



Evaluation of Antimicrobial Activity of Caraway Extract Against Dental Plaque Pathogens

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ABSTRACT

Dental diseases are recognized as major public health problems throughout the world. The human mouth is colonized by a variety of microorganisms, including bacteria, fungi, and viruses. Dental caries is most prevalent in early childhood and requires expensive restorative treatment. *Streptococcus mutans*, main pathogens that cause primary dental infections. Herbal medications turned to be a popular form of therapy throughout the world. Herbs have become a subject of importance as an alternative option to antibacterial agents. Caraway has played a very important role in removing dental plaque. Now a days, Caraway has been used as medicinal herb in treatment of oral cavity. Dental plaque pathogens were isolated using Nutrient agar supplemented with 5% sucrose and Brain heart infusion agar and isolates were screened for biofilm formation. The results obtained in the study clearly indicates the potential of caraway as natural, inexpensive herb for maintaining the oral health.

Keywords: Dental plaque, *Streptococcus mutans*, Caraway, biofilm formation.

INTRODUCTION

The mouth cavity contains more than 700 different bacterial species. The most common type of microorganism found in the mouth is bacteria. The mouth of a newborn is microbiologically sterile at birth, but within a few hours after delivery, streptococci colonize the mouth. Dental plaque is a soft, non-mineralized bacterial buildup on the surface of teeth or another hard surface in the mouth cavity, such as an intraoral prosthesis (Mazaheri *et al.*, 2017). Drugs that are cytotoxic can potentially cause mucositis and raise the risk of both local and systemic infections. The impact of cancer and anticancer medications on the risk of dental caries has been extensively studied.

Dietary carbohydrates are fermented by the oral flora, which results in the creation of acid that damages tooth tissues. *Streptococcus mutans* has been reported as a prominent oral streptococcal species in mouth and it produces soluble and insoluble glucans from sucrose in the diet through the action of glycosyltransferases enzymes, allowing the formation of extracellular aggregation for the biofilm formation on the tooth surfaces. D-cysteine treatment has reported to reduce the production of extracellular polysaccharides by biofilm forming *S. mutans*.

Extracellular polysaccharides aid bacteria in adhering to tooth surfaces, protecting them from external pressure and providing them with sustenance that creates an ideal habitat for microorganisms. The development of the biofilm required several processes that entail cellular adhesion to solid surfaces and cell-to-cell communication in tiny colony formations (Jorge Jesus. V. *et al.*, 2019). Dental caries has been known to be primarily caused by *Streptococcus mutans*, and strains that express the cell surface collagen-binding protein. (Zhiyan He *et al.*, 2019).

The glucan matrix is the most significant and well-characterized component of the biofilm. It offers a platform for *S. mutans* to cling to the tooth surface, mechanical stability and acidic microenvironments, The extracellular glycosyltransferases can create and organize the glucan matrix. Moreover, *S. mutans* generates mutacins, a bacteriocin that plays a significant role in the colonization of dental biofilm by *S. mutans*.

Natural products, such as herbal plants, were discovered to have fewer negative effects and a higher economic value than chemically created antibiofilm chemicals. There are several herbal components that aid in removing tooth plaque. As a dietary supplement, herbal medications are increasingly being used to treat or prevent common illnesses, including those that affect the mouth. Caraway has been proven to provide several health advantages, including anti-inflammatory, antioxidant, anticarcinogenic, and antibacterial properties. Nutraceuticals made from medicinal plants like caraway have been shown to have physiological advantages and offers some degree of protection against illnesses of the mouth.

MATERIALS AND METHODS

1. Isolation and Characterization of organisms associated with dental plaque

Sample Collection: Dental plaque samples were collected from young adults (20–25 age group).

Isolation of plaque pathogens: The plaque sample was streaked on Nutrient Agar with 5% sucrose to isolate organisms associated with plaque. General media was used, such as Nutrient Agar with 5% sucrose, Tryptone Yeast Extract Cysteine Agar, Mitis Salivarius Agar, and Brain Heart Infusion Agar.

Maintenance and characterization of isolates: Bacterial isolates were maintained on Nutrient Agar with 5% sucrose and Tryptone Yeast Extract Cysteine Agar for morphological characteristics (Kaustubh S. *et al.*, 2015).

2. Screening of biofilm forming dental plaque isolates

For qualitative biofilm formation, the isolated plaque pathogens were inoculated on a Congo red agar (CRA), is supplemented with 0.8g/l of Congo red dye. The biofilm producers give black colonies while the non-biofilm producers give red or colourless colonies after incubating at 37 degree C for 24 hrs anaerobically (Kaustubh S. *et al.*, 2015).

3. Evaluation of Antimicrobial activity of Caraway against dental plaque pathogens

Caraway procured from spice market from Mumbai, India. Aqueous extract of caraway seed was prepared by taking 10% in distilled water and mixture was refluxed for two hours. Extract was stored in dark bottle and kept in a refrigerator at 4°C for further use. Antimicrobial activity of Caraway was studied using agar well diffusion method. Muller Hinton Agar molten butt was added with culture suspension of plaque isolates and the mixture was then poured in sterile Petri plates. Using cork borer, wells were constructed and extract was added in well. The plates were kept at 37 degree C for 24 hrs. After incubation, plates were observed for zone of inhibition i.e. antimicrobial activity of the extract (Rawee T. *et al.*, 2016).

RESULTS AND DISCUSSION

Dental plaque and conditions linked to tooth caries are now serious public health issues in today's generation. The dental plaque pathogens were isolated on Nutrient Agar with 5% sucrose after enrichment in Brain Heart Infusion Broth. A total 10 isolates were obtained after screening 23 different plaque samples. During the development of the biofilm, sucrose significantly alters the biochemical and physiological processes, which enhances its caries-inducing capabilities (J.P.Woelber. *et al.*, 2017). Dental caries is a diet-dependent bacterial illness and the varied caries patterns observed was explained by the characteristics of the biofilm that has grown on the tooth surfaces. Regarding the biochemical and morphological changes that sucrose (sugar) causes in the composition of dental plaque, it is thought to be the most important dietary component. Members of the genera *Streptococcus*, *Actinomyces*, *Haemophilus*, *Neisseria*, and *Veillonella* have been reported as early colonizers of the tooth surfaces (Katherine R. *et al.*, 2019). The morphological features of the isolated plaque pathogens were studied by microscopic observation and were found to be Gram positive cocci in chain.

Congo red agar is used to identify biofilm producers as colonies that are black, crystalline and dry while non-biofilm producers as colorless colonies. Seven isolates showed black crystalline colonies on congo red agar indicating their capacity to form biofilm. Kaustubh S *et al.*, (2015) used a similar technique for characterization of oral biofilm-forming bacteria from healthy persons and validated biofilm development using Congo red agar. Anaerobes and facultative anaerobes make up the majority of the oral microbiota; hence, these organisms are typically catalase negative (Neelam B. *et al.*, 2017). In the present study, the selected Dental plaque pathogens were found to produce catalase enzyme and showed Methyl red test positive and Vogues Proskauer test negative.

Caraway was collected from local market and aqueous extract was prepared and studied for its antimicrobial activity against isolated dental plaque isolates. The Gram positive bacteria were pre-cultured in Brain Heart Infusion broth (BHI) overnight in a rotatory shaker at 37°C. Afterward the cell density was adjusted to 10⁸ cells/ml using 0.5 McFarland standard.

The caraway extracts showed promising antimicrobial activity against all the dental plaque isolates however, the highest activity was observed against isolate no.3 (Figure 1). The agar disk diffusion method offers many advantages over other methods: Simplicity, low cost, the ability to test enormous number of microorganisms and antimicrobial agents and ease to interpret results provided. Murugan P. *et al.*, (2021) and Rawee T. *et al.*, (2016) have reported the antimicrobial activity of caraway extract against dental plaque causing organisms.

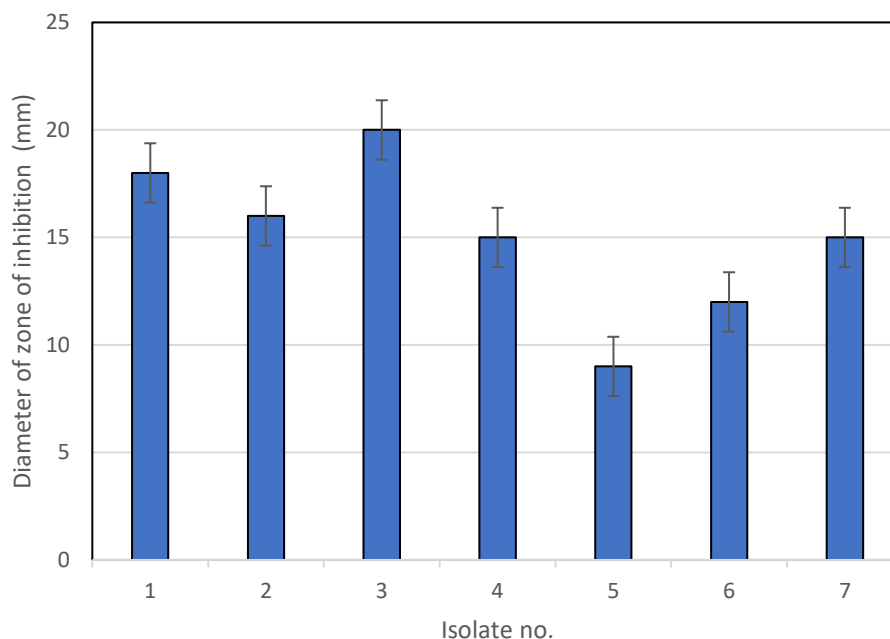


Figure 1: Antimicrobial activity of caraway extract against dental plaque isolates

CONCLUSION

Due to a lack of awareness, dental plaque issues in young adults are getting worse every day. For less developed nations, the cost of treating dental cavities is prohibitive. So, there is a need to comprehend and promote traditional ways, which can be advantageous, successful, less hazardous, readily available and inexpensive. Thus, the identification of dental plaque pathogens with biofilm formation ability would help in the identifying the actual cause of dental problems. In addition, this study would help to provide low cost-effective techniques to prevent plaque by using Caraway as herbal remedy for oral health.

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