



## COMPARISON OF ANTIMICROBIAL EFFICACY OF LIQUORICE AND SODIUM HYPOCHLORITE AGAINST STAPHYLOCOCCUS AUREUS.

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### ABSTRACT

**Aim-**To compare the antimicrobial efficacy of liquorice and sodium hypochlorite against staphylococcus aureus.

**Materials And Method-** The present study was conducted in the Department of Pediatrics and preventive dentistry, Swargiya Dadasaheb Kalmegh Smruti Dental College Nagpur, in collaboration with Department of Microbiology. Ethical clearance for the trial was obtained from the Institutional Ethics Committee of Swargiya Dadasaheb Kalmegh Smruti Dental College. Liquorice roots were obtained from herbal medical shop in Nagpur Maharashtra. Liquorice roots were dried in shade and coarse ground were made with the electric blender. Sample of the powder were soaked in distilled water for 24 h with intermittent shaking. The active ingredients leached out in the solvent were subsequently filtered using several folds of muslin cloth and Whatman No. 1 filter paper. The filtrate of extract was concentrated using a rotavapor and freeze dried using lyophilization, following which the residues were finely ground, weighed, and stored at 4°C for further experiments.

**Results-** At the end of 48hr, statistically significant anti-microbial activity was demonstrated by 50% Liquorice extract and 3% Sodium Hypochlorite used in this study ( $P = 0.002$ ).

**Conclusion-** The inhibitory effect shown by 50% Liquorice extract against Staphylococcus aureus was superior when compared with that of 3% Sodium Hypochlorite.

### KEYWORDS :

#### INTRODUCTION

Dental caries is a dynamic process, promoted by a various multifactorial discrepancy between demineralization and remineralizing factors in the oral environment. Although oral health education and prevention are priorities in contemporary dentistry, pulp changes in deciduous teeth are very frequent and cannot be avoided at all times. Thus curative dentistry should simultaneously evolve and improve concepts and therapeutic procedures. The success of an endodontic treatment depends on many factors with the reduction or elimination of bacterial infection being the most important one. However for this to occur, precise identification of microorganisms participating in the pathogenesis of irreversible pulpitis is important in order to understand disease process and to provide effective antimicrobial treatment<sup>1</sup>

Even with the standard antimicrobial regimens reinfections are not uncommon. Studies have shown that some species of microorganisms found in root canals are resistant to routine therapy and cause persistent infections. Staphylococcus aureus are capable of surviving for extended periods because of resistance to drying and temperature changes<sup>2</sup>.

Sodium hypochlorite (NaOCl) is the most common and effective intracanal irrigants used in root canal treatments, because of its low-cost and a very effective antimicrobial activity against microbiota of infected root canals.

At these concentrations, it is highly hypertonic and strongly

alkaline with pH 11 to 13. Despite its safe properties, serious complications can result from inadvertent use due to its cytotoxic features. Most of the complications are the result of accidental extrusion of the solution from the apical foramen or accessory canals or perforations into the periapical area<sup>3</sup>. Staphylococcus aureus which are gram positive facultative anaerobes are able to remain viable for extended periods because of resistant to drying and temperature changes. A case of refractory endodontic lesion due to Staphylococcus aureus was reported by Chad M et al and the possible pathogenicity of this microorganism was discussed. The majority of Staphylococcus aureus also have the ability to develop resistance to antimicrobials rapidly thus of concern if found in inflamed pulps.<sup>4</sup>

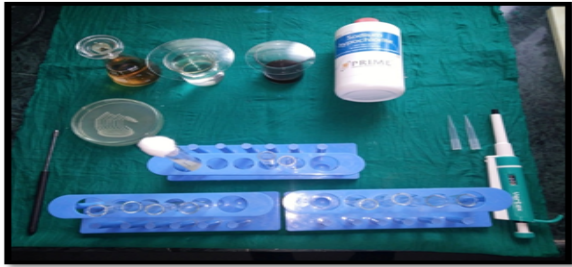
The success of endodontic treatment depends on the reduction or elimination of bacteria present in the root canal system. Residual pulpal tissue, dentine debris and bacteria like Staphylococcus aureus may persist in the irregularities of root canal systems, even after meticulous mechanical preparation.

The present study was undertaken to determine the cariostatic potential and antimicrobial efficacy of aqueous extracts of liquorice roots against Staph. aureus. The present study also aimed to compare the antimicrobial efficacy of liquorice and sodium hypochlorite.

#### MATERIALS AND METHODS

The present study was conducted in the Department of

Pediatrics and preventive dentistry, Swargiya Dadasaheb Kalmegh Smruti Dental College Nagpur, in collaboration with Department of Microbiology. Ethical clearance for the trial was obtained from the Institutional Ethics Committee of Swargiya Dadasaheb Kalmegh Smruti Dental College.



(Fig. 1- Armamentarium used for study)

**Preparation of liquorice extracts:**

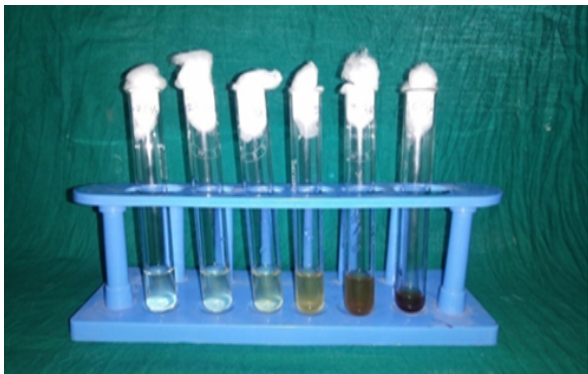
Liquorice roots were obtained from Herble medical shop in Nagpur Maharashtra. Liquorice roots were dried in shade and coarse ground were made with an electric blender. Sample of the powder were soaked in distilled water for 24 h with intermittent shaking. The active ingredients leached out in the solvent were subsequently filtered using several folds of muslin cloth and Whatman No. 1 filter paper. The filtrate of extract was concentrated using a rotavapor and freeze dried using lyophilization, following which the residues were finely ground, weighed, and stored at 4°C for further experiments. 50g powder of Liquorice root was dissolved in 100ml of distilled water for 24 hours. The active ingredients leaching out in the solvent were then filtered using Muslin cloth followed by Whatman's filter paper and the aqueous extract was stored at 4°C (Fig. 2)



(Fig. 2- Aqueous extract of Liquorice root extract)

**MIC of liquorice extract**

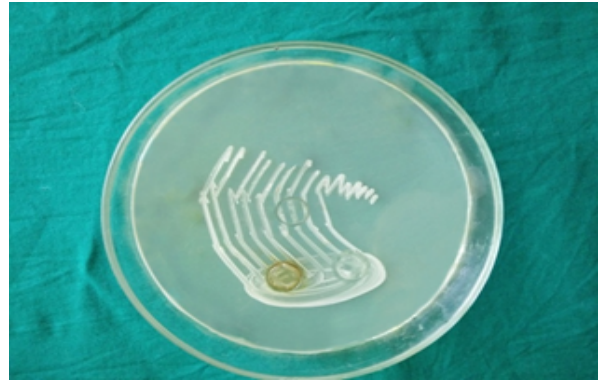
200 micro L Nutrient broth was dissolved in 200 micro L of SA. The serial dilution was then done at 100%, 50%, 25%, 12.5%, 6.25%, 3.12% concentrations respectively and the dolution was then incubated at 37 C for 24hr. MIC value was determined by using visual inspection & McFarland standard. MIC was 50%. (Fig. 3)



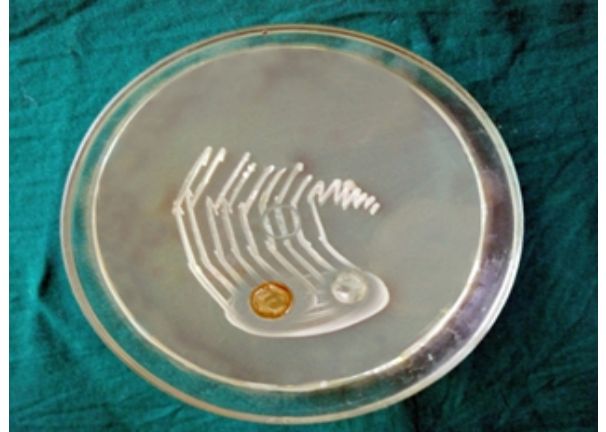
(Fig. 3- MIC of liquorice root extract)

**Zone of inhibition**

Three wells of 8 mm diameter were set on a Petri dish (Strain of SA+ Nutrient agar medium).The first well consisted of 100 l of 50% concentration of liquorice root extract. The second well consisted of 100 l of 3% Sodium Hypochlorite. The third, i.e. saline was used as the control was pipetted and the whole preparation was incubated at 37° Celsius for 48 hours. All the measurements of zone of inhibitions were carried out by a single examiner. (Fig. 4)



(Fig. 4-No evidence of zone of inhibition before incubation)



(Fig. 5- Evidence of zone of inhibition after incubation)

**RESULTS**

At the end of 48hr, statistically significant anti-microbial activity was demonstrated by 50% Liquorice extract and 3% Sodium Hypochlorite used in this study (P = 0.002). Since the data were continuous type, parametric tests were used for analysis. Mean ( ) and standard deviation were calculated. (Table 1, Fig.5)

**Table 1-Anti-microbial activity of the Liquorice extracts and Sodium hypochorite against S. aureus at 48 h.**

Sample	Meanzoneof Inhibition(in mm)±SD	P-value	Tukey post-hoc
Liquoricerootextract (50%)	13.8±0.27	0.002*	1>2>3
Sodiumhypochlorite (3%)	7.5±0.42		
Control(Saline)	0		

**ANOVA:Analysisofvariance,Significantat0.05level,SD: Standarddeviation, S.aureus:Staphylococcus aureus**

**DISCUSSION**

Liquorice is commonly known as Mulethi in Indian culture. It is hugely used to cure sore throat and cough<sup>1</sup>. The present study was conducted to evaluate and compare the antimicrobial efficacy of liquorice and 3% sodium hypochlorite against Staphylococcus aureus. The finding of

present study indicates that antimicrobial property of liquorice is superior as compare to sodium hypochlorite.

The antimicrobial activity of the liquorice extracts was assessed by estimating their inhibitory action against *Staphylococcus aureus* and determining their MIC. Results of the in vitro experiment revealed that aqueous extract of liquorice had better antimicrobial activity than the sodium hypochlorite.

In the present study, sodium hypochlorite was used in a concentration of 3%. Biomechanical preparation with NaOCl has been proven effective in eliminating microorganisms present in the root canal system.

Sodium hypochlorite (NaOCl) has been widely used as irrigation solution used from 0.5 to 5.25 concentrations for the chemo-mechanical treatment of root canals<sup>5</sup>. Concentration and toxicity of NaOCl are directly proportional<sup>6</sup>. At low concentrations of NaOCl, reducing cytotoxic and irritating properties benefit treatment but decrease antibacterial effects<sup>7</sup>.

Even though the antimicrobial action of NaOCl has not yet been fully understood, hypochlorite acid is formed in the presence of water containing active chlorine, a powerful oxidizing agent that produces an antimicrobial effect by irreversible oxidation of hydro-sulphuric groups of essential enzymes, disturbing the metabolic functions of the bacterial cell. Chlorine can also adhere to bacterial cytoplasm components forming highly toxic N-chloro composites that destroy the microorganisms<sup>8</sup>. Bystrom and Sundqvist<sup>9</sup> reported that 0.5% NaOCl was more effective than saline as an irrigant, confirming the antimicrobial properties of that substance. Although less concentrated solutions have shown antimicrobial effectiveness<sup>8</sup>.

*S. Aureus* is frequently isolated from infected root canals and it is also important bacteria among the bacteria that are resistant to normal intracanal medicaments. It is also responsible to cause endodontic infections and steady infections in follow-up visits during root canal treatment if root canal was left open<sup>10,11</sup>.

The methicillin-resistant *s. Aureus*, are highly tolerant to common antimicrobial treatments. Biocompatibility of NaOCl is inversely proportional to its concentration. According to Goldmanb et al (1981) toxicity of NaOCl has gained more importance in terms of undesirable complications throughout the treatment period<sup>12</sup>. In the lights of these problems, antimicrobial efficacy of alternative material such as liquorice root extract have been tried against *s. Aureus* and compared it with 3% sodium hypochlorite.

In present study around 13.8mm zone of inhibition was seen around 50% liquorice root extract. In present study around 7.5mm zone of inhibition was seen around 3% sodium hypochlorite.

Recent research suggests that antimicrobial, and anti-inflammatory properties of liquorice root extract and its potential benefits in oral diseases, including dental caries. Gupta V.K. et al. (2008) states that both aqueous and ethanolic liquorice extracts are potent cariostatic agents and are found to be palatable in child patients<sup>13</sup>. Eeshajain et al (2013) Khidir Agab Mohammed Hassan, Salwa Khalil (2013) Salehi M (2018) stated that liquorice is effective in the reduction of pain and inflammation of stomatitis mouth ulcers<sup>1, 14, 15</sup>. In other hand Due to the cytotoxic nature of NaOCl, occasional complications have been cited in the literature. When it comes into contact with tissue, it causes

hemolysis and ulceration, inhibits neutrophil migration, and damages endothelial and fibroblast cells<sup>16, 17</sup>. According to Chaugule VB (2015) concluded that as a irrigant sodium hypochlorite have some adverse effect specially in pediatric patient because of slippage of irrigant in periapical area due to open apex, resorb roots etc<sup>18</sup>.

So there is a need to modify root canal treatment modalities by including herbal product. *Liquorice* root extract shows antimicrobial property against *staphylococcus aureus*. We can use *liquorice* root extract as an irrigation solution in pediatric dentistry after checking its antimicrobial property against other bacterial species and toxicity of *liquorice* on the human body.

## CONCLUSION

The inhibitory effect shown by 50% Liquorice extract against *Staphylococcus aureus* was superior when compared with that of 3% Sodium Hypochlorite. Cytotoxicity of Liquorice needs to be evaluated. There is a need to carry out further research to promote Liquorice as an irrigation solution in dentistry specially in Pediatric Dentistry.

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