Republic of Iraq Ministry of Higher Education And scientific research University of Baghdad College of Dentistry



Anti-Bacterial effects of *Commiphora Myrrha* and *Ziziphus spina-christi* leaves extracts against primary colonizers of dental plaque (*Streptococcus* species) *(in vitro study)*

Thesis submitted to the Council of the College of Dentistry / university of Baghdad in partial fulfillment of the requirements for the degree of Master of Science in periodontics

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Abstract

Background

Dental plaque plays a major role in the etiology of periodontal diseases and the early colonizers of dental plaque are of great importance in the succession stages of biofilm formation. *Streptococci* constitute about 60% to 80% of oral bacteria present in the biofilm formed on tooth surface. Mechanical aids for oral hygiene in addition to a number of anti-plaque agents mostly used for plaque control as well as to prevent gingivitis which is the main goal to prevent periodontal diseases, hence, Chlorhexidine mouth wash is considered as gold standard control of the anti-plaque agents, but many side effects discourage long periods of its usage. This has led researchers to direct their attention onto plants to be able to discover new possible antimicrobial compounds alternative to synthetic chemicals such as Chlorhexidine with fewer side effects and because traditional medicinal are easily accessible, cheaper and relatively safer than other conventional medicines, of these herbal formulations Commiphora Myrrha and Ziziphus spina-christ leaves extracts are widely investigated for their antimicrobial properties.

Aims of the study

Test the antibacterial effect of alcoholic *Myrrha* and *Ziziphus* leaves extracts separately and in Combination against *Streptococcus* species (*Streptococcus sanguinis, Streptococcus mitis* and *Streptococcus oralis*) in comparison to 0.2% Chlorohexidine gluconate and distilled water *in vitro*, determination of minimum inhibitory concentration and minimum bactericidal concentration of alcoholic *Myrrha* and *Ziziphus* leaves extracts separately and in Combination.

Materials and methods

The Streptococcus species were isolated by careful collection of supragingival plaque samples from 15 (males and females) with gingivitis-dental biofilm-induced to be cultured under anaerobic conditions for 48 hours in suitable culture media using anaerobic jar in the incubator. Presence of the target microorganisms is confirmed using morphological characteristics, Gram stain, biochemical tests (Catalase, Arginine dihydrolase ability, Glucan production on both sucrose agar and

broth), hemolytic ability, antibiotic sensitivity and Vitek 2 Test. The *Myrrha* and *Ziziphus* leaves were extracted by using ethanol alcohol 70% concentration. This study involved two experiments *in vitro*. For the first experiment, agar disk diffusion technique was used to study the sensitivity of *Streptococcus* species to different concentrations of alcoholic *Myrrha* and *Ziziphus* leaves extracts (20%, 40%, 60%, 80% and 100%) separately and in Combination compared with Chlorhexidine gluconate 0.2% as a positive control and deionized water as a negative control on Mueller Hinton Agar media. The second experiment involved determination of the minimum inhibitory concentration of the extracts and then determination of the minimum bactericidal concentration of the extracts.

Results

Alcoholic Combination extracts showed highest antibacterial activity with all concentration against 3 *Streptococcus* species followed by *Myrrha* then *Ziziphus* leaves extracts. The *Streptococcus* species were sensitive to different concentration of alcoholic *Myrrha* and Combination extracts starting from 20% to 100% and to alcoholic *Ziziphus* leaves extract starting from 60% to 100% concentration. The Combination and *Myrrha* extracts at 60%,80% and 100% concentration showed almost higher antibacterial effect against almost all *Streptococcus* species than Chlorhexidine.

The minimum inhibitory concentration and minimum bactericidal concentration for *Myrrha* and Combination extracts against both *Streptococcus sanguinis* and *Streptococcus mitis* were 20% and 40% while against *Streptococcus oralis* were 40% and 60% respectively, hence they were lower than that for *Ziziphus* leaves extract against all *Streptococcus* species which were 60% and 80% respectively.

Conclusion

All the three extracts were effective against three *Streptococcus* species with bacteriostatic action and bactericidal action. Alcoholic Combination extracts with highest antibacterial activity at all concentrations against the three *Streptococcus* species then *Myrrha* and even higher than Chlorohexidine when used at high concentration, so they can be used as an alternative to Chlorohexidine.