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Evaluation of The Antibacterial Activity of Nasturtium officinale (watercress) Essential Oil with Calcium Hydroxide against Enterococcus faecalis Isolated from Root Canal (In vitro study)

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ABSTRACT

Background: The application of herbal extracts in dentistry is considered as an interesting alternative to synthetic antimicrobials due to their lower side effects and ease of availability and for the effort to overcome the increased resistance to the common antimicrobial agents.

The aim of this study: to investigate the antibacterial activity of Nasturtium officinale (watercress) essential oil in combination with calcium hydroxide against Enterococcus faecalis as intracanal medicament.

Materials and Methods: Enterococcus faecalis was isolated from patients with necrotic root canal or failed root canal treatment. The sensitivity of Enterococcus faecalis to different concentrations of Nasturtium officinale essential oil and compared with calcium hydroxide paste was determined by using agar well diffusion method. The agar plate method was used to determine the minimum bactericidal concentration (MBC) of the tested oil against the bacteria. The combination of Nasturtium officinale essential oil with calcium hydroxide was evaluated and compared to calcium hydroxide paste with iodoform by using agar well diffusion method.

Results: The essential oil of Nasturtium officinale exhibited antibacterial activity against Enterococcus faecalis, this activity was found to be increased as the concentration of extract increased and the difference was highly significant ($P \leq 0.01$) among all concentrations and calcium hydroxide paste. The tested combination revealed larger inhibition zones than the ones formed by each tested agent individually.

Conclusion: The essential oil extract of Nasturtium officinale is active against Enterococcus faecalis suggesting its potential to be used as an intracanal medicament alone or in combination with calcium hydroxide.

Key words: Nasturtium officinale, Essential oil, Enterococcus faecalis, Intracanal medicaments

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INTRODUCTION

Root canal treatment failure may occur due to different reasons such as survival of bacteria inside root canals, poor disinfection measures, failed coronal seal and untreated missed canals [1]. Root canal retreatment aims to achieve complete disinfection of the contaminated root canal system by chemo-mechanical means, that creates a favorable condition for periradicular healing and resolution of any infection that may affect the periodontal support of teeth [2]. However, due to complex anatomy of root canal system, presence of ramifications and penetration of bacteria into dentinal tubules, the complete eradication of bacteria is hard to be achieved by mechanical preparation and irrigation protocols. Intracanal medicaments with anti- microbial action are required to maximize the disinfection of root canal system especially in infected cases. In the field of endodontics, calcium hydroxide is the most commonly used intracanal medicament and a plethora of vehicles have been used with calcium hydroxide to enhance its antimicrobial properties significantly. [3] Enterococcus faecalis is regarded the most prevalent bacterial species isolated from cases with periapical infection or failed root canal treatment. This bacterium has specific characteristics such as the ability to form biofilms and colonizing in remote unreachable areas inside the root canal system that make it able to escape any disinfection measure by irrigants and to live in the presence of intracanal medication. The constant increase in resistant bacterial strains and side effects caused by synthetic antimicrobial agents has

prompted researchers to seek for herbal alternatives. In endodontics, herbal extracts can be used as irrigants and intracanal medicaments to overcome the potential side effects caused by conventional chemical agents. Such herbal preparations are derived from seeds, stems, flowers, roots and the leaves of medicinal plants. Nasturtium officinale (watercress) plant is now explored as a promising herb of great medicinal value. Many studies provide a good knowledge about the therapeutic potential of this plant and recommended its application in the field of pharmaceutical industry [4].

MATERIAL AND METHOD

Essential oil extract preparation

Nasturtium officinale (watercress) was purchased from local market in Baghdad city, Iraq. After cleaning and washing, only the leaves were collected, air dried, grinded and stored in airtight containers and sent to extraction. The extraction was prepared according to the method described in the study reported by Mahdavi.

Patient selection and sample collection

Enterococcus faecalis was isolated from 30 patients aged (25-45) during dental treatment. Single rooted teeth were only selected. Access opening to the root canal was made by using a sterile diamond round bur. Instrumentation was made by using Iso type k-files of different sizes then by sterile rotary files without use of any irrigants. The canal was flooded with sterile saline solution and agitation vertically with file was done in order to form a suspension of bacteria. Then, many paper points were introduced into the canal and kept for one minute until the canal was dry. They were transferred immediately to a tube containing sterile transport media (AMIES). The root canal samples were transported to the laboratory for isolation and identification.

Isolation of Enterococcus faecalis

The transported samples were mixed well by vortex then by use of a sterile inoculating loop some of this suspension was streaked in duplicate on plates containing blood agar and Pfizer agar (Bile Esculin Agar) an enterococcus selective media. The plates were incubated anaerobically at 37°C for 48 hours.

Identification of Enterococcus faecalis

Enterococcus faecalis identification was based on microscopical examination (Gram stain), colony morphology (on blood agar and Bile Esculin Agar) and Vitek 2 system.

In vitro experiments

Antibacterial activity of different concentrations of Nasturtium officinale essential oil against Enterococcus faecalis

Agar well diffusion method was employed to assess the antibacterial activity of the extract. The tested oil was prepared in four concentrations of 5, 10, 15 and 20 mg/ml in 10% dimethyl sulfoxide (DMSO). Six wells were prepared in Mueller Hinton Agar (MHA) plates, four of them filled with 100µl of the different concentrations of the essential oil and one with calcium hydroxide paste and the last one with DMSO as a negative control. The plates were incubated aerobically for 24hrs. at 37 ◦ C.

Determination of Minimum Bactericidal Concentration (MBC) of the tested oil on Enterococcus faecalis

Final concentrations of 10, 5, 2.5, 1.25, 0.625 were prepared from Nasturtium officinale essential oil and incorporated into a sterile BHI.A to get 10 ml of agar and the tested oil. The experimental bottles were poured into sterile petridishes and wait to become hard then inoculated by streaking loop full from activated bacteria. The petridishes were incubated for 24 hrs at 37°C including the control plate (positive control) which contained BHI.A with microbial inoculum without the addition of the tested essential oil, and (negative control) plate which contained BHI.A with different concentration of the tested oil without bacterial inoculum. The MBC was determined as the lowest concentration of the essential oil that killed the microorganism.

Test the combination effect of Nasturtium officinale essential oil with calcium hydroxide on Enterococcus faecalis in comparison to calcium hydroxide paste with iodoform

Agar well diffusion method was employed to assess the antibacterial activity of the combination of essential oil with calcium hydroxide paste. The experiment involved mixing equal amount 1:1 of minimum bactericidal concentration (MBC) value of the tested oil with calcium hydroxide paste. MBC of essential oil, calcium hydroxide paste with iodoform (ready made)were included in the wells.

Statistical analysis

Data were analyzed using SPSS (statistical package of social science) software version 25. One-way ANOVA test was used to compare the inhibition zone among different concentrations of the essential oil.

Tukey's HSD test was used to test any statistically significant difference between each two concentrations of the tested extract.

Independent sample t-test was used to compare the inhibition zone between the study and control group.

RESULTS

Identification of Enterococcus faecalis

The selected colonies of Enterococcus faecalis under the microscope appeared as Gram positive cocci. On the blood agar appeared as circular or convex colonies, white gray in color, with no hemolysis (gamma hemolysis). Bile Esculin Agar show positive reaction by Esculin hydrolysis demonstrated by black haloes and any black area around

the colonies. Vitek 2 system confirmed the presence of E. faecalis with 99% probability.

Antibacterial activity of different concentrations of Nasturtium officinale essential oil against Enterococcus faecalis

Enterococcus faecalis was sensitive to all concentrations of Nasturtium officinale essential oil and growth inhibition zones were formed as clear areas of no bacterial growth around the wells. The absence of inhibition zone had indicated the resistance of bacteria to the tested agent. In contrary to DMSO, calcium hydroxide paste revealed antibacterial activity against Enterococcus faecalis and inhibition zone was formed.

The antibacterial activity (diameter of the growth inhibition zone) found to increase as the concentrations of the Nasturtium officinale essential oil increased. By using ANOVA test, the difference was highly significant ($P \leq 0.01$) among the studied concentrations of essential oil and calcium hydroxide paste (Table 1 and Figure 1).

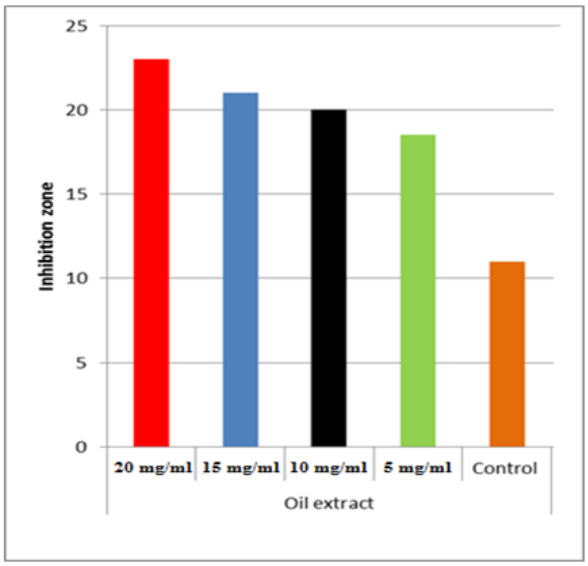

Figure 1: Antibacterial activity of Nasturtium officinale essential oil against Enterococcus faecalis and calcium hydroxide paste (control) against Enterococcus faecalis.

Extract	Conc.	Descriptive statistics					Conc. Difference	
		N	Mean	S.D.	Min.	Max.	F-test	p-value
Oil extract	20 mg/ml	10	23	2	20	26	74.241	0
	15 mg/ml	10	21	1.333	19	23		
	10 mg/ml	10	20	1.886	16	22		
	5 mg/ml	10	18.5	2.068	15	22		
	Control (calcium hydroxide paste)	10	11	0.816	10	12		

Table1: Comparison among the antibacterial activity (inhibition zone diameter in mm) of Nasturtium officinale essential oil against Enterococcus faecalis and calcium hydroxide paste (control).

Since the difference was highly significant between extract concentrations and the control, a statistical comparison had been done by using Tukey's HSD test between each two concentrations of Nasturtium officinale essential oil and the results had demonstrated that there was a highly significant difference ($P \leq 0.01$) among essential oil concentrations except between 15 mg/ml and 5 mg/ml the difference was significant ($P > 0.01$) and between 20 mg/ml and 15 mg/ml, 15 mg/ml and 10 mg/ml, 10 mg/ml and 5 mg/ml the difference was non-significant ($P > 0.05$) (Table 2).

Extract	Conc.		Mean difference	p-value
Oil extract	20 mg/ml	15 mg/ml	2	0.079
		10 mg/ml	3	0.002
		5 mg/ml	4.5	0
		Control	12	0
	15 mg/ml	10 mg/ml	1	0.678
		5 mg/ml	2.5	0.015
		Control	10	0
	10 mg/ml	5 mg/ml	1.5	0.29
		Control	9	0
	5 mg/ml	Control	7.5	0

Table2: Comparison among the antibacterial activity (inhibition zone diameter in mm) between each two concentration of Nasturtium officinale essential oil against Enterococcus faecalis and calcium hydroxide paste (control).

Determination of Minimum Bactericidal Concentration (MBC) of the tested oil on Enterococcus faecalis

The Minimum Bactericidal Concentration of Nasturtium officinale essential oil was 1.25 mg/ml.

The combination effect of Nasturtium officinale essential oil with calcium hydroxide on Enterococcus faecalis in comparison to calcium hydroxide paste with iodoform

Enterococcus faecalis was sensitive to the combination of minimum bactericidal concentration of Nasturtium officinale essential oil and calcium hydroxide paste and growth inhibition zones were formed.

Independent sample t-test was used to compare the inhibition zone between the studied combination and calcium hydroxide paste with iodoform as a control.

The results revealed that there was a highly significant difference (P ≤ 0.01) between the study and the control (Table 3).

Inhibition zones against Enterococcus faecalis	Descriptive Statistics				Group difference	
	Study		Control			
	Mean	S.D.	Mean	S.D.	t-test	p-value
Combination of oil extract with calcium hydroxide	16	2	8.4	0.966	10.82	0

Table3: Comparison among the antibacterial activity between combination of Nasturtium officinale essential oil with calcium hydroxide (study) in comparison to calcium hydroxide with iodoform (control).

Another statistical comparison had been done by using Independent sample t-test to study the effect of the studied combination and compare the inhibition zone of the tested oil at MBC value with and without calciumhydroxide. Essential oil with calcium hydroxide revealed antibacterial activity against Enterococcus faecalis higher than that of the tested oil alone (larger inhibition zones diameters formation) and the statistical difference was highly significant (P ≤ 0.01) (Table 4).

Inhibition zones against Enterococcus faecalis	Descriptive Statistics				Group difference	
	Without calcium hydroxide		With calcium hydroxide			
	Mean	S.D.	Mean	S.D.	t-test	p-value
Oil extract	12	1.414	16	2	-5.16	0

Table4: Comparison between the antibacterial activity of essential oil with and without calcium hydroxide against Enterococcus faecalis.

DISCUSSION

The use of herbal extracts in dentistry has been documented by many investigators. They have been reported to be effective in the management of many oral diseases due to their antimicrobial potential against oral bacteria.

Herbs are rich in antioxidant, antimicrobial, sedative, anxiolytic, and anti-inflammatory properties, thereby making them better alternatives for root canal disinfection. The results of this study revealed the antibacterial activity of Nasturtium officinale essential oil against Enterococcus faecalis.

This finding coincide with the study of in which the essential oil of Nasturtium officinale reported antibacterial activity against gram-positive bacteria.

Sensitivity of Enterococcus faecalis to the tested oil found to be increased as the concentrations increased, this came in agreement with the findings reported in an extensive documentation on the antimicrobial properties of essential oils. In agar diffusion method, the tested concentrations of watercress oil revealed a greater antibacterial activity than calcium hydroxide paste and the statistical analysis showed that there was a highly significant difference (P ≤ 0.01) among them. Calcium hydroxide showed a minimal antibacterial activity against Enterococcus faecalis, this finding is consistent with the study of Mozayeni el al.[5] The combination of essential oil with calcium hydroxide showed a larger growth inhibition zones of Enterococcus faecalis than the inhibition zones formed by each individual agent with a highly significant difference (P ≤ 0.01). This finding implies that a synergistic or additive effect could be achieved if higher concentration of watercress essential

oil used. The antibacterial property of watercress essential oil may be attributed to the active biological compounds reported in many studies such as alkaloids, flavonoids, saponins, terpenoids/steroids, protein, glycosides, tannins, organic alcohols and folic acid. Flavonoids are proven antimicrobial agents and several studies reported that their antibacterial mechanism of action attributed to the inhibition of nucleic acid synthesis, cytoplasmic membrane function and energy metabolism. While tannins are able to inhibit microbial extracellular enzymes, deprive the substrates necessary for microbial growth and interrupt the active transport and electron flow.

CONCLUSION

Nasturtium officinale essential oil exhibited a promising antibacterial activity suggesting its potential to be used as an endodontic irrigant and intracanal medicament. Future studies should investigate its penetration capabilities into dentinal tubules in ex-vivo models and further investigations regarding its safety, cytotoxicity and biocompatibility are needed.

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