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Research Article

A Comparison of Two Herbal Medicinal Plants' Anti-Inflammatory and Anti-Microbial Effects When Used as Root Canal Irrigation

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Abstract

Background: Necrotic substances such as microbes, and organic and inorganic elements make up the smear layer, and this layer lining the root canal walls and holes of the dentinal tubules. It creates a barrier that restricts intracanal medications and sealant from entering the dentinal tubules effectively.

Aim of the Study: The aim of the present study was to compare the anti -inflammatory and the anti-microbial effect of the *Matricaria chamomilla* Fam. Asteraceae and the *Trigonella foenum-graecum* L. Fam. Leguminosae.

Methodology: From October 2020 through May 2022, this study was conducted *in vitro* in Saudi Arabia. 5% marginal error accuracy with a 95% confidence level can be attained with the smallest sample size. The scanning electron microscope (SEM) was used to inspect and take pictures of five samples from each group. After being gold sputter-coated, each specimen was analyzed under a 30 kV scanning electron microscope. Using a 2000X magnification, the root canal of each specimen was studied at the coronal, intermediate, and apical levels. Using image analysis software SPSS 27, which was utilized for data entry and analysis, the images were evaluated subjectively for the level of cleanliness and the existence or absence of the smear layer.

Results: Total extract, the fractions of petroleum ether, chloroform, ethyl acetate, and n-butanol were synthesized for an anti-inflammatory activity test and an anti-microbial test against *Staphylococcus aureus* and *Enterococcus faecalis* bacteria, and the results are shown as follows: Regarding the anti-inflammatory results: Comparison between the two herbal extracts Fenugreek showed higher mean edema thickness with chloroform F1 (10.16 ± 0.16), total extract F2 (9.32 ± 0.24), ethyl acetate F3 (8.28 ± 0.19), n-butanol F4 (7.31 ± 0.25) and petroleum ether F5 (10.31 ± 0.15), while chamomile showed lower mean edema thickness with chloroform F1 (8.28 ± 0.54), total extract F2 (6.28 ± 0.35), ethyl acetate F3 (5.62 ± 0.35), n-butanol F4 (6.46 ± 0.24) and petroleum ether F5 (8.15 ± 0.14). On comparing the results, Fenugreek showed statistically significantly higher mean edema thickness than chamomile.

The mean values of edema thickness of control and Indomethacin were (10.19 ± 0.39), (7.25 ± 0.13) respectively for the Fenugreek and (10.21 ± 0.40), (7.25 ± 0.13) respectively for the chamomile. There was no statistically significant difference between the two herbal extracts.

Conclusion: The study concluded under the limitations of this investigation the following conclusions could be drawn we can use the total extract of *Matricaria chamomilla* L. and ethyl acetate of *Trigonella foenum-graecum* L. fractions as irrigants during the root canal treatment for their anti-inflammatory and anti-microbial effects and benefits.

Keywords: *Anti-Inflammatory; Anti-Microbial Effect; Root Canal Irrigants*

Introduction

Endodontic therapy's effectiveness depends on removing germs from the root-canal system (if any are present) and avoiding reinfection. To eliminate inflammatory and necrotic tissue, microbes/biofilms, and other debris from the root canal space, the root canal is shaped with manual and rotational instruments while being constantly irrigated. Effective filling, irrigation, and disinfection are the key aims of instrumentation [1].

Currently, antibacterial, and decalcifying chemicals, or their mixtures, are employed as irrigants during cleaning and shaping. They consist of ethylenediaminetetraacetic acid (EDTA), chlorhexidine, hydrogen peroxide, sodium hypochlorite (NaOCl), and a combination of tetracycline, an acid, and a detergent (MTAD). These chemical irrigants have the potential to cause numerous unpleasant incidents during treatment, including injury to skin, eye injury, allergic reaction, hematoma, ecchymosis, swelling, and nerve damage. Herbal medicine, often known as herbal medicine or botanical medicine, has developed recently. As a result of their strong antibacterial activity, biocompatibility, anti-inflammatory, and antioxidant qualities, herbal extracts are frequently used in dentistry and medical procedures [2].

All living things produce a wide variety of chemical compounds known as natural products. Carbohydrates, proteins, and lipids are examples of primary metabolites, which are organic compounds that are present in all living things. As a result, many of the chemical components of primary metabolites are discovered [3].

Throughout all pharmaceutical plants (e.g. amino acids, common sugars such as glucose, and fatty acids). In addition to main metabolites, plants also produce secondary metabolites, which are compounds with a more restricted distribution. Several of them have pharmacological effects that have been utilized to make medicines, regardless of their roles within plants. The most frequent secondary metabolites include alkaloids, which have a secondary or tertiary amine function within a heterocyclic ring, glycosides, which are made up of an aglycone (a non-sugar part) and a sugar part, phenols, which are found in many aromatic plant components, terpenes, flavonoids, and tannins [4].

Fenugreek, which has a long history of medical applications in Chinese medicine and has been used for a variety of medical purposes, is an example of a medicinal plant that is employed. Saponins, alkaloids, flavonoids, proteins, and amino acids are the primary components of fenugreek. Fenugreek is used as an emollient and digestive aid. It is combined with insulin for treating diabetes. Also, it brings down blood pressure. As an anti-inflammatory and antibacterial agent, fenugreek is employed. Mucilage, which is known for calming and relaxing inflamed tissues, is present in fenugreek. As mucosal fluids are produced more readily because of fenugreek, allergens and poisons are more easily removed from the respiratory tract [5].

The German chamomile, often known as camomile, is another plant that is employed. It is an annual plant in the Asteraceae family. This plant is most known for its ability to produce tea, which is frequently served with either honey or lemon and is frequently used as a sedative. German chamomile's primary chemical components, coumarins, flavonoids, and volatile oils, are used medicinally to treat irritable bowel syndrome, sore stomach, and other conditions. It also has modest laxative, anti-inflammatory, and antibacterial properties. *In vitro* tests have shown that chamomile possesses antibacterial, antioxidant, and cancer-prevention potential [6].

Endodontic success depends on the meticulous removal of germs, microbial toxins, and traces of both viable and necrotic pulp tissue from the root canal system. This can be aided by chemical-mechanical instruments. Although difficult to clean, root canals are complicated, unusual structures. Irrigation is therefore an essential auxiliary. Irrigation facilitates cleaning of the root canal system by flushing away debris and serving as a tissue solvent, antibacterial agent, and lubricant [7].

Dentin chips and remaining viable and necrotic pulp tissue that is weakly linked to the root canal wall and is typically infected were described as debris [8]. Additionally, upon instrumentation, a smear layer is created that obstructs the dentinal tube apertures and is composed of pulp tissue, bacteria, dentin particles, and residual irrigants. While chelating chemicals are needed to remove the smear layer, the chemo-mechanical action of sodium hypochlorite (NaOCl) eliminates loosely adherent detritus or organic material.

Fenugreek (*Trigonella foenum-graecum* L.) Fam. Leguminosae

Fenugreek, or *Trigonella foenum-graecum* L., is an annual plant of the Leguminosae family. Fenugreek has proven to be a source of phytochemicals with a distinctive chemical structure and novel biological and pharmacological effects. Fenugreek is believed to have come from India or the Middle East, where it naturally flourishes in some regions. It was originally described during the reign of the Pharaohs [9]. When it was combined with other plants or resin to embalm mummies or used to manufacture a particular form of incense. The oldest known treatise on agriculture, written by Cato the Elder in 200 BC, first used the name fenugreek and was the first to refer to it as foenum-graecum [10].

Using scanning electron microscopy analysis, Carvalho (2011) [11] evaluated the cleaning capacity of irrigants and additional chemical compounds in biomechanical preparation. Eight root canal procedures were carried out on 32 single-rooted human teeth using four different irrigants: group 1 used 2.5% NaOCl and 17% ethylenediaminetetraacetic acid (EDTA); group 2 used 2% chlorhexidine gel and 17% EDTA; group 3 used Canal Plus and 2.5% NaOCl; and group 4 used saline and 17% EDTA. In 2000, the cervical, middle, and apical thirds of the teeth were subjected to SEM analysis in order to assess cleaning quality. The teeth were instrumented before being made ready for this test. There was a statistically significant difference between groups 1 and 3 in terms of the number of open tubules, according to the data. With the chlorhexidine group showing a statistically significant difference, the cervical third was cleaned better than the middle and apical thirds in all groups. The root canal walls were completely cleansed by irrigation with Canal Plus combined with NaOCl and NaOCl followed by EDTA.

In 2009, Sharififara [12] investigated the anti-inflammatory characteristics of topical therapies produced from fenugreek (*Trigonella foenum-graecum* L.) seeds in a cream basis. The anti-inflammatory effects of the plant's methanolic extract were examined using a carrageenan-induced edema method, and the outcomes were compared to those of dexamethasone and ibuprofen. Different plant extract concentrations were also created as creams, and their anti-inflammatory qualities were evaluated and compared to those of 1% hydrocortisone ointment, which served as the standard treatment. The results showed that, in comparison to the control group, plant extracts at doses of 100 and 200 mg/kg significantly decreased edema. The plant's activity was comparable to that of ibuprofen and dexamethasone at doses of 100 and 200. Among the plant's manufactured formulations, fenugreek 3 and 5% creams showed the strongest edema inhibition, whereas hydrocortisone ointment had no detectable impact. The results of this study therefore supported and pointed to the conventional uses of fenugreek for inflammations.

Materials and Methods

Plant material

The seeds of (*Trigonella foenum-graecum* L.) Fam. Leguminosae was purchased from herbalist Haraz and flower heads of (*Matricaria chamomilla* L.) Fam. Asteraceae were collected from Experimental Station of Medicinal and Aromatic Plants, and the taxonomical features of the plants were kindly confirmed by Prof. of Plant Taxonomy.

Material for biological study

Plant extracts

Total extract of (*Trigonella foenum-graecum* L.) seeds and flower heads of (*Matricaria chamomilla* L.) were prepared by crushing 500g of each separately in an electric mill, then percolated in ethanol (70%) till exhaustion. Ethanol (70%) extracts were evaporated under reduced pressure to give 100, 110 gram residues of (*Trigonella foenum-graecum* L.) and (*Matricaria chamomilla* L.) respectively. Five gram of Ethanol (70%) extracts of each plant were suspended in bi-distilled water containing Tween 80* for the anti-inflammatory activity and others containing DMF** for the antimicrobial test then refrigerated for biological study.

Name of plant	Weight of dried plant parts (g)	Weight % of total yield extract
Trigonella foenum-graecum L.	500	21%
Matricaria chamomilla L.	500	23%

Table 1: *Percentage of yield extract of each plant.*

Ninety grams and one hundred grams of (*Trigonella foenum-graecum* L.) seeds and flower heads of (*Matricaria chamomilla* L.) were separately suspended respectively in double distilled water. The suspensions were separately partitioned successively between petroleum ether (5 x 400 ml), chloroform (5 x 400 ml), ethyl acetate (5 x 400 ml), and butanol (5 x 400 ml). Each fraction was concentrated to dryness under reduced pressure using a rotary evaporator to give petroleum ether (18%), chloroform (10%), ethyl acetate (30%), butanol (10%), and the remaining aqueous fractions (32%) for fenugreek and petroleum ether (20%), chloroform (5%), ethyl acetate (40%), butanol (5%) and the remaining aqueous fractions (30%) for chamomile. These fractions were refrigerated for biological study and endodontic irrigation.

Activity-guided fractionation of plant extracts

Total extract, petroleum ether, chloroform, ethyl acetate, butanol, and the remaining aqueous fractions were subjected to study its anti-inflammatory activity and the antimicrobial test against *Staphylococcus aureus* and *Enterococcus faecalis*.

Solvents: The solvents used in this work include ethyl alcohol, petroleum ether (40 - 60), chloroform, ethyl acetate, butanol, and formalin were purchased from El-Nasr Pharmaceutical Chemicals Co. (ADWIC). They were purified by adopting the procedures [13].

Apparatus:

- Percolators for extraction (Figure 1).
- Rotatory evaporator* (Figure 2).
- Sensitive electric balance** (Figure 3).

*SINYCO Technology CO., Shanghai, China.

**Shimadzu, AEG-220, Japan.

Figure 1: *Percolators for extraction.*

Figure 2: *Rotatory evaporator.*

Figure 3: *Sensitive electric balance.*

Methods

The anti-inflammatory activity

The anti-inflammatory activity was carried out following the method of materials used:

- Indomethacin*
- Formalin**
- Saline***.

Group design

42 Male Albino rats' strains (180 - 200g) were divided into 7 different groups each of 6 animals for each plant extract:

- Group 1: Control group, the rats in this group were injected with formalin with no treatment.
- Group 2: Indomethacin Group, the rats in this group were injected with formalin (6%) and were treated orally with Indomethacin (60 mg/kg).
- Group 3: The rats in this group were injected with formalin (6%) and treated orally with chloroform (300 mg/kg).
- Group 4: The rats in this group were injected with formalin (6%) and were treated orally with butanol extract (300 mg/kg).
- Group 5: The rats in this group were injected with formalin (6%) and treated orally with ethanol (300 mg/kg).
- Group 6: The rats in this group were injected with formalin (6%) and treated orally with ethyl acetate (300 mg/kg).
- Group 7: The rats in this group were injected with formalin (6%) and treated orally with petroleum ether (300 mg/kg).

Anti-inflammatory procedure

Initially, a caliber was used to gauge the left paw's thickness. Different plant extracts were administered for 30 minutes before 0.1 ml of a 6% formalin solution in normal saline was injected intravenously to cause inflammation. Equal amounts of saline were injected into the right hind paw. The swelling brought on by formalin was caused by the two paws' different thicknesses. The effectiveness of the anti-inflammatory treatment was calculated by comparing the swelling between the treated and control groups. After 30, 60, 90, and 120 minutes, the thickness difference was measured.

The antimicrobial activity: Materials used

Preparation of culturing medium

Blood agar base No. 21 is an upgraded version of blood agar base with better nutritional qualities that make it ideal for the development of picky pathogens and other microorganisms.

Composition of blood agar base no.2

The typical formula of the blood agar base No.2 was Proteose peptone 15.0 gm/liter, Liver digest 2.5 gm/liter, Yeast extract 5.0 gm/liter, Sodium chlorite 5.0 gm/liter, Agar 12.0 gm/liter, and the pH 7.4 ± 0.2 at 25 Co.

Muller-Hinton Broth (MHB) HIMEDIA (Mumbal-400 086, India)

A weight of 38g of the powder was dispersed in one liter of distilled water, and allowed heating on a water bath, to warm gently to dissolve well. The medium was sterilized by autoclaving at 121°C for 15 minutes.

McFarland suspension

Continuous stirring was used to mix 0.5 ml of 0.048M $BaCL_2$ (1.17% w/v $BaCL_2.2H_2O$) with 99.5 ml of 0.18M H_2SO_4 (1% w/v). It was meticulously combined to provide an even suspension. Find the absorbance of water at a wavelength of 625 nm using matched cuvettes with a 1 cm light path, a spectrophotometer, and water as a reference blank. Using screw-cap tubes of the same size and capacity as those used to cultivate the broth cultures, the standard was distributed.

Bacterial culture

A standard culture of *Staphylococcus aureus* standard strain number ATCC 25923 and *Enterococcus faecalis* standard strain number ATCC 29212, which is a frozen cultured broth with 50% glycerol, was graciously donated by the microbiology and immunology department at Cairo University. This culture was subcultured aseptically and spread out across the blood agar plate. For aerobic incubation, the inoculation plates were held at 37°C.

Method for determination of the anti-microbial activity

Disc agar diffusion method sensitivity test determination: *Trigonella foenum-graecum* L. and *Matricaria chamomilla* L. herb extracts were examined for their antibacterial activity against *Staphylococcus aureus* and *Enterococcus faecalis* using petroleum ether, chloroform, ethyl acetate, and butanol. It was done using the disc agar diffusion technique [14].

The tested extracts were aseptically deposited onto sterile discs (500 g/disc) of Whatman filter paper after being dissolved in DMF at a concentration of 20 mg/ml (5 mm diameter).

The discs were then put on top of the previously prepared inoculation plates. For 24 hours, the plates were incubated at 37°C. Following incubation, the inhibition zones were measured in millimeters. No effect was indicated by a diameter of less than 5 mm. A disc impregnated with 20 l of DMF was used as a negative control, while discs that contained sodium hypochlorite** 2% and chlorhexidine* 2% were utilized as positive controls. The sodium hypochlorite was made by diluting 38.09 milliliters of sodium hypochlorite (5.25 percent) with 61.91 milliliters of water to produce 100 milliliters of sodium hypochlorite (2%).

Determination of minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) (Figure 4)

The national committee for clinical laboratory standards NCCLS method was used to determine the fractions of *Trigonella foenum-graecum* L., *Matricaria chamomilla* L., and their minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC) against *Staphylococcus aureus* and *Enterococcus faecalis* [14]. In this method, plant extracts were diluted in a Petri dish with 2 x 10^6 CFu-per-ml inoculums. It was necessary to cultivate the bacterium on blood agar the previous day. In 0.5 McFarland of regular saline, the newly cultivated organism was suspended.

100L of MHB solution, sterilized, was given to each well. 100L of each extract was poured into the first well of each row. Following serial dilution from column 1 to column 12, the last 100L were thrown away. 100L, or 0.1 mL, is the total capacity for each well. 5L of bacterial solution that had been adjusted to a 0.5 McFarland concentration was introduced starting from the wells with the highest dilution (lowest concentration) to the well with the lowest dilution (highest concentration). Row H had three control wells, well H1 (organism+broth), wells H2-H7 (each extract+broth), and well H8 (control) (broth only). Afterward, trays were covered and incubated for 16 to 20 hours at 37°C with fresh air.

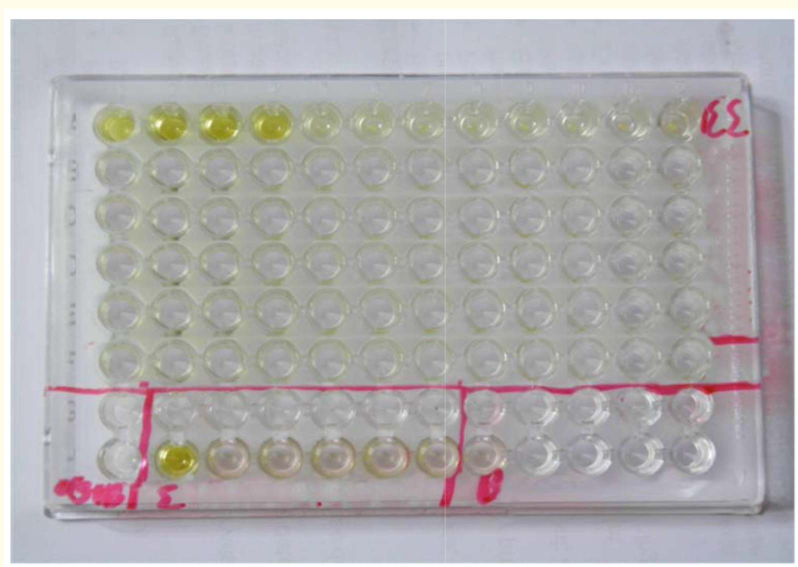

Figure 4: *MIC test result of ethyl acetate extract of Fenugreek against Enterococcus faecalis.*

Reading and recording the antimicrobial activity

1. The growth control well for organism viability was examined. The growth control should show heavy turbidity.
2. For all wells on the worksheet growth or no growth was recorded.
3. The MIC result of each extract was recorded.
4. Extracts showing the lowest MIC results were examined for the MBC.
5. The lowest concentration of antibiotic that totally prevents apparent growth turbidity is known as the MIC. The MBC is the lowest amount of an antibiotic that may kill 99.9% of a bacterial inoculum, according to definitions. MBC was evaluated by cultivating 10L from wells on an agar plate without any drugs that showed no apparent growth [15].

Statistical analysis

The mean standard error of the mean (SEM) for control and experimental animals was used to express all biological screening values. There were 7 animals in each mean. Following a one-way analysis of variance (ANOVA), the Tukey-Kramer multiple comparison tests were used to determine the statistical significance of values between groups.

The mean and standard deviation (SD) values of the data were displayed. The student's t-test was employed to compare the two groups. For comparisons between more than two groups, one-way ANOVA was employed. When the ANOVA test is significant, Tukey's post-hoc test was employed to compare the groups in pairs. The cutoff for significance was chosen at P 0.05. PASW Statistics 18.0® (Predictive Analytics Software) for Windows was used for statistical analysis. The average edema thickness was examined over time using repeated measures ANOVA.

Results

Following the completion of the sensitivity test, we chose to conduct the MIC and MBC tests on the ethyl acetate extract of fenugreek and the whole extract of chamomile. The findings are as follows.

With respect to *Enterococcus faecalis* and *Staphylococcus aureus*, the best extract for fenugreek had MICs of 1.25 mg/ml and 0.625 mg/ml, respectively, while the MBC findings were 5 mg/ml and 5 mg/ml, respectively.

The best extract in chamomile had MICs of 2.5 mg/ml and 1.25 mg/ml against *Enterococcus faecalis* and *Staphylococcus aureus*, respectively, whereas MBC values were 5 mg/ml and 2.5 mg/ml, respectively, against these pathogens.

The ethyl acetate fraction of *Trigonella foenum-graecum* L. and the total extract fraction of *Matricaria chamomilla* L. (most potent fractions) were chosen to be used as a natural irrigant which was used for the smear layer test during the endodontic treatment in teeth. This was done based on the previous antimicrobial and anti-inflammatory tests of all plant fractions and the SEM outcomes are as follows.

Regarding the anti-inflammatory results

Edema thickness (Anti-inflammatory activity)

Comparison between the two herbal extracts

After 30 minutes

Fenugreek showed higher mean edema thickness with chloroform F1 (10.16 ± 0.16), total extract F2 (9.32 ± 0.24), ethyl acetate F3 (8.28 ± 0.19), n-butanol F4 (7.31 ± 0.25) and petroleum ether F5 (10.31 ± 0.15), while chamomile showed lower mean edema thickness with chloroform F1 (8.28 ± 0.54), total extract F2 (6.28 ± 0.35),ethyl acetate F3 (5.62 ± 0.35), n-butanol F4 (6.46 ± 0.24) and petroleum ether F5 (8.15 ± 0.14).

On comparing the results, Fenugreek showed statistically significantly higher mean edema thickness than Chamomile.

The mean values of edema thickness of control and Indomethacin were (10.19 ± 0.39), (7.25 ± 0.13) respectively for the Fenugreek and (10.21 ± 0.40), (7.25 ± 0.13) respectively for the Chamomile. There was no statistically significant difference between the two herbal extracts.

After 60 minutes

Fenugreek showed higher mean edema thickness with chloroform F1 (10.06 ± 0.20), total extract F2 (9.14 ± 0.12), ethyl acetate F3 (8.00 ± 0.22), n- butanol F4 (7.16 ± 0.15) and petroleum ether F5 (10.17 ± 0.15), while chamomile showed lower mean edema thickness

Herbal extract	Chamomile		Fenugreek		P-value
Fractions	Mean	SD	Mean	SD	
Control	10. 1	0.40	10.19	0.39	0.954
Chloroform (F1)	8.28	0.54	10.16	0.16	< 0.001*
Total extract (F2)	6.28	0.35	9.32	0.24	< 0.001*
Ethyl acetate (F3)	5.62	0.35	8.28	0.19	< 0.001*
N-Butanol (F4)	6.46	0.24	7.31	0.25	< 0.001*
Petroleum ether (F5)	8.15	0.14	10.31	0.15	< 0.001*
Indomethacin	7.25	0.13	7.25	0.13	1.000

***Table 2**: The mean, standard deviation (SD) values and results of comparison between edema thickness after using the two herbal extracts after 30 minutes.*
**: Significant at P ≤ 0.05.*

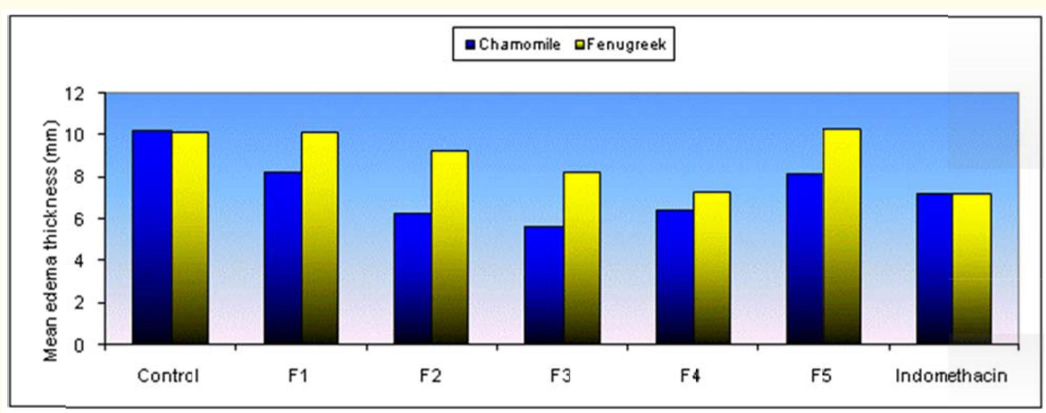

***Figure 5:** Bar chart representing mean edema thickness using the two herbal extracts after 30 minutes.*

with chloroform F1 (7.92 ± 0.83), total extract F2 (6.22 ± 0.32), ethyl acetate F3 (5.03 ± 0.26), n-butanol F4 (6.29 ± 0.17) and petroleum ether F5 (7.55 ± 0.27).

On comparing the results, Fenugreek showed statistically significantly higher mean edema thickness than Chamomile.

The mean values of edema thickness of control and Indomethacin were (12.52 ± 0.36), (7.15 ± 0.18) respectively for the Fenugreek and (12.51 ± 0.36), (7.15 ± 0.18) respectively for the Chamomile. There was no statistically significant difference between the two herbal extracts.

After 90 minutes

Fenugreek showed higher mean edema thickness with chloroform F1 (10.00 ± 0.25), total extract F2 (9.04 ± 0.15), ethyl acetate F3 (7.82 ± 0.24), n-butanol F4 (6.87 ± 0.19) lower mean and petroleum ether F5 (10.07 ± 0.16), while Chamomile edema thickness with

Herbal extract	Chamomile		Fenugreek		
Fractions	Mean	SD	Mean	SD	P-value
Control	12.51	0.36	12.52	0.36	1.000
F1	7.92	0.83	10.06	0.20	< 0.001*
F2	6.22	0.32	9.14	0.12	< 0.001*
F3	5.03	0.26	8.00	0.22	< 0.001*
F4	6.29	0.17	7.16	0.15	< 0.001*
F5	7.55	0.27	10.17	0.15	< 0.001*
Indomethacin	7.15	0.18	7.15	0.18	1.000

Table 3: *The mean, standard deviation (SD) values and results of comparison between edema thickness after using the two herbal extracts after 60 minutes.*
**: Significant at P ≤ 0.05.*

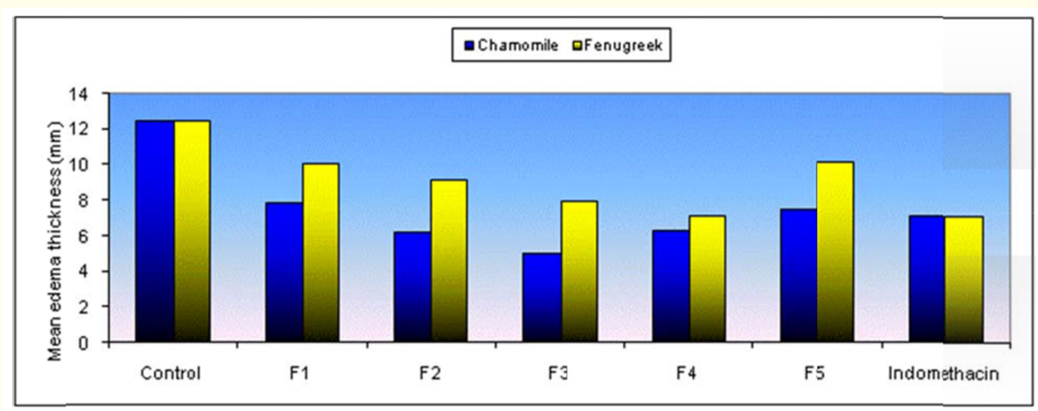

Figure 6: *Bar chart representing mean edema thickness using the two herbal extracts after 60 minutes.*

chloroform F1 (7.39 ± 0.33), total showed extract F2 (6.10 ± 0.23), ethyl acetate F3 (3.55 ± 0.35), n-butanol F4 (6.10 ± 0.31) and petroleum ether F5 (7.18 ± 0.13).

On comparing the results, Fenugreek showed statistically significantly higher mean edema thickness than Chamomile.

The mean values of edema thickness of control and Indomethacin were (13.07 ± 0.37), (7.03 ± 0.24) respectively for the Fenugreek and (13.07 ± 0.37), (7.03 ± 0.24) respectively for the Chamomile. There was no statistically significant difference between the two herbal extracts.

After 120 minutes

Fenugreek showed higher mean edema thickness with chloroform F1 (9.88 ± 0.19), total extract F2 (8.88 ± 0.16), ethyl acetate F3 (7.61 ± 0.22), n-butanol F4 (6.80 ± 0.21) and petroleum ether F5 (9.70 ± 0.20), while Chamomile showed lower mean edema thickness with

Herbal extract	Chamomile		Fenugreek		P-value
Reactions	Mean	SD	Mean	SD	
Control	13.07	0.37	13.07	0.37	1.000
F1	7.39	0.33	10.00	0.25	< 0.001*
F2	6.10	0.23	9.04	0.15	< 0.001*
F3	3.55	0.35	7.82	0.24	< 0.001*
F4	6.10	0.31	6.87	0.19	< 0.001*
F5	7.18	0.13	10.07	0.16	< 0.001*
Indomethacin	7.03	0.24	7.03	0.24	1.000

Table 4: *The mean, standard deviation (SD) values, and results of comparison between edema thickness after using the two herbal extracts after 90 minutes.*
**: Significant at P ≤ 0.05.*

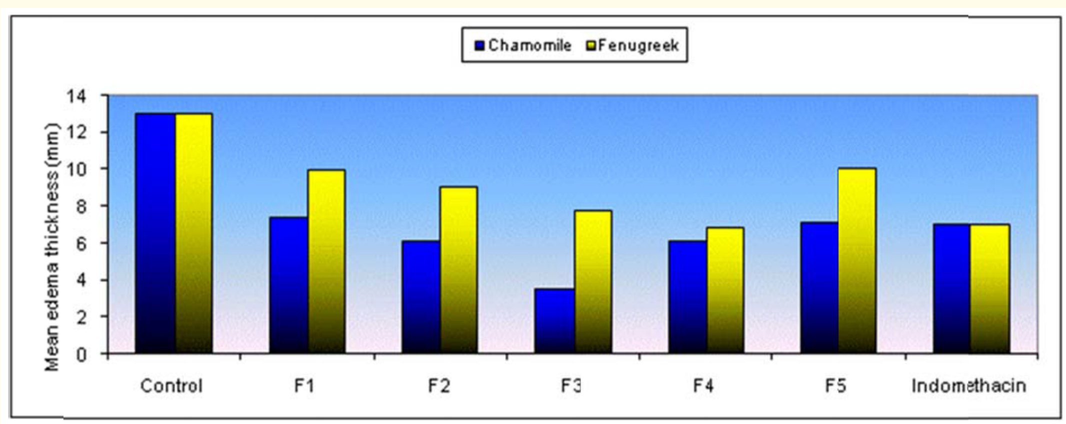

Figure 7: *Bar chart representing mean edema thickness using the two herbal extracts after 90 minutes.*

chloroform F1 (6.87 ± 0.19), total extract F2 (5.97 ± 0.34), ethyl acetate F3 (2.98 ± 0.19), n-butanol F4 (5.99 ± 0.31) and petroleum ether F5 (7.05 ± 0.15).

On comparing the results, Fenugreek showed statistically significantly higher mean edema thickness than Chamomile.

The mean values of edema thickness of control and Indomethacin were (11.77 ± 0.33), (6.49 ± 0.29) respectively for the Fenugreek and (11.77 ± 0.33), (6.49 ± 0.29) respectively for the Chamomile. There was no statistically significant difference between the two herbal extracts.

MIC and MBC tests

After we finished the sensitivity test the best results were shown by Ethyl acetate extract of Fenugreek and total extract of Chamomile, so we decided to make the MIC and MBC tests for them, and the following are the results.

Herbal extract	Chamomile		Fenugreek		P-value
Fractions	Mean	SD	Mean	SD	
Control	11.77	0.33	11.77	0.33	1.000
F1	6.87	0.19	9.88	0.19	<0.001*
F2	5.97	0.34	8.88	0.16	<0.001*
F3	2.98	0.19	7.61	0.22	<0.001*
F4	5.99	0.31	6.80	0.21	<0.001*
F5	7.05	0.15	9.70	0.20	<0.001*
Indomethacin	6.49	0.29	6.49	0.29	1.000

Table 5: *The mean, standard deviation (SD) values and results of comparison between edema thickness after using the two herbal extracts after 120 minutes.*

**: Significant at P ≤ 0.05.*

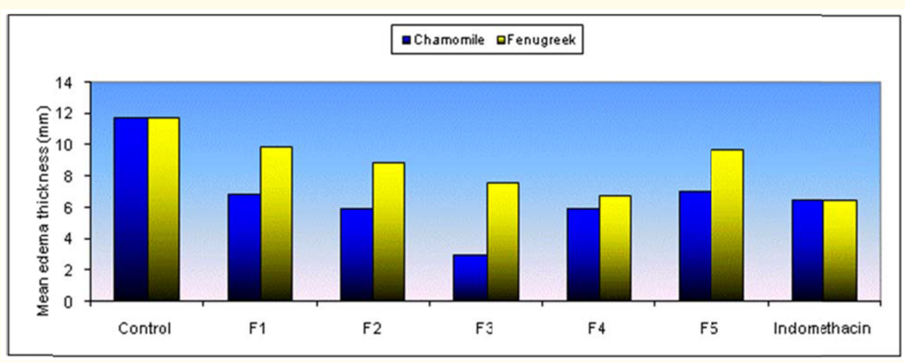

Figure 8: *Bar chart representing mean edema thickness using the two herbal extracts after 120 minutes.*

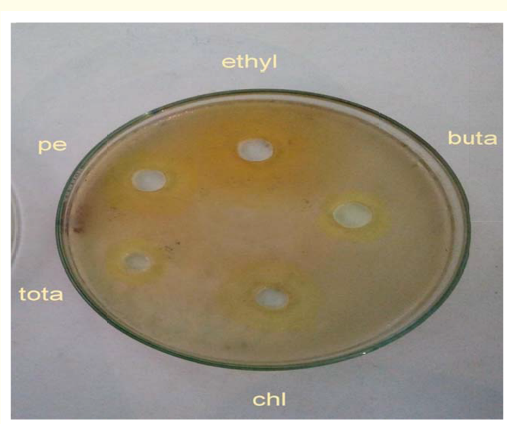

Figure 9: *Sensitivity test results of fenugreek extracts against Enterococcus faecalis.*

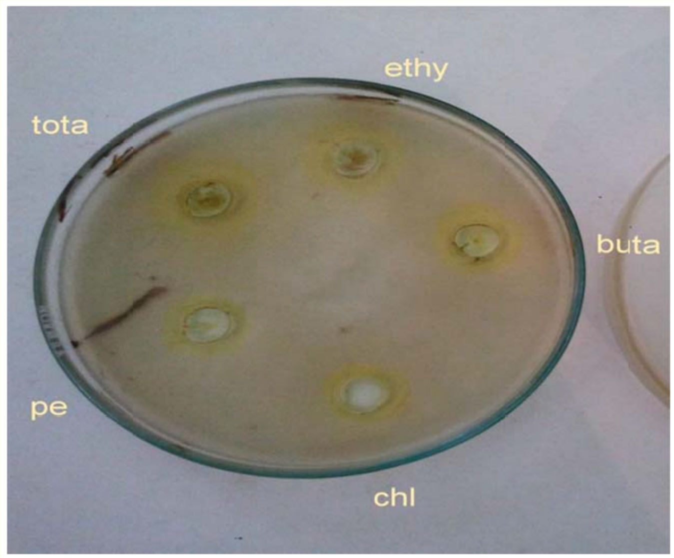

Figure 10: *Sensitivity test results of Chamomile extracts against Enterococcus faecalis.*

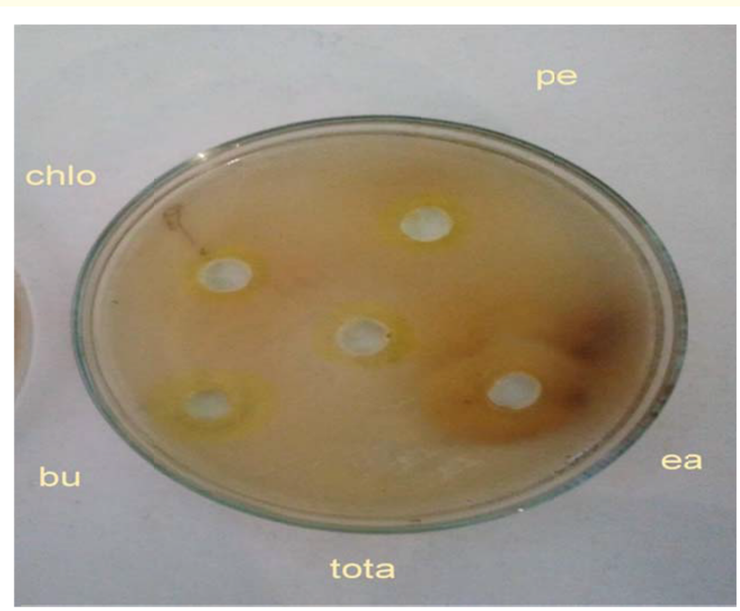

Figure 11: *Sensitivity test results of fenugreek extracts against Staphylococcus aureus.*

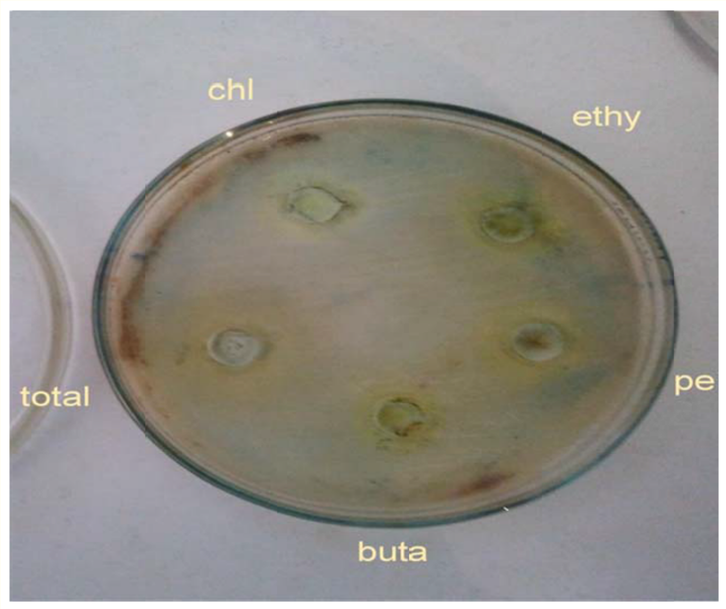

***Figure 12:** Sensitivity test results of Chamomile extracts against Staphylococcus aureus.*

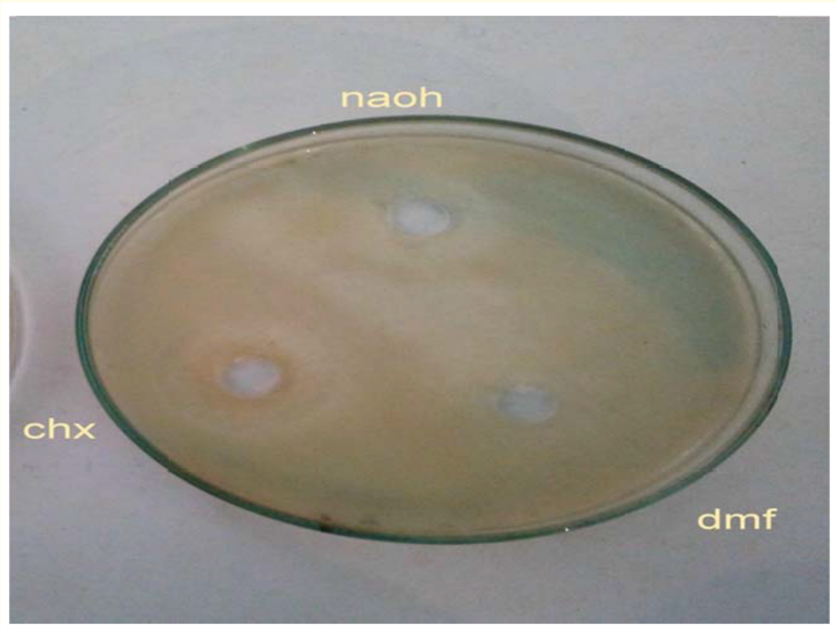

***Figure 13:** Sensitivity test results of chemical and control drugs against Enterococcus faecalis.*

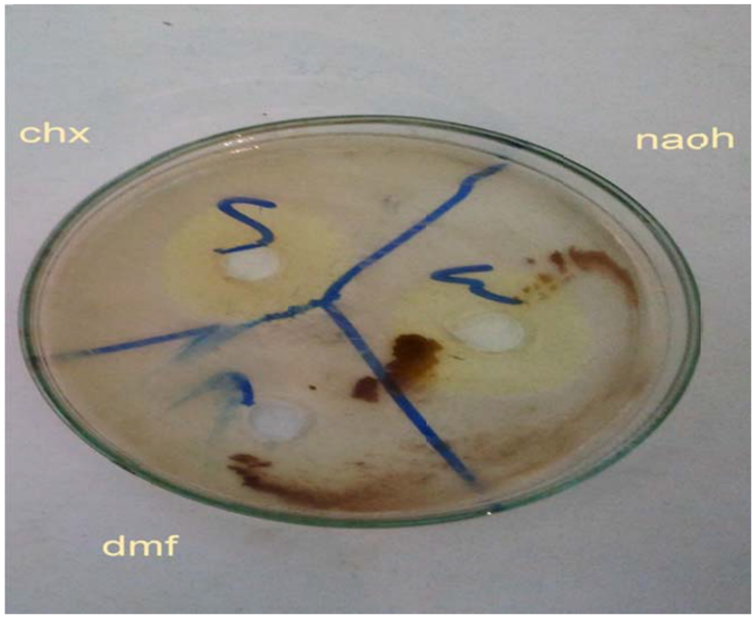

Figure 14: *Sensitivity test results of chemical and control drugs against Staphylococcus aureus.*

For Fenugreek: The best extract was Ethyl acetate with MIC of 1.25 mg/ml and 0.625 mg/ml against *Enterococcus faecalis* and *Staphylococcus aureus* respectively while the MBC results were 5 mg/ml and 5 mg/ml against *Enterococcus faecalis* and *Staphylococcus aureus* respectively.

In Chamomile the best extract was the total extract with MIC of 2.5 mg/ml and 1.25 mg/ml against *Enterococcus faecalis* and *Staphylococcus aureus* respectively while the MBC results were 5 mg /ml and 2.5 mg/ml against *Enterococcus faecalis* and *Staphylococcus aureus* respectively.

According to the previous antimicrobial and the anti-inflammatory tests of all plant fractions and as we depend to a greater extent on the antimicrobial effect in the endodontic treatment so the ethyl acetate fraction of *Trigonella* foenum- graecum L. and the total extract fraction of *Matricaria chamomilla* L. (most potent fractions) were chosen to be used as a natural irrigants which were used for the smear layer test during the endodontic treatment in teeth and the following are the SEM results.

Discussion

In endodontic therapy, the hollows are cleaned, shaped, and decontaminated using microscopic tools and irrigating fluids before being obturated with a filling. The two most commonly utilized irrigants are ethylenediaminetetraacetic acid and sodium hypochlorite (EDTA), hydrogen peroxide, chlorhexidine and others which are considered as chemical irrigants however they have harmful effects (swelling, ecchymosis, burning sensation, paralysis of muscles [16-18] therefore we chose to study alternative substances derived from plant origin (*Trigonella foenum-graecum* L. and *Matricaria recutita* L.) to reduce the harmful effects of the chemical irrigants as possible.

In this study, total extract, Petroleum ether, chloroform, ethyl acetate and butanol fractions of *Trigonella foenum-graecum* L. and *Matricaria chamomilla* L. were subjected to antimicrobial tests the anti- inflammatory test to get the most potent fractions and then use them as endodontic irrigants.

Irrigation of the root canals with antibacterial solutions is a mandatory step to reduce or to eliminate micro-organisms or their by-products from the root canal system [19].

Chemical irrigants have a number of negative consequences, including an inflammatory response [20]. It is crucial to examine the anti-inflammatory activities of *Trigonella foenum-graecum* L. and *Matricaria* recutita in order to minimize this effect. Because L.E. *faecalis* is the most resilient intracanal bacteria and, according to many authors, one of the primary reasons of endodontic failures and flare-ups, it was selected in this work as a model for investigating the antibacterial effect of several root canal irrigants [21-23]. It has also been demonstrated to possess the capacity to endure in root canals as a single organism without the assistance of the other bacteria [24] S. *aureus*, one of the most prevalent bacterial strains associated with endodontic therapy and resistant to a number of antimicrobial treatments, was also utilized to evaluate the antibacterial impact of several irrigants.

The sensitivity test results of all *Trigonella foenum-graecum* L. fractions in this study demonstrated that the ethyl acetate fraction significantly reduced the growth of *Staphylococcus aureus* and *Enterococcus faecalis* and had the highest zones of inhibition of both microorganisms (21 mm and 17 mm, respectively). This means that it had a stronger antimicrobial effect than chlorhexidine and sodium hypochlorite (23 mm and 25 mm respectively). There may be flavonoids and alkaloids present that have been shown to have antibacterial activity, which could explain this antimicrobial activity [25].

In comparison to sodium hypochlorite and chlorhexidine, it significantly inhibits both bacteria (23 mm and 17 mm, respectively) (26 mm and 23 mm respectively). The presence of volatile oils and flavonoids, both of which have been recognized as having antibacterial activity in accordance with [26] The ethyl acetate fraction of *Trigonella foenum-graecum* L. and the whole extract of *Matricaria chamomilla* L. underwent sensitivity, MIC, and MBC testing (Moody and Knapp 2004).

This study established that the best antibacterial extract for fenugreek had a minimum inhibitory concentration (MIC) of 0.625 mg/ml, whereas the best antimicrobial extract for chamomile had a maximum inhibitory concentration (MIC) of 1.25 mg/ml against *Staphylococcus aureus*.

The MBC findings for fenugreek ethyl acetate and chamomile entire extract against *Staphylococcus aureus* were 5 mg/ml and 2.5 mg/ml, respectively.

The best antibacterial extract for fenugreek had a MIC of 1.25 mg/ml and the best antimicrobial extract for chamomile had a MIC of 2.5 mg/ml against *Enterococcus faecalis*. The MBC findings against *Enterococcus faecalis* were 5 mg/ml for the ethyl acetate of fenugreek and 5 mg/ml for the whole extract of chamomile, respectively. However, our study demonstrated the antibacterial properties of chamomile, which are similar to those reported by Abdoul-Latif., *et al*. (2011) but with different values against *Staphylococcus aureus* (MIC = 2, MBC = 2) and *Enterococcus faecalis* (MIC = 4, MBC = 8). Our knowledge does not indicate that fenugreek has been previously reported to have antimicrobial properties against *Staphylococcus aureus* and *Enterococcus faecalis*, and this study may be the first of its sort [27].

Regarding the anti-inflammatory results

Both chamomile and fenugreek showed a good anti-inflammatory effect in accordance with the best result showed by the ethyl acetate fraction of chamomile (Ethyl acetate showed the statistically significant mean edema thickness after 120 minutes "2.98 ± 0.052") followed by the total extract showed the statistically significant mean edema thickness after 120 minutes "5.97 ± 0.045") in comparison to indomethacin. Such anti-inflammatory effect which may be due to terpenoids: α-bisabolol, α-bisabolol oxide A and B, chamazulene, sesquiterpenes; flavonoids: apigenin, luteolin, quercetin and coumarins: umbelliferone as recorded by [28].

The n-butanol fraction of fenugreek demonstrated the highest results (n-butanol showed the statistically significant mean edema thickness after 120 minutes, "6.800.054"), followed by the ethyl acetate fraction with no statistically significant difference, "7.610.069," in contrast to indomethacin. Protodioscin, trigoneoside, diosgenin, and yamogenin are some bioactive chemicals identified from fenugreek seeds that may have an anti-inflammatory impact (Murakami., *et al*. 2000). Diosgenin [(25R)-5-spirosten- 3h-ol], a steroid sapogenin component of fenugreek seeds, is a precursor to steroid hormones like progesterone and anti-inflammatory steroids like cortisone (Norton 1998).

According to the previous antimicrobial and anti-inflammatory tests of all plant fractions and as we depend to a greater extent on the antimicrobial effect in the endodontic treatment so the ethyl acetate fraction of *Trigonella* foenum- graecum L. and the total extract fraction of *Matricaria chamomilla* L. (most potent fractions) were chosen to be used as natural irrigants for the removal of the smear layer during endodontic treatment, While the ethyl acetate of *Matricaria chamomilla* L. and the butanol of *Trigonella foenum-graecum* L. were not chosen because they are not the most potent antimicrobial fractions.

In this study, matched pairs of single-canalled teeth were used to eliminate as many variations as possible in root canal size, shape, or length between the studied groups [29].

Decoronation of the specimens 2 mm coronal to the level of the cervical line was also done to minimize access cavity variations that may occur during root canal instrumentation. Longitudinal sectioning was used in this study to exclude the possibility of the occurrence of artificial debris and/or loss of original debris that could occur during cross sectioning. For the same reason, the disc was not allowed to penetrate the canal space during longitudinal grooving [30].

Apical foramina were sealed with sticky wax in order to simulate the clinical conditions of the periapical tissue as well as to prevent the escape of irrigating solutions during irrigation. According to this study's findings, preparation was carried out 1 mm shorter than the W.L. to reduce the amount of extruded debris and irrigant (Cunningham and Martin 1982). The 30-gauge irrigation needle was used to match the 35-size apical preparation file, which is thought to be the smallest instrumentation size required for irrigants to penetrate into the apical portion of the root canal [31].

We prepared the canal up to size 35 for the current study. According to reports, gauge irrigation needles with a diameter that was smaller than the prepared canal's diameter had more effectiveness for deeper penetration and, as a result, removed more material from the apical region of the canal (Abou-Rass and Piccinino 1982).

Equal amount volumes of 2 ml of irrigating solutions were used followed by flushing with 20 ml of distilled water to stop any further solvent action. The duration of exposure to irrigating solutions was selected on the basis of studies that were done by other investigators [32].

In this investigation, an environmental scanning electron microscope was utilized to assess how clean the coronal, middle, and apical thirds were (debris and smear layer removal). For a close-up view and to gauge the elimination of the smear layer, the SEM was employed. The use of 2000X magnification allowed for a more comprehensive and in-depth look of the canal wall surfaces. It made it possible to spot detritus, smear layers, and tubule orifices [33].

In the current study, Image J software was used to calculate and assess the percentages of debris in each canal. It seemed to offer a less arbitrary and more precise technique of assessment, which led to a more trustworthy comparison of the tested irrigation systems [34].

In this present study, we used either the plant material extract or the chemical irrigant alone and we mixed them together in a concentration of 2% to take the benefits of both materials and check their efficiency in smear layer removal.

Conclusion and Recommendation

According to the limitations of this study, we can utilize the whole extract of *Matricaria chamomilla* L. and ethyl acetate of *Trigonella foenum-graecum* L. fractions as irrigants during the root canal treatment for their anti-inflammatory and anti-microbial activities and advantages. To ascertain the impact of cleaning with these extracts on the sealing ability, adaptability, and push-out bond strength of various sealers and core materials used in root canal obturation, additional research should be conducted using these herbal extracts (ethyl acetate fraction of *Trigonella foenum-graecum* L. and the total extract fraction of *Matricaria chamomilla* L.). Future research might also use these herbal extracts to flush out the root canals after they have been irrigated, finishing with EDTA.

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