Research Article

Investigating the antibacterial effect of German chamomile flower extract as a root canal irrigation solution against *Enterococcus faecalis* biofilm: An *in vitro* study

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Abstract: Background: Enterococcus faecalis (E. faecalis) is a prototypical resistant bacterium in root canal infections and a leading cause of endodontic treatment failure. German chamomile (Matricaria chamomilla) flower extract has been used as a traditional medicine to treat infections. The aim of this study was to investigate the antimicrobial efficacy of chamomile extract on the removal of E. faecalis root canal biofilm. Materials and Methods: Chamomile flower extract was prepared and subjected to detailed chemical analysis. For the in vitro biofilm model, human mandibular premolars (n=48) with 18-20mm working length were used. Root canal preparation was performed using the ProTaper® Next system. Each sample was split longitudinally and reassembled within a pre-prepared silicone matrix, and a mature E. faecalis biofilm was grown for seven days. Samples were randomised into four groups (n=12 for each arm) based on the type of irrigant protocol comprising normal saline, 3% sodium hypochlorite (NaOCl), dual irrigation with 17% ethylenediaminetetraacetic acid (EDTA) and 3% NaOCl, and chamomile extract. After irrigation, residual biofilms were measured in (n=9/group) using densiometric measurements. Observational analysis of residual biofilm was performed in (n=3/group) using Scanning Electron Microscopy (SEM). Results: Phytochemical investigations showed that chamomile extract is rich in antimicrobial components such as flavonoids, polyphenolic compounds, tannins, and others. Spectrophotometry analysis showed a statistically significant difference between the normal saline group with mean optical density (OD)= 0.63044; ±0.106 and each of EDTA and NaOCl group (mean OD=0.35922; ±0.091), chamomile group (mean OD=0.37611; ±0.106) at $p<0.001$, and the NaOCl group (mean OD=0.46344; ±0.096) at $p<0.01$. No statistically significant difference between chamomile group and NaOCl and the dual EDTA and NaOCl irrigation protocol. SEM images showed removal of E. faecalis biofilm and smear layer was in the order: dual EDTA and NaOCl irrigation>chamomile extract>NaOCl. Conclusions: Chamomile flower extract has an apparent removal efficacy of E. faecalis biofilm from root canals. It may be a promising antimicrobial material to be used as a natural endodontic irrigation solution.

Keywords: Bacterial biofilm, Chamomile, Endodontic Microbiology, *Enterococcus faecalis*, Root canal irrigation.

Introduction

The main objective of endodontic treatment is the total eradication of bacteria, which is mainly accomplished by chemomechanical root canal preparation using antimicrobial irrigants [(1)].

Enterococcus faecalis (*E. faecalis*) is the most resistant bacterium commonly encountered within infected root canals, especially in cases where apical periodontitis is persistent because of its capacity to infiltrate and persist in dentinal tubules [2].

Sodium hypochlorite (NaOCl) is the most commonly used endodontic irrigant, a strong proteolytic agent that exhibits powerful tissue-dissolving ability [3]. However, it has several disadvantages, such as tissue toxicity, risk of emphysema, and allergic potential, and it might not completely cleanse the root canal [3, 4]. Ethylenediaminetetraacetic acid (EDTA) is a chelating agent that dissolves inorganic components within the root canal. However, it can cause dentin erosion with prolonged exposures [4]. Also, chelating substances may significantly impact the free available chlorine content of NaOCl and, in turn, its capacity to dissolve tissues [5].

Chamomile (*Matricaria recutita*) is an ancient medicinal herb commonly seen in the monuments of Mesopotamian civilisations as it was regarded as a gift from the earth. This plant's extracts are rich in phenolic acids, flavonoids, and coumarins [6]. It has anti-inflammatory, analgesic, anti-spasmodic, and antimicrobial effects [7]. It was found that chamomile is capable of treating oral sores resulting from chemotherapy, and its oil is an effective therapy for malaria and parasite infections [8]. In addition, chamomile showed effective antimicrobial function against multidrug-resistant pathogens and their biofilm [6]. Many previous studies have examined the antimicrobial effect of chamomile [8-11]; however, it is essential to provide information about its antimicrobial effect against the most resistant bacterial biofilm within the root canal system in comparison to traditional synthetic irrigants.

In an attempt to seek a biocompatible material that can be used as a substitute for traditional irrigants, the chamomile flower extract can be a good candidate for such a purpose. Therefore, in this study, German chamomile flower extract was investigated for its antibacterial activity against *E. faecalis* bacterial biofilm prepared within extracted human premolars.

Methods and Methods

Ethical approval was obtained before conducting the study from the ethics committee in the College of Dentistry, University of Baghdad, reference number 760, on January 12, 2023.

Preparation of chamomile flower crude extract

The extraction process of crude chamomile was performed by cold maceration method [12] through immersing 300gm of dried chamomile flower powder in 2 litters of 70% ethyl alcohol (Chem-Lab, Belgium) for three days at room temperature with magnetic stirring. The extract was filtered by gauze and then using filter paper No. 1 (Whatman, United Kingdom) before being transferred to a rotary evaporator at 40-50°C to obtain the concentrated dried extract. The dry extract was weighed and kept in a dark and dry container at 4°C until use. The percentage of the yield of extract was calculated by the following equation [13]:

(%) yield of extract= [weight (g) of dried extract/ weight (g) of dried plant sample] × 100

The obtained crude chamomile extract was then redissolved in sterile distilled water at the desired concentration (10%, 20%, 30%, and 40% w/v) in a borosilicate glass beaker with the aid of a magnetic stirrer

(Labinco, Netherlands). Then the extract solution was filtered with filter paper No. 1 (Whatman, United Kingdom) and further filtered by syringe filter with 0.22μm pore size (Sartorius, Germany) [14, 15].

Preliminary phytochemical investigations of chamomile flower extract

This procedure was done in order to detect the active ingredients present within chamomile extract containing potential antimicrobials. A weight of 600mg of concentrated dry extract was dissolved in 50mL of distilled water and filtered by filter paper No. 1 (Whatman, United Kingdom). The filtrates were used to conduct the following chemical tests [15], which are presented in Table 1.

Table 1: Preliminary phytochemical screening of German chamomile extract

	Components to be detected	Type of test	The procedure
1	**Tannins**	1% lead acetate test	Mixing 2mL of extract with 1mL of 1% lead acetate solution [16].
2	**Polyphenols**	Ferric chloride test	Mixing 2mL of extract with 2mL of 5% ferric chloride solution [17].
3	**Flavonoids**	Alkaline reagent test	Mixing 2mL of extract with 1mL of sodium hydroxide solution [15].
4	**Alkaloids**	Dragendorff's test	Mixing 5mL of extract with 1mL of Dragendorff's reagent (solution of potassium bismuth iodide) [13, 18].
5	**Reducing sugars**	Benedict's test	1mL of extract was mixed with 2mL of Benedict's reagent, and then the mixture was boiled in a water bath [13, 15].
6	**Saponins**	Foam test	5mL of extract was agitated manually in a test tube for 15 minutes [13].

Qualitative Thin Layer Chromatography (TLC) analysis of the extract

Flavonoids and phenolic contents of the extract were identified by thin-layer chromatography (TLC), using aluminum TLC plate (SILICYCLE, Canada) [19]. The mobile phase consis of toluene, ethyl acetate, formic acid and methanol (ratio 55: 30: 10: 5) for separating extract components. 100mL of the solvent was placed into a glass tank. A drop from each extract and standard was applied to the TLC plate using capillary tubes. The dry plate was examined by ultraviolet (UV) light at 254 nm. Retardation factor (R_f) value for each constituent that was visualised as a fluorescent spot under UV light was calculated.

R_f value = Distance traveled by the spot (the constituent)/Distance traveled by the solvent [20].

The standard phenolic compounds applied were chlorogenic acid, caffeic acid, gallic acid, cinnamic acid, hydroquinone, p-coumaric acid, and pyrogallol. Standards for flavonoids were; apigenin, catechin, epicatechin, kaempferol, luteolin, rutin, quercetin, quercitrin, and hesperidin.

Tooth sample collection

Sample size estimation was conducted using G*Power 3.1.9.7 software (Heinrich Heine University, Düsseldorf, Germany) based on a previous study [21]. The calculation threshold selected was an effect size of (0.50), α error (0.05), power (0.80), and number of groups (4). As a result, 48 extracted human sound mandibular premolars, which had straight, single root canals with fully formed apices, were collected. Selected teeth had no evidence of root caries, calcifications, internal resorption, previous filling or previous root canal treatment. The teeth were stored at room temperature in plastic containers with 0.1% thymol then in distilled water to prevent bacterial growth and avoid dehydration until completion of collection [22].

Tooth sample preparation

Inside a biological safety cabinet hood (Telstar Bio-II-A, Pennsylvania, USA) with strict aseptic conditions, the coronal portion of each tooth was cut in a standardised manner to make all teeth of similar length (18-20mm), and preserving the pulp chamber. The point of cutting was marked by using a permanent marker, and cutting was made perpendicular to the tooth's long axis by a diamond disk (Kerr, Germany) mounted on a straight handpiece (NSK, Japan) under constant water cooling [23]. An access cavity was made, and the working length was measured using a size 10 K-file (Thomas, France) in the canal until it protruded from the apical foramen. Then, 1mm was subtracted from the recorded length.

Root canal preparation and the procedure of root splitting were performed according to a previous study [24]. Canals were prepared by ProTaper® NEXT rotary NiTi system (Dentsply Sirona, Ballaigues, Switzerland) according to the manufacturer's instructions up to size X3 (30/0.07). Irrigation was performed with distilled water after each instrument change using a 30-gauge irrigation needle (SinaliDent, China). Each sample was then embedded in silicone putty (Protesil putty, Italy) to produce a set matrix, which allows the reassembly of the tooth for the irrigation procedure after splitting. Each tooth and its own matrix was given a specific number by using a permanent marker. Then, by diamond disk, each tooth was grooved on buccal and lingual surfaces along the entire length. Tooth sectioning was made by placing the tooth longitudinally on a silicone pad for cushioning and using a surgical blade and mallet. Sterile ProTaper® NEXT X3 gutta-percha (Dentsply Sirona, Ballaigues, Switzerland) was inserted into the canal during this procedure to minimise contamination of the canal. The two halves of each tooth were marked as side A or B. Each split tooth was reassembled during *E. faecalis* biofilm preparation and irrigation, then finally disassembled for residual biofilm investigations.

Biofilm development on the root canal walls

Enterococcus faecalis was isolated by swabs from root canals of patients having failed previous endodontic treatment, identified as *E. faecalis* by growth in bile-esculin and brain heart infusion agar medium (HIMEDIA, India) containing 6.5% salt, gram stain, catalase, VITEK 2 system (bioMérieux, France), and 16S rRNA gene sequencing. The biofilm formation capacity of the isolated bacteria was confirmed by a microtiter plate and crystal violet stain with the use of enzyme-linked immunosorbent assay plates, which confirmed that the produced biofilm showed strong adherence to the internal well wall.

Brain heart infusion broth inoculated with *E. faecalis* at 1.5×10^{8}CFU/ mL concentration. Forty-eight teeth were included in this experiment. The teeth halves and the silicone moulds were first sterilised by UV light of a class II biological safety cabinet (NuAire™, Minnesota, USA) for 30 minutes [25]. Each tooth was

held inside its own silicone mould and then injected with the inoculum inside the root canal by using a sterile plastic syringe with a 30-gauge irrigation needle (SinaliDent, China). The needle tip was inserted up to the apical third, and the inoculated broth was injected gently inside the canal until the canal was filled. Then, samples were incubated in an aerobic environment at 37°C for one week, and the medium was refreshed every 72 hours [21, 26].

Irrigation experiments

The extract was investigated at four concentrations (10%, 20%, 30%, and 40% w/v) on *E. faecalis* biofilm adherence using microtiter plate assay and after all concentrations showed strong biofilm inhibition, a concentration of 20% was selected for irrigation tests as it has a consistency close to that of NaOCl. After biofilm development on the samples, they were randomly divided into four groups (n=12) depending on the irrigant type to be used. Irrigation was made using a 10mL plastic syringe and a 30-gauge closed-end, side-vented irrigation needle (SinaliDent, China). Each sample was irrigated with 9mL of irrigation solution for 90 seconds while the needle tip was inserted up to 3mm short of working length [21]. In the first group (control group), normal saline (PiONEER, Iraq) irrigant was used, while in the second group, 3% NaOCl (AMECLEAN, UAE) was used. In the third group, each sample was irrigated with 17% EDTA (CERKAMED, Poland), followed by normal saline, then 3% NaOCl. In the second and the third groups, after completion of irrigation, both halves of each sample were immersed in 4mL of 5% sodium thiosulphate (HIMEDIA, India) solution for 5 minutes to neutralise the remaining NaOCl on the sample [21]. In the fourth group, irrigation was made with a 20% chamomile hydroalcoholic extract solution, followed by irrigation with normal saline. After irrigation, nine samples from each group were tested using a spectrophotometric device set at 595nm, while three samples were examined by Scanning Electron Microscopy (SEM) to identify the remaining biofilm on the canal walls.

Bacterial adherence test by spectrophotometer

To remove planktonic, non-adherent bacteria, samples were rinsed twice with phosphate-buffered saline for a minute while being gently rocked, then dried on filter paper [27]. The root canal wall of every tooth half was stained for ten minutes with 0.1% crystal violet, followed by a phosphate-buffered saline rinse [28]. Both halves of each individual tooth were immersed in 4mL of 96% ethanol in a plain tube for 3 minutes, vortexed (Labnique Inc., Maryland, USA) for 1 minute before removing the tooth halves and measuring the optical density within the washing ethanol using a spectrophotometer (APEL PD-303, Japan) set at 595nm [29].

Scanning Electron Microscopy testing

Using a sharp scalpel, horizontal marks were made on the split dentin surface out of the root canal at the midpoints of the coronal, mid-root, and apical parts to locate the centre of each canal section. The halves of 12 teeth samples were affixed on aluminium pin stubs, then inserted into a Gold plasma sputter coater under a vacuum (YKY, China) for 10 seconds before examination using SEM (Inspect F50, FEI, Netherlands). The appearance of remaining biofilm was observed at three points (apical, middle, and coronal) of each tooth half [21].

Statistical analysis

Optical density values were analysed by parametric one-way Analysis of Variance (ANOVA). Shapiro-Wilk and Kolmogorov–Smirnov tests were used to discover the normality distribution of data. The Bonferroni test was used to identify the significance level between the groups (multiple comparisons). *P* value<0.05 was considered as the lowest limit of significance. The data were analysed by SPSS (IBM SPSS Statistics for Windows, Version 23.0. Armonk, New York, USA).

Results

Plant extract residue

The percentage yield of ethanolic extract of 300g chamomile flowers was 76.566g (25.522% w/w).

Preliminary phytochemical tests

The results of these tests are shown in Table 2. It is apparent that the extract sufficiently contains most of the tested components.

Table 2: Results of preliminary phytochemical screening tests of the chamomile flower extract

Test	Result*	Comment
Tannins detection	++ ve	White precipitate
Polyphenolic compounds detection	++ ve	Deep blue precipitate
Flavonoids detection	++ ve	Bright yellow color
Alkaloids detection	++ ve	Orange-brown precipitate
Reducing sugars detection	++ ve	Orange-red precipitate
Saponins detection	+ ve	Foam formation

*(++ ve) result indicated that the active compound is adequately presented, while (+ve) indicated that the active compound is weakly presented.

Thin Layer Chromatography (TLC) analysis of the extract

This qualitative analysis showed (see Table 3 and Figure 1) that the chamomile flower extract contains different phenolic compounds, including chlorogenic acid, caffeic acid, gallic acid, cinnamic acid, hydroquinone, p-coumaric acid, and pyrogallol. In addition, several flavonoids were also detected, including apigenin, catechin, kaempferol, epicatechin, hesperidin, luteolin, quercetin, rutin and quercitrin. The retardation factor (R_f) for each component is shown in Table 3, and the thin layer chromatogram is shown in Figure 1.

Spectrophotometry analysis for bacterial adherence

According to statistical tests, the data were normally distributed. As shown in Fig. 2, the lowest mean of optical density, which represents the residual *E. faecalis* biofilm on root canal walls after irrigation, was associated with (EDTA and NaOCl) group (0.35922; ±0.091) followed by chamomile group (0.37611; ±0.106), NaOCl group (0.46344; ±0.096) and finally normal saline group (0.63044; ±0.106). Analysis of Variance (ANOVA) test showed a statistically significant difference present between the tested groups

(p<0.001). The Bonferroni test showed a statistically significant difference present between the normal saline group and all other groups. There was no significant difference between chamomile and (EDTA and NaOCl), nor between chamomile and NaOCl groups.

Table 3: R_f values of standards and separated phenolic components and flavonoids from the extract

Standards of phenols	R_f of the standards	R_f of the ethanolic extract	Standards of flavonoids	R_f of the standards	R_f of the ethanolic extract
Chlorogenic acid	A- 0.220	A- 0.220	**Catechin**	0.314	0.314
	B-0.118	B-0.118			
Caffeic acid	0.511	0.511	**Epicatechin**	0.299	0.299
Cinnamic acid	0.748	0.748	**Kaempferol**	0.472	0.472
Hydroquinone	0.157	0.157	**Rutin**	0.062	0.062
Gallic acid	0.354	0.354	**Apigenin**	0.614	0.614
p-Coumaric acid	0.590	0.590	**Luteolin**	0.527	0.527
Pyrogallol	0.496	0.496	**Quercetin**	0.519	0.519
			Quercitrin	0.236	0.236
			Hesperidin	0.078	0.078

*R_f: Retardation factor.

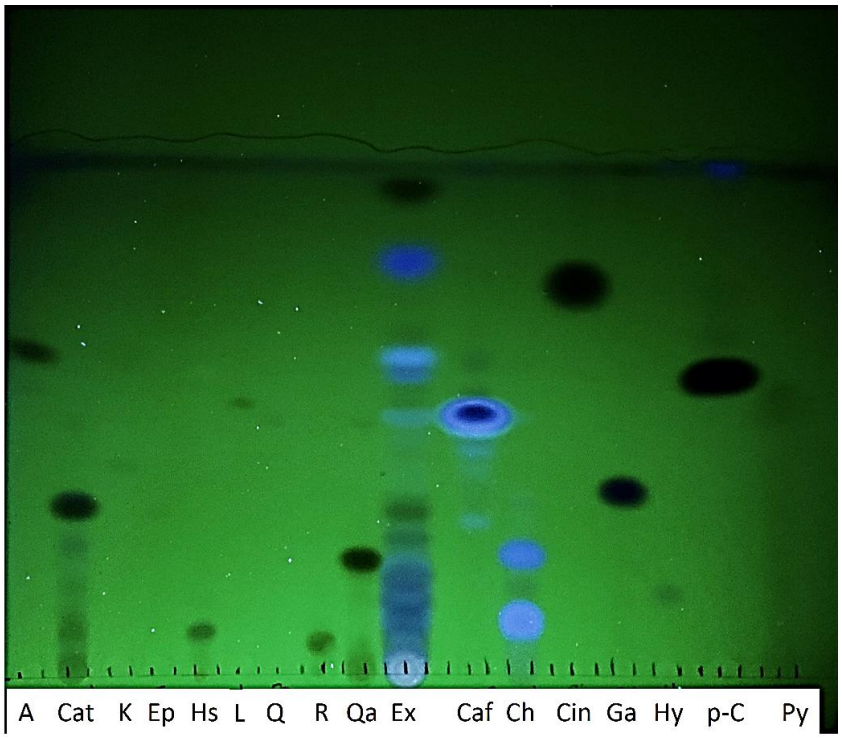

Figure 1: Thin layer chromatogram of chamomile flowers ethanolic extract. (Ex) represents the extract (appears in the centre). The standard phenolic compounds are apparent on the right side to Ex, which included caffeic acid (Caf), chlorogenic acid (Ch), cinnamic acid (Cin), gallic acid (Ga), hydroquinone (Hy), p-coumaric acid (p-C), and pyrogallol (Py). Standards for flavonoids that appear on the left side to Ex include quercitrin (Qa), rutin (R), quercetin (Q), luteolin (L), hesperidin (Hs), epicatechin (Ep), kaempferol (K), catechin (Cat), and apigenin (A).

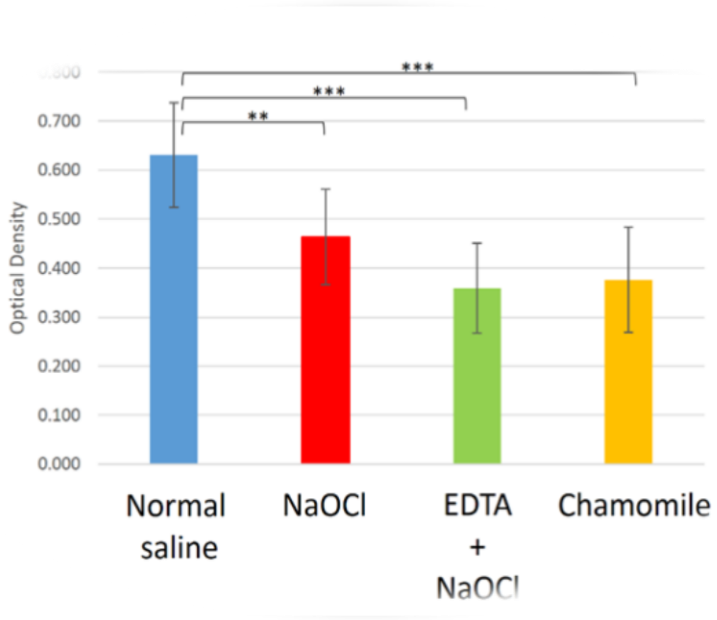

Figure 2: Bar chart of mean optical density representing residual biofilm after irrigation (vertical line inside each bar representing SD). ** means there is a significant difference between the two groups at ($p<0.01$), while *** means there is a significant difference at ($p<0.001$).

Observation of Scanning Electron Microscopy images

Images by SEM for residual biofilm on canal walls are presented in Fig. 3. Images of the normal saline group (Fig. 3, a and b) displayed the morphology of bacterial cells as cocci. These cells are arranged in colonies and are completely encapsulated by an extracellular polymeric matrix that holds the cells together. In the NaOCl group (Fig. 3, c, and d), there are signs of damaged bacteria with residual biofilm and enterococcal surface protein (ESP). There were also unaffected, morphologically intact bacteria still enclosed in extracellular polymeric substances. Complete removal of biofilm was associated with (EDTA and NaOCl) group. However, some sparse viable bacterial cells were detected (Fig. 3, e and f). In the chamomile group (Fig. 3, g and h), there is obvious biofilm damage with ESP destruction. Regarding the smear layer, in the normal saline group, the smear layer covered the canal walls completely. No dentinal tubules are visible even at high magnification (Fig. 3, a and b). NaOCl samples are also completely covered with a smear layer (Fig. 3, c, and d). On samples irrigated with (EDTA and NaOCl), no smear layer was observed (Fig. 3, e, and f). In the chamomile group, it is obvious that the smear layer was less than the normal saline group and NaOCl group. The locations of dentinal tubules are apparent at both low and high magnifications (Fig. 3, g and h).

Discussion

The antibacterial effect of German chamomile flower extract as an endodontic irrigation solution, specifically against *E. faecalis* biofilms, was evaluated and compared with the antibacterial capacity of 3% NaOCl, the traditional endodontic irrigant. The main finding in this research is that there was no statistically significant difference in the biofilm disrupted, as determined by mean optical density present between chamomile and (EDTA and NaOCl), nor between chamomile and NaOCl groups. This can be considered a promising result regarding the use of this natural herbal extract as an endodontic irrigation solution.

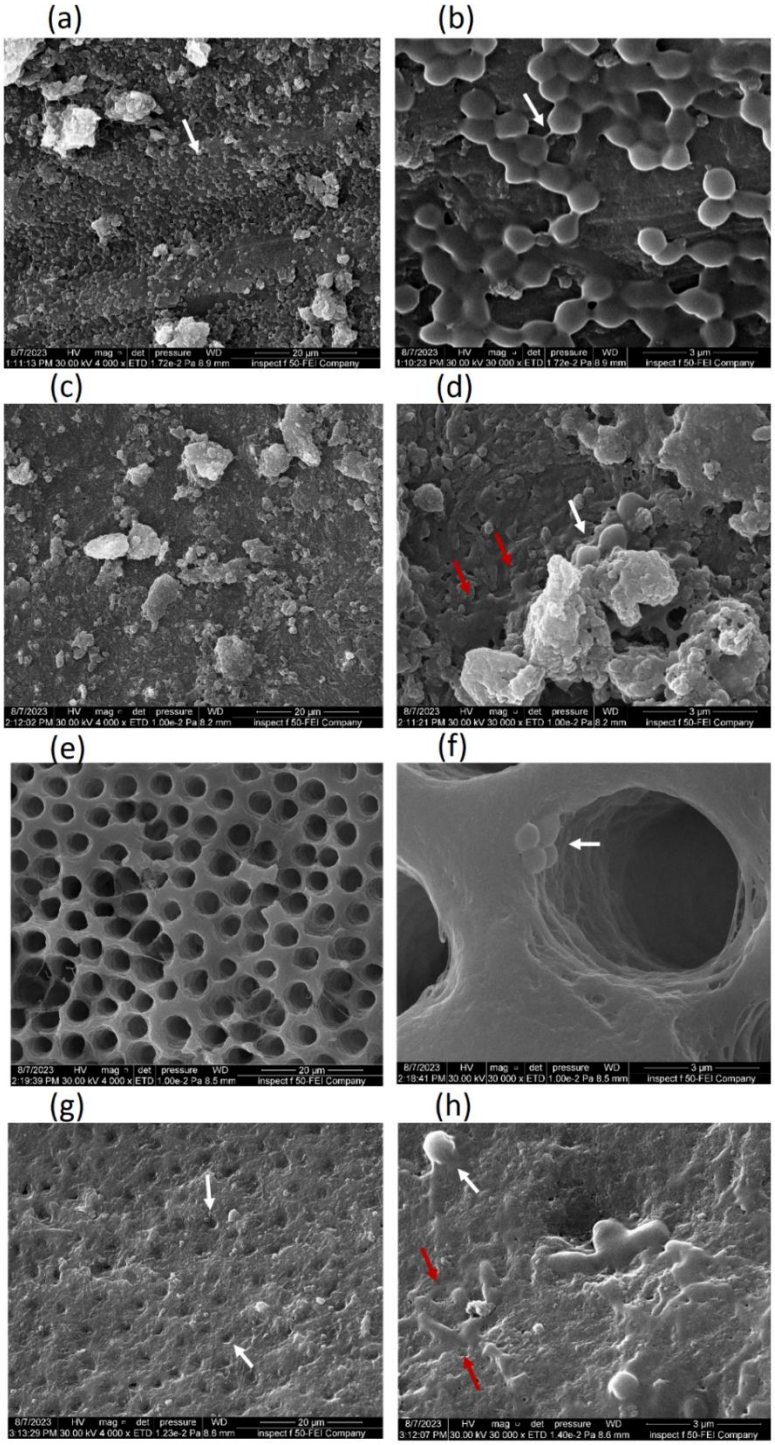

Figure 3: SEM images illustrate canal walls after irrigation with normal saline (a and b), NaOCl (c and d), (EDTA and NaOCl) (e and f), and chamomile (g and h). Red arrows in the figure point to a disrupted biofilm. White arrow in (a) pointing to bacterial cells within the extracellular matrix (ECM), in (b) to ECM, in (d), (f), and (h) to viable bacteria, and in (g) to apertures of dentinal tubules. (a), (c), (e), and (g) have a magnification of 4000x and scale bar 20µm, while (b), (d), (f), and (h) have a magnification of 30 000x and scale bar 3µm.

Cold maceration methods were used to obtain chamomile extract, as using heat during the extraction procedure could affect the active components, especially antioxidant compounds [30, 31]. The phytochemical screening for chamomile extract was performed through chemical detectors, and then identities were confirmed by a TLC-based methodology. The first method is a routine, low cost and simple procedure to give an overview screening of the active ingredients within the extract [16]. In contrast, TLC analysis is a quick and simple qualitative method to detect the presence of different types of flavonoids and phenolic compounds, which are important antimicrobials [13, 32, 33]. Different types of flavonoids and phenolic compounds were detected within this study; however, there are further flavonoids and phenolic compounds which have not been investigated due to the absence of their standards within the working laboratory. Therefore, further analysis with different standards could be suggested.

Because *E. faecalis* is difficult to remove from infected root canals and dentinal tubules and can lead to refractory apical periodontitis [34], it was chosen as the current study's microorganism. The virulence factors of *E. faecalis* include several, including lipoteichoic acid, lytic enzymes, cytolysin, and aggregation substance. By producing collagen-binding proteins such as adhesins, Ace, and serine proteases, this bacteria can adhere to dentine or cementum collagens and penetrate between these structures [35]. It can endure after careful canal instrumentation and disinfection. This bacterium tolerates extremely high pH levels by activating its enzymatic systems and proton pump and can withstand starvation for up to 12 months [36, 37].

Bacteria in biofilm exhibit significant resistance to both the host immune system and antimicrobial agents. Compared to the planktonic phase, biofilm bacteria are up to a thousandfold more resistant [38, 39]. In a previous study, the ability to form biofilm was demonstrated by 75.5% of *E. faecalis* strains isolated from root canals with failed endodontic treatment [40]. Another study showed that isolates of *E. faecalis* from failed endodontically treated teeth have high biofilm-forming rates [41]. To replicate the clinical condition and evaluate the efficacy of the tested irrigants throughout the entire canal, the experimentally formed biofilm in this study was grown to the full canal length. Furthermore, biofilm was grown for seven days, as it has been found that this period is enough to allow biofilm development on canal walls [26]. Also, biofilm resistance is inherent, and after five days of incubation, mature wild bacterial biofilm can be formed [42]. Since it has been found that 9mL of irrigation solution is enough to improve solution penetration and remove bacterial biofilm from root canals [21], a similar volume was used in each sample irrigation in the current study.

In terms of spectrophotometry method and measuring the optical density, besides its simplicity, accuracy, and time saving, it can provide quantitative evaluation about the entire residual bacterial biofilm in the whole root canal, this is because only the viable bacterial cells can adhere to the canal walls in a biofilm [29].

According to this study's results, German chamomile extract showed better *E. faecalis* biofilm removal than NaOCl, as determined by optical density measurements. In addition, it was observed in the available SEM images of the current study that this extract has an obvious effect in smear layer removal better than NaOCl but less effective than (EDTA with NaOCl), as shown in Fig. 3. This outcome is in agreement with the findings of a previous study regarding smear layer removal by chamomile extract [7]. Chelator agents have been found to play a role in the detachment of biofilms, most likely by sequestering calcium, which

is essential for the stability of the biofilm extracellular matrix [43]. This indicates that chamomile's effect on smear layer removal is very important in its antibacterial action.

The antibacterial efficacy of chamomile extract has been supported by previous studies, which revealed that alcoholic chamomile extract exterminates aerobic organisms and inhibits the growth of bacteria such as *Escherichia coli* and *Helicobacter pylori* [8, 44]. The antimicrobial activity of chamomile is primarily related to its active components, including flavonoids (such as apigenin, luteolin, quercetin, patuletin, and others), polyphenolic compounds (such as chlorogenic acid, p-coumaric acid, and others), tannins and essential oil components such as α-bisabolol with its oxides and azulenes [45, 46]. There is a possibility that smear layer removal by chamomile, which has been observed in SEM images of this study, may be related to the effect of these active ingredients.

While the precise mechanism of action of chamomile against bacteria is still not well known, prior research has shown that chamomile can have dual antimicrobial effects: bacteriostatic through bisabolol and bactericidal through chamazulene [8]. It can act as a protein synthesis inhibitor, and its broad-spectrum antibacterial effects may be by inhibiting cell membrane enzymes and disrupting the permeability barrier of cell walls [8]. Typically, active components in chamomile extracts exhibit notable activity against bacterial cell membranes, resulting in an increase in the diffusion of outer cell membranes. This permits the buildup of hazardous concentrations of monoterpenes in the cytoplasm of bacteria, which causes membranes to expand, increase in membrane fluidity, and inhibition of enzymes that are embedded in the membrane [8]. Active ingredients in chamomile, specifically bisabolol, inhibit cyclooxygenase, and this action mimics the antibacterial action of common drugs like diclofenac and aspirin by preventing DNA synthesis in the bacterial cells [47].

As a result, the majority of earlier research findings agreed with those of the current study. However, this study is an *in vitro* study and investigated only a single bacterial species. Biofilms of infected canals consist of multiple bacterial species. In addition, surrogates to the traditional methods that measure the bacterial growth were used in this study; this should be considered in future studies.

Conclusion

German chamomile flower extract has an apparent effect in removing *E. faecalis* biofilm from root canal walls better than NaOCl and can be considered a promising material to be used as an endodontic irrigation solution. Further studies about chamomile extract cytotoxicity, its effects on restorative materials, and irrigation protocols when using this extract have to be undertaken to employ this natural herbal extract effectively for root canal irrigation.

Conflict of interest

The authors have no conflicts of interest to declare.

Author contributions

The study's conception and design were prepared by A.S.Y., A.F.M., and S.M. Data collection and methodology were performed by A.S.Y. Statistical analysis and interpretation of the results were performed by A.F.M. The original draft of the manuscript was prepared by A.S.Y. with the supervision of A.F.M., G.K.,

and S.M. G.K. critically revised the work. The previous manuscript versions were reviewed and edited by A.F.M., G.K., and S.M. All authors approved the final manuscript version for publication.

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Informed consent

Informed consent was obtained from all individuals or their guardians included in this study.

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دراسة التأثير المضاد للبكتيريا لمستخلص زهرة البابونج الألماني كمحلول لري قناة الجذر ضد الأغشية الحيوية لـ*Enterococcus faecalis* دراسة في المختبر:

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المستخلص:

الخلفية: *Enterococcus faecalis* (*E. faecalis*) هي بكتيريا مقاومة نموذجية في التهابات قناة الجذر وسبب رئيسي لفشل العلاجات اللبية. تم استخدام مستخلص زهرة البابونج الألماني (*Matricaria chamomilla*) كدواء تقليدي لعلاج الالتهابات. كان الهدف من هذه الدراسة هو التحقق من الفعالية المضادة للميكروبات لمستخلص البابونج في إزالة الأغشية الحيوية لـ*E. faecalis* لقناة الجذر. المواد والطرق: تم تحضير مستخلص زهرة البابونج وإخضاعه للتحليل الكيميائي التفصيلي. بالنسبة لنموذج الأغشية الحيوية في المختبر، تم استخدام ضواحك الفك السفلي البشرية (عدد = 48) بطول عمل يتراوح بين 18-20 ملم. تم إجراء تحضير قناة الجذر باستخدام نظام ProTaper® Next. تم تقسيم كل عينة طوليًا وإعادة تجميعها داخل مصفوفة سيليكون مُعدة مسبقًا، وتم زراعة غشاء حيوي ناضج لـ *E. faecalis* لمدة سبعة أيام. تم تقسيم العينات بصورة عشوائية إلى أربع مجموعات (العدد = 12 لكل ذراع) بناءً على نوع بروتوكول الري الذي يشتمل على محلول ملحي عادي، و3% هيبوكلوريت الصوديوم (NaOCl)، والري المزدوج ب17% حمض الأسيتيك الإيثيلي الديامين (EDTA) و3% NaOCl، ومستخلص البابونج. بعد الري، تم قياس الأغشية الحيوية المتبقية في (عدد = 9 / مجموعة) باستخدام قياسات الكثافة. تم إجراء التحليل الرصدي للأغشية الحيوية المتبقية في (عدد = 3 / مجموعة) باستخدام المجهر الإلكتروني الماسح (SEM). النتائج: أظهرت الدراسات الكيميائية النباتية أن مستخلص البابونج غني بالمكونات المضادة للميكروبات مثل المركبات الفلافونويدية، الفينولية المتعددة، التانينية، وغيرها. أظهر تحليل القياس الطيفي وجود فرق ذو دلالة إحصائية بين مجموعة المحلول الملحي العادي ذات متوسط كثافة بصرية= 0.63044 ؛±0.106 وكل من مجموعة EDTA وNaOCl (متوسط الكثافة البصرية=0.35922 ؛ ±0.091)، ومجموعة البابونج (متوسط الكثافة البصرية=0.37611 ؛ ±0.106) عند 0.001 >p، ومجموعة NaOCl (متوسط الكثافة البصرية=0.46344 ؛ ±0.096) عند0.01<p. لا يوجد فرق ذو دلالة إحصائية بين مجموعة البابونج وNaOCl وبروتوكول الري المزدوج EDTA وNaOCl. أظهرت صور SEM إزالة الأغشية الحيوية لـ *E. faecalis* وطبقة اللطاخة كانت بالترتيب: الري المزدوج EDTA و NaOCl< مستخلص البابونج< NaOCl. الاستنتاجات: مستخلص زهرة البابونج له فعالية واضحة في إزالة الأغشية الحيوية لبكتيريا *E. faecalis* من قنوات الجذر. قد تكون مادة مضادة للميكروبات واعدة لاستخدامها كمحلول طبيعي لري قنوات الجذور.