

The antibacterial evaluation of dandelion extracts as root canal irrigating solutions (A comparative study)

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ABSTRACT

Background: Irrigation has a central role in endodontic treatment. Several irrigating solutions have the antimicrobial activity and actively kill bacteria and yeasts when introduced in direct contact with the microorganisms.

The purpose of this study was to evaluate the antimicrobial effectiveness of Dandelion (*Taraxacum officinale*) root and leaf extracts as possible irrigant solutions, used during endodontic treatments, and both were compared to Sodium hypochlorite, Propolis and Ethyl alcohol.

Materials and Method: Forty seven human extracted single rooted teeth were selected. The teeth were decoronated using a diamond disk to have a length of 15 mm \pm 1 mm and they were instrumented using the hybrid technique. All roots were sterilized by an autoclave, five roots without bacterial inoculation served as the negative controls, the rest were inoculated with *Enterococcus faecalis*, then five roots were selected randomly as the positive controls, then the remaining 37 roots were divided into five groups of 8 samples each except group V with 5 roots. Group I: irrigated with Propolis extract. Group II: irrigated with Dandelion leaf extract. Group III: irrigated with Dandelion root extract. Group IV: irrigated with Sodium hypochlorite. Group V: irrigated with Ethyl alcohol. Bacterial swabs were taken from each root and cultured. Bacterial growths were calculated by counting the number of colonies appeared on the cultures.

Results: the results were statistically analyzed; within the limitation of this in vitro study, the Dandelion leaves extract and Dandelion root extract proved to have some antimicrobial properties. Sodium hypochlorite has the best antimicrobial effect, followed by Propolis, Dandelion root, Ethyl alcohol then Dandelion leaf.

Conclusion: Dandelion root and leaf extracts are possible irrigant solutions that can be used successfully during endodontic treatments, to aid disinfection of the root canal system.

Keywords: *Taraxacum officinale*, *Enterococcus faecalis*, Propolis. (J Bagh Coll Dentistry 2014; 26(3):35-40).

الخلاصة

ان غسل قنوات جذر الاسنان له دور اساسي خلال معالجتها. هنالك مواد مختلفة تستعمل لغسل قنوات الجذور وهي مضادة للبكتريا وتستطيع القضاء على البكتريا و الفطريات عندما تستعمل وتوضع بصورة مباشرة مع هذه الكائنات المرضية الدقيقة.

الغرض من هذه الدراسة هو تقييم القابلية المضادة للميكروبات لمستخلص الجذور ومستخلص الاوراق لنبات الهندباء لاستعماله كمادة لغسل قنوات جذور الاسنان خلال عملية معالجة قنوات جذور الاسنان. ومقارنة هذه المستخلصات مع هابيوكلورايت الصوديوم و العكبر (صمغ النحل) و الكحول الايثيلي.

لهذه الدراسة تم اختيار سبع واربعون سن بشري. الاسنان قصرت الى طول 15 ملم عن طريق قطع تاج السن وتم تحضير القنوات باستخدام التقنية الهجينة. تم تعقيم الجذور بواسطة جهاز التعقيم. ثم تم زراعة قنوات الاسنان ببكتيريا المكورات المعوية وتم تقسيم الاسنان الى 5 مجاميع.

مجموعة I : عشرة اسنان تم غسل قنواتها باستعمال مستخلص العكبر.

مجموعة II : عشرة اسنان تم غسل قنواتها باستعمال مستخلص اوراق نبات الهندباء.

مجموعة III : عشرة اسنان تم غسل قنواتها باستعمال مستخلص جذور نبات الهندباء.

مجموعة IV : عشرة اسنان تم غسل قنواتها باستعمال هابيوكلورايت الصوديوم.

مجموعة V : سبعة اسنان تم غسل قنواتها باستعمال الكحول الايثيلي.

بعد ذلك تم اخذ مسحة بكتيرية من كل قناة جذر وتم زراعتها. تم حساب عدد البكتيريا المزروعة بواسطة حساب عدد المستعمرات البكتيرية التي ظهرت على الوسط الزرعوي. ومعالجة النتائج احصائيا.

ضمن الظروف المختبرية التي تمت خلالها هذه الدراسة. وجد ان مستخلص اوراق ومستخلص جذور نبات الهندباء يحتوي على بعض الخواص المضادة للبكتيريا. وان هابيوكلورايت الصوديوم اظهر افضل خاصية مضادة للبكتيريا مقارنة مع كل المواد المستعملة في هذه الدراسة يتبعه العكبر ثم جذور نبات الهندباء واوراق نبات الهندباء

INTRODUCTION

The success of endodontic treatment depends on the elimination of microbes (if present) from the root-canal system and prevention of reinfection. Chemomechanical preparation of the infected root canal using antimicrobial agents, followed by obturation and coronal restoration, provides a favorable outcome. The root canal system is instrumented using hand or rotatory instruments, a study using advanced techniques such as microcomputed tomography (CT) scanning have demonstrated that proportionally large areas of the main root-canal wall remain untouched by the instruments. This study showed the importance of chemical means of cleaning and disinfecting all areas of the root canal ⁽¹⁾.

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Disinfection of the root canal system, as part of endodontic therapy, by preparation and irrigation is the key in reducing the number of bacteria within the root canal and helping to control periapical disease ⁽²⁾. A variety of antibacterial irrigation solutions may be used to irrigate and disinfect root canals in conjunction with root canal preparation. Sodium hypochlorite is the most common irrigant used in endodontics until now.

It is an effective antimicrobial agent and an excellent organic tissue solvent ⁽³⁾, but has its numerous drawbacks like unpleasant odour and taste and, even more relevant, the high toxicity when it is extruded into the periapical tissues. Chlorhexidine gluconate is a broad-spectrum antimicrobial agent that has substantive antibacterial activity and relatively low toxic effects, but it does not dissolve organic tissues ⁽⁴⁾.

The *Enterococcus faecalis* is known to be an important resistant species in the infected root canals, and they may cause treatment failures⁽⁵⁾.

The search for alternative irrigating solutions has focused on the substances with antibacterial effect and capacity to clean dentin surfaces. Herbal products have been used since ancient times in folk medicine, involving both eastern and western medicinal traditions. Many plants with biological and antimicrobiological properties have been studied. Herbal or natural products have been used in dental and medical practice for thousands of years and have become even more popular today due to their high antimicrobial activity, biocompatibility, anti-inflammatory and antioxidant properties⁽⁶⁾.

The purpose of this, in vitro, study was to evaluate the effectiveness of Dandelion root & leaf extracts (*Taraxacum officinale*) as possible irrigants in endodontics; against *Enterococcus faecalis* in comparison with Sodium hypochlorite, Propolis and Ethyl alcohol.

MATERIALS AND METHODS

Preparation of the samples

Forty seven extracted single rooted teeth were collected from different dental centers in Iraq. The teeth were decoronated, using a diamond disk in a straight hand piece to have length of 15 mm \pm 1 mm and the length of each tooth was verified by digital vernier.

The roots were instrumented using the hybrid technique; with each file change, the canals were irrigated with 2 ml distilled water to remove the debris. The files were discarded after using with every five uses. The apical foramen was sealed with light-cured restorative glass ionomer cement. The other surfaces of the roots were covered with two layers of nail varnish.

Microbiological procedure

The method of bacterial inoculation and culturing, used in this study, was similar to the method used by Mehrvarzfar et al.⁽¹¹⁾

1. First step

- All roots were sealed with aluminum foil and placed in a container filled with distilled water, then sterilized by an autoclave for 30 minutes at 121°C under a pressure of 15 PSI.
- Brain Heart Infusion broth was prepared according to the manufacturer's instructions by weighting 37 gm of Brain Heart Infusion powder. The powder was dissolved in one liter of distilled water. Then it was mixed well and placed on a heater to complete the dissolution until

boiling, leaving the broth to boil for one minute. After it became colder, it was poured into glass tubes with screw cap (25 ml). The cover was sealed well then autoclaved for 15 minutes at 121°C under a pressure of 15 PSI.

- Each root was transported to a glass tube that contains sterile BHI broth by a sterile forceps; inside hood cabinet and beside the flame of a burner.
- The tubes containing the roots were incubated at 37°C for 48 hours to ensure the sterilization of the roots. The roots were daily examined for no turbidity.
- After 48 hours, each root was transported to a sterile Eppendorf tube using a sterile forceps under sterile conditions.

2. Second step

Five teeth were selected randomly to serve as the negative controls.

- *Enterococcus faecalis* suspension was prepared as follows:
- The pure isolate of *Enterococcus faecalis* was inoculated to Brain Heart Infusion broth and incubated at 37°C overnight. The BHI broth at a concentration of 1.5×10^8 CFU/ml was used for inoculation. The bacterial suspension was adjusted to match the turbidity of McFarland 0.5 scale. Bacterial suspension of 0.01 ml (10 μ l) was inoculated into each canal (42 roots) using sterile insulin syringes. Then the roots (47 roots) were incubated for three weeks under aerobic conditions at 37°C. The inoculum inside the canals was replaced with 0.01 ml (10 μ l) of fresh bacterial suspension every other day.
- After the incubation period, five inoculated roots were selected randomly to act as the positive controls.
- Experimental groups:
 1. GI Propolis: 8 roots for Propolis 12% (120 mg/1 ml).
 2. G II Dandelion Leaf: 8 roots for Dandelion leaf 0.7% (7 mg/ 1 ml).
 3. G III Dandelion Root: 8 roots for Dandelion root 0.7% (7 mg/ 1 ml).
 4. G IV NaOCl: 8 roots for Sodium hypochlorite 2.5 %.
 5. G V Ethyl alcohol: 5 roots for Ethyl alcohol 55%.

3. Third step

- Plain tubes, paper points, insulin syringes, 10 ml disposable syringe and forceps were sterilized by UV light for 30 minutes.

- Preparations of dilutions of the plants extracts, then all dilutions were mixed well by vortex device for 20 seconds.

4. Fourth step

- Group one were irrigated with 8 ml of the irrigant (1 ml for each root).
- After 5 minutes the irrigant was withdrew using a sterile syringe; a sterile paper point was grasped by sterile forceps then inserted inside the canal and moved in a circular direction for 10 seconds to allow collection of bacteria and remaining irrigant material.
- The paper point then transported to a plain tube containing sterile 10 ml of normal saline and mixed by vortex for 20 seconds.
- One ml of the mixture was drawn using a sterile syringe and transported to another plain tube containing 9 ml of normal saline which was then mixed by vortex for 20 seconds.
- Again 1 ml of the mixture was drawn using a sterile syringe and transported to another plain tube containing sterile 9 ml of normal saline, mixed by vortex for 20 seconds.
- After that 1ml of the mixture was collected from the last plain tube and discharged as this step done in accordance with procedure of serial dilutions.
- Five μ l were taken of the mixture of the last plain tube and applied to Bile Esculine agar culture plates and spread by a sterile loop and incubated at 37°C overnight.
- One ml of the irrigant that was collected is re-added to the canal and let for another 5 minutes and the steps were repeated from the withdrew of the irrigant from the canal using a sterile syringe and a sterile paper point insertion inside the canal to allow collection of the bacteria till the bacterial counting to obtain 10-min duration.
- Another 1 ml of the irrigant was re-added to the canal and left for another 5 minutes and the same procedure was repeated to obtain 15-min duration.

The above procedure was repeated with all the experimental groups. After the incubation periods of the Petri dishes that were inoculated with microbial swabs from the roots. Bacterial growths were calculated by counting the number of colonies appeared on each dish.

The results were statistically analyzed by Kruskal-Wallis H test and Mann-Whitney U test.

RESULTS

According to the results of this study, with the respect of the duration test, all the tested irrigation solutions reduced the number of *Enterococcus faecalis* colonies with different values.

Mann-Whitney U test was used to compare the paired groups, within each irrigation duration, (Table 1 and Figure 1), in which highly significant differences were recorded in most of the compared groups. However, in the 5-min duration there were no significant differences between the groups I vs. IV; II vs. III and a significant difference between the group IV and V; in the 10-min duration there were significant differences between the groups I vs. III and I vs. IV. Finally, in the 15-min duration there were no significant differences between the groups I vs. III and II vs. V.

Table 1: Mann-Whitney U test results comparing paired groups in each duration

Duration	Groups	P-value		
5 min.	I	II	0.000 (HS)	
		III	0.000 (HS)	
		IV	0.057 (NS)	
	II	V	0.003 (HS)	
		III	0.073 (NS)	
		IV	0.001 (HS)	
	III	V	0.003 (HS)	
		IV	0.001 (HS)	
	10 min.	I	V	0.011 (S)
			II	0.001 (HS)
			III	0.017 (S)
		II	IV	0.048 (S)
V	0.003 (HS)			
III	0.001 (HS)			
15 min.	I	IV	0.001 (HS)	
		V	0.003 (HS)	
		III	0.087 (NS)	
	II	IV	0.000 (HS)	
		V	0.003 (HS)	
		III	0.878 (NS)	
	III	IV	0.004 (HS)	
		V	0.007 (HS)	
IV	V	0.001 (HS)		

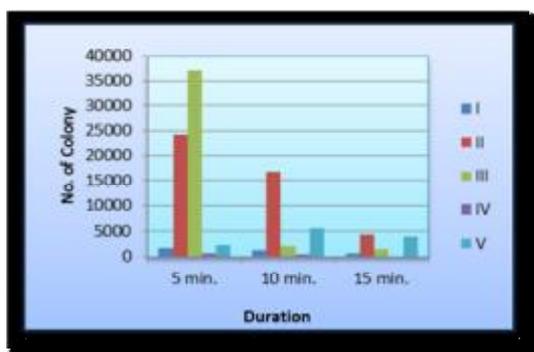


Figure 1: Bar chart of the mean of Enterococcus faecalis colonies versus irrigation duration for all tested groups

DISCUSSION

Bacterial infections and inflammation are among the ailments treated by traditional healers. The World Health Organization has expressed high interest in traditional medicine. World Health Organization data show that 80% of the world population use plant extracts and content in treatment of diseases⁽⁷⁾.

This study was an attempt to clarify whether intracanal irrigation with Dandelion leaf and root extracts would be able to eradicate *Enterococcus faecalis* contaminating root canals.

Enterococcus faecalis is one of the most frequently isolated species^(8,9); it can be found at depths up to 300 μm within dentinal tubules; where it is able to survive notwithstanding the scant available nutrients, unlike other bacterial species. Furthermore, *Enterococcus faecalis* appears to be very resistant to the action of endodontic dressing like $\text{Ca}(\text{OH})_2$, because of its capability to survive at a very high pH; it can resist heat, U.V., ethanol, hydrogen peroxide and acidity, therefore; it can persist and survive in treated root canal systems⁽¹⁰⁾.

The method of bacterial inoculation and culturing, used in this study, was similar to the method used by Mehrvarzfar et al.⁽¹¹⁾.

In the present study, the antimicrobial activity of hydroalcoholic extract of 12% Propolis, 0.7% hydroalcoholic extract of Dandelion leaf and Dandelion root, 2.5% Sodium hypochlorite and 55% Ethyl Alcohol were compared. The use of the most effective antimicrobial irrigant has a clinical importance for successful endodontic treatment.

A concentration of 2.5% Sodium hypochlorite had been used as an intracanal irrigant against *Enterococcus Faecalis*. Since a solution of 2.5% NaOCl is generally used when treating teeth with necrotic pulp and apical periodontitis. Also NaOCl solutions at higher concentrations have a greater irritating effect on the apical and

periapical tissues⁽¹²⁾. The acceptable cytotoxic level of NaOCl is 0.5%, but this concentration is less effective⁽⁵⁾.

Twelve percent of Propolis extract, which was used in this study, found to be an effective intracanal irrigant against *Enterococcus faecalis*. The antimicrobial activity of propolis extract on the microorganisms was verified by different studies^(13,14).

The antibacterial activity of *Taraxacum officinale* leaves and roots extracts had been evaluated, since they have been used for hundreds of years to treat liver, gallbladder, kidney, and joint problems. Recently the plant extracts were investigated in several studies to find possible antimicrobial effect against many pathogens⁽¹⁵⁻¹⁷⁾.

Dandelion root extract and leaf extract were used in this study; a concentration of 0.7% in accordance with a study used the aqueous extract of Dandelion and since it showed antibacterial properties at concentration 7.0 mg/ml.⁽¹⁵⁾

Dandelion extracts, in this study, proved to have antimicrobial properties, both extracts reduced the number of colonies of the tested pathogen, this is in agreement with many studies which verified the antimicrobial activity of this plant extracts against many pathogenic microorganisms^(15,17,18).

Fifty five percent of ethyl alcohol was used, as an intracanal irrigant, and its antimicrobial effect was compared with the other tested irrigants. It is used in this study as Alcohols exhibit rapid broad-spectrum antimicrobial activity against vegetative bacteria (including mycobacteria), viruses, and fungi but are not sporicidal⁽¹⁹⁾.

When comparing among these groups, 2.5% Sodium hypochlorite, found to reduce the bacterial colonies very effectively. This antimicrobial activity may be related to bacterial essential enzymatic sites promoting irreversible inactivation by hydroxyl ions and the chloramination reaction, Sodium hypochlorite when comes in contact with organic tissue, releases chlorine that combine with the protein amino group to form chloramine which interferes with the cellular metabolism⁽¹²⁾.

The results of this study, regarding the effectiveness of 2.5% of Sodium hypochlorite, are in agreement with several studies, such as Mehrvarzfar et al.⁽¹¹⁾.

According to this study, Propolis possess good antimicrobial properties against the tested pathogen which is in agreement with Mattigatti et al. and Ehsaniet al.^(20,21). It has a higher antibacterial action in 5, 10 and 15 minutes than Dandelion leaf extract, dandelion root extract and Ethyl alcohol. This may be due to some active

components present in Propolis. The real mechanism of the antimicrobial action of Propolis appears to be complex and not yet fully understood, however, its active agents, include flavonoids, phenolic and aromatic compounds like caffeic acid⁽²²⁾.

While insignificant difference was found between Propolis and Sodium hypochlorite in 5 minutes duration indicating that both irrigants were effective and any one of them can be used, this is in agreement with a study conducted by Mattigatti et al.⁽²⁰⁾. Propolis was more effective on *Enterococcus faecalis* as the contact time increased.

Dandelion leaf and dandelion root extract, used in this study, proved to have some inhibitory effect on the growth of the tested pathogen. The inhibitory effects may be due to the glycosides and/or phenolic compounds and/or tannins and/or flavonoids and/or alkaloids and/or proteins presence in the plant extracts. Such compounds had been reported to have an active effect on the bacterial cells membrane, which might destroy these microorganisms⁽²³⁾. The alkaloids interact with the DNA, the tannins inhibit the carrier enzymes and proteins present in the cells membrane, while the phenolic compounds form a complex with the dissolved protein out of the cells or with cells membrane which may destroy the bacteria, another possible reason for antibacterial effect is that *Taraxacum officinale* had higher saponin content with higher antimicrobial potential as Kannabiran et al.⁽²⁴⁾ reported that there was linear relation with the saponin content and antimicrobial activity⁽²⁵⁾.

The screening of phytochemical constituents of this plant's ethanolic extract by Lateef and Issah⁽¹⁶⁾ revealed the presence of saponin, phenolics, reducing sugar, anthracenosides, triterpenes, steroids, tannins, and phlobatanins. These compounds are known to be biologically active and, therefore; aid in the antimicrobial activities of Dandelion. Moreover, these classes of compounds are reported to have activity against several pathogens, therefore; the results support the use of *taraxacum officinale* as antimicrobial agents during endodontic treatment.

The antibacterial findings of this study regarding Dandelion disagree with Khan et al. study⁽²⁶⁾ as they found that methanolic extract of *Taraxacum officinale* did not show antibacterial activity against *Enterococcus faecalis* but it had antifungal activity. These differences in antimicrobial susceptibility tests may be due to variations in methodology, since several factors (inoculum amount, medium composition, pH, incubation, type of the extraction whether it is

methanol, ethanolic or aqueous) can influence the interaction between microorganisms and antimicrobial agents, thus affecting the results.

Dandelion leaf and roots showed no significant difference between both extracts at 5 minutes duration but there was a difference between them at 10 and 15 minutes with the root extract being slightly more effective and this may be due to the presence of a little difference in the percentage of the active phytochemicals (alkaloid, flavonoid, saponin and phenols) found in the leaf or root extracts of Dandelion as found in a study by Mir et al.⁽²⁷⁾. Both extract showed better activity than Ethyl alcohol with the time.

Ethyl alcohol showed antibacterial effect at 5 minutes duration. Little is known about the specific mode of action of alcohols, but based on the increased efficacy in the presence of water; it is generally believed that they cause membrane damage and rapid denaturation of proteins, with subsequent interference with metabolism and cell lysis⁽¹⁹⁾.

In this study, Ethyl alcohol irrigant found to be less effective than Propolis, Sodium hypochlorite with the last one had the best antibacterial effect. This result was in agreement with a similar pervious study used 21% alcohol along with various endodontic irrigants against six selected microorganisms, the study found that Sodium hypochlorite is the most effective irrigant⁽²⁸⁾. The evaporation of alcohol may be the reason behind decreasing Ethyl alcohol effectiveness after 10 and 15 minutes duration.

At 15 minutes, there were no significant differences between Ethyl alcohol and Dandelion leaf extract and between Propolis and Dandelion root extract, this is may be caused by the loss of effect of the biologically active components in these irrigants with the time.

According to the results obtained in this study, the highest antibacterial irrigant, at 5 minutes, was Sodium hypochlorite followed by Propolis, Ethyl alcohol, dandelion leaf, dandelion root being the least effective. While at 15 minutes, Sodium hypochlorite followed by Propolis then Dandelion root which showed increased activity followed by ethyl alcohol along with the dandelion leaf and these findings may indicate, increasing the contact time of Dandelion extracts has better antibacterial effect.

Within the circumstances of this study, the following conclusions could be withdrawn:

- All the irrigants used in this study had variable antimicrobial properties against *Enterococcus faecalis*.
- Both Dandelion leaf and Dandelion root extracts can be used as possible irrigant

solutions during endodontic treatment to aid disinfection of the root canal system.

- The antimicrobial effect of Dandelion extracts was improved with increasing the contact time.
- Sodium hypochlorite and Propolis had better antimicrobial properties than Dandelion extracts.

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