

## Comparative Antibacterial Efficacy of Endemic *Satureja Khuzistanica* Jamzad Essential Oil, Sodium Hypochlorite and Chlorhexidine Gluconate Solutions as Root Canal Irrigations

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### ABSTRACT

**Background:** The aim of this study was to compare the antibacterial efficacy of endemic *Satureja Khuzistanica* Jamzad (SKJ) essential oil as root canal irrigation versus 2.5% sodium hypochlorite and 2% chlorhexidine gluconate.

**Methods:** In current in vitro experimental study, fifty four single-rooted teeth were randomly divided into 6 groups of 9 samples: 2.5% sodium hypochlorite (NaOCl), 2% chlorhexidine gluconate (CHX), 0.31 mg/ml SKJ, 0.62 mg/ml SKJ, positive and negative controls. Each tooth was instrumented, sealed and autoclaved. Then, test groups were inoculated with *E. faecalis*, treated with irrigation solution and viable bacterial counts in intracanal dentin chips were determined. Utilizing SPSS 18 software, collected data were analyzed by Kruskal-Wallis one way analysis of variance ( $P = 0.05$ ).

**Results:** 99.94 % and 99.50% reduction in bacteria load after 5 min treatment with NaOCl and CHX were detected, respectively. Similarly, 99.97% and 99.96% reduction in bacterial counts were observed after 5 min application of 0.62 mg/ml and 0.31 mg/ml SKJ essential. No significant differences were detected among the four irrigation solutions ( $P = 0.755$ ).

**Conclusion:** SKJ essential oil with the minimum inhibitory concentration (MIC) of 0.31 mg/ml could be an effective antibacterial irrigation solution.

**Keywords:** Anti-Bacterial agents, Essential, Oils, Root canal irrigants, *Satureja*.

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### Introduction

Role of bacteria and their byproducts in the pathogenesis of pulp and periodontium is well documented.<sup>1</sup> Main challenge during root canal therapy would be to maintain asepsis in vital pulp cases and antiseptics in nonvital pulp cases.<sup>2</sup> Using various irrigation solutions, chemo-mechanical instrumentation has become the preferred protocol by most clinicians. Flushing the irrigation solution in the canal space is reported to be ineffective in elimination of bacteria from canal space; therefore, antibacterial action of the irrigation solution might be the desired property of the solution.<sup>3</sup>

Phytotherapeutic potential of herbal essential oils as irrigation solutions and interappointment intracanal medications is gaining more popularity

in recent years. Herbal irrigation solutions are generally considered as safe and nontoxic for the host and some have proved to be strong antibacterial materials in-vitro. Antibacterial effect of green tea, *Morinda citrifolia*, *Zataria multiflora* Boiss extract in infected canals has been investigated.<sup>4-6</sup> *Satureja Khuzistanica* Jamzad (SKJ), an endemic plant in southern parts of Iran, has been used by the nomads to relieve the tooth pain for hundreds of years.<sup>7</sup> Antibacterial, antifungal, antiviral activity and also antiinflammatory effect of SKJ essential oil are reported.<sup>8,9</sup> Seghatoleslami et al.<sup>10</sup> tested SKJ essential oil against eleven common oral bacteria and came to the interestingly low concentration of 0.31 mg/ml as the minimum inhibitory con-

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centration (MIC) of the essential oil. SKJ was found to have substantive property up to 21 days on the bovine dentin.<sup>11</sup> Therefore, the aim of this in-vitro study was to evaluate the antibacterial effect of endemic SKJ essential oil, sodium hypochlorite and chlorhexidine gluconate solutions as root canal irrigation solutions within root canal space.

## Materials and Methods

### Collection and preparation of the samples

In this in vitro experimental study, fifty four single canal maxillary and mandibular bicuspids were collected and stored in normal saline to prevent dehydration. Number of the canals was verified by periapical radiographs and samples were visually inspected for existence of defects such as external resorption, crack and caries. All samples were decoronated at CEJ level using high speed diamond fissure bur (Tizkavan, Tehran, Iran). Working lengths were determined with K-file #10 (FKG Dentaire, La Chaux-de-Fonds, Switzerland) and range of root length was between 12-15 mm. Rotary root canal instrumentation was performed as outlined below: race #40, 10 (FKG Dentaire, La Chaux-de-Fonds/Switzerland) in 1/3 coronal section, race #35, 0.08 for 1/3 middle part and finally, race #30, 0.06 for 1/3 apical section. Irrigation was performed after each instrument application by flushing 2 ml of normal saline. To eliminate smear layer, 10 ml of 17% EDTA (Aria Dent, Asia ChemiTeB, Tehran, Iran) was used for one minute followed by 5 ml of 5.25 % NaOCl (Golrang, Pakshoo Co. Tehran, Iran), subsequently. To prevent apical leakage, apex of the roots was sealed with composite resin (Amelogen, UltraDent, Utah, USA). Each tooth was autoclaved in a cryo tube containing brain heart infusion (BHI) medium (Merck Co., Germany).

### Irrigation solutions

The essential oil of SKJ was obtained from Khorraman Co. Lorestan, Iran. Chemical analysis of the essential oil by gas chromatography/mass spectrometry (GC/MS) revealed that the major components of the SKJ essential oil are carvacrol (93%), eugenol (1%), P-cymene (0.8%) and thymol (0.6%).<sup>7</sup> Our previous in-vitro study assessing the antibacterial activity of SKJ essential oil provided MIC value of 0.31 mg/ml against nine oral pathogens.<sup>10</sup> SKJ essential oil was diluted in Di methyl sulfoxide (DMSO) to reach the final concentrations of 2 MIC and MIC which were compared to 2.5% sodium hypochlorite (Pars Co., Tehran, Iran) and 2% chlor-

hexidine gluconate (Consepsis, Ultradent, Utah, USA) as two common irrigation solutions in root canal environment in-vitro.

### Antibacterial evaluation

*Enterococcus faecalis* ATCC 29212 was used as challenging microorganism. The bacterial suspension was prepared by suspending overnight colonies from Caso agar medium (Merck Co., Germany) in 0.9% saline. The bacterial suspension was adjusted photometrically at 600 nm to a cell density equivalent to approximately 0.5 McFarland standard ( $1.5 \times 10^8$  CFU/ml).

The teeth were randomly divided into 6 groups of 9 samples. The groups consisted of 2.5% sodium hypochlorite (NaOCl), 2% chlorhexidine gluconate (CHX), 0.31 mg/ml SKJ, 0.62 mg/ml SKJ, positive and negative controls.

Under aseptic conditions, each tooth (except negative controls) was removed from the cryo tube and the canal was dried with a sterile #30 paper point (Sendoline, Solna, Sweden). Then, the canal was filled with bacterial suspension by using a sterile insulin syringe. The inoculated tooth was transferred to the cryo tube containing BHI medium and incubated at 35°C under microaerophilic condition (MART system, 5% CO<sub>2</sub>, 5.9% O<sub>2</sub>, 7.2% H<sub>2</sub>, 79% N<sub>2</sub>) for 48 h.

After incubation, the tooth was removed from cryo tube and the canals were washed 3 times with 10 ml sterile saline using a sterile insulin syringe and dried with a sterile #30 paper point (Sendoline, Solna, Sweden). Then, each canal was filled with irrigation solution using a sterile insulin syringe. After 5 minute exposure, the canal was dried with sterile paper point and filled with soy-lecithin-poly sorbate 20 (Merck Co., Germany) for inactivation of the antibacterial agent. The inactivator was removed after 5 min with sterile paper point. The positive controls were inoculated with bacterial suspension but were exposed to sterile saline instead of irrigation solution.

For determination of viable bacterial count after each treatment, dentin chips were collected by intracanal sampling using #40 hand Hedström files. The mean amount of collected dentin chips from each canal was 0.1 grams. Dentin chips were transferred into 10 ml sterile saline containing 1% Tween 80 and were shaken vigorously for 30 sec to separate the bacteria. The viable bacterial counts were determined by plating 1 ml aliquots of resulting suspension using

caso agar medium. After 48 h incubation of the plates at 35°C under microaerophilic condition, the bacterial colonies were counted and expressed as CFU (colony forming unit)/dentin chips.

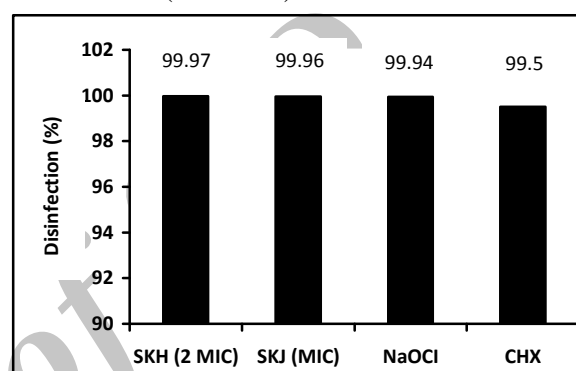
### Statistical Analysis

Statistical analysis was performed by the SPSS 18 software. Statistical differences among solutions were determined by one-way ANOVA. To compare several groups, Tukey post-hoc test was applied and the mean differences with  $p < 0.05$  were considered statistically significant. The data was not normally distributed; therefore, the comparison between groups was done using Kruskal-Wallis one-way analysis of variance. The differences between the ranks of the groups with  $p < 0.05$  were considered statistically significant.

### Results

Mean number of bacteria in positive control group ( $n=9$ ) after a period of 48 h incubation was  $2.45 \times 10^4$  CFU/dentin chips (Table 1). Mean number of bacteria after five minutes contact with NaOCl 2.5 % was  $1.5 \times 10^1$  CFU/dentin chips and  $12.3 \times 10^1$  CFU/dentin chips for CHX 2% group which showed 99.94 % and 99.50% reduction in bacterial load, respectively (Figure 1). Mean number of bacteria after five mi-

minutes contact with MIC and 2 MIC concentrations of SKJ essential oil was  $0.67 \times 10^1$  CFU/dentin chips and  $0.95 \times 10^1$  CFU/dentin chips with 99.97% and 99.96% reduction in bacterial load, respectively. All 9 samples from the positive control group demonstrated bacterial growth. However, no visible bacterial growth was observed in negative control group. While there were differences in bacterial counts among all four experimental groups in comparison with the control group ( $P < 0.05$ ), no significant differences among the four irrigation solutions were detected ( $P = 0.755$ ).



**Figure 1.** Comparison of disinfectant activity of *Satureja Khuzistanica* essential oil (SKJ) at MIC and 2 MIC values (0.31 and 0.62 mg/ml), sodium hypochlorite 2.5% (NaOCl) and chlorhexidine gluconate 2% (CHX) as root canal irrigants against *E. faecalis*.

**Table 1.** *E. faecalis* recovered from dentin chips after irrigation of tooth canals ( $n = 9$ ) with 0.31 mg/ml *Satureja Khuzistanica* Jamzad (SKJ), 0.62 mg/ml SKJ, 2.5% sodium hypochlorite or 2% chlorhexidine gluconate.

Sample	Viable count (CFU/dentin chips)				
	Positive Control group	SKJ 0.31 mg/ml	SKJ 0.62 mg/ml	sodium hypochlorite 2.5%	chlorhexidine gluconate 2%
1	$3.0 \times 10^4$	0	0	0	0
2	$1.07 \times 10^4$	0	0	0	0
3	$2.82 \times 10^4$	0	0	0	0
4	$2.86 \times 10^4$	20	25	40	240
5	$2.66 \times 10^4$	40	0	0	0
6	$2.24 \times 10^4$	0	0	0	0
7	$2.25 \times 10^4$	0	60	0	745
8	$2.14 \times 10^4$	0	0	0	0
9	$3.02 \times 10^4$	0	0	95	125
Mean	$2.45 \times 10^4$	$0.67 \times 10^1$	$0.94 \times 10^1$	$1.5 \times 10^1$	$12.33 \times 10^1$

## Discussion

Level of effectiveness of antibacterial agents might differ in root canal environment than what has been detected in laboratory tests; therefore, in a continuation of our previous investigation, SKJ essential oil was evaluated against *E. faecalis* in root canal environment in the current study.

Gentil et al.<sup>12</sup> found the *Arctium lappa* extract to completely inhibit intracanal microorganisms' growth at 14 and 30 days which seems to be rather a long period in clinical practice. Various green teas were tested against 24 bacterial strains in-vitro and found to be effective on many of them including obligative anaerobes and the action was contributed to the high contents of poly phenols which is mainly catechin.<sup>4</sup> Murray et al.<sup>5</sup> inoculated canals with *E. faecalis* and found that the *Morinda citrifolia* effectively removed the smear layer, and suggested it as an alternative to NaOCl, but did not mention the probable antibacterial property of Noni juice. Compared to NaOCl 2.5 %, no significant difference of fifteen minutes contact of 1% and 2% *Zataria multiflora* against intracanal *E. faecalis* was detected, however such concentrations seem to be rather high and indication of weaker antibacterial property of the extract.<sup>6</sup>

It was shown that an over the counter product of SKJ essential oil (Dentol, Khorraman Co. Lorestan, Iran) reduced *E. faecalis* count on bovine dentin blocks up to 99.3% at the day 28, which is a promising point of substantivity property for the SKJ extract.<sup>11</sup> But, the tested product was 10% solution of SKJ essential oil with di methyl sulfoxide (DMSO) solvent which is different from pure carvacrol and seems to be an unnecessarily high concentration and therefore, unsuitable to be used as intracanal irrigation solution. Although DMSO is considered to be neutral in bacterial activity studies, it is not considered as a safe agent for the host tissue cells.

In their pilot study, Nosrat et al.<sup>13</sup> came to similar MIC values and reported that 6% SKJ to be as effective as *E. faecalis* as final flush and suggested as an acceptable alternative for NaOCl solution.

SKJ is an endemic plant with proven antibacterial, antifungal and antiviral effects. The major component of the plant is carvacrol, with wide range of antibacterial spectrum.<sup>14</sup> Carvacrol inhibits the ATPase activity and leads to increased nonspecific permeability of bacterial cell membrane and lysis.<sup>15</sup> Spectral analysis of methanolic extract of SKJ revealed the presence of flavones, triterpenoids and

steroids, with excessive amounts of ursolic acid with known anti-inflammatory effects.<sup>16</sup>

Although sodium hypochlorite solution with various concentrations from 0.5% up to 5.25% has been widely used as intra-canal irrigation, its toxicity to vital periodontium tissues has been widely reported.<sup>17</sup> Phenolic compounds such as Eugenol, CMCP, and Cresol have a long history of usage as intracanal medicament in endodontic practice but gradually disappeared from modern practice due to high irritation potential to periapex tissues. Contrary to other former phenolic compounds, the major advantage of the SKJ extract is its' effectiveness in extremely low concentration. SKJ essential oil with MIC value as low as 0.31 mg/ml, has proved to be as effective as routine solutions such as NaOCl and CHX against resistant pathogens such as *E. faecalis*. Essential oils are generally considered as safe materials and biocompatibility studies have found SKJ essential oil to be highly safe for the vital host tissues.<sup>15</sup> Considering the complexity of root canal system and the importance of eliminating necrotic pulp remnants in areas that mechanical instrumentation has limitations, soft tissue solubility of NaOCl seems to be advantageous to SKJ essential oil, and however, such property of the SKJ remains to be investigated.

## Conclusion

SKJ essential oil with the MIC of 0.31 mg/ml might be considered as an effective antibacterial irrigation solution.

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