disk No. 1 impregnated with Hanse Dent Endomix	Active	Active	Active
Disk No. 2 impregnated with Ledermix	Inactive	Inactive	Inactive
Disk No. 3 impregnated with Metapaste	Inactive	Inactive	Inactive
Disk No. 4 impregnated with Distaled water (the control)	Inactive	Inactive	Inactive

Result of the second experiment: determining the (MIC) of Hanse Dent Endomix medicament using (the filter paper disk agar diffusion) method's:

The results were taken after 24 hours:

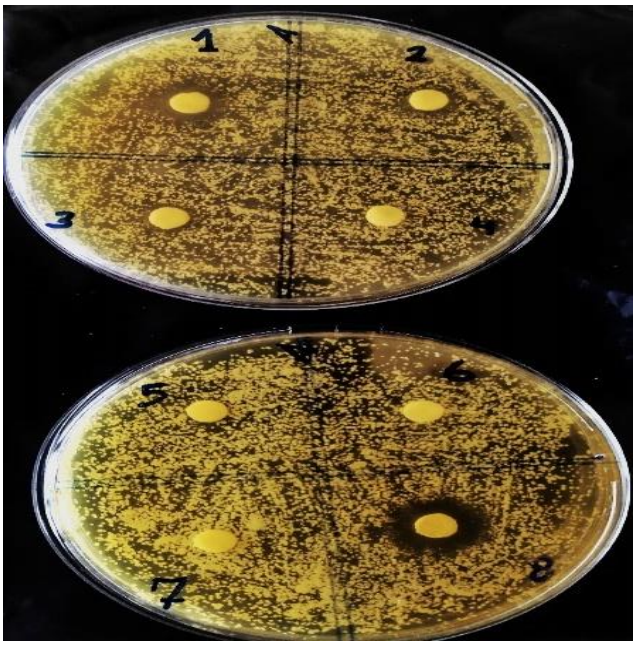

Figure NO.11: Trial A

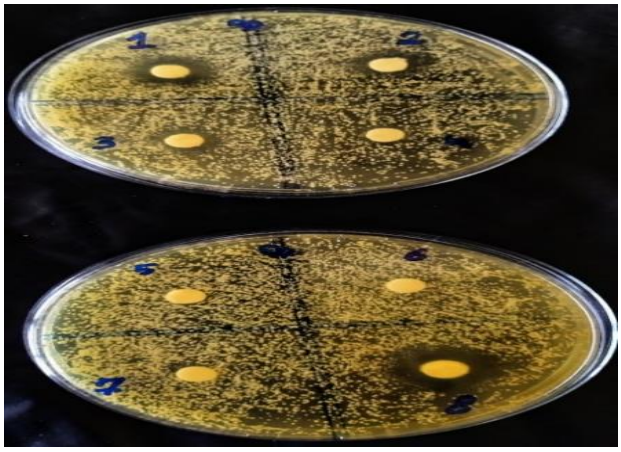

Figure NO.12: Trial B

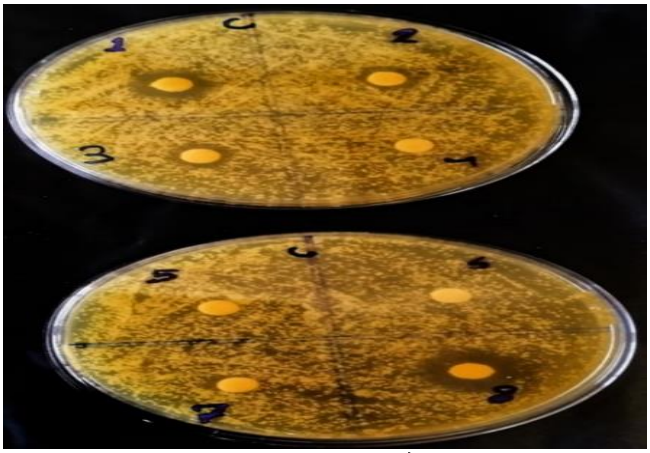

Figure NO.13: Trial C

Table NO.4								
Concentrations in µg\ml	36	18	9	4.5	2.25	1.125	0 (sterility control)	1:1 (effectiveness control)
Inhibition Zone (Trial A)	YES	YES	YES	NO	NO	NO	NO	YES
(MIC) (Trial A)			⬆					
Inhibition Zone (Trial B)	YES	YES	YES	NO	NO	NO	NO	YES
(MIC) (Trial B)			⬆					
Inhibition Zone (Trial C)	YES	YES	YES	NO	NO	NO	NO	YES
(MIC) (Trial C)			⬆					

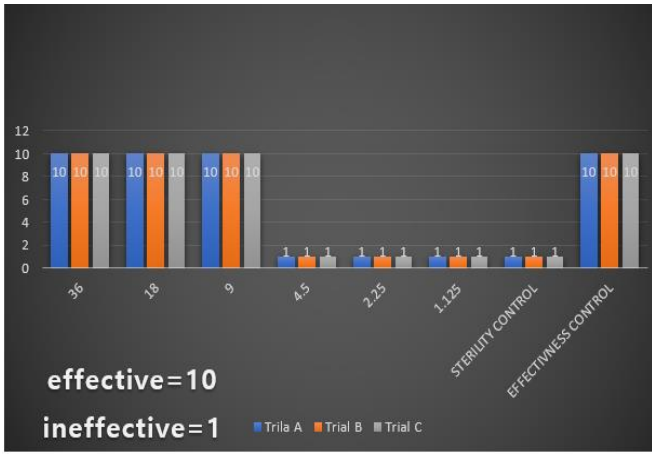

Figure NO.14

4. Discussion

- Within the limits of this study, the effect of Hanse Dent Endomix medicament was discussed in comparison with Metapaste and Ledermix medicaments on Candida albicans fungi growing on PDA medium and within a Petri dish, That is, the fungi are present within a medium that promotes their growth, and this medium differs from the medium within the root canal system of the tooth, where we can notice the clear difference in the effect of each of the three medicaments.
- The most likely reason for Hanse Dent Endomix's effect on Candida albicans is that it contains several effective antifungals such as tea tree oil and chamomile extract, in addition to nystatin. The efficacy of these compounds covers most fungal groups [13, 14, 15, 16], including Candida albicans, as seen in this study.
- Metapaste contains Calcium Hydroxide, which has an effect on most microorganisms and good efficacy against a wide range of microbes. The mechanism of action of Calcium Hydroxide is by raising the alkalinity of the medium around it and thus changing the environmental conditions important for the growth of many microorganisms and thus eliminating them.[17]
- Our study agreed with Ferguson and colleagues study [18], they reported ineffectiveness of saturated Calcium Hydroxide solution against C. albicans. Because C. albicans is able to survive in a wide range of pH, alkalinity of Calcium Hydroxide solutions may not have any effect on this organism. In addition, saturated Calcium Hydroxide solution readily presents calcium ions necessary for growth and morphogenesis of Candida [19, 20].
- About Ledermix, there was no clear effect on candida albicans, in contrast to many studies like Paul and colleagues[21], they found a clear efficacy of Ledermix on candida albicans and they attributed this to the effect of demeclocycline present in its composition, the mechanism of action may have differed according to the study method, where they studied Ledermix in extracted human teeth, While we conducted our study in Petri dishes in a suitable growth medium for fungi.
- Regarding the determination of the minimum inhibitory concentration, we weighed an amount equivalent to one scale of an insulin syringe, which is an estimate of the average amount suitable for filling one root canal, which is equivalent to 36 µg, we dissolved this amount in 1 ml of peptone water to obtain a concentration of 36 µg\ml, then we diluted Several concentrations to study the effect of these concentrations on the inhibition of Candida albicans after 24 hours to obtain the minimum inhibitory concentration for the growth of these fungi.
- the last concentration effective in inhibiting Candida albicans compared to the effectiveness control was (9 µg\ml) in trials A, B, and C and it is the minimum inhibitory concentration(MIC).

5. Conclusion

This study showed the effectiveness of Hanse Dent Endomix® in inhibiting the growth of *Candida albicans*, while there was no clear effect of Metapaste® and Ledermix® *on Candida albicans* growth, and the minimum inhibitory concentration of Hanse Dent Endomix® against Candida albicans was determined to be 9 µg / ml.

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