***ISSN:* 1812–1217**

The Antimicrobial Effect of Alcoholic Extract of Olive Leaves as a Root Canal Irrigant.

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ABSTRACT

Aims: The study aims to evaluate the antimicrobial effect of different concentrations of alcoholic extract of Olive Leaves solution (0.1%, 0.2%, 0.4%, 0.6%, 0.8%) in an in vitro.It also to determines the time required for the most effective concentration of Olive Leaves to start the effect in selected microorganisms, and to evaluate the antimicrobial effect of most effective concentrations of alcoholic extract of Olive Leaves solution in an in vivo study as a root canal irrigant. **Materials and Methods:** The antimicrobial effect of Olive Leaves (0.1%, 0.2%, 0.4%, 0.6%, 0.8%), 2.5% sodium hypochlorite, and normal saline were determined in vitro by using broth microdilution method. The direct exposure test was used to evaluate the time required for 0.8% of Olive Leaves to start their antimicrobial effect on the selected microorganisms. In an in vivo study, 36 uniradicular teeth with necrotic pulps were chosen. The patients were divided randomly into three groups, 12 patients for each group as in the following: Group I: alcoholic extract of Olive Leaves at 0.8%. Group II: sodium hypochlorite solution at 2.5% as a positive control. Group III: normal saline as a negative control. Bacteriological samples were obtained from the canal at the beginning of the first appointment; at the beginning of the second appointment; at the end of the second appointment; at the beginning of the third appointment using sterile wet paper point. **Results:** The Results showed that Olive Leaves at (0.2%, 0.4%, 0.6%, 0.8%), and sodium hypochlorite had a significant antimicrobial effect against aerobic and anaerobic bacteria recovered from teeth with necrotic pulps. While the normal saline and 0.1% Olive Leaves had no significant antimicrobial effect. As for Olive Leaves exract. The best antimicrobial effect was noticed at 0.8%, which showed a significant difference from other concentrations of Olive Leaves,but no significant difference from that of sodium hypochlorite. The results of this test showed that 2.5% sodium hypochlorite and 0.8% Olive Leaves had immediate effect on all selected microorganisms. The results revealed that 0.8% alcoholic extract of Olive Leaves solution had a significant antimicrobial effect when utilized clinically as an endodontic irrigant, which was not significantly differ from sodium hypochlorite but significantly different from normal saline. **Conclusions:** Olive Leaves alcoholic extract solution at 0.8% was an effective antimicrobial agent when used as an irrigant in endodontic treatment of teeth with necrotic pulps.

Key Words: Antimicrobial Effect, Alcoholic Extract, Olive Leaves, Root Canal Irrigant.

Al-Sabawi NA, Al-Shakir NM, Kamel JH. Sealing Ability Of Different Retrograde Filling Materials. *Al–Rafidain Dent J*. 2009; 9(1): 136-148.

Received: 4/3/2008 ***Sent to Referees***: 4/3/2008 ***Accepted for Publication:*** 20/4 /2008

INTRODUCTION

The elimination of bacteria present in the root canal system is the fundamental objective of endodontic treatment as they play an important role in the development and maintenance of periapical lesions[1,2].

The irrigant solutions are very important during the root canal preparation, because they aid in the cleaning of root canal, lubricate the files, flush out debris, and have an antimicrobial effect and tissue dissolution, without damaging the periapical tissues. The selection of an ideal irrigant depends on its action on microorganisms and periapical tissues[3,4].

Sodium hypochlorite solution has been largely used in the endodontic treatment of teeth with necrotic pulp and periapical

lesions. The antimicrobial activity, capacity to dissolive organic tissue, low surface tension and detergent action are important properties attributed to the sodium hypochlorite [5,6,7,2].

The *Olive* tree has been called the Tree of Life. Olive Leaves Extract has a natural antibiotic action. Researchers have found that olive leaves extract destroys virus, bacteria and fungus. This means that it is beneficial for bacterial infections and conditions where antibiotics are ineffective, such as viral infections like colds, flu, herpes and fungal problems like candida. It is a non-toxic way to strengthen the immune system. Olive leaves is an effective killer of harmful bacteria, virusis, yeast and fungi [8].

This study was carried out to evaluate the antimicrobial effect of different concentrations of alcoholic extract of Olive Leaves solution (0.1%, 0.2%, 0.4%, 0.6%, 0.8%) in an in vitro, also to determine time required for the most effective concentration of Olive Leaves to start its effect in selected microorganisms, and to evaluate antimicrobial effect of most effective concentration of alcoholic extract of Olive Leaves solution in an in vivo study as a root canal irrigant.

MATERIALS AND METHODS

Alcoholic Extraction of Olive leaves:

Five hundred grams of Olive leaves were cleaned and washed, then ground to the powder with the commercially available food blender (S.S/ GLASSCO), and then 120ml of 60% ethanol were added to 40g of powder in a sterile well capped flask, left for 3 days at room temperature and then filtered through several layers of gauze. The resultant mixture was again passed through No.1 filter paper to get ridoff the gross remnants of mixture. The resultant extract was then dried at very low temperature and high vacuum in the lyophilizer (EDWARDS/ Moduly/ England) machine and stored in sterile screw capped vials in the refrigerator until needed for use and then freshly prepared in distilled water

Patient Selection:

Patients included in this study are from those attending Department of Conservative Dentistry, College of Dentistry, University of Mosul. Twenty patients of both sexes were included in this study. Their age ranged from 18-40 years, and have no history of systemic diseases. None of the patients were treated with antimicrobial agents at the time of sampling. Twenty uniradicular teeth with opened necrotic pulp were chosen. Pulp necrosis was determined by radiographic presence of apical rarefaction and lack of response to pulp vitality test by using electric pulp tester (Dentotest TB 09, Germany). There should be no fistula or sinus at the time of selection [10,11].

Sample Collection:

Microbiological samples were obtained from the canal at the first appointment. At the beginning of the first appointment, the tooth was isolated with rubber dam (Digflex, India). The tooth surrounding and the clamp (Ash, England) were disinfected with 70% ethanol (Wadi Al-Rafidain Factory for Pharmaceutical Product, Iraq). An access opening was made with a sterile round bur at high speed. Canal length was determined by placing No.10 or No.15 K-type file (Union Broach Co., Long Island City, N.Y., USA) inside the canal. A radiograph was taken and the file length was adjusted within 1mm of the radiographic apex. Sterile absorbent paper points (Produits Dentaires S.A., Vevey) soak previously in distled water were prepared, the two paper points one after one was inserted into the root canal to the apical foramen and left for one minute. With a sterile tweezers, the paper points were removed from the root canal. One of the paper point was placed in the screw capped vial containing 5ml of thioglycollate broth (Oxiod LTD, Basigstoke, Hants/ England) and the other paper point was placed in another screw-capped vial containing 5ml of brain heart infusion broth (Oxiod LTD, Basigstoke, Hants/ England). The samples were then transferred for incubation (Fisher Scientific/ Russia) at 37^{o}C for 18 hours [12,13].

Microbiological Study:

The microbiological study was done in the Microbiology Unit, Department of Dental Basic Sciences, College of Dentistry, University of Mosul. The antimicrobial effect of root canal irrigants and

Olive leaves extract used in this study was determined through using a broth microdilution method. Two types of media were used for this purpose, brain heart infusion broth for aerobic bacteria and thioglycollate broth for anaerobic bacteria. The examined solutions were alcoholic extract of Olive Leaves at concentrations of (0.1%, 0.2%, 0.4%, 0.6%, 0.8%), 2.5% sodium hypochlorite, and normal saline. At least three replicates of each treatment were inoculated as well as the untreated microorganism culture. The same procedure was repeated for thioglycollate broth. The turbidity (microorganism growth) of each vial was measured using spectrophotometer (CEIL CE 1021/ England) at 590 nm W.L. So, in this method there was a control positive vial, control negative vial and treatment vial. A control negative vial represents a turbidity which is caused by examined solution itself, while control positive vial represents a turbidity caused by microorganisms' growth. In order to determine the exact antimicrobial effect of each examined solution, the turbidity of examined solution itself must be excluded [14,15,16,17,18,19].

Direct Exposure Test:

The irrigants used were 0.8% Olive leaves extract solution, 2.5% sodium hypochlorite, and normal saline. This test was carried out to analyze the immediate antimicrobial effect of the tested irrigating solutions. The microbial strains were used in this study include*: Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Klebseilla spp., Pseudomonas aeruginosa,* and *Candida albicans*. All these microorganisms were isolated and identified from clinical samples at the Microbiology Labrotory of College of Dentistry, Mosul Univesity. Unidentified samples from root canals with necrotic pulp also used in this study. Samples of root canal contents were taken in same manner as a previously described. Solutions were evaluated at different time intervals; immediately, 5 min, 10 min and 15 min after exposure and repeated five times. All bacterial samples were cultivated and maintained in proper atmosphere and medium.

The suspension of each bacteria was obtained by inculating a loop full from its culture in 4ml brain heart infusion broth for aerobic microorganisms and in 4ml of thioglycolate for anaerobic microorganisms and incubated at 37 ^{o}C for 18 hours. To standardize the bacterial suspensions, the samples were diluted and counted to obtain a suspension of approximately 10^8 cells/ml. Twenty eight # 80 presterilized absorbent paper points were used for each expermintal sample. Twenty four points were submerged in beakers containing 20 ml of the inculom for at least 5 minutes. The remaining four paper point used as controls. Ten milliliter of one of the sterile test solutions were placed into each of 24 sterile beakers. One paper point from those that had been submerged in the inculom was then placed into each of the 24 beakers containing the test solution. Eight of the points were removed from test solution at interval of immediately, 5 min, 10 min and 15 min. Upon removal, the points were placed individually into a screw capped vial containing 10 ml of brain heart infusion broth for aerobic microorganisms. Each vial was then vortexed for 10 second to minimize the germicidal effect of the residual test solution. After 48 hours incubation at 37 ^{o}C, the presence of turbidity was recorded for each vial. Each vial including those that had a negative and positive growth response in the broth was then subcultured into a blood agar plates for confirmation. These plates were inoculated using sterile cotton swabs. The blood agar plates were incubated at 37^{o}C for 48 hours. The presence of colonies of test microorganism on the pour plates, confirmed by Gram stain, was considered to be positive evidence of growth in the corresponding vial. Two of the remaining four sterilized paper points which were used as a control were placed into the beaker containing the bacterial culture for 5 minutes and then placed into separate vial containing 10ml of brain heart infusion broth. The two remaining sterilized points were placed directly into two vials containing 10ml of brain heart infusion broth. In addition a 0.5ml aliquot of each of the stok test solutions was placed into separate vial that containing 10ml of the broth. After vortexing, the control vials were then incubated and subcultured as described before and results were re-

corded. The same procedure was repeated for thioglycolate broth in which anaerobic bacteria can be growth, except that the blood agar plate which was inoculated with thioglycolate broth was incubated under CO_2 (candle jar) in the incubater [20].

In Vivo Study of Antimicrobial Effect of Olive Leaves Extract and Root Canal Irrigants:

Patient Selection:

Additional thirty six uniradicular teeth were chosen. Patient and teeth have the same criteria as mentioned befor (in an in vitro study). The patients were divided randomly into three groups:

Group I : Twelve patients. In this group, alcoholic extract of Olive Leaves at 0.8% concentration (because it shows the best antimicrobial *effect* among used concentrations of alcoholic extract of Olive Leaves in the in vitro study), was used as a root canal irrigant solution.

Group II: Twelve patients. In this group, sodium hypochlorite at 2.5% concentration was used as a root canal irrigant solution (positive control).

Group III: Twelve patients. In this group, normal saline (0.9% sodium chloride) was used as a root canal irrigant solution (negative control).

Sample Collection:

Microbiological samples were obtained from the canal at each appointment, as follows: At the beginning of the first appointment, samples of the root canal contents were taken in the same manner as mentioned before (in in vitro study). Then the canal was debrided and irrigated with 5ml alcoholic extract of Olive Leaves solution (0.8%), for group I; 5ml sodium hypochlorite solution (2.5%), for group II; and 5ml normal saline solution (0.9% sodium chloride), for group III, for about 30 second. After that the canal was dried with sterile paper points. Then a sterile cotton pellet (without any root canal medicament) was placed in the pulp chamber and sealed with zinc phosphate cement as an inter appointment seal. The samples were then transferred for microbiological study. At the beginning of the second appointment, (one week later), the tooth was isolated, the temporary dressing was removed, and samples of the root canal contents were taken in the same manner as previously described. At this appointment, root canal instrumentation and irrigation procedures were performed. The canal was instrumented with k-type files at least three sizes larger than the first file that fit snuggly within 1mm of the apex, cheked by radiograph. Circumferential filing action was used for instrumentation. The canal was irrigated after each file size with 5 ml of the irrigant related to the group of patients for about 30 second. Canal instrumentation was continued until a clean white dentin was obtained in the flutes of the file. Then the canal was finally irrigated with 10 ml of the related irrigant solution. The canal was then dried with sterile paper points, and samples of its contents were taken again. The samples were then transferred for microbiological study. At the beginning of the third appointment (one week later), samples of root canal contents were taken in same manner as previously described. At this appointment root canal obturation was performed if there are no signs or symptoms contraindicating the procedure. The samples were then transferred for microbiological study [10,21,22,23].

Microbiological Study:

Each screw capped vial was shaken to disperse the sample content evenly. 0.1ml inoculum was taken from the inoculated thioglycollated broth, using micropippete, and inoculated on one blood agar plate, streaked and incubated under CO_2 (candle jar) at 37^oC for 24 hrs. Also 0.1ml inoculum was taken from the inoculated brain heart infusion broth, using micropippete, and inoculated in other blood agar plate, streaked and incubated under aerobic condition at at 37^oC for 24 hrs [10,22,24].The plates were then examined and the number of bacterial colonies were counted.

RESULTS

Results of the In Vitro Studies:

The Antimicrobial Effect of Olive Leaves Extract and Root Canal Irrigants Against Aerobic Microorganisms:

In this study, the analysis of variance at level ($p<0.01$) was performed. The mean

absorbance values in (nm) of the replicates were measured and compared with control group by Duncan's New Multiple Range Test. This was shown in Table (1).

Table (1): Duncan's New Multiple Range Test for Antimicrobial Effect of Olive Leaves Extract and Root Canal Irrigants Against Aerobic Microorganisms.

Examined Solutions	Absorbance Mean (nm)± SD	Duncan's Grouping*
Control +ve Aerobic Microorganisms	0.82±0.07	E
Normal saline	0.80±0.08	E
Sodium hypochlorite 2.5%	0.05±0.03	A
Olive Leaves 0.1%	0.76±0.08	E
Olive Leaves 0.2%	0.52±0.13	D
Olive Leaves 0.4%	0.42±0.16	C
Olive Leaves 0.6%	0.28±0.10	B
Olive Leaves 0.8%	0.06±0.04	A

*= The different letters mean significant difference exists.

The results revealed that Olive Leaves Extract at concentrations of (0.2%, 0.4%, 0.6%, 0.8%), 2.5% sodium hypochlorite had antimicrobial effect significantly different from control group, however, Olive Leaves Extract at 0.1% and normal saline failed to show any significant effect.

For Olive Leaves Extract, the best antimicrobial activity was noticed at 0.8% concentration which showed a highly significant difference from other concentrations of Olive Leaves. 0.4% had antimicrobial effect lower than 0.6% and higher than 0.2% which was significantly different from them. Results also showed that sodium hypochlorite at 2.5% concentration had a highest antimicrobial effect which was significantly not different from Olive Leaves Extract at concentration of 0.8%.

The Antimicrobial Effect of Olive Leaves Extract and Root Canal Irrigants Against Anaerobic Microorganisms:

The same statistical analysis procedure was repeated as mentioned before. The mean absorbance values in (nm) of the replicates were measured and compared with control group by Duncan's New Multiple Range Test. This was shown in Table (2).

Table (2): Duncan's New Multiple Range Test for Antimicrobial Effect of Olive Leaves Extract and Root Canal Irrigants Against Anaerobic Microorganisms.

Examined Solutions	Absorbance Mean (nm)± SD	Duncan's Grouping*
Control +ve Anaerobic Microorganisms	0.87 ±0.05	E
Normal saline	0.83±0.05	E
Sodium hypochlorite 2.5%	0.05±0.03	A
Olive Leaves 0.1%	0.83±0.07	E
Olive Leaves 0.2%	0.65±0.11	D
Olive Leaves 0.4%	0.45±0.11	C
Olive Leaves 0.6%	0.29±0.11	B
Olive Leaves 0.8%	0.06±0.03	A

*= The different letters mean significant difference exists.

The results indicated that all different concentrations of Olive Leaves Extract at concentrations of (0.1%, 0.2%, 0.4%, 0.6%, 0.8%), 2.5% sodium hypochlorite and normal saline had nearly similar results as mentioned before.

Direct Exposure Test:

Both 2.5% sodium hypochlorite and 0.8% Olive Leaves extract eliminated all the microorganisms that were used in this experiment at all times period, while normal saline did not show any antimicrobial effect. This was shown in Table (3).

Table (3): Direct Exposure Test of the Olive Leaves and Irrigating Solution on all Tested Microorganisms and Unidentified Samples from Root Canals.

Time after contact	0.8% OL	2.5% NaOCl	Normal Saline
Immediate	-	-	+
5 min	-	-	+
10 min	-	-	+
15 min	-	-	+

NaOCl=Sodium hypochlorite. OL= Olive Leaves. + = Growth. - = No Growth.

Results of the In Vivo Study:

From each root canal in group I (irrigation with 0.8% Olive Leaves extract solution), group II (irrigtion with 2.5% sodium hypochlorite solution), and group III (irrigation with normal saline solution), both aerobic and anaerobic bacterial counts were taken at each appointment as follows:

1. At the beginning of the first appointment.
2. At the beginning of the second appointment.
3. At the end of the second appointment.
4. At the beginning of the third appointment.

The data were tabulated according to the number of bacterial colonies counts on each culture media. The mean and standard deviation for both aerobic and anaerobic bacteria at different appointments were calculated.

All 36 teeth contained bacteria before treatment, as indicated by positive culture results are obtained from the initial root canal contents after aeobic and anaerobic culturing.

The percentages of reduction of the aerobic bacterial count, at the beginning of the second appointmant; at the end of the second appointment, and at the beginning of the third appointment; for Olive Leaves extract were 31.53%, 99,17% and 98.64% respectively; for sodium hypochlorite were 40.07%, 99.10%, and 98.61% respectively; and for normal saline were 13.68%, 53.57%, and 30.60% respectively.

The reduction of the anaerobic bacterial count, at the beginning of the second appointmant; at the end of the second appointment, and at the beginning of the third appointment; for Olive Leaves extract were 28.91%, 98.88%, and 98.38% respectively; for sodium hypochlorite were 38.40%, 99.00%, and 98.47% respectively; and for normal saline were 61.01%, 53.01%, and 22.42% respectively (Figure 1 and 2).

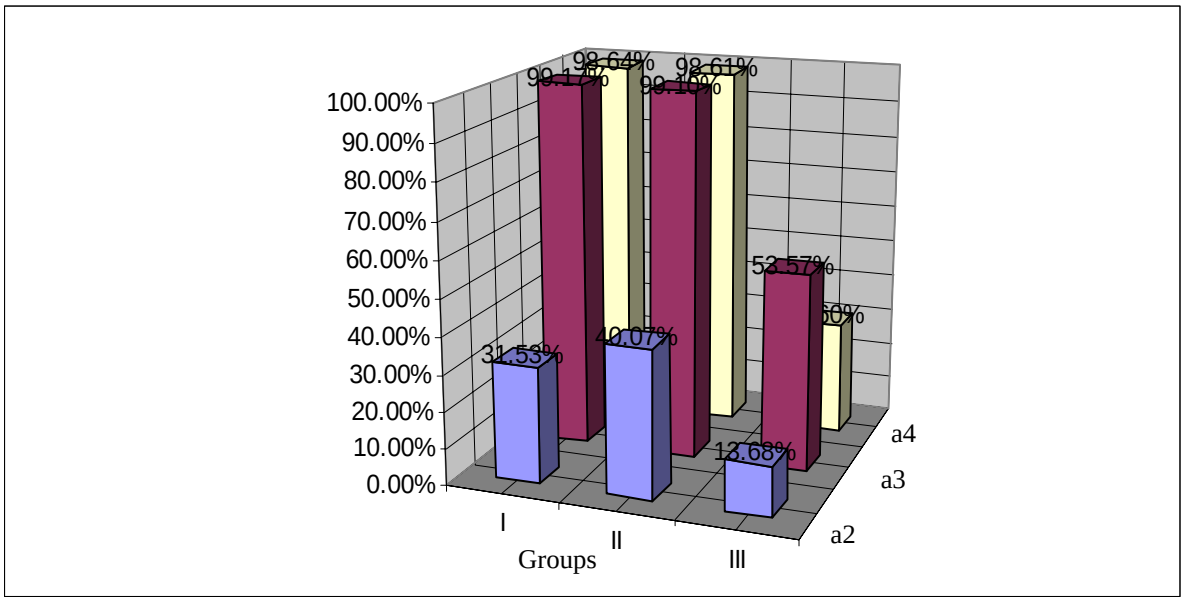

Figure (1): The Mean of the Percentage of Aerobic Bacterial Count Reduction of Group I (0.8% Olive Leaves), Group II (2.5% Sodium Hypochlorite), and Group III (Normal Saline) at Different Appoinntment.

a2= Aerobic bacterial count at the beginning of second appointment.
a3 = Aerobic bacterial count at the end of second appointment.
a4= Aerobic bacterial count at the beginning of third appointment.

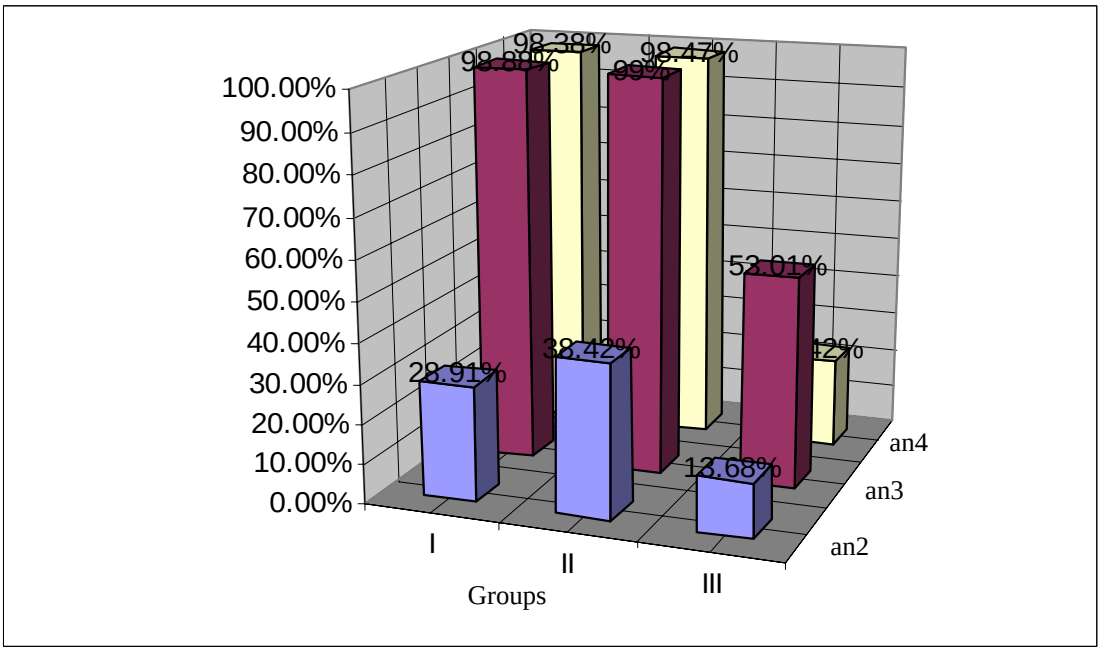

Figure (2): The Mean of the Percentage of Anaerobic Bacterial Count Reduction of Group I (0.8% Olive Leaves), Group II (2.5% Sodium Hypochlorite), and Group III (Normal Saline) at Different Appoinntment.

an2= Anaerobic bacterial count at the beginning of second appointment.
an3 = Anaerobic bacterial count at the end of second appointment.
an4= Anaerobic bacterial count at the beginning of third appointment.

Comparing the results at different appointments for each group, it was found that the mean bacterial counts (both aerobic and anaerobic), at the beginning of second appointment, less than that at the beginning of first appointment was statisticaly significant ($p<0.05$) for Olive Leaves and sodium hypochlorite, but statistically not significant ($p>0.05$) for normal saline. Result revealed that the mean bacterial counts (both aerobic and anaerobic), at the end of second appointment, less than that at the beginning of second appointment which statistically significant ($p<0.05$) for Olive Leaves, sodium hypochlorite, and normal saline. Result also found that the mean bacterial counts (both aerobic and anaerobic), at the beginning of third appointment, greater than that at the end of second appointment, which was statistcally not significant ($p>0.05$) for Olive Leaves, and sodium hypochlorite, while statistically significant ($p<0.05$) for normal saline. This was shown in Tables (4) and (5).

Table (4): Comparison for the Antimicrobial Effect of 0.8% Olive Leaves Extract, 2.5% Sodium Hypochlorite, and Normal Saline on Aerobic Bacteria at Different Appointment.

Groups	***Mean ± SD***			
	Order of Appointment			
	a1	***a2***	**a3**	**a4**
I	561.42±77.93	378.92±72.91	4.67±1.92	7.50±1.56
	C	**B**	**A**	**A**
II	568.67±105.57	335.58±74.38	5.08±2.31	7.67±2.77
	C	**B**	**A**	**A**
III	536.92±143.32	509.33±93.88	250.92±30.77	366.33±104.26
	C	**C**	B	**A**

The different letters horizontally mean significant difference exist.
I=Olive Leaves. II=Sodium Hypochlorite. III= Normal Saline.

Table (5): Comparison for the Antimicrobial Effect of 0.8% Olive Leaves Extract, 2.5% Sodium Hypochlorite, and Normal Saline on Anaerobic Bacteria at Different Appointment.

Groups	***Mean ± SD***			
	Order of Appointment			
	a1	***a2***	**a3**	**a4**
I	603.33±92.57	420.67±56.49	4.67±1.92	7.50±1.56
	C	**B**	**A**	**A**
II	568.67±105.57	335.58±74.38	5.67±2.77	8.75±2.56
	C	**B**	**A**	**A**
III	597.58±101.35	508.25±92.40	273.83±50.32	459.50±92.64
	C	**C**	**B**	**A**

The different letters horizontally mean significant difference exist.
I=*Olive Leaves*. II=Sodium Hypochlorite. III= Normal Saline

Comparing the results at different appointments among the four group, it was found that the mean bacterial counts (both aerobic and anaerobic) at the beginning of first appointment was significantlly not different ($p>0.05$) for all groups (this indicat that the bacterial counts for all groups were comparable before treatment); but the mean bacterial counts at the beginning of second appointment, at the end of second appointment, and at the beginning of third appointment; for Olive Leaves statistically had significant different

($p<0.05$) from normal saline, but statistically had no significant different ($p>0.05$) from sodium hypochlorite. However, sodium hypochlorite showed least bacterial counts among other groups used in this study, which statistically had significant different ($p<0.05$) from normal saline.This was shown in Tables (6) and (7).

Table (6): Comparison between the Antimicrobial Effect of 0.8% Olive Leaves Extract, 2.5% Sodium Hypochlorite, and Normal Saline on Aerobic Bacteria at Different Appointment.

Groups	***Mean ± SD***			
	Order of Appointment			
	a1	***a2***	**a3**	**a4**
I	561.42±77.93	378.92±72.91	4.67±1.92	7.50±1.56
	A	**A**	**A**	**A**
II	568.67±105.57	335.58±74.38	5.08±2.31	7.67±2.77
	A	**A**	**A**	**A**
III	536.92±143.32	509.33±93.88	250.92±30.77	366.33±104.26
	A	**B**	**B**	**B**

The different letters vertically mean significant difference exist.
I=Olive Leaves. II=Sodium Hypochlorite. III= Normal Saline.

Table (7): Comparison between the Antimicrobial Effect of 0.8% Olive Leaves Extract, 2.5% Sodium Hypochlorite, and Normal Saline on Anaerobic Bacteria at Different Appointment.

Groups	**Mean ± SD**			
	Order of Appointment			
	a1	***a2***	**a3**	**A4**
I	603.33±92.57	420.67±56.49	4.67±1.92	7.50±1.56
	A	**A**	**A**	**A**
II	568.67±105.57	335.58±74.38	5.67±2.77	8.75±2.56
	A	**A**	**A**	**A**
III	597.58±101.35	508.25±92.40	273.83±50.32	459.50±92.64
	A	**B**	**B**	**B**

The different letters vertically mean significant difference exist.
I=*Olive Leaves*. II=Sodium Hypochlorite. III= Normal Saline

Using t-test, the percentage of reduction in the counts of aerobic and anaerobic bacteria were compared at each appointments for all group. Result found that the percentage of reduction in the counts of aerobic bacteria were greater than that of anaerobic bacteria at different appointments for all group but statistically no significant different exist at level of significance ($p>0.05$). This was shown in Table (8).

Table (8): Comparison of the Percentage of Reduction in the Counts of Aerobic and Anaerobic Bacteria at Different Appointments for all Groups.

Groups	Order of Appointment	Mean ± SD		* Significant
		Aerobic	Anaerobic	
I	1	561.42±77.93	603.33±92.57	*P > 0.05*
	2	378.92±72.91	420.67±56.49	*P > 0.05*
	3	4.67±1.92	6.50±2.84	*P > 0.05*
	4	7.50±1.56	9.67±1.45	*P > 0.05*
II	1	568.67±105.57	607.08±121.91	*P > 0.05*
	2	335.58±74.38	366.92±87.78	*P > 0.05*
	3	5.08±2.31	5.67±2.77	*P > 0.05*
	4	7.67±2.77	8.75±2.56	*P > 0.05*
III	1	536.92±143.32	597.58±101.35	*P > 0.05*
	2	509.33±93.88	508.25±92.40	*P > 0.05*
	3	250.92±30.77	273.83±50.32	*P > 0.05*
	4	366.33±104.26	459.50±92.64	*P > 0.05*

* P>0.05=Not significant.; 1=Beginning of the first appointment. 2=Beginning of second appointment; 3= End of the second appointment. 4=Beginning of third appointment; I=Olive leaves. II= Sodium Hypochlorite. III=Normal Saline.

DISCUSSION

Various methodologies can be used to assess the antimicrobial activity of endodontic irrigants. Indeed, the methodology could be apossible explantion for the differences in the results between different studies [25].

Olive Leaves extract is effective for gum disease, infections, herpes, hepatitis, colds and flu, fungus, diabetes, arthritis, chronic fatigue syndrome, allergies, vaginal yeast infections, dental abscess, skin conditions and inflamed tonsils[26]. However, the potential antimicrobial effect of Olive Leaves as a root canal irrigant is not yet currently known. This study was performed for evaluating the antimicrobial effect of different concentration of Olive Leaves on microorganisms present in the root canal, and compared with 2.5% sodium hypochlorite and normal saline.

In vitro, the antimicrobial activity of Olive Leaves as a root canal irrigant was evaluated using broth microdilution method. Olive Leaves extract exhibited different antimicrobial effect against aerobic and anaerobic microorganisms isolated from necrotic dental pulp at 0.1%, 0.2%, 0.4%, 0.6%, and 0.8%. The best antimicrobial activity was noticed at 0.8% which statistically had no significant difference form sodium hypochlorite at 2.5% and had significant different from normal saline. This revealed that its action is a concentration dependent.

In direct exposure test five facultative anaerobic bacterial strains were tested (Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Klebseilla spp., Pseudomonas aeruginosa) because these are presenting all phases of the development of endodontic infection. One yeast was also tested (Candida albicans) because it was present in necrotic pulps [23,27,28,29,30]. In this test, the time required for each irrigant to start its antimicrobial effect was determined. The result revealed that Both 2.5% sodium hypochlorite and 0.8% Olive Leaves extract eliminated all the microorganisms that were used in this experiment at all times period, while normal saline did not show any antimicrobial effect.

The antibacterial, antiviral compo-nent derived from Olive Leaves is called oleuropein. Studies demonstr-ated that the components of Olive Leaves extract are

also toxic to a wide range of bacteria, prtozoa, yeasts, parasites and fungi. An in vitro study revealed that oleuropein and its derivative hydroxytyrosol act as natural antibiotics against a wide range of gram-negative and gram-positive bacteria. Most impressively, these two components of Olive Leaves inhibited Staphylococcus aureus. Components in Olive Leaves extract also have inhibited the growth of Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, and Candida albicans [31]. As recently as 1998, researchers investigated oleuropeins antibacterial action and concluded that it can enhance nitric oxide production in mouse macrophages. By increasing nitric oxide production, oleuropein appears to arm the macrophages against endotoxins (bacterial poisons generated by gram-negative bacteria). Interestingly, oleuropein only increased nitric oxide production when endotoxins were present [32].

Worthington and Druker (2004) [33] studied the effect of different concentrations of sodium hypochlorite (0.5, 2.5,and 5.25%) on different microorganisms (Actimyces naeslundii, Candida albicans, and Enterococcus faecalis). They found that all solutions started their effect after 10 seonds.

Markin et al., (2003) [34] studied the antimicrobial effect of Olive Leaves against bacteria and fungi. The microorganisms tested were inoculated in various concentrations of Olive Leaves water extract. Olive Leaves 0.6% (w/v) water extract killed almost all bacteria in 3-hours. Scanning electron microscopic observations of Candida albicans exposed to 1.25% Olive Leaves extracts showed invaginated amorphous cells. Escherichia coli cells subject to only 0.6% showed complete distruction.

In an in vivo study statistically there was no significant difference between 0.8% Olive Leaves and 2.5% sodium hypochlorite on aerobic and anaerobic bacteria isolated from infected root canal. These findings explain that the biochemical root canal irrigation with alcoholic extract of Olive leaves and sodium hypochlorite had antibacterial effect which appeared to be sufficient to reduce the bacterial population of necrotic root canals, while sterile normal saline had no antibacterial effect. These finding may therefore, enforce the need for a combination of mechanical instrumentation and chemical irrigation to effectively remove most root canal microrganisms. Many studies had found that irrigation with antibacterial irrigant was significantly more effective than saline solution in elimination of bacteria from root canals [35,36,37].

Olive Leaves extract is an extraordinary 100% natural herbal antibacterial/antiviral. Extract obtained from specific parts of the Olive tree, this new properietry phytochemical extract is not only safe, but also a non toxic immune system build[26].

CONCLUSIONS

1. The alcoholic extract of Olive Leaves solution had the best antimicrobial effect at (0.8%) concentration, which shows immediate antimicrobial effect on tested microorganisms.
2. Olive Leaves alcoholic extract solution at 0.8% was an effective antimicrobial agent when used as an irrigant in endodontic treatment of teeth with necrotic pulps in an in vivo study.

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