Iraqi Journal of Agricultural Sciences –2026:57(2):639-650
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/0bbwap73

EVALUATING GERMAN CHAMOMILE'S ACTIVITY AGAINST *ENTEROCOCCUS FAECALIS* ISOLATED FROM ROOT CANALS

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ABSTRACT

This study was aimed to investigate German chamomile extract's active ingredients and their antibacterial activity against *Enterococcus faecalis* (*E. faecalis*) which was previously isolated from root canals. Chamomile hydroalcoholic extract was obtained by maceration process. Phytochemical tests and thin layer chromatography (TLC) used to detect active ingredients in the extract. Agar well diffusion method was used to compare antimicrobial effect of 3% sodium hypochlorite (NaOCl) and 4 extract's concentrations (100mg/mL-400mg/mL) against *E. faecalis*. Chamomile's anti-biofilm effect against *E. faecalis* biofilm was tested by microtiter plate assay with ELISA reader. Phytochemistry showed that, tannins, glycosides, alkaloids, flavonoids, and polyphenolic chemicals were abundant. TLC revealed presence of phenolic compounds including; caffeic acid, chlorogenic acid, and others, and flavonoids such as; apigenin, catechin, and others. Agar well diffusion showed higher inhibition zone diameter for 200mg/mL, 300mg/mL, and 400mg/mL chamomile in comparison to 3% NaOCl. The extract showed strong *E. faecalis* biofilm inhibition by all its concentrations, while moderate inhibition exerted by NaOCl. It can be concluded that German chamomile extract appears rich antimicrobial and effective inhibitor against *E. faecalis* biofilm.

Keywords: antimicrobial tests, bacteria, endodontic irrigation, endodontic Microbiology, herbal extract , Phytochemistry
***Part of M.Sc. thesis of the 1st author.**

Received: 23/1/2024, Accepted: 14/4/2024, Published: 28/2/2026

INTRODUCTION

Oral microorganisms, especially bacteria, are the source of primary endodontic infections. These microorganisms are typically opportunistic and can infiltrate a root canal that has necrotic tissue in order to initiate an infectious process (Siqueira, 2002). In general, chemomechanical root canal preparation with the use of antimicrobial irrigation solutions provides total eradication of microorganisms however this may not always be successful. One of the bacteria that is most resistive to treatment in infected root canals is *E. faecalis*, particularly when there is persistent apical periodontitis. This bacteria is the most pathogen that persists in root canal therapy due to its capacity to infiltrate dentinal tubules, survive in hard canal conditions, and adapt to fatal challenges (Shafiq *et al*, 2024). It is a facultative type of bacteria which enables this microorganism to endure within biofilm Sodium hypochlorite (NaOCl) is recommended to be used as an antibacterial irrigant solution in the endodontic treatment. It is a powerful proteolytic agent, which exhibits tissue dissolving ability as an endodontic irrigant (Adham *et al*, 2022). However, NaOCl has numerous unfavorable features including tissue toxicity, the potential to induce allergic reaction, the possibility of emphysema when overfilled, unpleasant taste and smell and it might not entirely clean the root canal (Mohmmed & Mahdee,2019).The attention in the use of herbal medicines in endodontics has been developed (Shafiq & Mahdee, 2023). The chamomile plant's flower has numerous active components and is thought to be a source of many therapeutic

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uses. It is an ancient medicinal herb commonly seen in the monuments of Mesopotamian civilizations as it was regarded as a gift from the earth.One of the herbal preparations is chamomile hydroalcoholic extract which has been investigated for a number of pharmacological characteristics including antimicrobial, anti-oxidant, anti-inflammatory, sedative, analgesic, antispasmodic, anticancer, and other effects (Sadr Lahijani *et al*,2006). The most often utilized type is German chamomile (*Matricaria recutita L.)* which is an annual plant used as table tea in several regions of the world (Sadr Lahijani *et al*,2006). For antibacterial activity, crude ethanolic extract from chamomile flowers was suggested (Alkuraishy *et al*,2015). Although there were many research about antibacterial action of chamomile, few of them focused on their antibacterial effect on *E. faecalis* bacteria. So it is necessary to do deep research on the relevant material as it may be suggested to be used as an endodontic irrigation solution. This study aimed to characterize the active ingredients of German chamomile flower extract and investigate its antibacterial effect against *E. faecalis* bacteria in comparison to NaOCl irrigant.

MATERIALS 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 at January 12, 2023.

Preparation of chamomile flower crude extract: The extraction process of crude chamomile was conducted in the Biotechnology Research Center, Al-Nahrain University, by cold maceration method through immersing 300gm of dried, powdered chamomile flowers (which were growing in Iraq) in 2 litters of 70% ethyl alcohol for three days at 25ºC with magnetic stirring. The extract was filtered by gauze then using filter paper No. 1 (Whatman, United Kingdom), then transferred to a rotary evaporator (Heidolph, Germany) at 45ºC to obtain the dried extract. This was weighted, 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 (AL-Qaralusi & Al-Mizraqchi,2023; Shafiq *et al*,2024). (%) yield of extract= [weight (g) of dried extract/ weight (g) of dried plant sample] × 100

The obtained chamomile extract was then dissolved in sterile distilled water at different concentrations (100mg/mL, 200mg/mL, 300mg/mL, and 400mg/mL) 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, then filtered by syringe filter with 0.22µm pore size (Sartorius, Germany) (Phuyal *et al* ,2020; Shafiq *et al*, 2024).

Preliminary phytochemical screening of German chamomile flower extract

This procedure was done in order to detect the active ingredients present within chamomile extract which could be potent antimicrobials. 600mg of dry extract was dissolved in 50mL of distilled water and filtered by filter paper No. 1. The filtrates were used to conduct the following chemical tests (Morsy,2014).

Detection of tannins: By using a glass pipette, 1mL of 1% lead acetate solution was added to 2mL of plant extract present in a glass test tube. If a gelatinous or white precipitate was formed, this indicates the presence of tannins (Shaikh & Patil, 2020).

Detection of glycosides: Aliquot of 1mL of the extract was mixed with 2mL of the Benedict's reagent (alkaline solution containing cupric citrate complex). The mixture was boiled in water bath for 5 minutes, then allowed to cool at room temperature. If a red deposit was formed, this indicates the presence of glycosides (Jassim Al-khafagi & Mohammed,2023).

Detection of alkaloids (Dragendorff test

In a borosilicate glass measuring cup, 60mg of bismuth sub-nitrate were dissolved in 0.2mL HCl, this is solution A. In a graduated glass cylinder, a mixture of 600mg potassium iodide in 1mL of distilled water was prepared, this is solution B. The solution A was added to solution B in the glass cylinder and mixed. 1mL of the mixture was added to 5mL of plant extract in a test tube then poured in a petri dish. If an orange to brown color was formed, this indicates the presence of alkaloids (Shafiq *et al*,2024).

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Detection of the saponins: Five milliliters of the extract in a glass graduated cylinder was manually shaken for 15 minutes. The formation of a foam at the top of the extract indicates the presence of saponin (Shafiq *et al*,2024).

Detection of flavonoids: Alkaline reagent test was carried out by adding 2mL of 2% sodium hydroxide solution, with the aid of glass pipette, to 2mL of the extract solution in a glass test tube. When a bright yellow color was obtained, this indicates the presence of flavonoids

Detection of polyphenolic compounds

Aliquot of 2mL of 5% ferric chloride solution were added to 2mL of the plant extract solution in a glass test tube. If a deep blue or deep green deposition was formed, this indicates the presence of polyphenols (kumar Bargah,2015).

Qualitative thin layer chromatography (TLC) analysis: This procedure was performed to investigate the presence of phenolic compounds and flavonoids. These compounds have been proven to be active ingredients for the antibacterial effect (Farhadi *et al*, 2019; Maisuria *et al*,2015). A stock solution was prepared by dissolving 5mg of the extracted residue in 1mL of absolute methanol. A standard phenolic compounds and standard flavonoids were prepared in the same concentration in methanol including: chlorogenic acid, gallic acid, cinnamic acid, p-coumaric acid, caffeic acid, pyrogallol, and hydroquinone as phenolic standard compounds. The following were the standard flavonoids: apigenin, catechin, kaempferol, epicatechin, hesperidin, luteolin, quercetin, rutin, and quercitrin. TLC was performed with the use of an aluminum TLC plate (SILICYCLE, Canada) with a size 20×20cm and thickness 0.1mm. This was coated with silica gel containing fluorescent indicator F_{254}, which represented the stationary phase in the chromatography separation method. While for the mobile phase, toluene, ethyl acetate, formic acid and methanol (ratio 55: 30: 10: 5) were used. The type of flavonoids and phenolic compounds separated was identified corresponding to the standard flavonoids and phenolic compounds spots in their distance (by obtaining retention factor value). Retention factor (R_f) value has been derived from dividing the distance moved by each flavonoid or phenolic compound in each model phase to the distance moved by the solvent (Shafiq *et al* ,2024): R_f value=distance moved by each flavonoid or phenolic compound/distance moved by the mobile phase When the silica plate exposed to ultraviolet (UV) light, each flavonoid and phenolic component can be identified individually as a colored spot. The silica plate should be covered with fluorescent material, which flashes under UV light at a wavelength of 254nm when it binds to the active groups of flavonoids and phenolic compounds. The result can be shown under UV light as bright spots.

Bacterial isolation: *Enterococcus faecalis* bacterial isolate was obtained from the Microbiology Laboratory in the College of Dentistry/ University of Baghdad, which was previously isolated from root canals of teeth with failed previous endodontic treatment. Then, the obtained isolated *E. faecalis* was grown on blood agar (Neogen, England) and Brain Heart Infusion (BHI) agar (HIMEDIA, India) and the plates were incubated at 37°C for 24 hours (Shafiq *et al* ,2024).

Identification of *Enterococcus faecalis*

Colonies of *E. faecalis* were chosen from the blood agar plate under microscope and subjected to the Gram's stain, then examined under light microscope. Also, colonies of *E. faecalis* were inspected by the naked eye according to their morphological characteristics on the blood agar and BHI agar plates (Zoletti et al, 2006). Hemolytic activity of *E. faecalis* isolates was investigated on blood agar which was mixed with human blood (Patidar et al,2013). The VITEK 2 Compact system (bioMérieux, France) was utilized for *E. faecalis* identification. The GP cards were inoculated with the bacterial isolates and subsequently processed on the VITEK 2 system to identify *E. faecalis* colonies (Yaaqoob, 2022).

Antimicrobial testing of German chamomile flower extract: Agar well diffusion : Mueller Hinton Agar (HIMEDIA, India) plate surface was inoculated by *E. faecalis* bacterial suspension with turbidity of 0.5 McFarland

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(1.5×10^8 CFU/ mL). Six-millimeter diameter holes were made in the seeded agar using a sterile cork borer. In a sterile condition, 100µL of each of the following was poured into each well; 100mg/mL, 200mg/mL, 300mg/mL, and 400mg/mL of chamomile extract, and 3% NaOCl. Ten plates for each concentration were prepared and incubated for 24 hours at 37°C, before measuring the inhibition zones diameters with a caliper (Yaaqoob, 2022).

Measuring the extract's minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

This test was done according to a previous study (Raoof et al,2019). For the serial dilution of the extract, ten tubes were utilized; and one tube served as a control, containing BHI broth including bacteria only. The broth dilution method was used to prepare extract solutions at varying concentrations. There was 1mL of BHI broth in each tube. After adding the extract to the first tube to reach an extract concentration of 300mg/mL and thoroughly mixing it, 1mL of the first tube's solution was added to the second tube. In the end, 1mL of the last tube was thrown away, bringing each tube's dilution to half its predecessor's concentration (making the extract concentration in the tubes as follows: 300 mg/mL, 150 mg/mL, 75 mg/mL, 37.5 mg/mL, 18.75 mg/mL, 9.375 mg/mL, 4.687 mg/mL, 2.343 mg/mL, 1.171 mg/mL, 0.585 mg/mL). Each tube received 50 µL of the already made bacterial suspension at 0.5 McFarland turbidity (1.5×10^8 CFU/ mL). After being incubated for 24 hours at 37°C, the tubes were checked for turbidity resulting from the growth of the injected bacteria. The lowest concentration that inhibits bacterial growth (MIC) was found in the first tube, which had no visible turbidity. 10µL of that tube was then cultured on BHI agar without plant extract and incubated for 24 hours at 37°C. This concentration was regarded as MIC if the growth of *E. faecalis* was positive. Ten microliters were taken from each tube that showed no signs of bacterial growth and spread out on the surface of BHI agar that was devoid of extract in order to calculate the MBC of the extracts. The presence of microbial growth was monitored in cultured plates after 24 hours of incubation at 37°C. MBC was the first extract concentration that did not result in any bacterial growth on agar.

Anti-biofilm test of the extract

Using colorimetric crystal violet and microtiter plates, the extract's anti-biofilm action was investigated (Raoof et al,2019); Firstly, the biofilm formation capacity of the used *E. faecalis* isolate was confirmed by adding 200µl of BHI broth with inoculum at 0.5 McFarland turbidity (1.5×10^8 CFU/ mL) to a well (Bulacio et al,2015). Then in another wells, 100µl of BHI broth were added to the wells and 100µL of different concentrations of the extract (100mg/mL, 200mg/mL, 300mg/mL, and 400mg/mL) were tested with 10µL of the prepared bacterial suspension diluted by BHI broth to 0.5 McFarland turbidity (1.5×10^8 CFU/ mL) which was put to the wells. In another control well BHI broth was added only, and in other extract and BHI broth only. The plates were incubated at 37°C for 24 hours. Then each well was washed three times with 300µl of sterile phosphate buffered saline to remove planktonic bacteria, and dried at room temperature. Each well received 200µl of 96% ethanol for 5-10 minutes to stabilize the adhered bacteria on the internal surface before removing the ethanol and drying the plate at room temperature. Each well was stained with 200µL of 0.1% crystal violet for 5 minutes, rinsed with distilled water and dried at room temperature. Biofilm was assessed by measuring dye absorbance using enzyme-linked immunosorbent assay (ELISA) reader (HumaReader HS, Germany) (Bulacio *et al*,2015). The process was performed in triplicate and repeated three times. The calculation of biofilm formation was accomplished according to previous studies (Mirzaee *et al*,2014).

RESULTS AND DISCUSSION

Plant extract residue: The residue from extraction of 300g chamomile flowers was 76.566g (25.522% w/w).

Preliminary phytochemical screening of German chamomile flower extract: The results of the preliminary phytochemical tests are illustrated in Table 1 which shows that the plant is rich with almost all of the detective active ingredients.

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Table 1. Results of preliminary phytochemical screening tests of chamomile flower extract

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

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

Qualitative thin layer chromatography (TLC) analysis: In Figure 1, the thin layer chromatogram appears with the extract components labelling in the centre, and the standards for both flavonoids and phenolic compounds on its left and right sides respectively. It is apparent that the extract shows the presence of almost all of the tested compounds in comparison to their standards. At the same time, the retention factor (R_f) for each compound present in the extract was calculated and compared to its corresponding value of the standard. The R_f values are the same for both, which means that the compounds detected within the extract are typically the same in comparison to their standards

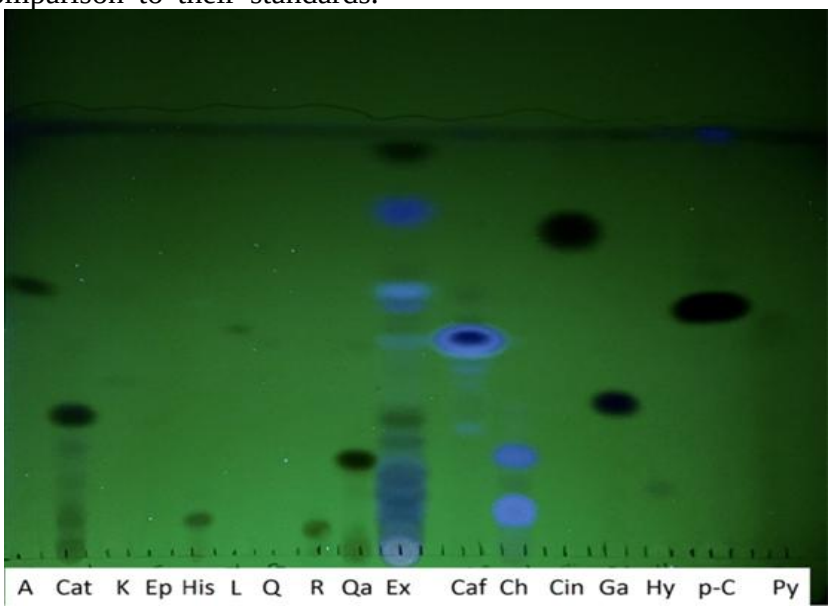

Figure 1. Thin layer chromatogram of chamomile flowers ethanolic extract. (Ex) represents the extract (appears in the center). The standard phenolic compounds are apparent on the right side to the Ex which include; caffeic acid (Caf), chlorogenic acid (Ch), cinnamic acid (Cin), gallic acid (Ga), hydroqinone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

Identification of *Enterococcus faecalis*

Enterococcus faecalis colonies appeared similar to Gram-positive cocci when viewed using a light microscope. It is apparent that they are grouped in pairs, small chains, and large chains (Figure 2, A). *E. faecalis* colonies appeared on blood agar as circular colonies, white gray in color, 0.1-0.5mm in diameter (Figure 2, B). Hemolysis on blood agar was seen by the development of a clear zone surrounding the colonies (β-hemolysis), (Figure 2, C). While *E. faecalis* colonies on BHI agar appeared as white circular colonies ranging from 0.1-0.5mm in diameter (Figure 2, D). Vitek 2 system showed the presence of *E. faecalis* with 99% probability and excellent identification.

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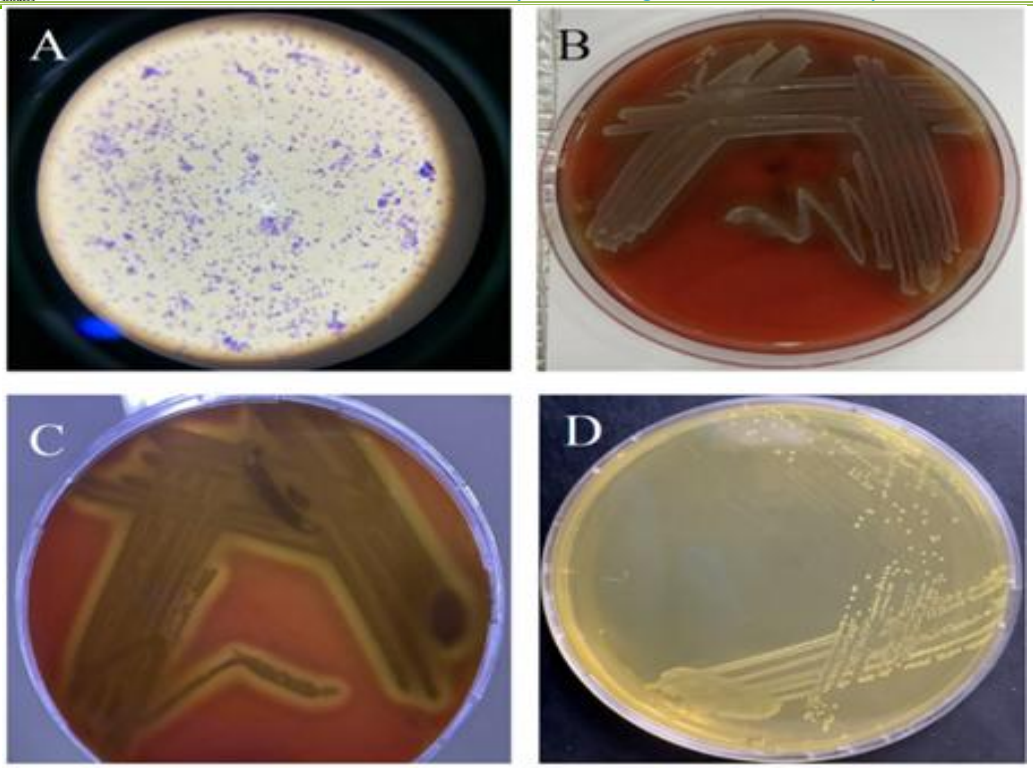

Figure 2. *Enterococcus faecalis* Identification. (A): *E. faecalis* stained with crystal violet stain under light microscope. (B): *E. faecalis* colonies on blood agar. (C): β-hemolysis by *E. faecalis* which is seen as a clear zone around the colonies. (D): *E. faecalis* colonies on BHI agar

Antimicrobial testing of German chamomile flower extract: Agar well diffusion

The inhibitory effect of 100mg/mL, 200mg/mL, 300mg/mL, and 400mg/mL chamomile extract and 3% NaOCl on *E. faecalis* growth has been shown in Table 2 and Figure 3 (A). It is apparent that chamomile extract in 200mg/mL, 300mg/mL, and 400mg/mL concentrations demonstrated bacterial inhibition better than NaOCl. Kruskal-Wallis test showed significant difference between the groups (2-sided test), p=0.000. Post-hoc analysis is shown in Table 2, the significance level is 0.05.

Table 2. Descriptive statistics and comparisons between groups for inhibition zones diameters in agar well diffusion test.

Groups	Median inhibition zone diameter (cm)	Interquartile rage
NaOCl 3%	**1** [a, e]	**0**
Chamomile 100mg/mL	**0**	**0**
Chamomile 200mg/mL	**1.2** [b, e, f]	**0.1**
Chamomile 300mg/mL	**1.4** [c, f, g]	**0.1**
Chamomile 400mg/mL	**1.5** [d, g]	**0.1**

***Similar superscript letters mean there is no statistical significant difference (p>0.05). Not similar superscript letters mean there is statistical significant difference (p<0.05).**

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC): It is apparent in Figure 3 (B) that extract concentration of 75mg/mL starts to show

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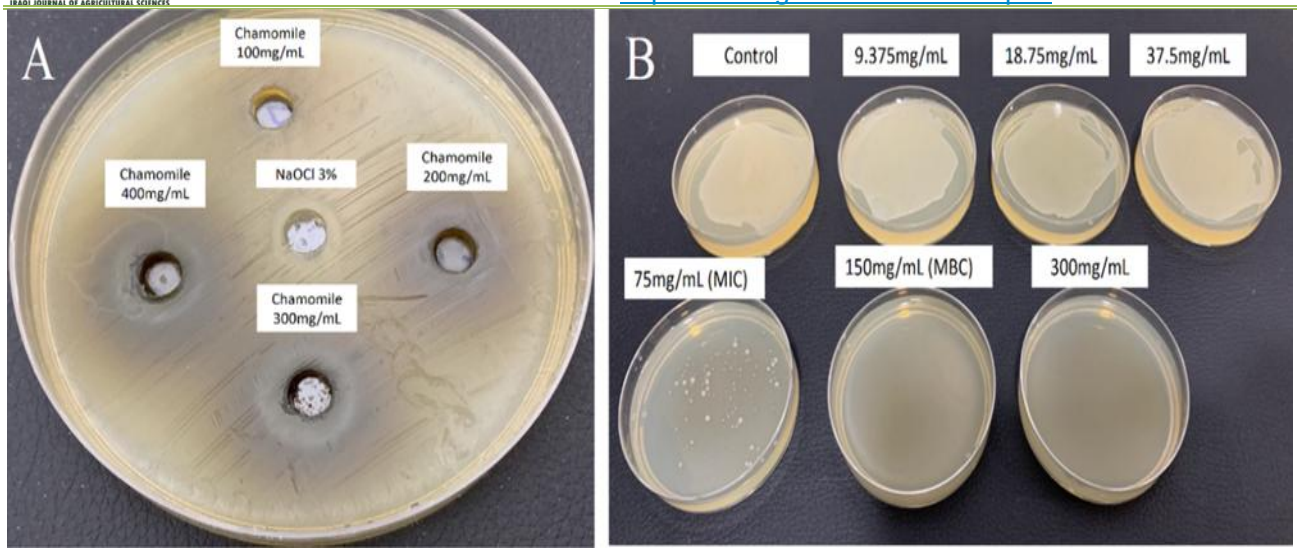

Figure 3. Antimicrobial testing of chamomile extract. (A): Inhibition zones of 100 mg/mL, 200 mg/mL, 300 mg/mL, and 400 mg/mL chamomile, and 3% NaOCl after 24 hours incubation. (B): *E. faecalis* growth on BHI agar after being tested with different extract concentrations (labelled in the image) to determine MIC and MBC

Anti-biofilm test of the extract

Table 3 reveals that *E. faecalis* biofilm is weakly adherent in the presence of chamomile extract at all the tested concentrations. While, the biofilm appears moderately adherent in the presense of 3% NaOCl.

Table 3. The adherence condition of *E. faecalis* biofilm in the presence of 100mg/mL, 200mg/mL, 300mg/mL, and 400mg/mL chamomile and 3% NaOCl.

Sample	Mean optical density (nm)	Adherence condition
Culture medium and bacteria only, without extract	0.592	SA*
Culture medium only without bacterial inoculation	0.005	NA*
100mg/mL chamomile	0.130	WA*
200mg/mL chamomile	0.132	WA
300mg/mL chamomile	0.135	WA
400mg/mL chamomile	0.117	WA
3% NaOCl	0.207	MA*
Extract and culture medium only	0.065	NA

***SA: Strongly Adhered, *NA: Non Adhered, *WA: Weakly Adhered, * MA: Moderately Adhered (Mirzaee *et al*,2014).**

Since insufficient cleaning of the canal system is the primary cause of endodontic failure, *Enterococcus faecalis* was chosen as the subject of this research. This decision was primarily made because it can be difficult to eradicate *E. faecalis* in the infected root canals and dentinal tubules, which can result in refractory apical periodontitis (Love, 2001). Due to the side effects of the chemical materials used as endodontic irrigants, various plants with antimicrobial activity have been the focus of numerous studies, and they may be effective agents for eradicating microbes that are resistant to therapy (Shafiq *et al*,2024). The main finding in this study is that, German chamomile extract can exert better antimicrobial action against *E. faecalis* bacteria than NaOCl. However, the chamomile concentration which provided this purpose was different between the tests which were used in this study. In agar well diffusion, chamomile concentration of 100mg/mL did not produce inhibition zone, while this concentration was effective in inhibiting the biofilm formation in anti-biofilm test, and the MIC was less than 100mg/mL (MIC was 75mg/mL). The different approaches used in the investigations may be the cause of the disparity in the reported results (Raoof *et al*,2019). *Enterococcus faecalis* can survive even after precise root canal instrumentation and disinfection, because it has the ability to form a biofilm on the root canal surface which increases its antimicrobial resistance (Halkai *et al*,2012). Through the initiation of the proton pump and

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its enzymatic systems, this bacteria can resist extremely high pH levels and survive for a full year under conditions of starvation (Sedgley *et al*,2005). However, not all strains of *E. faecalis* have the same biofilm-producing capacity (Bulacio *et al*,2015). Therefore, isolates from failed endodontically treated teeth with strong capacity to produce biofilm on microplates were used in the current study. In this study, the most popular classes of therapeutic chemicals found in plants were identified qualitatively using straightforward and trustworthy procedures. The assays employed in this work can provide a useful framework for identifying biologically active components and providing ways to explore the significance of a plant as a source of these compounds. According to the results of this study, flowers extract of German chamomile has been shown to be enriched with different active ingredients such as tannins, alkaloids, flavonoids, polyphenolic compounds, glycosides and saponins. These components could be contributed to the antibacterial activity of this extract. According to previous studies, tannins have a potent antibacterial effect and may have pharmacological significance. It was discovered that when a plant extract's tannin content increases, the antibacterial efficacy increases (Raoof *et al*,2019). This component demonstrated antibacterial activity against *Escherichia coli, Staphylococcus aureus* (*S. aureus*), *Salmonella typhi, Streptococcus pyrogens, Proteusvulgaris* and *Pseudomonas aeruginosa*, by using agar diffusion method (Raoof *et al*,2019). In addition, numerous alkaloids inhibit the formation of and/or disperse bacterial biofilms, which could be due to bacterial quorum sensing (QS) disruption, or inhibition of sortase or adhesins (Cushnie *et al*,2017). Furthermore, flavonoids showed up to six-fold stronger antibacterial activities than standard antimicrobial drugs in the market *(*Farhadi *et al*,2019). According to a previous study, apigenin can reduce *E. faecalis* viability in biofilms in a dose-dependent way (Kim & Min,2023). Catechin which is derived from herbal sources, has a powerful capacity to inhibit *E. faecalis* and considered as an anti-QS and antibacterial agent (Kurnia *et al*,2021). Luteolin reduced the quantities of biofilm matrix components generated by *Candida albicans* and *E. faecalis* in both single and dual species, when examined by confocal laser scanning microscope (CLSM) (10). According to a previous study, quercetin simultaneously affects multiple proteins resulting in inhibition of *E. faecalis* biofilm formation when was examined by scanning electron microscopy (SEM) and CLSM (Qayyum *et al*,2019). Through a potent synergistic impact, naturally occurring polyphenolic compounds demonstrated the capacity to enhance the efficacy of conventional antibiotics against planktonic and biofilm pathogenic bacterial cells. The synergy between the antibacterial and antibiofilm properties is facilitated by the capacity of polyphenolic compounds to cause membrane permeabilization and efflux pump inactivation in the bacterial cells (Maisuria *et al*,2020). A phenolic compound, chlorogenic acid demonstrated an antimicrobial activity against *S. aureus* in both planktonic and biofilm situations (Luís et al,2014). Another active ingredients found in chamomile are glycosides which are secondary metabolites found in many natural plants. They have a range of pharmacological characteristics, including the ability against *S. aureus* bacteria which has the ability to generate resistance to almost all available antibiotics (Zou & Fang,2021). The results of this study indicate that German chamomile extract has apparent antibacterial properties against *E. faecalis*. Consequently, the majority of earlier research findings agreed with the results of the current study.

CONCLUSIONS

German chamomile extract is rich in antimicrobial ingredients. It has an obvious antibacterial effect against *E. faecalis* and strong inhibition of its biofilm. Therefore, it is a promising material to be considered as a root canals disinfectant for endodontic use. However, further studies about using this material inside the root canals of the teeth, and cytotoxicity investigations are necessary.

ACKNOWLEDGEMENT

Authors appreciate Prof. Dr. Zainab Y. Mohammed Hasan for her efforts in the preparation of the herbal extract. Also, special

thanks to Asst. Prof. Dr. Fadia A. Al-Khayat for her help in the bacteriology work.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DECLARATION OF FUND

The authors declare that they have not received a fund.

REFERENCES

Adham, A. H., Ali, A. H., & Mannocci, F. 2022. Continuous chelation concept in endodontics. Journal of Baghdad College of Dentistry, 34(4):59-69 .
DOI:10.26477/jbcd.v34i4.3287

Alkuraishy, H., Al-Gareeb, A., Albuhadilly, A., & Alwindy, S. 2015. In vitro assessment of the antibacterial activity of *Matricaria chamomile* alcoholic extract against pathogenic bacterial strains. British Microbiology Research Journal, *7*(2): 55-61 .
DOI: 10.9734/BMRJ/2015/16263

AL-Qaralusi, D. M., & Al-Mizraqchi, A. S. 2023. Investigating the impact of non-nutritive sweeteners on the antifungal potential of alcoholic and aqueous *Eucalyptus* extracts against salivary *candida albicans* (An *in-vitro* study). Journal of Baghdad College of Dentistry, 35(3):1-9 .
DOI:10.26477/jbcd.v35i3.3446

Bulacio, M. d. l. Á., Galván, L. R., Gaudioso, C., Cangemi, R., & Erimbaue, M. I. 2015. *Enterococcus faecalis* biofilm:

Cushnie, T. T., Cushnie, B., & Lamb, A. J. 2014. Alkaloids: An overview of their antibacterial, antibiotic-enhancing and antivirulence activities. International Journal of Antimicrobial Agents, 44(5): 37 . 386-7.
DOI:https://doi.org/10.1016/j.ijantimicag.2014.06.001

DOI: 10.3233/MGC-130117

DOI:https://doi.org/10.1016/j.micpath.2018.11.013

DOI:https://doi.org/10.1080/08927014.2013.845878

DOI:https://doi.org/10.36103/ijas.v55iSpecial.1910

Doss, A., Mubarack, H. M., & Dhanabalan, R. 2009. Antibacterial activity of tannins from the leaves of *Solanum trilobatum* Linn. Indian J Sci Technol, 2(2): 41-43 .
https://ischolar.sscldl.in/index.php/indjst/article/view/29391

Farhadi, F., Khameneh, B., Iranshahi, M., & Iranshahy, M. 2019. Antibacterial activity of flavonoids and their structure–activity relationship: An update review. Phytotherapy Research, 33(1): 13-40.
DOI: https://doi.org/10.1002/ptr.6208

Formation and development *in vitro* observed by scanning electron microscopy. Acta Odontologica Latinoamericana,28(3):210-214
http://www.scielo.org.ar/scielo.php?pid=S1852-48342015000300002&script = sci_arttext

Fu, Y., Wang, W., Zeng, Q., Wang, T., & Qian, W. 2021. Antibiofilm efficacy of luteolin against single and dual species of *Candida albicans* and *Enterococcus faecalis*. Frontiers in Microbiology, 12, 715156.
DOI:https://doi.org/10.3389/fmicb.2021.715156

Halkai, R., Hegde, M. N., & Halkai, K. 2012. *Enterococcus faecalis* can survive extreme challenges-overview. Journal of Health and Allied Sciences NU, 2(03): 49-53.10.1055/s-0040-1703595
DOI:https://doi.org/10.36103/ijas.v54i5.1818

Jhajharia, K., Parolia, A., Shetty, K. V., & Mehta, L. K .2015. Biofilm in endodontics: a review. Journal of International Society of Preventive & Community Dentistry, 5(1): 1 .
DOI: 10.4103/2231-0762.151956

Kim, M.-A., & Min, K.-S. 2023. Combined effect of apigenin and reduced graphene oxide against *Enterococcus faecalis* biofilms. Journal of Oral Science, 22-0459 .
DOI:https://doi.org/10.2334/josnusd.22-0459

kumar Bargah, R. 2015. Preliminary test of phytochemical screening of crude ethanolic and aqueous extract of *Moringa pterygosperma* Gaertn. Journal of Pharmacognosy and Phytochemistry, 4(1):07-09.
https://www.phytojournal.com/archives?year=2015&vol=4&issue=1&ArticleId=536&si=false

Kurnia, D., Ramadhanty, Z .F., Ardani, A. M., Zainuddin, A., Dharsono, H. D. A., & Satari, M. H. 2021. Bio-mechanism of catechin as pheromone signal inhibitor: prediction of antibacterial agent action mode by *in vitro* and *in silico* study. Molecules, 26(21): 6381 .

DOI:https://doi.org/10.3390/molecules26216381

Love, R. 2001. *Enterococcus faecalis*–a mechanism for its role in endodontic failure. International Endodontic Journal, 34(5): 399-405 . DOI:https://doi.org/10.1046/j.1365-2591.2001.00437.x

Luís, Â., Silva, F., Sousa, S., Duarte, A. P., & Domingues, F. 2014. Antistaphylococcal and biofilm inhibitory activities of gallic, caffeic, and chlorogenic acids. Biofouling, 30(1): 69-79 .

Maisuria, V .B., Hosseinidoust, Z., & Tufenkji, N. 2015. Polyphenolic extract from maple syrup potentiates antibiotic susceptibility and reduces biofilm formation of pathogenic bacteria. Applied and Environmental Microbiology, 81(11): 3782-3792 .
DOI:https://doi.org/10.1128/AEM.00239-15

Mirzaee, M., Najar Peerayeh, S., & Ghasemian, A.-M. 2014. Detection of icaABCD genes and biofilm formation in clinical isolates of methicillin resistant *Staphylococcus aureus*. Iranian Journal of Pathology, 9(4): 257-262 .
https://ijp.iranpath.org/article_7049_9.html

Mohmmed, S. A., & Mahdee, A. F. 2019. Assessment of the effect of three agitation techniques on the removal efficacy of sodium hypochlorite for the organic films. World, 10(6): 441.
DOI:10.5005/jp-journals-10015-1676

Morsy, N. 2014. Phytochemical analysis of biologically active constituents of medicinal plants. Main Group Chemistry ,13(1): 7-21.

Patidar, R .K., Gupta, M. K., & Singh, V. 2013. Phenotypic detection of virulence traits and antibiotic susceptibility of endodontic *Enterococcus faecalis* isolates. American Journal of Microbiological Research, 1(1): 4-9 .DOI:10.12691/ajmr-1-1-2

Phuyal, N., Jha, P. K., Raturi, P. P & ,. Rajbhandary, S. 2020. Total phenolic, flavonoid contents, and antioxidant activities of fruit, seed, and bark extracts of *Zanthoxylum armatum* DC. The Scientific World Journal, 2020
DOI: https://doi.org/10.1155/2020/8780704

Qayyum, S., Sharma, D., Bisht, D., & Khan, A. U. 2019. Identification of factors involved in *Enterococcus faecalis* biofilm under quercetin stress. Microbial Pathogenesis, 126, 205-211 .

Raoof, M., Khaleghi, M., Siasar, N., Mohannadalizadeh, S., Haghani, J., & Amanpour, S. 2019. Antimicrobial activity of methanolic extracts of *Myrtus communis* L. and *Eucalyptus galbie* and their combination with calcium hydroxide powder against *Enterococcus faecalis*. Journal of Dentistry, 20(3): 195
DOI: 10.30476/DENTJODS.2019.44898

Sadr Lahijani, M., Raoof Kateb, H., Heady, R., & Yazdani, D. 2006. The effect of German chamomile (*Marticaria recutita* L.) extract and tea tree (*Melaleuca alternifolia* L.) oil used as irrigants on removal of smear layer: a scanning electron microscopy study. International Endodontic Journal, 39(3): 190-195 .
DOI:https://doi.org/10.1111/j.1365-2591.2006.01073.x

Sedgley, C., Lennan, S., & Appelbe, O. 2005. Survival of *Enterococcus faecalis* in root canals *ex vivo*. International Endodontic Journal, 38(10): 735-742
DOI:https://doi.org/10.1111/j.1365-2591.2005.01009.x

Shafiq, N. E., & Mahdee, A. F. 2023. *Moringa oleifera* use in maintaining oral health and its potential use in regenerative dentistry. The Scientific World Journal,
DOI: https://doi.org/10.1155/2023/8876189

Shafiq, N. E., Mahdee, A. F., & Mohammed Hasan, Z. Y. 2024. Leaf extracts of *Moringa oleifera* cultivated in Baghdad: characterization and antimicrobial potential against endodontic pathogens. The Scientific World Journal, 2024
DOI: https://doi.org/10.1155/2024/6658164

Shaikh, J. R., & Patil, M. 2020. Qualitative tests for preliminary phytochemical screening: An overview. International Journal of Chemical Studies, 8(2): 603-608 .DOI: https: // doi .org / 10.22271 / chemi .v8. i2i .8834

Siqueira Jr, J. F. 2002. Endodontic infections: concepts, paradigms, and perspectives. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology, 94(3): 281-293 .
DOI:https://doi.org/10.1067/moe.2002.126163

Yaaqoob, L. 2022. Evaluation of the biological effect synthesized iron oxide nanoparticles on *Enterococcus faecalis*. Iraqi Journal of Agricultural Sciences, 53(2): 440-452

Zoletti, G., Siqueira Jr, J., & Santos, K. 2006. Identification of *Enterococcus faecalis* in root-filled teeth with or without periradicular lesions by culture-dependent and—independent approaches. Journal of Endodontics, 32(8): 722-726 . DOI:https://doi.org/10.1016/j.joen.2006.02.001

Zou, Y., & Fang, W. 2021. Naturally derived glycosides with potential activity against *Staphylococcus aureus*. Current Topics in Medicinal Chemistry, 21(27): 2500-2512 . DOI:https://doi.org/10.2174/1568026621666211015091539

Iraqi Journal of Agricultural Sciences –2026:57(2):639-650
P-ISSN: 0075- 0530 /E-ISSN: 2410-0862
DOI: https://doi.org/10.36103/0bbwap73

تقييم تأثير البابونج الألماني ضد *Enterococcus faecalis* المعزولة من قنوات جذور الاسنان

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المستخلص

تهدف هذه الدراسة فحص المكونات الفعالة لمستخلص البابونج الألماني ونشاطها المضاد ضد المكورات المعوية الوازية المعزولة سابقا من قنوات جذور الاسنان. تم الحصول على المستخلص الكحولي المائي للبابونج بطريقة النقع. استخدمت الاختبارات الكيميائية النباتية و كروماتوغرافيا الطبقة الرقيقة للكشف عن المكونات الفعالة في المستخلص. استخدمت طريقة انتشار الاكار لمقارنة الفعالية المضادة للميكروبات لهيبوكلوريت الصوديوم 3٪ و 4 تراكيز من المستخلص (100ملغم/مل-400ملغم/مل) ضد المكورات المعوية الوازية. تم دراسة تاثير البابونج المضاد للأغشية الحيوية ضد الأغشية الحيوية للمكورات المعوية الوازية باستخدام فحص لوحة معايرة دقيقة مع قارئ ELISA. أظهرت الكيمياء النباتية أن المركبات التانينية والجليكوسيدية والقلويداتية والفلافونويدية والمواد الكيميائية الفينولية المتعددة كانت وفيرة. كشفت كروماتوغرافيا الطبقة الرقيقة عن وجود مركبات فينولية مثل؛ حمض الكافيك، وحمض الكلوروجينيك، وغيرها، و فلافونويدات مثل؛ أبيجينين، كاتشين، وغيرها. أظهر انتشار الاكار قطر منطقة تثبيط أعلى لـ 200ملغم/مل، 300ملغم/مل، و400ملغم/مل بابونج مقارنة بـ 3% هيبوكلوريت الصوديوم. أظهر المستخلص النباتي تثبيطًا قويًا للأغشية الحيوية للمكورات المعوية الوازية بجميع تراكيزه بينما تثبيط هيبوكلوريت الصوديوم كان متوسطًا.

الكلمات المفتاحية: الكيمياء النباتية ,ري قناة الجذر، علم الأحياء الدقيقة اللبية، فحوصات مضادات الميكروبات، مستخلص نباتي، بكتريا.

***جزء من رسالة الماجستير للباحث الأول.**