

Evaluation of the Antimicrobial Effect of Ethanolic Extracts of Sage and Rue as a Root Canal Irrigants (An In Vivo Study).

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الخلاصة

الأهداف: تهدف هذه الدراسة الى تقييم التأثير المضاد للجراثيم للمستخلصات الكحولية للسعد و الميريمية بتركيز (0.2%) و مقارنة النتائج مع التركيز الذي هو عينه من مادة الكلورهكسيدين كلوكونت (0.2%) و المحلول الماحي الفسلجي سريريا". **المواد وطرائق العمل:** تم اختيار 35 سنا احادي الجذر و ذول لب ميت، ثم تقسيم المرضى عشوائيا الى اربع مجاميع، 10 مرضى للمجاميع الأولى و الثانية و الثالثة و ه مرضى للمجموعة الرابعة كالتالي: 0.2% من المستخلص الكحولي للميريمية، 0.2% من المستخلص الكحولي للسعد، 0.2% مادة الكلورهكسيدين كلوكونت و المحلول الماحي الفسلجي بالتسلسل. تم الحصول على عينات جرثومية من قناة الجذر في بداية الزيارة الأولى، في بداية و نهاية الزيارة الثانية، و في بداية الزيارة الثالثة باستخدام الأقماع الورقية الرطبة المعقمة (paper points). **النتائج:** أوضحت النتائج ان محلول المستخلص الكحولي بتركيز 0.2% له قابلية فعالة لقتل الجراثيم عندما يستخدم سريريا كغاسل لقناة الجذر، و لم يكن مختلفا معنويا عن 0.2% من محلول الكلورهكسيدين كلوكونت، ولكنه مختلف معنويا عن المحلول الماحي الفسلجي الأستنتاجات: أظهرت النتائج ان السعد و الميريمية تمتلك قابلية فعالة لقتل الجراثيم الهوائية و اللاهوائية لقناة الجذر عند استخدامها كغاسل لقناة الجذر سريريا و مقارنتها بمحلول الكلورهكسيدين كلوكونت.

ABSTRACT

Aims: To evaluate the antimicrobial effect of the ethanolic extracts of *Ruta graveolens* (Rue) and *Salvia officinalis* (Sage) in a concentration of 0.2% and compare the results with the same concentration of chlorhexidine 0.2% (CHX) and normal saline on root canal bacteria using the above plant extracts as an irrigating solutions clinically. **Materials and Methods:** Thirty five uniradicular teeth with necrotic pulps were chosen. The patients were divided randomly into four groups, 10 patients for groups I, II and III and 5 patients for group IV. Using 0.2% ethanolic extract of Sage, 0.2% ethanolic extract of Rue, 0.2% chlorhexidine gluconate (CHX) and normal saline, respectively. Samples were obtained from the canal at the beginning of the first and second appointments, at the end of the second appointment and at the beginning of the third appointment using wet sterile paper points. **Results:** The results revealed that 0.2% of the ethanolic extract of both Sage and Rue have a significant antimicrobial effect when used clinically as an endodontic irrigant, and was significantly not different from 0.2% chlorhexidine gluconate (CHX) and significantly different from normal saline. **Conclusions:** Rue and Sage demonstrated antimicrobial effects on the root canal bacteria (both aerobic and anaerobic) used as endodontic irrigants compared with CHX.

Key words: Antimicrobial effect, Rue, Sage, root canal bacteria.

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INTRODUCTION

The problem of microbial resistance is growing and the outlook of using antimicrobial drugs in the future is still uncertain.⁽¹⁾ Therefore, actions must be taken to reduce this problem, for example, to control the use of antibiotic, develop research to better understand the genetic mechanisms of resistance, and to continue studies to develop new drugs, either synthetic or natural. The ultimate goal is to offer appropriate and efficient antimicrobial agents to the patient.⁽²⁾

For along period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. The use of plant compounds for pharmacological purposes has gradually increased.⁽³⁾

According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants.

Therefore, such plants should be investigated to better understand their properties, safety and efficiency.⁽⁴⁾

Plant species have been utilized as a source of food, fragrance and medicine throughout the world.⁽⁵⁾ The family Lamiaceae has been extensively known to have immense medicinal, pharmacological and industrial properties. *Salvia* (sage family) is one of the large genus of Lamiaceae family and feature prominently in the pharmacopoeias of several countries of the world. It contains about 500 species of which is *Salvia officinalis*.⁽⁶⁾

The prevailing components in the plant extract obtained by ultrasound extraction were alpha - thujone (48.4%) and camphor (14.2%), 1,8 - cineole (10.9 — 43.1 %) and beta —thujone (4.9 — 25.8%). Some components of the plant extract and the essential oils have antimicrobial activity.⁽⁷⁾ It is believed that these essential oils contain complex mixture of different volatile oils which are associated with anti - microbial properties as well as flavanoids.⁽⁸⁾

Ruta graveolens (family Rutaceae) is another medicinal plant that have been used since time immemorial. Traditionally, Rue is considered to be emmenagogue, ebolic, anthelminthic and antispasmodic. It has been approved by Food and Drug Administration (FDA) as a flavouring agent. Anti - inflammatory⁽⁹⁾, antifungal⁽¹⁰⁾, antibacterial⁽¹¹⁾, and hypotensive⁽¹²⁾ activities of *Ruta graveolens* have been scientifically proven but at higher concentrations it has been reported to exhibit toxicity.⁽¹³⁾

More than 120 compounds of different classes of natural products such as acidone alkaloids, coumarins essential oils, flavonoids and furoquinolines have been isolated from *Ruta graveolens*. The medicinal properties of this plant have been attributed to the presence of these biologically active principles.^(14,15)

On the basis of the common uses of these plants in traditional folk medicine and their above reported activities in the literature, the antimicrobial effect of 0.2% concentration of the ethanolic extract of Sage and Rue on root canal bacteria (in - vivo) was evaluated and compared the results with the same concentration of the most commonly used root canal irrigant

solution (chlorhexidine gluconate 0.2%) as a positive control and normal saline solution as a negative control.

MATERIALS AND METHODS

A. Preparation of Plants Extract:

1. Aqueous extract of Sage and Rue:

Sage and Rue were purchased from a local market. Dried ariel parts of the plants were reduced to a fine powder with a mechanical grinder. The powder plant material (200 g) of each plant. was soaked in 3 liters of 80% ethanol and stored for 3 days. The extract was concentrated to dryness and stored at a temperature of 4°C until use.⁽¹⁶⁾

The dilutions was made using Dimethyl sulfoxide (DMSO), because this solution has no effect on bacteria and also help to dissolve the active ingredients of the plants and according to the following equation advocated by Summerlin (1981)⁽¹⁷⁾ (law of dilution); volume(1) x concentration(1) = volume(2) x concentration(2). 0.2% dilution of each plant was prepared.

Normal saline (0.9 w/v sodium chloride) (Mosul I.V. plant, Iraq) was used as a negative control, while 0.2% chlorhexidine (ENECA Limited UK) was used as a positive control.

Thirty five patients were selected from those attending a private clinic, needing endodontic treatment. Uniradicular teeth with necrotic pulps were chosen. Pulp necrosis was determined by radiographic presence of apical rarefaction and by lack of response to pulp vitality using electric pulp tester (Dentotest TB O9 Germany). A detailed medical and dental history was obtained from each patient and all patients gave informed consent to participate in the study. The teeth involved in this study were mostly carious.

The patients were divided randomly into four groups (10 patients for group I, II and III and 5 patients for group IV). With each group; canals were irrigated with 0.2% ethanolic extract of Sage for group I, 0.2% ethanolic extract of Rue for group II, 0.2% CHX (as positive control) for group III and normal saline (as a negative control) for group IV.

Sampling procedure:

Microbiological samples were obtained from the canal at each appointment, as follows⁽¹⁸⁾:

A. First Appointment

Following rubber dam isolation, the tooth, its surroundings and the clamp were disinfected by 70% ethanol for 1 minute. A high speed hand piece and a sterilized round no.2 bur were used to remove the carious tissue. The coronal necrotic pulp tissue was carefully removed and subsequent enlargement of the coronal third of the root canal was performed to prevent contamination of the sampling by the content of the coronal pulp and after access surgery to the root canal, new antisepsis of the operating field was carried out. Canal length was determined by placing a sterile no.10 or 15 file that fits in the canal within the average working length. A radiograph was taken, and the file length was adjusted to within 1mm of the radiographic apex. Then a sterile paper point was introduced into the full working length of the canal by means of a sterile tweezers and left for 1 minute absorb the content of the canal. In case where a dry canal was identified, a further sterile paper point moistened with sterile saline and used to ensure a viable sample acquisition. In case of a wet canal as many paper points as necessary were used to absorb all the fluid inside the canal. The paper point sample from a root canal was immediately transferred to a screw capped vial containing 5 ml thioglycollate broth (Oxoid LTD, Basigstoke, Hants / England), as a transporting media for the anaerobic microorganisms. Another paper point was placed in the canal also for one minute and then placed in another screw capped vial containing a brain heart infusion broth (BHI) (oxoid), as a transport media for the aerobic microorganisms.⁽¹⁹⁾ The canal was then debrided and irrigated with 5 ml of 0.2% ethanolic extract of sage (EES) for group I, 5 ml of 0.2% ethanolic extract of Rue (EER) for group II, 5 ml of 0.2% chlorhexidine (CHX) for group III, 5 ml of normal saline for group IV, for about 30 seconds.

The canal was then enlarged with two sizes beyond the initial measurement file and again was irrigated with 5 ml of the tested irrigating solution after each change in file size. Then the canal was dried with

paper points. 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.

B. The Second Appointment (5 days later):

It was devoted to the completion of the canal preparation. Canal preparation was achieved when it is adequately cleaned and shaped to facilitate the condensation of gutta – percha and sealer at subsequent appointment. The second appointment was identical to the first one, but the sample of the root canals contents were obtained at the beginning and at the end of this appointment.

C. Third appointment:

At the beginning of this appointment (5 days later), samples of the root canals contents were taken in the same manner as previously described. Root canal obturation was performed if there is no sign or symptoms contraindicating the procedure. The samples were again taken directly to the laboratory for microbiological work.

Microbiological Study:

Each screw capped vial was shaken to disperse the sample contents evenly. (0.1) ml inculum was taken from the inoculated thioglycollate broth and inoculated on one blood agar plate. Another (0.1) ml inoculum was taken from the inoculated brain heart infusion broth, using micropipette and inoculated on another blood agar plate.

The inoculum was streaked by a sterile cotton swab on the culture media. The blood agar plate that was inoculated with thioglycollate broth, was incubated under anaerobic conditions and the one that was inoculated with brain heart infusion broth was incubated under aerobic condition.

Both plates were incubated at 37°C for 24 hours⁽²⁰⁾. The plates were then examined and the number of bacterial colonies were counted.

Statistical Analysis:

The experimental designs which were used by the aid of computer program (SAS) were:

One Way ANOVA test.

Duncan's New Multiple Range test at level of significance 0.05.

Two sample t – test at level of significance 0.05.

RESULTS

From each root canal in all groups, both aerobic and anaerobic bacterial counts were taken at each appointment. This in vivo study showed that there was no significant difference between the antimicrobial effects of 0.2% ethanolic extract of Sage, 0.2% ethanolic extract of

Rue and 0.2% CHX on the aerobic and anaerobic microorganisms, while normal saline failed to show any antimicrobial effects. The statistical analysis for the percentages of reduction of bacterial counts reduction of both aerobic and anaerobic bacteria is shown in Tables (1-6).

Table (1): Descriptive statistics of the effects of the four irrigants solutions against aerobic and anaerobic bacteria.

Materials	Bacteria	No.**	Minimum	Maximum	Mean	Standard deviation
EES	Aerobic	10	31.11	71.95	46.410	12.576
	Anaerobic	10	0.51	86.01	36.239	25.481
EER	Aerobic	10	13.63	76.57	41.997	19.879
	Anaerobic	10	24.37	74.68	44.701	15.700
CHX	Aerobic	10	33.00	77.33	47.758	14.858
	Anaerobic	10	75.00	86.50	39.401	46.925
Normal sa-line	Aerobic	5	0.00	17.36	8.780	6.202
	Anaerobic	5	6.15	20.79	13.634	5.446

EES: Ethanolic extract of Sage. EER: Ethanolic extract of Rue. CHX: Chlorhexidine gluconate.

Table (2): Comparison between effects of the four irrigants solutions against aerobic and anaerobic bacteria.

Materials	Bacteria	No.	Mean	Standard deviation	T - test	P - value
EES	Aerobic	10	46.410	12.576	1.132	0.273
	Anaerobic	10	36.239	25.481		
EER	Aerobic	10	41.997	19.879	-0.338	0.740
	Anaerobic	10	44.701	15.700		
CHX	Aerobic	10	47.758	14.858	0.537	0.598
	Anaerobic	10	39.401	46.925		
Normal sa-line	Aerobic	5	8.780	6.202	-1.315	0.225
	Anaerobic	5	13.634	5.446		

EES: Ethanolic extract of Sage. EER: Ethanolic extract of Rue. CHX: Chlorhexidine gluconate.

Table (3): ANOVA test to compare between effects of the four irrigants solutions against aerobic bacteria.

Groups	Sum of square	df	Mean square	F - value	P - value
Between groups	5925.189	3	1975.063	8.598	0.000
Within groups	7120.915	31	229.707		
Total	13046,104	34			

Table (4): Duncan's Multiple Range Test to find the significant effect of the four irrigants solutions against aerobic bacteria.

Materials	No.	Mean	Standard deviation	Duncan's group*
EES	10	46.410	12.576	B
EER	10	41.997	19.879	B
CHX	10	47.758	14.858	B
Normal saline	5	8.780	6.202	A

*Different letters mean significant difference at $p \leq 0.05$.

Table (5): ANOVA test to compare between effects of the four irrigants solutions against anaerobic bacteria.

Groups	Sum of square	df	Mean square	F - value	P - value
Between groups	3370.672	3	1123.557		
Within groups	27998.626	31	903.181	1.244	0.011
Total	31369.298	34			

Table (6): Duncan's Multiple Range Test to find the significant effect of the four irrigants solutions against anaerobic bacteria.

Materials	**No.	Mean	Standard deviation	Duncan's group*
EES	10	36.239	25.481	B
EER	10	44.701	15.700	B
CHX	10	39.401	46.925	B
Normal saline	5	13.634	5.446	A

*Different letters mean significant difference at $p \leq 0.05$. **No.=number of samples

The results also showed that the antimicrobial effect of the ethanolic extract of Sage and Rue on the aerobic microorganisms was higher than the anaerobic but significantly not different. Also, the effect of 0.2% ethanolic extract of Rue was better than Sage extract on the aerobic microorganisms, but was significantly not different.

DISCUSSION

In recent years, drug resistance to human pathogenic bacteria has been commonly and widely reported in literature. Because of the side effects and resistance that pathogenic microorganisms build against antimicrobial agents, many scientists have recently paid attention to extracts and biologically active compounds isolated from plant species in herbal medicines.⁽²¹⁾

For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies, the use of plant compounds for medicinal purposes has gradually increased.

It has been reported that the higher plants have shown to be a potential source for new antimicrobial agents. The antimicrobial compounds from plants may inhibit bacterial growth by different mechanisms than those presently used. The antimicrobial activity of plant oils and extracts have formed the basis of many applica-

tions including alternative medicine.⁽²²⁾

Lamiaceae and Rutaceae species are important for the antimicrobial activities among plants, which are used in researches of antimicrobial activity. In this study Sage species of the family Lamiaceae and Rue species of the family Rutaceae were investigated for the antimicrobial activity on root canal microorganisms (in – vivo) and compared with commonly used root canal irrigant solution that is CHX.

Microorganisms play a key role in the pathogenesis of pulpal and periapical diseases. Successful endodontic therapy depends upon reduction or elimination of these microorganisms. Failure in endodontic therapy may be due to the persistence of infection.⁽¹⁹⁾

The statistical analysis revealed that the reduction of bacterial counts (both aerobic and anaerobic) for group I, II and III, at the end of the second appointment had no significant difference ($p > 0.05$), while it was significantly different ($p < 0.05$) from group IV (normal saline). These findings explain that the biochemical root canal irrigation with 0.2% ethanolic extract of Rue and Sage had antimicrobial effect which appeared to be sufficient to reduce the bacterial population of necrotic root canals, while sterile normal saline had no antibacterial effect. These findings may therefore, enforce the need of a combination of mechanical instrumentation and chemical irrigation to effectively remove root canal microorganisms.

In this study, 0.2% of Rue and Sage ethanolic extracts were investigated. Activities of both plants have been scientifically proven but at higher concentrations they have been reported to exhibit toxicity. It was found that the activity of these plants was reduced when higher concentration was used. This reduction in activity may be due to toxicity of the extracts at high concentrations.⁽¹³⁾

An absolute increase in bacterial counts has been demonstrated between appointments. This is probably a consequence of the ideal conditions existing for bacterial multiplication on the remaining tissue substrate within the root canal system. Several studies have shown that microorganisms may replicate in deep areas of the root canal between appointments.⁽²³⁾

CONCLUSIONS

Rue and Sage demonstrated antimicrobial effects on root canal microorganisms (both aerobic and anaerobic) when these materials are used as an endodontic irrigants in vivo.

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