ORIGINAL ARTICLE

Can Ethanolic Leaf Extract of Olive or Black Mulberry Substitute Sodium Hypochlorite as a Root Canal Irrigant? An *In Vitro* Study

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Abstract

Aim: This study aimed to evaluate *Olea europaea* (olive) leaves and *Morus nigra* (black mulberry) leaves as potential natural alternatives to sodium hypochlorite (NaOCl) endodontic irrigant. Their antimicrobial activity against *Enterococcus faecalis* (*E. faecalis*) and their effects on both root dentin microhardness and push-out bond strength of resin sealer/root dentin were assessed.

Methodology: Fifty-four extracted teeth were selected. Samples were grouped according to the irrigant used: group I (control): 2.5% NaOCl, group II: 8% ethanolic extract of *Olea europaea*, and group III: 2% ethanolic extract of *Morus nigra*. Antibacterial activity ($n = 6$) was evaluated after each canal was autoclaved, inoculated with *E. faecalis*, and incubated. Canals were sampled before and after chemicomechanical canal preparation with 2 mL of irrigant. The colony-forming units (CFUs) were counted at 1/10 and 1/100 broth concentrations. Vickers hardness number (VHN) of root dentin ($n = 6$) was measured before and after root canal preparation and irrigation. Push-out bond strength testing ($n = 9$) was carried out following preparation, irrigation, obturation, and thermocycling. Results were considered statistically significant at $p \leq 0.05$.

Results: Following irrigation, the CFUs of *E. faecalis* were significantly reduced with no significant difference in the CFU count between all groups at both broth concentrations. A significant reduction in root dentin microhardness resulted in all groups following irrigation, with *Morus nigra* resulting in the lowest percentage reduction (26.42 $\pm$ 1.12). The lowest significant mean push-out bond strength was revealed in the *Olea europaea* group (3.372 $\pm$ 1.513 MPa).

Conclusion: The use of 2% mulberry (*Morus nigra*) leaf extract and 8% olive (*Olea europaea*) leaf extract as alternatives to NaOCl provides promising antimicrobial action against *E. faecalis*.

Clinical significance: 2% *Morus nigra* extract may represent a promising natural endodontic irrigant.

Keywords: Endodontic irrigant, *Enterococcus faecalis*, Herbal extracts, Microbiology, Microhardness.

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Introduction

Successful endodontic treatment is dictated by removing all tissue remnants and microorganisms from the root canal system and preventing recurrent infection. Instrumentation removes microorganisms from the accessible canal parts, whereas irrigation removes tissue debris and disinfects the root canal space.[1] If microorganisms are left behind, they may recolonize within the canal increasing the risk of failure.[1,2] *Enterococcus faecalis* (*E. faecalis*), gram-positive facultative anaerobe, is among the microorganisms isolated from root canals with both primary and recurrent infections.[3,4] This was attributed to its ability to survive in environments with poor nutrition and in extreme environments with low pH and high temperatures.[4] Therefore, using endodontic irrigants, with antimicrobial properties against *E. faecalis*, is mandatory for successful endodontic treatment.

Sodium hypochlorite (NaOCl), with a concentration from 0.5 to 5.25%, is considered the gold standard root canal irrigant owing to its excellent antimicrobial activity[2,5] and its ability to dissolve pulpal remnants and organic components of dentin.[1,6] However, it is toxic at high concentrations and can cause tissue irritation and chemical burns upon extrusion beyond the root apex.[2,5,7,8] Moreover, long-term exposure of root dentin to high concentrations of NaOCl can impair dentin microhardness and flexural strength, which may eventually lead to vertical root fracture.[9,10] Furthermore, 1% NaOCl may compromise the bond strength of a total-etch adhesive.[11]

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Due to the side effects of synthetic irrigants, natural alternatives have been investigated.[12–14] Thousands of plant species have potential medical uses owing to their high flavonoid content. Flavonoids are a class of plant secondary metabolites that protect plants from microorganisms, fungi, and insects. Several flavonoid-containing natural products (e.g., green tea and chamomile) have been investigated for use as endodontic irrigants.[12]

Olive leaf (*Olea europaea*) extract has been used for medicinal purposes for decades. Its therapeutic effect is attributed to the synergistic effect of the different plant constituents.[15]

Olive leaf extract was reported as an effective treatment for oral infections such as candida infections and Epstein-Barr virus. Al-Sabawi et al. (2009) evaluated the antimicrobial effects of 0.8%

olive leaf extract as an endodontic irrigant vs 2.5% NaOCl. They reported that both 2.5% NaOCl and 0.8% *Olea europaea* had a comparable antimicrobial effect against *E. faecalis in vitro*.[16] However, the effect of *Olea europaea* on the mechanical properties of root dentin has not been investigated.

Black mulberry *(Morus nigra)* is another flavonoid-containing widely distributed natural product.[17] It has a long history of medical use in Chinese medicine.[18] Studies reported that black mulberry is rich in anthocyanins and alkaloid compounds that are responsible for its different biological and medical properties.[19]

Souzaa et al. (2018) reported that ethanolic extracts of *Morus nigra* were bactericidal against *E. faecalis* and *E. coli* with a minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) less than 0.195 mg/mL for both bacteria.[17] Moreover, *Morus nigra* was reported effective against four bacterial cariogenic strains.[20]

Despite its wide use in the medical field,[21–23] to the best of our knowledge, *Morus nigra* has not been investigated for use as an endodontic irrigant. Therefore, the present study evaluated both *Olea europaea* and *Morus nigra* extracts as natural irrigant alternatives to NaOCl in terms of their antimicrobial activity against *E. faecalis* and their effects on both root dentin microhardness and push-out bond strength of resin sealer/root dentin. The null hypothesis was that there is no significant difference between NaOCl, *Morus nigra,* and *Olea europaea* in terms of their antimicrobial activity against *E. faecalis*.

Materials and Methods

Preparation of the Phytochemical Ethanolic Extracts

Two phytochemicals—*Olea europaea* (olive leaves) and *Morus nigra* (mulberry leaves)—were obtained from the National Agricultural Research Centre and were used in the current study. A voucher specimen for the *Olea europaea* (NGB-12107) is available in the National Gene Bank and Genetic Resources Center, Giza, Egypt, and was identified by H Sayed. For the Morus nigra, the voucher specimen (CAI-78b) is available in the Cairo University Herbarium and was identified by S Sisi.

The phytochemical ethanolic extracts were prepared using the cold simple maceration technique.[24] For each irrigant, 500 g of leaves were washed, dried overnight, and ground using a mechanical grinder. Powder from olive and mulberry leaves was diluted into 3.5 and 2.5 L of 99% ethyl alcohol, respectively. Following extraction, excess alcohol was evaporated using a rotary flash. The concentrated extracts were stored at –5°C until needed. Immediately before use, olive and mulberry leaf extracts were diluted to a concentration of 8 and 2%, respectively.

Sample Size Calculation and Sample Selection

Sample size was calculated using G*Power to detect a large effect size ($f = 0.40$) with a power of 80 and 5%.[11] Caries-free permanent incisors or premolar teeth extracted due to periodontal or orthodontic reasons were utilized following the approval of the Research Ethics Committee, Faculty of Dentistry, Cairo University (REC# 13-12-41). The teeth were cleaned and stored in distilled water. They were randomly grouped into three groups according to the type of irrigant applied: group I (control): 2.5% NaOCl, group II: 8% ethanolic extract of *Olea europaea*, and group III: 2% ethanolic extract of *Morus nigra*.

Chemical Analysis of the Active Compounds of the Phytochemical Extracts

Determination of the Total Flavonoid and Phenolic Contents of the Prepared Solutions

Total flavonoid content was determined using the aluminum chloride colorimetric method.[25] Each dried plant extract was mixed with methanol, 10% aluminum chloride solution (Qualikems Fine Chem. Pvt., Ltd., India), potassium acetate, and distilled water. The absorbance of the mixture was measured at 415 nm using a spectrophotometer (Jasco V-630 Spectrophotometer, Japan). The total flavonoid content was calculated from a calibration curve for quercetin. Results were expressed in terms of mg of quercetin equivalent per gram of dry extract.

The total phenolic content was determined by the Folin–Ciocalteu method.[26] One gram of the natural extract was mixed with 1 mL of Folin–Ciocalteu (Qualikems Fine Chem. Pvt., Ltd., India), followed by the addition of 20% saturated sodium carbonate solution. The absorbance of the mixture was measured at 725 nm. The total phenolic content was calculated with reference to the standard curve for gallic acid. Results were expressed in terms of mg of gallic acid equivalent per gram of dry extract weight.

Determination of Antioxidant Properties

The antioxidant activity of the natural extracts was determined via 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay.[27] A blank solution of 0.1 mM of DPPH (Sigma Aldrich, Germany) in methanol was prepared. One milliliter of this solution was added to 3 mL of the dried natural extract. The absorbance of both solutions—the blank and the one with the active extract—was measured using a spectrophotometer at 517 nm. The ability of the extracts to scavenge the DPPH radical was calculated using the following equation:

$$\textbf{DPPH scavenging effect}\ (\%) = (A_0 - A_1)/\ A_0 \times 100$$

where A_0 is the absorbance of the blank solution containing all the reagents except the investigated test compounds and A_1 is the absorbance in the presence of all tested extracts.

Antimicrobial Activity

For this test, an additional group utilizing absolute ethanol as an irrigant was included: group IV. Antimicrobial activity was evaluated by means of the "serial dilution test" described by Vijaykumar et al. (2010).[28] Twenty-four teeth, six for each irrigant ($n = 6$), were used to evaluate the antimicrobial activity of each irrigant.

Root Canal Contamination with E. faecalis

The teeth were decoronated and autoclaved, and then, each root canal was inoculated with a standardized strain of *E. faecalis* suspension (ATCC 29212) using a micropipette. After inoculation, the teeth were incubated at 37°C for 48 hours. Before instrumentation, three sterile paper points, size 15 or 20, were used to obtain samples from the most apical part of each root canal. The obtained samples were transferred to test tubes containing brain heart infusion (BHI) broth (Lab M Limited, UK). One milliliter of broth was aspirated and evenly distributed into each bile esculin (Himedia, France) agar media plate. The plates were incubated at 37°C for 48 hours and the number of colony-forming units (CFUs) was counted manually (N_1).

Root Canal Preparation

Mechanical root canal preparation was performed using the step-back technique[1] coupled with the application of the

corresponding irrigants: group I (control): 2.5% NaOCl, group II: 8% ethanolic extract of *Olea europaea*, group III: 2% ethanolic extract of *Morus nigra*, and group IV: absolute ethanol. After each file was used, 2 mL of the irrigant was introduced inside the canal at a rate of 1 mL irrigant over 15 seconds.[29] With approximately 15 files used during the canal preparation, the total irrigation time was equal to an average of 450 seconds (7.5 minutes).

Sample Collection

Samples from each root canal were obtained using three paper points, size 35 or 40, inserted to the depth of each canal, and transferred to test tubes containing BHI broth. Two broth dilutions, 1/10 and 1/100, were separately incubated in bile esculin agar plates. The number of CFUs after irrigation was manually counted (N_2 and N_3, respectively) as described in Section "Root canal contamination with *E. faecalis*."

Measurement of Dentin Microhardness

The roots of nine teeth were bisected longitudinally. The sections were randomly grouped ($n = 6$) for each irrigant and were embedded in resin blocks, leaving the root canal dentin exposed. The specimen surface was cleaned, finished, and polished. Before irrigation, the baseline Vickers hardness number (VHN) of the exposed dentin was measured for each specimen using a Vickers microhardness testing machine (BUEHLER® OmniMet® MHT) by applying a perpendicular 100 g load at three different sites. For each site, two readings were taken and averaged (MH_1). The specimens of each group were immersed in the corresponding irrigant, which was replaced every minute for a total immersion time of 10 minutes. The specimens were finally flushed with distilled water and dried. Dentin microhardness value was then measured in the same way as the baseline measurement (MH_2), and the percentage change was calculated.

Resin Sealer/Dentin Bond Strength Testing

Twenty-seven teeth ($n = 9$/group) were decoronated and mechanical root canal preparation was performed using the same technique described in Section "Root canal preparation". The canals were then dried and obturated using gutta percha points (Meta Biomed, Korea) and AH 26 silver-free resin sealer (Dentsply, Germany). The obturated roots were stored in saline-moistened sterile gauze at 37°C for 7 days.

Artificial aging was performed by thermocycling (5,000 cycles in a water bath between 5 and 55°C, 20 seconds of immersion, and 3 seconds of transfer time).[30] Each root was then horizontally sectioned into three 2-mm-thick segments. The sections were aligned over the Instron Universal Testing Machine (Model 3345, England) support plate. Push-out test was performed by applying a compressive load (at a cross-head speed of 0.5 mm/minutes using a 500 Newton load) to the apical aspect of each slice until the first dislodgment of the obturating material. The push-out bond strength in megapascals (MPa) was calculated using the following equation: [31]

Push-out bond strength (MPa) = maximum failure load (N)/bonded area of root canal filling material (mm^2)

Statistical Analysis of the Results

Data were listed as means and standard deviation (SD), except when nonnormality was detected, where means, medians, and interquartile ranges were reported. Descriptive statistics were calculated using Minitab 19. Normality was assessed using the Shapiro–Wilk test. One-way analysis of variance (ANOVA) was used to test the differences among groups when a normal distribution of results was obtained. Comparison of means was carried out using the Tukey's *post hoc* multiple comparisons test at $p \leq 0.05$. The statistical analysis of nonparametric findings was carried out using the Kruskal-Wallis test. Pairwise comparisons using Dunn's test were performed at $p \leq 0.05$. The effect of treatment on root dentin microhardness was analyzed using a paired *t*-test.

Results

Active Compounds of the Phytochemical Extracts

Compared to *Morus nigra* (group III), *Olea europaea* (group II) possessed higher phenolic (10.095 vs 5.555 g/100 g) and flavonoid (1.597 vs 1.348 mg/100 g) content, as well as greater DPPH-free radical scavenging activity (83.101 vs 70.049%); (Table 1).

Antimicrobial Activity of the Investigated Irrigants

Following inoculation of *E. Faecalis*, the number of bacterial colonies was uncountable. After irrigation, the number of colonies within the root canals decreased. Kruskal–Wallis one-way ANOVA revealed that there was no statistically significant difference ($p = 0.942$) in the number of CFUs at broth concentration 1/100 between all four groups: group I (2.5% NaOCl) (median 0, mean 0.4 ± 0.5), group II: 8% *Olea europaea* (median 0, mean 0.4 ± 0.5), group III: 2% *Morus nigra* (median 0, mean 0.2 ± 0.4), and group IV: Ethanol (median 0, mean 1.2 ± 2.7); (Figs 1 and 2).

On the contrary, at 1/10 broth dilution, Kruskal–Wallis one-way ANOVA revealed a statistically significant difference in CFUs count, among the different groups ($p = 0.025$). Dunn's test pairwise comparisons revealed that *Olea europaea* resulted in a statistically significantly lower number of CFUs compared to ethanol ($p = 0.015$). However, there was no significant difference in the CFU count of 2.5% NaOCl (median 10, mean 10.2 ± 10.8), 8% *Olea europaea* (median 2, mean 1.6 ± 0.9), 2% *Morus nigra* (median 4, mean 3.4 ± 1.5), and ethanol (median 0, mean 16.4 ± 8.4) groups (Fig. 3).

Root Dentin Microhardness

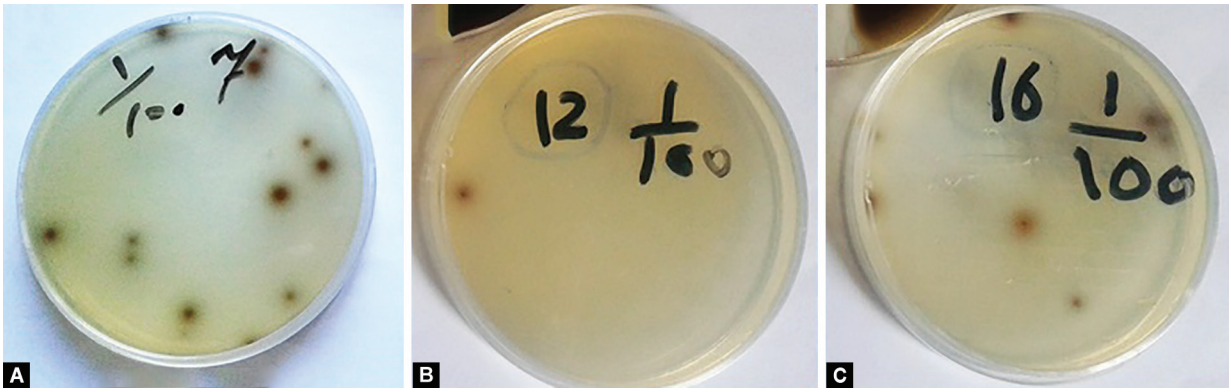
A paired *t*-test revealed a significant reduction in root dentin microhardness in all test groups after treatment ($p \leq 0.001$) (Table 2). One-way ANOVA revealed that the percent reduction in microhardness values of root dentin (Table 3), calculated before and after VHN values listed in Table 2, was significantly different among the groups ($p = 0.008$), where *Morus nigra* (group III) resulted in the lowest percent (%) reduction (26.42 ± 1.12) compared to both group I: NaOCl (29.16 ± 2.79) and group II: *Olea europaea* (31.13 ± 1.13).

Resin Sealer/Dentin Push-out Bond Strength

One-way ANOVA revealed a statistically significant difference in the resin sealer push-out bond strength between the groups ($p \leq 0.001$). Tukey's pairwise multiple comparisons revealed that group II (8% *Olea europaea*) had the lowest mean push-out bond strength value (3.37 ± 1.51 MPa) compared to group I 2.5% NaOCl

Table 1: Mean value of total phenols, total flavonoids, and percentage of DPPH-free radical scavenging activity for the prepared extracts

Extract	*Total phenols, g/100 g*	*Total flavonoids, mg/100 g*	*DPPH%*
Olea europaea	10.095	1.597	83.101%
Morus nigra	5.555	1.348	70.049%



Figs 1A to C: Colony-forming units on bile esculin agar plates. (A) 2.5% NaOCl; (B) 8% *Olea europaea;* and (C) 2% *Morus nigra*

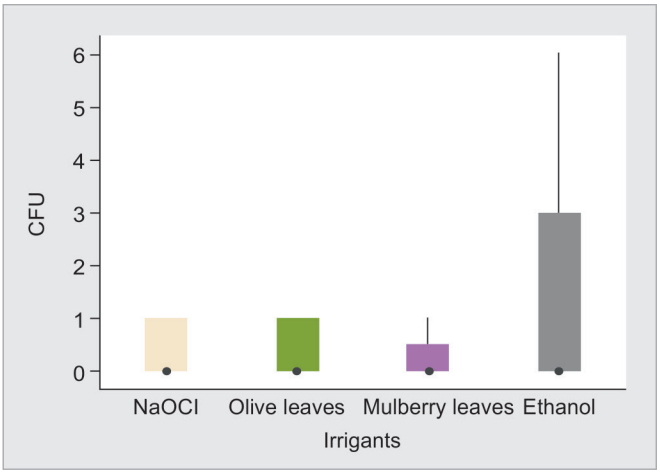


Fig. 2: Box plot representing the bacterial count in CFUs of the different groups at broth concentration (1/100) showing no statistical difference between groups at $p \leq 0.05$. The lower and upper boundaries of the box represent the 25th and 75th percentiles, respectively. Each dotted line represents the median of the corresponding irrigant

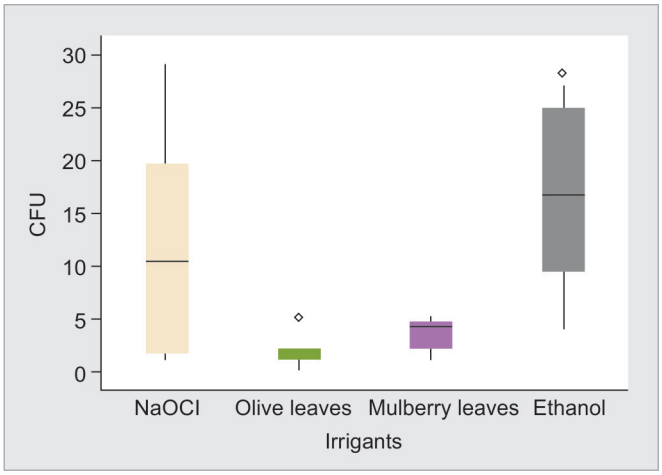


Fig. 3: Box plot representing the bacterial count in CFUs of the different groups at broth concentration (1/10). The groups sharing similar symbols (◊) are statistically significant different at $p \leq 0.05$

Table 2: Descriptive statistics of root dentin microhardness (VHN) before and after irrigation

Group	*Root dentin microhardness (VHN) before irrigation**	*Root dentin microhardness (VHN) after irrigation**	*Reduction* (VHN)*	*t value*	*p value*
I NaOCl	83.56 ± 2.97	59.15 ± 1.76	24.41 ± 3.06	19.562	0.001**
II *Olea europaea*	85.52 ± 1.06	58.90 ± 1.25	26.62 ± 1.0	65.895	0.001**
III *Morus nigra*	82.66 ± 1.23	60.82 ± 1.135	21.84 ± 1.06	50.278	0.001**

*Means ($n = 6$) ± standard deviations: p = probability level for the effect of irrigation (paired t-test), ** = significant at $p \leq 0.001$

Table 3: Results of one-way ANOVA for comparison between the percentage (%) reduction in root dentin microhardness of different test groups after irrigation

Group	*% Reduction* Mean ± SD*	*F value*	*p value*
Group I NaOCl	29.16[a] ± 2.79		
Group II *Olea europaea*	31.13[a] ± 1.13	9.011	0.003**
Group III *Morus nigra*	26.42[b] ± 1.12		

*Means ($n = 6$) ± standard deviations: **Means with the same letter within the column are not significantly different at $p \leq 0.05$

(6.29 ± 1.49 MPa) and group III: 2% *Morus nigra* (5.62 ± 1.96 MPa), which were not significantly different (Fig. 4).

Discussion

Since the failure of properly cleaned and shaped root canals has been attributed to resistant microorganisms, the effectiveness of endodontic irrigants their usually measured by its antimicrobial activity against resistant microorganisms as *E. faecalis*.[1] Despite its side effects, NaOCl is still considered the gold standard for endodontic irrigation.[2,9,32] Natural flavonoid-containing

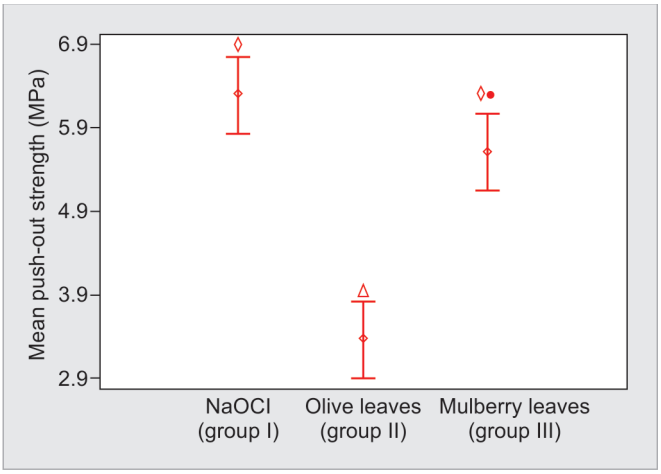


Fig. 4: An interval plot illustrating the mean values of the push-out bond strength of resin sealer to irrigated root dentin (MPa) and 95% least significant difference (LSD) intervals for the investigated irrigants: group I (NaOCl: 6.29 ± 1.49), group II (olive leaves: 3.37 ± 1.51), and group III (mulberry leaves: 5.62 ± 1.96). Different symbols (Δ●◊) denote statistically significant differences between groups at ($p \leq 0.05$)

phytochemicals may provide safer alternatives to NaOCl.[12] In the present study, two natural flavonoid-containing plants—olive (*Olea europaea*) leaves and mulberry (*Morus nigra*) leaves—were assessed as potential endodontic irrigants. Both mulberry leaf and olive leaf extracts have been reported effective against *E. faecalis*.[17,33] Despite such antimicrobial effect, Bavabeedu et al. (2018) reported that upon using extra virgin olive oil as an intracanal medicament, all plates revealed a lack of direct antibacterial activity.[34]

Several methods (such as maceration, percolation, decoction, digestion, and infusion) have been reported for the preparation of medicinal plants extracts. In the present study, the ethanolic extracts of the natural products were prepared using the "simple cold maceration" technique. The simple maceration is the technique of choice in preparing extracts made from plant roots, stems, or leaves. It involves soaking the prepared dry plant powder in a suitable solvent at room temperature.[24] Dehydration of plant leaves may be achieved by different drying methods, most commonly thermal drying through natural convection (such as shade and open sun drying).[35] Most authors who reported the use of ambient temperature in the shade for drying did not specify the exact drying time. Such time will be strongly affected by the climate temperature since complete dryness of the leaves is reached when no further change in their weight, owing to water loss, takes place. Iwansyah et al. (2020) reported that the extract resulting after tray drying of olive leaves in the shade at 45°C for 4 hours had higher total phenolic contents, total flavonoids contents, and free-radical scavenging capability compared to that after sun drying.[36] Since the present study was conducted during the summer where the temperature reached 45°C, overnight drying was found sufficient as the leaves maintained constant weight thereafter.

Ethanolic solvents are more suitable for extracting the active components of medicinal plants.[37] They were used to prepare the phytochemical extracts. Although long-term alcoholic exposure may result in cytotoxicity above certain threshold concentrations,[38,39] in the case of endodontic irrigation, such toxicity is not a potential hazard because the total time of irrigation does not usually exceed an average of 450 seconds. Moreover, ethanol will dry out rapidly from the canal, owing to its volatile nature, hence, reducing the time of direct contact to tissues.

The concentration of the investigated olive leaf extract irrigant was selected based on an average MIC value for olive leaf extract on *E. faecalis*.[33,40] The MIC of *Morus nigra* on *E. faecalis* was less than 0.195 mg/mL.[17] The 2.5% concentration of NaOCl was selected because concentrations higher than 2.5% are reported to be highly toxic,[41] whereas concentrations as low as 1% possess minimal antimicrobial action.[42]

In the current study, the serial dilution method was used to evaluate the antibacterial activity of the irrigants against *E. faecalis*. In order to better simulate the clinical condition, the technique described by Vijaykumar et al. (2010) was modified. Two ml of irrigant was introduced in the canal over 30 seconds following each file size rather than keeping the irrigant in contact with root dentin for 5 minutes. The antimicrobial activity against *E. faecalis* of both 8% *Olea europaea* and 2% *Morus nigra* irrigants was equivalent to that obtained by 2.5% NaOCl (Figs 2 and 3). Thus, our results failed to reject the null hypothesis regarding the antibacterial activity of the investigated irrigants. In order to confirm that the obtained results were due to the antimicrobial effect of the natural products rather than their ethanolic content, an additional group of teeth was prepared and tested (group IV: 99% absolute ethanol). Since the results of antimicrobial effect at broth concentration 1/10 revealed that the only significant difference was between 8% *Olea europaea* (group II) and ethanol (group IV); therefore, the higher antimicrobial action of the tested natural products could be attributed to their active constituents (Table 1) rather than their alcoholic content. The antimicrobial effect of olive leaf extract (group II) was similar to that reported by Al-Sabawi et al. (2009), who reported that the 0.8% olive leaf extract had an antimicrobial effect on *E. faecalis* similar to that of 2.5% NaOCl.[16]

For all test groups, a reduction in the VHN values was evident after irrigation (Tables 2 and 3). These findings are in accordance with those of Saleh et al. (1999), Slutzky-Goldberg et al. (2004), and Das et al. (2014) who reported that the use of endodontic irrigants decreased the root dentin microhardness values. It is worth noting that the lowest % reduction was recorded after irrigation with *Morus nigra* (group III) (Table 3). However, the clinical reliability of these results is still questionable since no reported *in vitro* values of root dentin microhardness have been correlated with clinical performance. Despite a significant reduction in dentin microhardness following irrigation with NaOCl, it is still considered the gold standard of endodontic irrigation. This suggests that the investigated irrigants may perform in a similar manner clinically.

In order to evaluate the effect of the irrigants on resin sealer bonding to root dentin, a push-out bond strength test was performed. This is an efficient, reproducible, and easily interpreted test.[43] According to the ISO TR 11450 standard (1994), thermocycling (500 cycles in water between 5 and 55°C) is an appropriate artificial aging test. However, such a number of cycles was considered too low to achieve a realistic aging effect.[44] Therefore, to obtain more clinically reliable results, we increased the number to 5,000 cycles.

In the current study, black mulberry leaf extract resulted in a higher sealer/dentin bond strength compared to *Olea europaea*. This may be attributed to its anthocyanin proanthocyanidin (PA) content.[45] PA increases the dentin/adhesive resistance to biodegradation because it functions as a dentin-collagen matrix stabilizer.[46] Liu et al. (2013) reported that treating dentin with PA for as little as 10 seconds

enhanced the resistance of collagen toward enzymatic challenge.[47] This may also be attributed to its vitamin C content[48] which has been reported to act as a matrix metalloproteinase inhibitor maintaining the dentin adhesive interface.[49]

Conclusion

The present research provides a baseline in the study of the antimicrobial properties and effect of olive leaf and mulberry leaf extracts on root dentin. To the best of our knowledge, this is the first study to evaluate the effects of 2% mulberry (*Morus nigra*) leaf extract and 8% olive (*Olea europaea*) leaf extract on both root dentin microhardness and the push-out bond strength of resin sealer/root dentin. Both phytochemicals have antibacterial properties together with acceptable effects on root dentin in terms of microhardness and push-out resin/sealer bond strength.

Additional studies are needed to conclude whether mulberry leaf extract can successfully replace NaOCl or can only serve as an adjunctive treatment. In-depth studies on the ability of mulberry leaf extract both to dissolve organic tissues and to remove the smear layer are essential. Preclinical and clinical trials are also required to evaluate its biocompatibility and safety prior to clinical use.

Clinical Significance

Not only do these provide natural irrigant alternatives, but also they act on minimizing the waste such as that arising from the olive oil industry. Among the investigated phytochemicals, 2% mulberry leaf extract may be a promising endodontic irrigant in terms of its antimicrobial activity together with its effects on both root dentin microhardness and rein sealer/dentin push-out bond strength.

Credit Author Statement

RO Ibrahim carried out the conceptualization and methodology and prepared the original draft. A Amin supervised and managed the project. RA Salama did the conceptualization and formal analysis, and she reviewed and edited the draft and also supervised the project with A Amin. All authors approved the manuscript and gave consent for publication.

Data Availability Statement

The data set used in the current study is available on request from RA Salama (rania.salama@dentistry.cu.edu.eg).

Ethical Statement

All experiments were conducted following the approval of the Research Ethics Committee, Faculty of Dentistry, Cairo University (REC# 13-12-41).

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