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Antibacterial effect of bioceramic root canal sealer (BioRoot^{TM}) modified with *Thymus Kotschyanus* Boiss

Khalid Mohammed Sharif Zebari[1], Abdullkareem Ramazan AL-Mezory[2]*, Hadar Saaeed Faizy[2]

[1]*Department of Conservative Dentistry, College of Dentistry, University of Duhok, Iraq.*
[2]*College of Agricultural Engineering Science, Duhok University, Iraq.*

***Corresponding author**: abdullkareemramazan@gmail.com

Original Research

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Abstract:

The present study aimed to evaluate the antibacterial efficacy of bioceramic root canal sealer modified with herbal extracts, because there are insufficient scientific reports that indicate the antibacterial effects of herbal extracts added to endodontic root canal sealers. The essential oils of the aerial parts of *Thymus Kotschyanus* Boiss were obtained by hydrodistillation (HD) method and analyzed by Gas Chromatography-Mass Spectrometry (GC/MS) Analysis, and the major compounds were carvacrol (53.61) and thymol (12.78). The herbal extracts were added in different percentages to BioRoot^{TM} root canal sealer to know the effective concentration against the *E. faecalis* bacteria by placing into the agar base, and the zones of inhibitions were measured. The groups were assessed as AH Plus (control), BioRoot^{TM}, and (BioRoot^{TM}+*Thymus Kotschyanus* Boiss) Exp. Material (1-4)%, Exp. Material (1-2)%, Exp. Material (1-1)% and for evaluating the antibacterial activity the agar diffusion test (ADT) was used. The AH Plus (control) exhibited the largest inhibition zone 10.05 (0.99) mm, followed by Exp. Material (1-1)% 7.82(0.77) mm, Exp. Material (1-2)% 4.96 (0.60) mm, while the least inhibition zone was found in BioRoot^{TM} 4.39 (0.55) mm and Exp. Material (1-4)% was 4.36 (0.59) mm. Using ANOVA one-way test, there was a statistically significant difference of inhibition zone among root canal sealers. Based on results, statistical analysis and observation of the AH Plus sealer and Exp. Material (1-1)% had higher antibacterial effects than BioRoot^{TM} sealer, so the oil of *Thymus Kotschyanus* Boiss can be added as one of the components of root canal sealer, but farther research is needed before recommending it as sealers.

Keywords: *Thymus Kotschyanus* Boiss; BioRoot^{TM}; AH Plus, Agar diffusion test; *E. faecalis*; Antibacterial activity

1. Introduction

A successful root canal therapy relies on proper preparation of the access cavity, appropriate shaping and cleaning and obturation of the root canal space. The elimination of 100% of bacteria in the root canal system is not achieved even with the different protocols. For that reason, the proper obturation procedures should be carried out to prevent the ingress of bacteria and entomb the remaining bacteria to provide peri apical healing. The root canal obturation involves the use of gutta percha in combination with root canal sealer, as there is no adhesion between gutta percha and dentin, the sealer must fill the space between the dentinal walls of root canal and the gutta percha cones [1]. The type of endodontic sealer is an important factor in successful of root canal treatment [2, 3]. Bioceramic based sealers are considered one of the most effective sealer in conjunction with gutta percha for obturation of the root canal system [4] and the developments in root canal filling materials have focused on the chemical and physical properties of the sealer [2].

Herbal medicines and natural phytochemicals are considered useful replacements to synthetic medicines and have become more popular due to their high antimicrobial, anti-inflammatory and biocompatible properties. Several

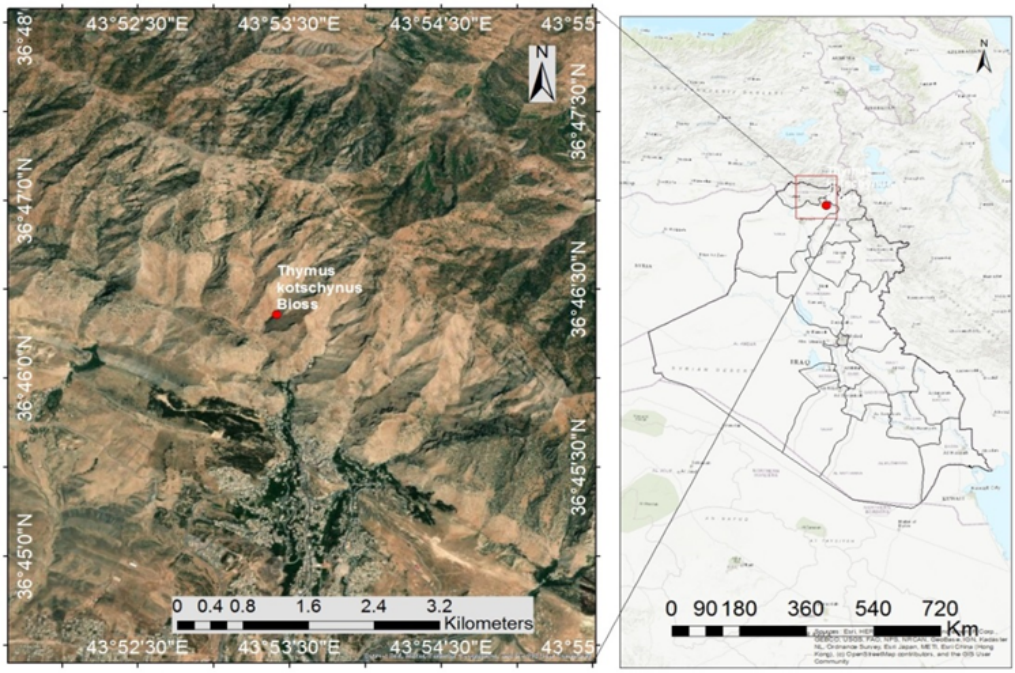

Figure 1. Dinarta- Akre district map at Duhok province, Iraqi Kurdistan region.

medicinal plants have been investigated to prevent and treat numerous oral diseases [5, 6].

As the result of several advantages associated with herbal products, such as antibacterial, anti-inflammatory, astringents, anesthetic, and anti-cariogenic effects, the field of dentistry also has begun to exploit herbal products in the form of toothpastes, mouthwashes, root canal irrigants, storage media for avulsed tooth, tooth whiteners, *Thymus Kotschyanus* Boiss plant is one of the most well-liked plants in the world due to its fragrant and therapeutic qualities, Thymus essential oils and extracts are used in the pharmaceutical field, because of its microbial and antioxidant properties [7].

Due to the lack of existing studies in the dentistry field, this study was conducted to evaluate and compare the antimicrobial efficacy of root canal sealers modified with herbal extracts (*Thymus Kotschyanus* Boiss) against microbes found in the root canal system to increase the antibacterial effect of BioRoot^{TM} root canal sealer because sometimes there are periapical infections with bioceramic root canal sealer and till now there are insufficient scientific reports that indicate the antibacterial effects of herbal extracts mixed with endodontic root canal sealers.

2. Methods

2.1 Collection of plant materials

The aerial portions of *Thymus Kotschyanus* Boiss at vegetative stage plants were collected from fields in the Dinarta-Akre district (Fig. 1) at latitude 902 m S/L, longitude 43.890254° and latitude 36.772601° in Duhok city, Iraqi Kurdistan Region from April 20th to 27th May 2022. The collected aerial parts (leaves and stems) were transferred to the Agricultural Engineering Science College/Duhok University laboratory.

The aerial parts (leaves and stems) were freshly weighted (3100 kg) had been taken and shade drying was done in the laboratory to get (2000 kg) of dry material (Fig. 2), after that the dry weight of plant materials was taken the dried plant materials was ground to a powder by milling, then kept in the dark glass containers for preparing the

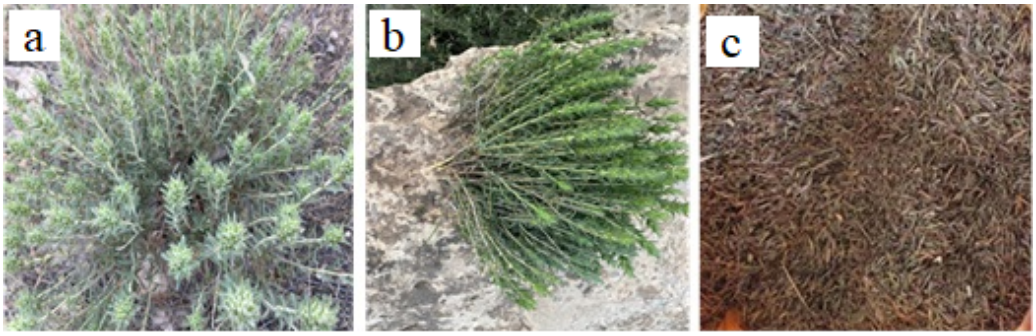

Figure 2. *Thymus Kotschyanus* plant a) in the field; b) after collection; c) after drying.

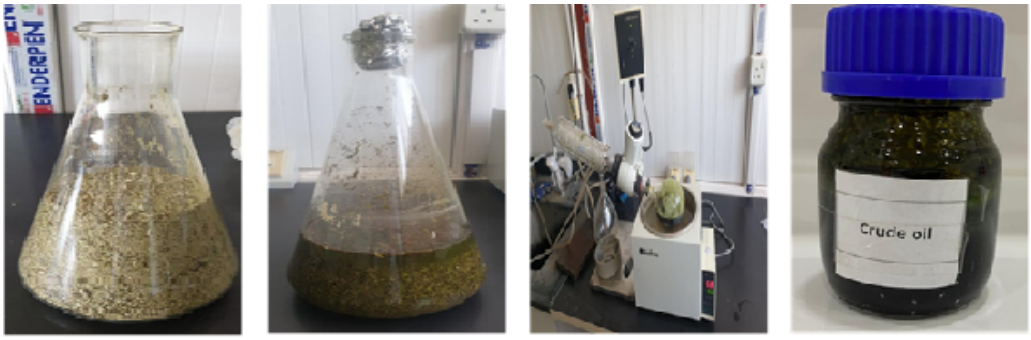

Figure 3. *Thymus Kotschyanus* Boiss, a) dry plant material; b) dry plant material covered with ethanol solvent; c) extraction processing in a rotary evaporator; d) ethanol crud extracted.

extract by methanol extraction as well as performing the phytochemical tests to establish the following experiments. The percentage of dry matter (%) was measured according to the following formula below [8–10]:

$$\text{Dry matter}(\%) = \frac{\text{Dry weight}}{\text{Fresh weight}} \times 100$$

The dry powder plant material collected from the field was extracted by ethanol solvent; 1 kg of plant material was extracted three times using ethanol (1000 mL) at room temperature overnight on an automatic shaker. The extract was concentrated under reduced pressure in a rotary evaporator, then located in a water bath for 4 hours and incubated in the laminar for 24 hours to remove all the solvent residue and yield crude (Fig. 3) ethanol extract [5, 11].

2.2 Extraction of essential oil

For essential oil extraction, the 500 g of dried aerial parts of *Thymus Kotschyanus* Boiss plant was put in the Clevenger (Fig. 4). The sample was covered with distilled water in the round flask placed on the heater then the water reached the boiling point; after 6 hours, the quantity of essential oil 3 mL was collected [9–11].

2.3 Gas chromatography-mass spectrometry (GC/MS) analysis

The essential oil was analyzed by gas chromatography-mass spectrometry (GC/MS). Thermo Finnigan Trace 2000 GC/MS, made in the USA, was employed with an HP-5MS capillary column (30 m long and 0.25 mm wide, and a 0.25 μm of film thickness) at a 250 °C of injector chamber. The initial column temperature was at 120 °C for 5 min, then raised to 280 °C at 10 °C/min. Helium was used as a carrier gas at 35 mL/min. MS parameters as follows: ionization energy, 70 eV; ion source temperature, 200 °C; voltage, 3000 V; and mass range, 30 to 600. The compositions of the essential oil were identified by comparison of their retention indexes (RI), retention times (RT), and mass spectra with those of authentic samples in the Wiley library [9–15].

2.4 Anti-bacterial assay

The standard strains of *Enterococcus Fecalis* ATCC (29212) were purchased from private Media Laboratory/ Erbil in sterile brain heart infusion (BHI) and incubated at 37 °C in the Golan Hospital/ Akre laboratory.

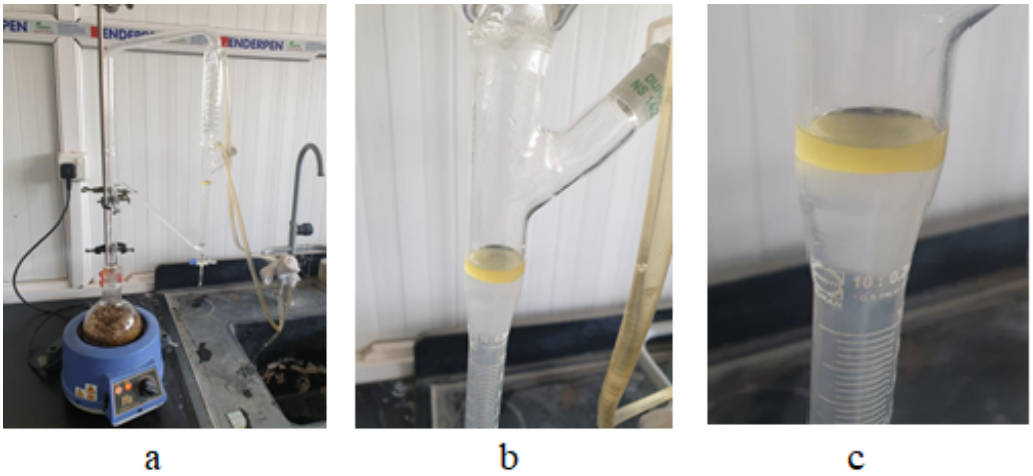

Figure 4. a) Plant Material inside the Clevenger; b) essential oil extraction, and c) collecting the essential oil from the plant.

Figure 5. a) Preparation of HiCrome^{TM} Entrococci broth; b) measuring the turbidity by DensiChek.

2.5 Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) test

For the preparation of HiCrome^{TM} Entrococci Broth suspend 37.18 grams in 1000 mL distilled water, heat to dissolve the medium completely (Fig. 5), dispense into tubes as desired, sterilized by autoclaving at 15 lbs pressure (121 °C) for 15 minutes. An amount of 10 mL of *Thymus Kotschyanus* Boiss oil was diluted by distal water by serial dilution method to (100, 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78) %.

After that 10 mL of HiCrome^{TM} Entrococci Broth was added to each dilution, the tube that contained 100% concentration of *Thymus Kotschyanus* Boiss oil was used as a negative control. In contrast, the tube that contains only bacteria and broth without *Thymus Kotschyanus* Boiss oil served as a positive control, a sterile swab was used to transfer a sufficient number of *Enterococcus Faecalis* colony of a pure culture and to suspend in 5 mL of sterile saline in a 12 × 75 mm clear plastic test tube. The turbidity is adjusted accordingly (0.5 McFarland) and measured using a turbidity meter called the DensiChek (Fig. 5) [16].

5 mL with bacteria suspension adjusted to 0.5 McFarland was added to the tubes containing (*Thymus Kotschyanus* Boiss and broth) and this mixture was incubated at 37 °C for 24 hrs. After 24 hrs the tubes were visually checked for turbidity (bacterial growth), the lowest dilution that inhibiting the growth of bacteria was taken as MIC, a loopful of the broth dilutions were taken and streaked on HiCrome^{TM} *Entrococcus Faecium* Agar Base. The concentrations (100, 50, 25, 12.5, 6.25, and 3.125)% inhibit bacterial growth (no turbidity or blue color), while the concentrations (1.26 and 0.78)% a turbidity and blue color is indicating the growth of bacteria (Fig. 6)[17].

The concentration (25)% inhibits the bacterial growth (no turbidity or blue color of broth) and its not change the color of sealer (BioRoot^{TM}) so this concentration was selected in the present study, while the higher percentages (50 and 100)% changed the color of sealer to darker color.

2.6 Agar diffusion test

Preparation of Enterococcus selective media, the present in vitro microbiological study was conducted in (Golan Hospital Laboratory/ Akre). In the present study, the antimicrobial efficacy of Thymus's herbal extracts in combination with BioRoot^{TM} sealer was evaluated against *Enterococcus Faecalis* using an agar diffusion test.

Suspend 27.1 g powder of Enterococcus agar base from (HI Media® laboratories, India) was added to 500 mL purified distilled water in the flask, heat to boiling to dissolve the medium completely (Fig. 7) with frequent agitation. The medium was cooled 45-50 °C and dispensed into disposable Petri- dishes under complete aseptic conditions depending on Alkhalidi et al., [18] and Maghded [19]. When the medium was solidified, the Petri- dishes were inverted to avoid excess moisture and the prepared medium was stored at 8-15 °C.

The antibacterial effect of AH Plus sealer, BioRoot^{TM} sealer and Experimental material, were evaluated against *Enterococcus Faecalis*, the antibacterial effect of these materials

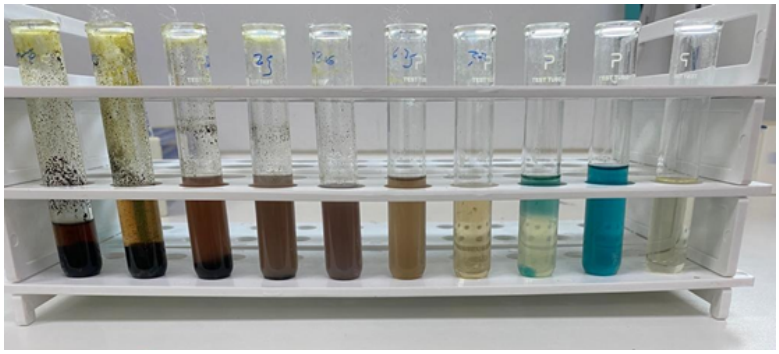

Figure 6. Tubes of *E. faecalis* broth showing bacterial growth.

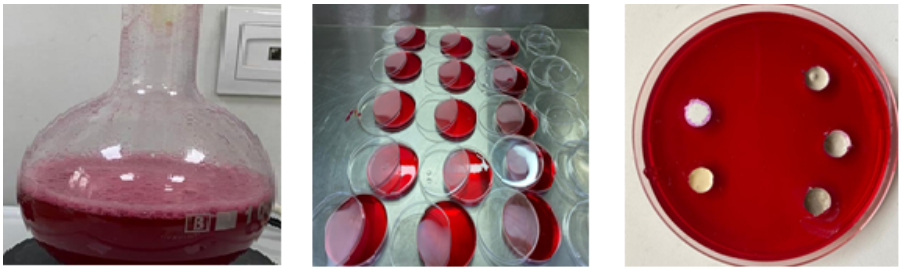

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Figure 7. a) Preparation of HiCrome ™ *Entrococcus Faecium* Agar. b) Petri dishes contain HiCrome ™ *Entrococcus Faecium* Agar Base. c) Petri dishes with two wells filled (AH Plus and BioRoot) sealers, three wells filled with experimental material in different concentrations (1-1%, 1-2% and 1-4%).

was measured by agar diffusion tests as described by Bauer et al., [20].

150 Petri dishes (30 for each group) with 25 mL of HiCrome™ *Entrococcus Faecium* Agar Base (Fig. 7).

Enterococcus agar base were inoculated with the microbial suspensions using sterile swabs that were spread to *Enterococcus Faecalis* homogeneously onto the Petri dishes, obtaining growth injunction. Five wells in each Petri dish (Fig. 7), 6 mm in diameter and 3 mm in depth were made with an adapted punch by removing the agar [1]. Each material was mixed on a dry pre-sterilized glass slab using a cement spatula at room temperature, each material was backloaded into a sterile 5 mL syringe and placed into the wells.

Two wells were each filled with the different sealers (AH Plus and BioRoot™), three further wells filled with experimental material in different concentrations 1-1 (one drop of sealer liquid to one drop of *Thymus Kotschyanus* liquid), 1-2 (one drop of *Thymus Kotschyanus* liquid to tow drops of sealer) and 1-4 (one drop of *Thymus Kotschyanus* liquid to four drops of sealer) and incubated aerobically at 37 °C for 24 hours [1].

After incubation, the diameter of the inhibition zone was measured by a digital caliper (Fig. 8) at 3 different points and the mean value was used as the result of the specimen, growth inhibitory zones around each tested material were evidenced by lack of bacterial colonization (clearing of agar) adjacent to each group. The most uniform diameter segment of the zone of inhibition was measured, and 6 mm (diameter of the well) was extracted from the measurement as the cutoff value. All measurements above this value were considered indicative of significant bacterial growth inhibition. Wider zones of inhibition were interpreted to indicate greater antimicrobial activity of the involved sealers and the sealers that mixed with herbal extracts [21], the size of the inhibition zones for each materials were calculated from the diameters of the halo of inhibition produced, and the disc's diameter as follows [18–25];

Size of inhibition zone=
Diameter of halo-Diameter of the disc.

3. Results

3.1 Chemical compositions of essential oils of *Thymus Kotschyanus* Boiss.

The analysis of essential oils of *Thymus Kotschyanus* Boiss by GC-MS lead to the identification of different compounds,the identified compounds and their percentages

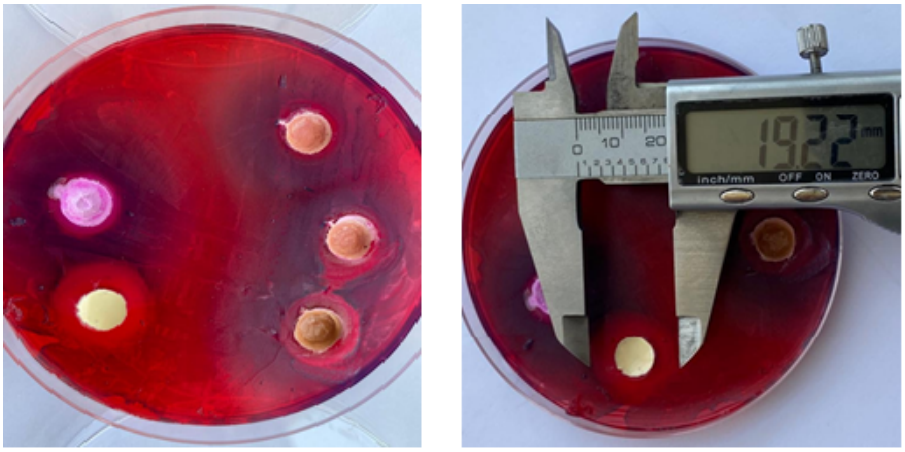

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Figure 8. a) Inhibition zones around tested materials were evidenced by lack of bacterial colonization (clearing of agar), and b) measurement the diameter of inhibition zone by digital caliper.

Table 1. Chemical compounds of essential oils of *Thymus Kotschyanus* Boiss.

No	Compound	Percentage	Ri
1	Hexane	0.14	600
2	α.-Pinene	0.85	932
3	β.-Pinene	0.9	974
4	β.-Myrcene	0.13	988
5	α.-Phellandrene	0.58	1005
6	δ-3-carene	2.01	1008
7	$(+)$-3-Carene	1.57	1013
8	α.-Terpinene	0.45	1014
9	*P*-Cymene	9.14	1020
10	γ.-Terpinene	8.57	1054
11	*Trans*-Sabinene Hydrate	0.03	1098
12	Endo-Borneol	0.23	1165
13	(-)-4-Terpineol	0.69	1174
14	(-)-α-Terpineol	1.41	1186
15	Thymol Methyl Ether	0.4	1232
16	Carvacrol Methyl Ether	6.52	1241
17	Thymol	12.78	1289
18	Carvacrol	53.61	1298

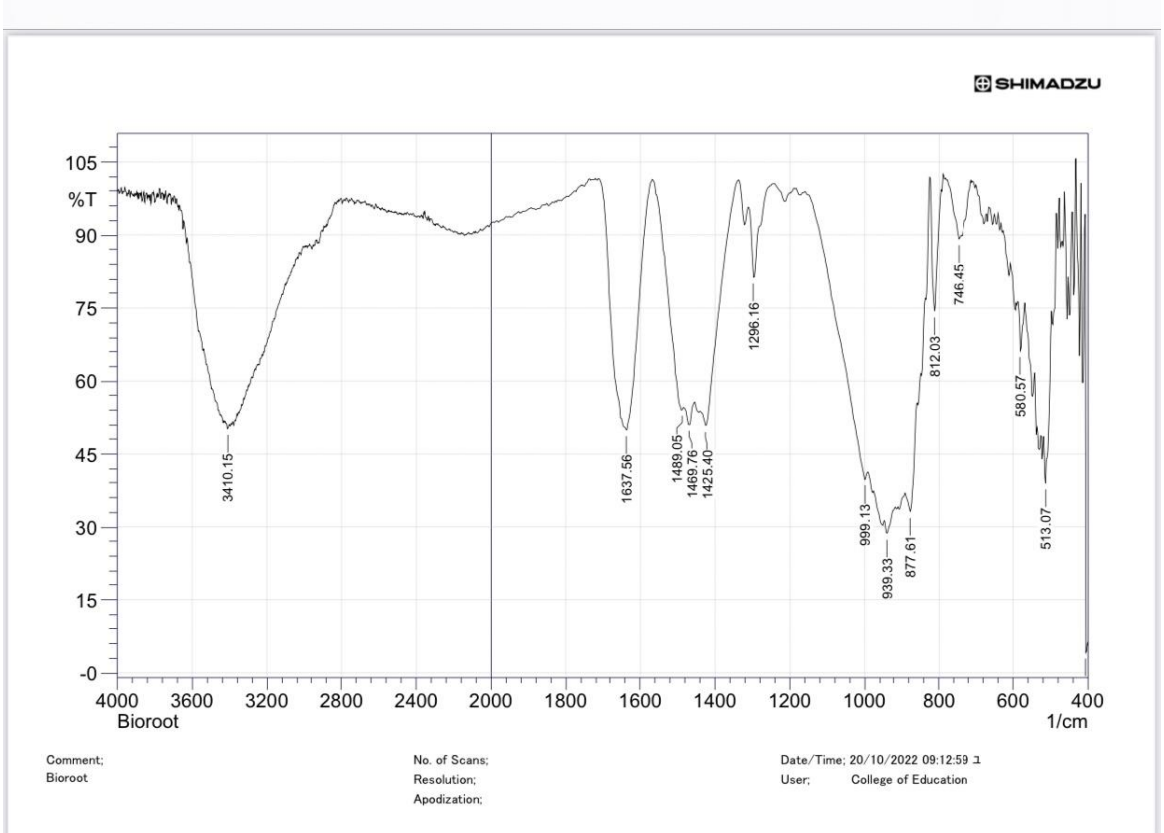

Figure 9. FTIR analysis of BioRoot^{TM} root canal sealer.

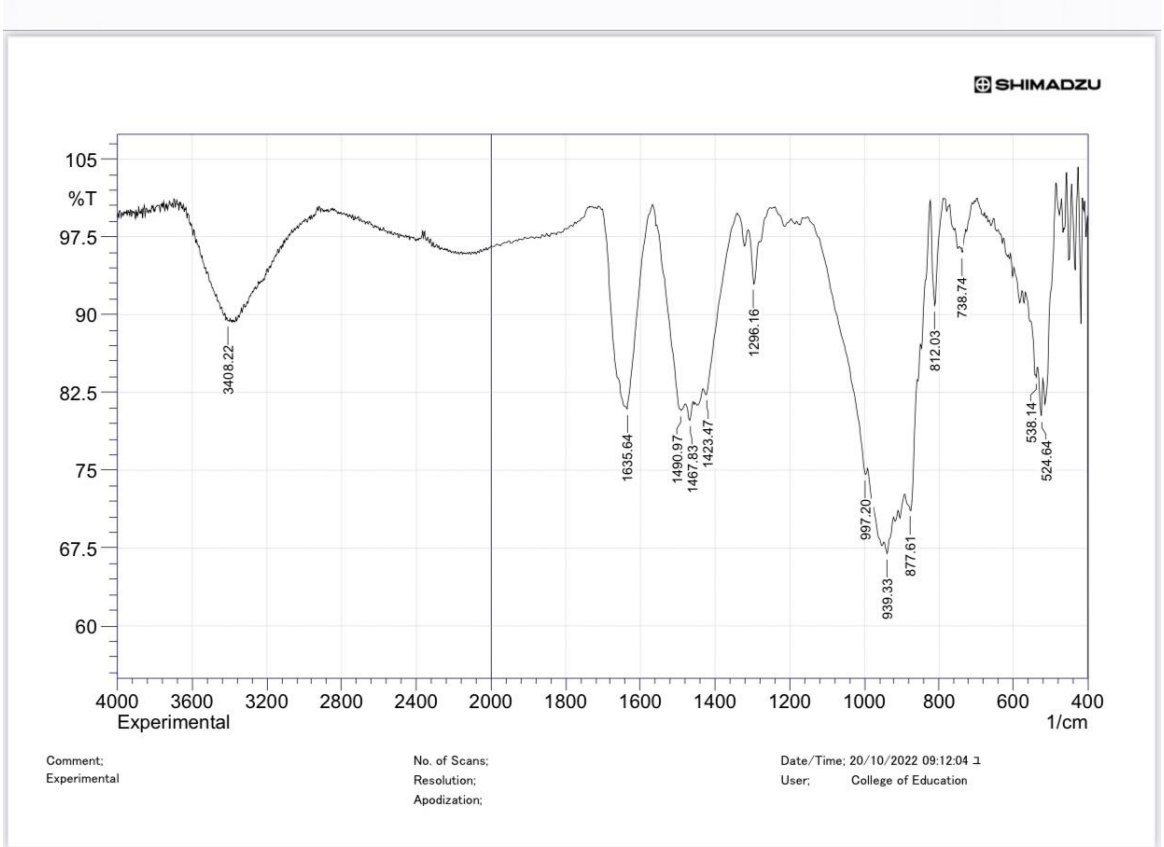

Figure 10. FTIR analysis of Exp. Material (BioRoot^{TM} + *T. Kotschyanus* Boiss).

have been given in (Table. 1). 18 compounds were identified in the oils of *Thymus Kotschyanus* Boiss.

3.2 Results of Fourier Transform Infrared spectroscopy (FTIR)

The FTIR analysis was done to compare BioRoot^{TM} root canal sealer and the Exp. After setting it grind to powder, the FTIR was done, and the result was not changed in the chemical structure of BioRoot^{TM} and Exp. material (Fig. 9) and (Fig. 10) after adding of herbal extract *Thymus Kotschyanus* Boiss to BioRoot^{TM} root canal sealer.

3.3 Results of antibacterial efficacy of AH Plus, BioRoot^{TM} root canal sealer and Experimental material

The data in the (Table. 2) and (Fig. 11) presented the mean inhibition zone diameter among root anal sealers. It was found that the AH Plus (control) exhibited largest inhibition zone 10.05(0.99) mm, followed by Exp. material (1-1)% 7.82(0.77) mm, Exp. material (1-2)% 4.96 (0.60) mm, while the least inhibition zone was found in BioRoot^{TM} 4.39 (0.55) mm and Exp. Material (1-4)% was 4.36 (0.59) mm.

Using ANOVA one-way test, there was a statistically significant difference of inhibition zone among root canal sealers. (Table. 3) presents the comparisons of inhibition zone between root canal sealers. There was a significant difference between all groups except the BioRoot^{TM} and Exp. There was no significant difference in material (1-4)%.

4. Discussion

The important factor for the success of root canal treatment is eliminating bacteria from the root canal system. Many protocols has been done for disinfection of the root canal chemo-mechanically, but all of them cannot provide the sterility of the root canal disinfection completely, because none of the elements of root canal treatment (host defense systems, instrumentation, irrigation, intracanal medications, obturations and final restorations) can provide guarantee for complete disinfection.

The main purpose of endodontic sealer in root canal treatment is to fill the gap between the dentinal wall of the canals and the core root canal filling materials to minimize leakage or prevent penetration of fluids into the root canal which can be a nutrient for the microorganisms that remain in the canal and the antimicrobial properties of root canal sealers may control the infections by preventing recolonization of microorganisms in the root canal after chemo-mechanical preparation. The antimicrobial activity of a root canal sealer increases the success rate of endodontic treatments by eliminating residual intraradicular infections that might have survived root canal treatment or have invaded the canal later through microleakage [26, 27].

In the failed root canal treatment, there are numerous aerobic bacteria species such as *Staphylococcus aureus*, *Streptococcus mutans* and *Fusobacterium nucleatum*. However,

Table 2. Comparisons of inhibition zone among Root Canal Sealers.

Study group	Statistics		
	Mean/mm	Std Dev	P (two-sided)
AH Plus (Control)	10.05	0.99	
BioRoot	4.39	0.55	
Exp. Material (1-1)%	7.82	0.77	<0.0001
Exp. Material (1-2)%	4.96	0.60	
Exp. Material (1-4)%	4.36	0.59	

ANOVA one-way was performed for statistical analyses.

the bacteria that usually related to the aetiologic factor of persistent periradicular infections was *Enterococcus faecalis*. Because it has several virulence factors such as it Gram-positive, facultative anaerobic bacteria, it can survive in the root canal either alone or with other microbes and it can invade the dentinal tubules and bind to dentinal collagen so it is difficult to be eradicated from the root canal completely [26, 28].

Usually, the failure of root canal treatment is related to the persistence of bacteria in the root canal system especially *E. faecalis*. [[29]].

For these reasons *E. faecalis* was the microorganism selected in the present research to evaluate the antimicrobial properties of the root canal sealers,

The bioactive compounds present in the extracts of herbal plants are potent antimicrobial agents so the antimicrobial properties of this agents could be beneficial in dentistry. So extracts of thyme can mixed with root canal sealer (BioRoot^{TM}), enhancing the antibacterial properties of this sealer [21, 25, 30].

The antimicrobial efficacy of any natural or synthetic agents can be evaluated by many methods such as Broth dilution method, Agar dilution method, Disc diffusion method, Agar well diffusion method; in the present study the Broth dilution method used for selecting the percentage of thyme that added to BioRoot sealer, while the Agar well diffusion method (ADT) used for evaluating the efficacy of antibacterial effect of sealer against *E. faecalis* bacteria by measuring the inhibition zone because ADT is the most commonly used method for evaluating the antimicrobial activities of dental materials and the results of this method influenced by the contact between material and agar, the possibility of material to diffusion into agar and setting time, the solubility of material, and temperature ect, so highly diffusible material can produce a large growth inhibition zone. However ADT technique have many advantages and disadvantages, this technique is widely used, the benefits of this technique are the antimicrobial activities of these materials can be easily compared directly and variables can also be easily controlled, while the disadvantages are it cannot differentiate the bacteriostatic and bactericidal effects of materials and it depend on the standardization of inoculum, medium content, size, density, agar viscosity and number of specimens per plate [21, 25, 30, 31].

It is not easy to compare the different results of researches, because many studies investigated the antimicrobial efficacy of root canal sealer with different techniques and different microbes, the most significant difference of this study from other studies was evaluating the antibacterial effect of bioceramic sealer (BioRoot^{TM}) to specific bacterial (*E. faecalis*) in specific agar medium (*E. faecalis* agar) and in the present study the antibacterial effects of root canal sealers where different from each other and by adding different percentages of herbal extracts *Thymus Kotschyanus* Boiss.

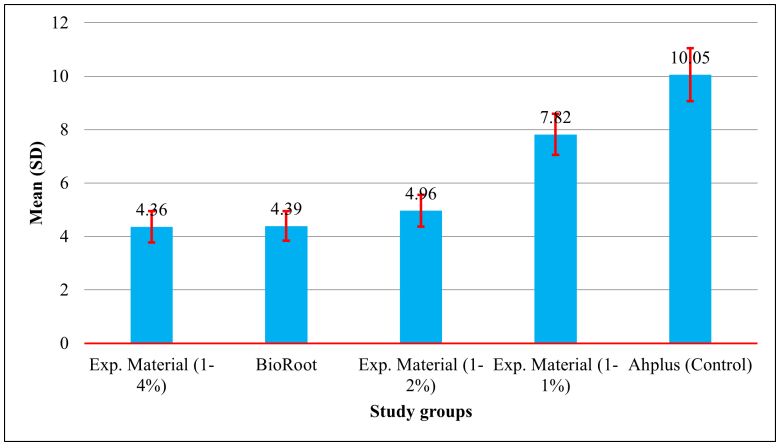

Figure 11. Inhibition zone of Root Canal Sealers.

Table 3. Pairwise comparisons of inhibition zone between Root Canal Sealers.

Group	- Group	Difference	p-Value
AH Plus (Control)	BioRoot	5.66	<0.0001
AH Plus (Control)	Exp. Material (1-1)%	2.23	<0.0001
AH Plus (Control)	Exp. Material (1-2)%	5.09	<0.0001
AH Plus (Control)	Exp. Material (1-4)%	5.69	<0.0001
BioRoot™	Exp. Material (1-1)%	-3.43	<0.0001
BioRoot™	Exp. Material (1-2)%	-0.57	0.0210
BioRoot™	Exp. Material (1-4)%	0.03	0.9998
Exp. Material (1-1)%	Exp. Material (1-2)%	2.86	<0.0001
Exp. Material (1-1)%	Exp. Material (1-4)%	3.46	<0.0001
Exp. Material (1-2)%	Exp. Material (1-4)%	0.60	0.0122

Tukey test was performed for statistical analyses.

In the present study, the AH Plus sealer has significantly higher antibacterial effect among the tested materials (10.05mm) this antibacterial activity may be explained by the presence of antibacterial components in epoxy resin [26], due to its release of bisphenol-A-diglycidyl ether during polymerization [32, 33].

The un polymerized components of epoxy resin sealers (epoxide and amine) may be released into surrounding area during the setting process, increasing the antimicrobial effects of epoxy resin sealers [27].

Studies by Leonardo et al. 1999 and Teixeira et al. 2019 explained the antimicrobial activities of epoxy resin sealer due to bisphenod A diglycidyl ether and formaldehyde during polymerization process [34, 35]. The pH values of this type of sealer have no effect as antibacterial activity during setting reaction [36] and the AH Plus is a resin so it has a high flow, which provide plasticity lead to increase its antibacterial effects [37].

The results of the present study is in agreement with the studies by Munitić et al. 2020 that done for evaluating the antibacterial efficacy of three bioceramic root canal sealers (BioRoot™, Fillapex and Totalfill BC) AH Plus they showed both Fillapex and AH Plus has better antibacterial effect than BioRoot™ and Totallfill.

The study by Roshdy et al. 2021 was done to investigate the antimicrobial effect of AH Plus and Ceraseal sealers with and without incorporation of silver nanoparticles against *E. faecalis* they found the AH Plus had higher antibacterial effect than Ceraseal sealer.

Poggio et al. reported lower antibacterial activity of the BioRoot™ RCS compared with epoxy resin sealers against *E. faecalis*, which was explained due to its shorter working and setting time.

The BioRoot™ root canal sealer (Septodent, Saint-Maur-des- Fossés, France) is two components calcium silicate-based endodontic sealer consisting of powder and liquid; the powder contains tricalcium silicate and zirconium oxide which serve as a radiopaque filler and povidone which enhances adhesion to hard dental tissues, the liquid contains mostly sterile water and calcium chloride which serves as setting modifier [38].

The microbial effects of this types of sealer is based on the chemical reactions, the reaction of tricalcium silicate with water forms a calcium silicate hydrate gel and calcium hydroxide according to the chemical equation [39]

$$3CaO{\cdot}SiO_2 + H_2O \rightarrow C\text{-}S\text{-}H + Ca(OH)_2$$

The environment that formed during this chemical reaction has a high pH value which is the source of antibacterial properties of this sealer. Still, the lower antibacterial effect of calcium silicate-based sealer may be due to the high resistance of *E. faecalis* bacteria to calcium hydroxide [40] and the ability of *E. faecalis* to buffer the highly alkaline pH by activating its proton pump, but it's able to buffer the alkaline pH only to value 11.5 [41]. The difference in pH values of these types of sealers may be due to the different mixing ratio of powder and liquid during the sealer preparation, which may affect its antibacterial properties.

These in agreement with the studies by Castillo-Villagomez 2022, the antimicrobial activity of root canal sealers was evaluated against *E. faecalis* which is strongly associated with persistent periapical infection and endodontic failures, the root canal sealers BioRoot™ and EndoSquence (calcium silicate-based sealers) were more antimicrobial than AH Plus.

A study by Viana et al. 2021 showed the antimicrobial effect of bioceramic sealer (BioRoot™) greater than the resin–based sealers (AH Plus).

A study by Alsubait et al. 2019 showed the antibacterial activity of BiooRoot™ was significantly higher than that of the TotalfillBC and AH Plus sealer.

The antibacterial effect of experimental material (BioRoot™ + thyme) increased gradually by increasing the percentages of adding the thyme to BioRoot™ sealer (4.36 to 4.96 to 7.82 mm) these increasing of inhibition zone may be due the presence of phenolic components in thyme.

In the present study, the essential oils analysis of *Thymus Kotschyanus* Boiss by GC/MS showed thymol 12.78% and carvacrol 53.61%. The antibacterial effect of thyme due to their higher phenolic compound such as thymol and carvacrol [16, 42–44].

The present study's results agree with that of a previous investigation that showed the natural oils modified sealers against cariogenic microorganism showed an acceptable antimicrobial effect [30, 45].

The concentration (25)% inhibit the bacterial growth (no

turbidity or blue color of broth) and its not change the color of sealer (BioRoot^{TM}) so this concentration was selected in the present study, while the higher percentages (50 and 100)% changed the color of sealer to darker color.

5. Conclusion

Based on results, statistical analysis and observation of the AH Plus sealer and exp, material (1-1)% had a higher antibacterial effect than BioRoot^{TM} sealer, so the oil of *Thymus Kotschyanus* Boiss can be added as one of the components of root canal sealer as an antibacterial agents. Still, further research is needed before recommending it as sealers.

Ethical approval:
This manuscript does not report on or involve the use of any animal or human data or tissue. So the ethical approval does not applicable.

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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