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Antimicrobial Efficacy of Herbal Formulation Triphala and Manuka Honey for Prevention of Dental Plaque

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Abstract- *Dental Caries and periodontal diseases are among the most important global oral health problems. Dental plaque is the main cause of most of the periodontal diseases. The poor oral health can cause systemic diseases, such as cardiovascular diseases, rheumatoid arthritis and osteoporosis as well as periodontal diseases may also contribute to the risk of pregnancy complications such as preterm low birth weight. Regardless of the advancement of modern medical science, satisfactory treatment of oral diseases by newer drugs is not fully achieved yet, rather than the chemical compounds have exposed the patients to its different ill effects. Due to this, there is an interest to find out effective remedy by harmless herbal drugs. Our research paper is based on the development of anti-plaque herbal agents like Triphala and Manuka Honey-based products. Triphala is a traditional ayurvedic herbal formulation consisting of the dried fruits of three medicinal plants Terminalia chebula, Terminalia bellirica and Emblica officinalis, also known as 'three myrobalan'.Triphala means 'three'[tri] fruits [phala] is the herbal cocktail that may act in a synergetic manner within human body and can provide unique therapeutic properties with minimum or no undesirable side effects.ManukaHoney, a monofloral honeyderived from the manuka tree (Leptospermum scoparium) has greatly attained the attention of researchers for biological properties, especially its antimicrobial and antioxidant capacities. As many of the medicinal properties of plants can be transmitted through honey, so that honey could be used as a vehicle for transporting plant medicinal properties, Manuka Honey is a type of monofloral honey, a dark honey has greatly attracted the attention of the international community. This honey is derived from the Manuka tree, Leptospermum scoparium of the Myrtaceae family, which grows as a shrub or a small tree throughout New Zealand and eastern Australia[27].*

The aim of this study (Research work) is to develop the efficient use of mouthwashes based on Triphala and Manuka Honey (MH) for the prevention of dental plaque forming bacteria in the young kids and teenagers.This study was a double blind, randomized controlled field trial conducted in Patna city (Bihar), India.

Keywords: - *Dental plaque, Plaque forming bacteria, Triphala, Manuka honey,Plaque score, Antimicrobial*

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I. Introduction

Theold but time-tested proverb "If the eyes are a window to the soul, then the mouth is the doorway to the body" reflects the importance of oral health. Oral health touches every aspect of our lives but is often taken for granted. Our mouth can show signs of nutritional deficiencies or general infection. Systemic diseases: that affects the entire body, may first become apparent because of mouth lesions or other oral problems. Oral health totally depends on the teeth. Teeth are the hardest structure of the human body. The type, numbers and arrangement of a set of teeth represents the dentition. Chewing is the main function of teeth and it is the first stage of the process of digestion. Foods need to be broken down and chewed before entering the digestive system so that the body can easily absorb nutrients from them. In the absence of teeth, digestion is hampered leading to significant disturbances in food absorption. Dental plaque is a kind of structurally and functionally organized biofilm(Fig.1). Biofilms are a kind of complex communities of bacteria which typically enhances the component that's bacteria's resistance to both the host's defense system and anti-microbial agents. Bacteria live in a plaque, secrete acids, cause tooth decay, and irritate gums tissue. This irritation causes an inflammatory reaction by our body that can eventually lead to gingivitis and periodontal disease[18]. If plaque is notremoved regularly by tooth brushing and flossing, it hardens to create Calculus (also known as tartar)(Fig.2). Calculus cannot remove with a toothbrush, only a dental professional can remove it during an oral cleaning[22]. Bacterial plaque is one of the major causes for dental decay and gum disease. Different types of bacteria are normally present in the mouth. These bacteria, as well as leukocytes, neutrophils, macrophages, and lymphocytes, are part of the normal oral cavity and contribute to the individual health[4]. Several chemical agents are commercially available as treatment options for oral diseases. These chemicals can alter oral microbiota and have undesirable side effects such as vomiting, diarrhea and teeth staining. Other antibacterial agents used against oral pathogens,

including cetylpyridinium chloride, chlorhexidine, amine fluorides or products containing such agents, are reported to exhibit toxicity, cause staining of teeth or in the case of ethanol (commonly found in mouthwash) have been linked to oral cancer. The global need for alternative prevention andtreatment options and products for oral diseases that are safe, effective and economical comes from rise in diseases incidence, increased resistance by pathogenic bacteria to currently used antibiotics and chemotherapeutics opportunistic infections in individuals and financial consideration in developing countries. It has been observed that herbal products used against oral infection have more inhibitory effect on dental pathogen than synthetic drugs and have no side effects.

Fig.1- Dental plaque

Fig. 2-Calculus (tartar)in tooth

II. Materials and Methods

Nature has been an incredibly good source of medicinal treatments since millennia and plant-based systems continue to play an essential role. One such products are Triphala and Honey. According to the traditional Indian medicinal system, Triphala strengthens the different tissues of the body, prevents ageing, promotes health and shows immunomodulatory properties and helps in improving the body's defense system. It exhibits anti-viral, anti-bacterial, anti-fungal and anti-allergic properties[1][5]. Triphala is a traditional ayurvedic herbal formulation consisting of the dried fruits of three medicinal plants *Terminalia chebula,Terminaliabellirica* and *Emblica officinalis*, also known as 'three myrobalan'(Fig.3).Triphala means 'three'[tri] fruits [phala] is the herbal cocktail that may act in a synergetic manner within human body and can provide unique therapeutic properties with minimum or no undesirable side effects. Conditions for which Triphala is employed include headache, dyspensia, constipation, liver conditions, ascites and leucorrhoea. It is also used as a blood purifier that can improve the mental faculties and it possesses anti-inflammatory, analgesic, anti-arthmitic, hypoglycemic and anti-aging properties, strengthens the gums[6], prevents and treats several diseases of mouth such as dental caries, spongy and bleeding gums gingivitis and stomatitis. It has been observed that herbal products used against oral infection have more inhibitory effect on dental pathogen than synthetic drugs. Hence screening of crude plant extracts may be the best way to treat the oral diseases effectively and economically.

Fig. 3 – Triphala [Amla, Bibhitaki and Haritaki]

Just give a sweet smile, not all the sweet foods are bad for your teeth. Scientific research reveals that Manuka honey has real benefits for oral health and despite its high natural sugar contents behaves in quite different ways to refined sugar. The research also shows that high grade Manuka honey inhibits the oral bacteria associated with plaque formation, teeth decay and gum diseases. Unlike sugar, honey doesn't create an acidic demineralizing environment in the mouth (that is how the tooth decay starts). Manuka honey is showing similar effectiveness of reducing plaque formation when compared with other pharmaceutical antibacterial mouthwash chlorhexidine, being gentle on oral tissue and providing anti-inflammatory with healing properties are one of the added benefits of Manuka honey.ManukaHoney, a monofloral honey derived from the manuka tree (*Leptospermum scoparium*) has greatly attained the attention of researchers for biological properties, especially its antimicrobial and antioxidantcapacities (Fig.5).

Fig. 4: - Manuka flower

Fig. 5: - Raw Manuka Honey

MGO stands for methylglyoxal which is a chemical that occurs naturally in Manuka honey. MGO is the "Magic ingredient" in Manuka honey that grade the Manuka honey. MGO and K-factor standards ensure these following factors like Free of antibiotics, glyphosate and pesticides. Non-GMO, pH levels and antioxidant levels. High antibacterial activity and good healing properties[23][24].

The trial was conducted from Dec 2019 to Feb 2020 as a field trial in government school of Patna, Bihar. The present study was a double-blind, randomized controlled field trial conducted to evaluate the effectiveness of three types of mouthwash (MH, TR and CHx) on plaque and gingival scores of 30 students from 8–15 years. Permission was obtained to conduct the study from the principal of the selected school and their parents. Before the start of the study, both the honey and Triphala were testedfor purity at Ayurveda Research Institute for Infectious Diseases, CCRAS, Ministry of Ayush, Govt. of India and all the values were within the permissible level. The pH of mouthwash formulation was checked before conducting the study and the MH mouthwash was well above the critical pH of saliva (demineralizing action on teeth). pH of MH

mouthwash was 5.9. pH of TR mouthwash was 5.8. In Triphala, all dried powder was equally mixed for the best result.

Streptococcus is the early colonizer of plaque and after its adherence to the tooth surface it is significant in providing attachmentsubstrates for the subsequent colonizers and ultimately influencing the succeeding stages of biofilm formation. Therefore, both the mouthwashes were tested for their activity against it and final mouthwash formulations were made based on the findings of this result.

0.01g of sodium methylparaben and 0.01 g of sodium propylparaben were weighed separately and dissolved in 25 ml of distilled water. Stirring was continued till a uniform mix was obtained. To this, 0.5 g sodium benzoate was added and stirred for 15 min. This content was prepared for both the mouthwash based on Manuka and Triphala. A weighed quantity of MH (40 g/35 ml), and glycerine (5g) was added to the above contents and stirred for 10 min. For Triphala mouthwash A weighed quantity of TR(10g/100 ml) was added to the above contents and stirred for 90 minutes. All this preparation and trial were done in the supervision of Dentist Dr. Ashish Kunal Chaurasia.

All the participants were selected from a single hermitage to remove any bias to dietary and lifestyle pattern. There were some criteria like:

INCLUSION CRITERIA

1. Participants who were free from systemic diseases.
2. Having moderate gingival.
3. All the index teeth completely erupted.
4. Parents of the participants who gave informed consent.
5. Participants who gave assent.

EXCLUSION CRITERIA

1. Any participant who was suffering from any disease which might affect the salivary flow.
2. Having the history of antibiotic therapy in the previous 1 month till the start of the study.
3. Presented with retained deciduous teeth.
4. Suffering from any physical disability.

Standardization of the oral hygiene practices was done before the start of the study by teaching the participants to brush twice-daily using Colgate toothpaste and toothbrush. The MH group participants were trained to apply the Manuka honey mouthwash gently into the space between teeth and gum tissue and surround all the teeth and wait for fiveminutes before rinse. All the children were advised to do not eat or drink anything for 30 min after rinse the mouth. The procedure was repeated for 8 weeks twice a day for all three groups MH, TR and CHx. Each group having 10 children of different age group. All the children were examined at baseline (55^{th} day), just next to discontinuation of mouthwash and 62^{nd} day (1 week after discontinuation of mouthwash) for plaque index. All this procedure is done in the supervision of their parents.

III. Results

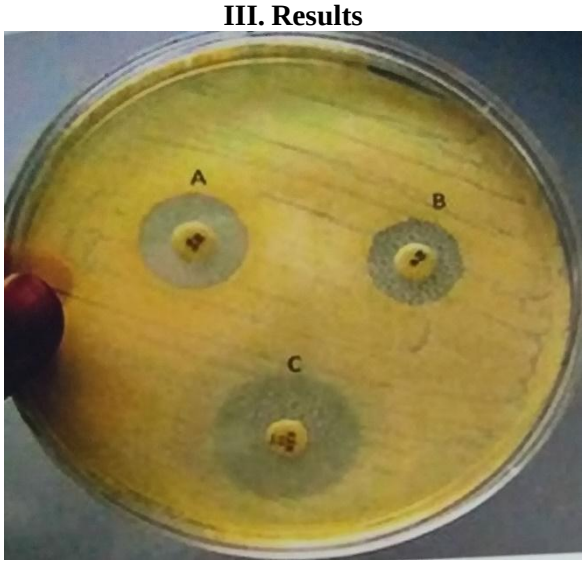

Fig. 6:-*Streptococcus mutans*MTCC 890 bacterial strain showing zone of inhibition for (A)Triphala (B) Manuka Honey (C) Chlorhexidine (CHx).

Minimum Inhibitory Concentration (MIC) used to determine the slowing down or preventing a process, reaction, or function during our experiment.

Table:1.MIC (mg/ml) of Triphala,Manuka Honey and Chlorhexidine against *Streptococcus mutans* MTCC 890.

S.No.	Mouthwashes	MIC
1.	Triphala	0.055
2.	Manuka Honey	0.045
3.	Chlorhexidine	0.039

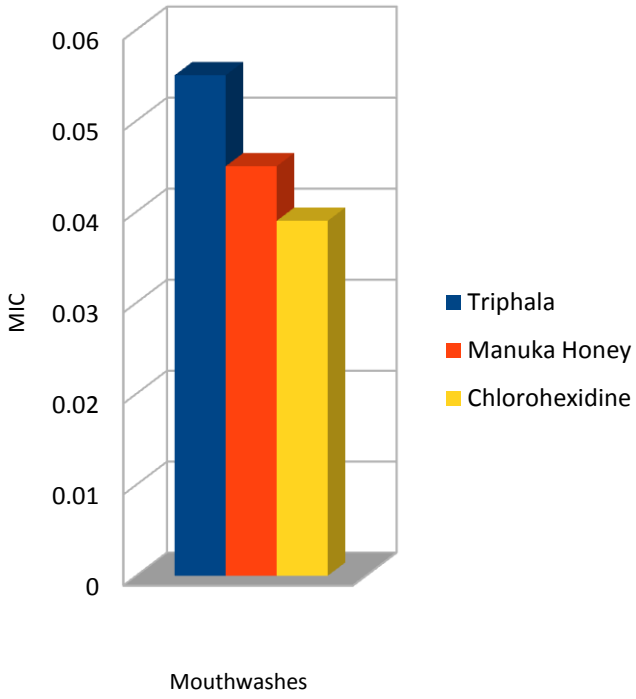

Fig.7: Comparison of Minimum Inhibitory Concentration (MIC) of different mouthwashes on *Streptococcus mutans* MTCC 890.

Table:2.Efficacy of CHx mouthwash on the reduction of dental plaque

Segment	*Average Baseline plaque Score*	*Average Experimental plaque score*	*Dental plaque reduction (%)*
Anterior	*2.95*	*1.19*	*59.95*
posterior	*3.10*	*1.34*	*56.40*
Facial	*3.96*	*1.35*	*65.99*
Lingual	*3.06*	*1.25*	*59.05*
Upper	*3.01*	*1.35*	*55.50*
Lower	*2.97*	*1.21*	*59.62*

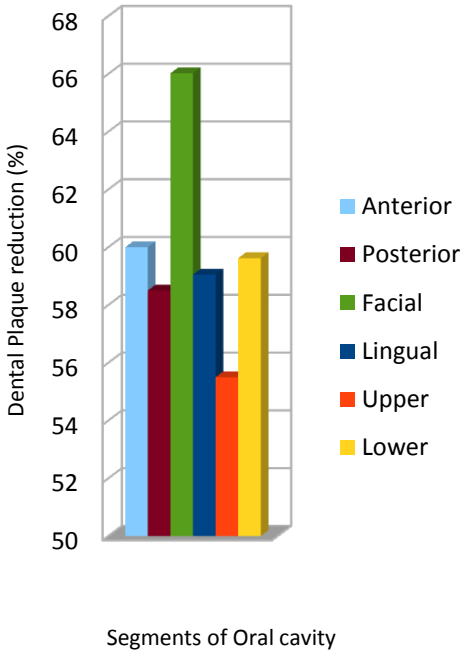

Fig.8. Efficacy of Chlorhexidine on reduction of Dental Plaque

Table:3.Efficacy of Manuka honey (MH)on the reduction of dental plaque

Segment	*Average Baseline plaque Score*	*Average Experimental plaque score*	*Dental plaque reduction (%)*
Anterior	*2.95*	*1.19*	*59.55*
posterior	*3.10*	*1.34*	*56.40*
Facial	*3.96*	*1.35*	*65.99*
Lingual	*3.06*	*1.25*	*59.05*
Upper	*3.01*	*1.35*	*55.50*
Lower	*2.97*	*1.21*	*59.62*

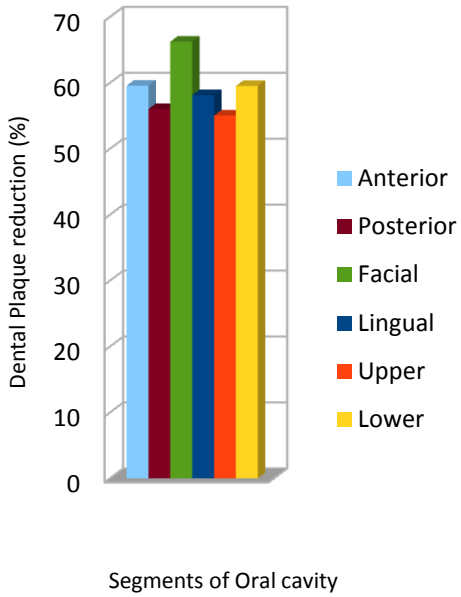

Fig.9. Efficacy of Manuka Honey on reduction of Dental Plaque

Table.:4.Efficacy of Triphala(TR) on reduction of dental plaque

Segment	*Average Baseline plaque Score*	*Average Experimental plaque score*	*Dental plaque reduction (%)*
Anterior	*2.95*	*1.20*	*59.32*
posterior	*3.20*	*1.40*	*56.25*
Facial	*3.95*	*1.35*	*65.82*
Lingual	*2.85*	*1.20*	*57.89*
Upper	*3.55*	*1.60*	*54.93*
Lower	*2.80*	*1.15*	*58.93*

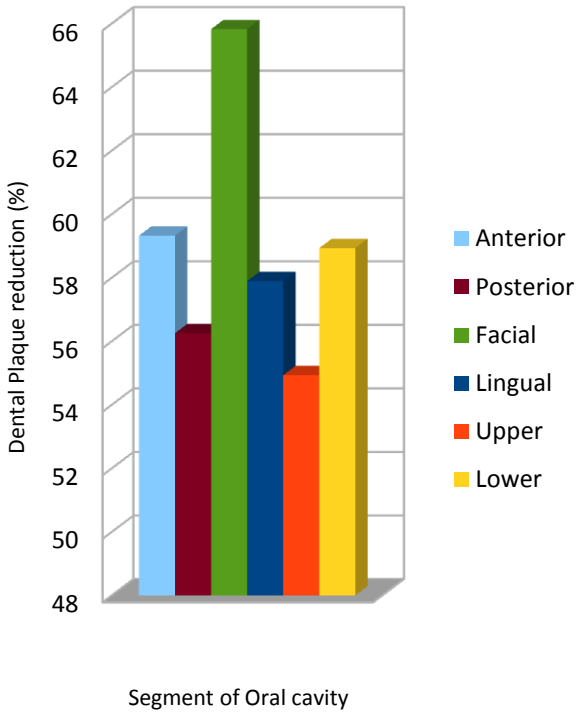

Fig.10. Efficacy of Triphala on reduction of Dental Plaque

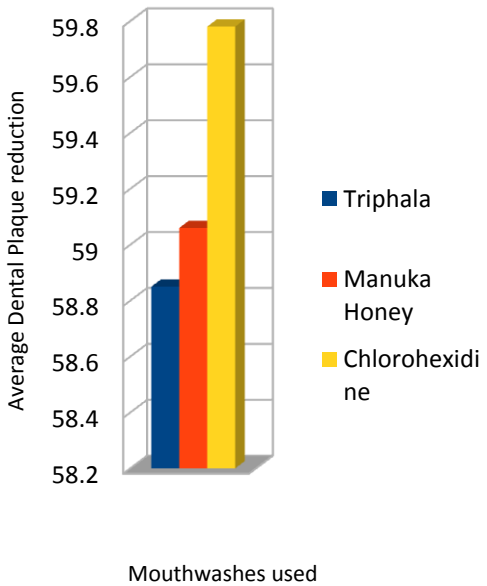

Fig:11. Comparative study of different mouthwashes for average Dental Plaque reduction

IV. Discussion

There are many antiplaque agents in the market. Of them, chlorhexidine is one of the most prescribed antiplaque agents. It has a long-lasting antibacterial activity with a broad-spectrum of action and it has been shown to reduce plaque, gingival inflammation, and bleeding (Gunsolley, 2010). Its use is considered as a powerful adjuvant to mechanical oral hygiene. Available as mouthwash, gel, aerosol, spray and disks, chlorhexidine is considered a safe compound, with minimal and transitory local and systemic side effects. Chlorhexidine mouthwash is also a useful alternative to mechanical oral hygiene procedures in those cases in which they are contraindicated, e.g. after a surgical procedure, or as a preoperative rinse (Gunsolley, 2010).
However, several clinical trials have described the occurrence of side effects associated with the use of chlorhexidine; however, all of these are completely reversible once the treatment is suspended (Addy et al., 2005). Given the incidence of oral disease, increased resistance by bacteria to antibiotics, adverse effects of some antibacterial agents currently used in dentistry and financial considerations in developing countries, there is a need for alternative prevention and treatment options that are safe, effective and economical. Hence, the search for alternative products continues and natural phytochemicals isolated from plants used as traditional medicines are considered as good alternatives.

V. Conclusion

The herbal formulation (Triphala) comprising three plants dry fruit extracts namely *Terminalia bellirica*, *Emblica officinalis* and *Terminalia chebula* have been evaluated in preventing Dental plaque formation.The qualitative determinations of active ingredients in aqueous extracts of dried fruit of different plants show that alkaloids, saponins, flavonoids, glycosides, fixed oils and fats and tannins are the main active class of phytochemicals with antimicrobial property through its free radical scavenging potential and hence, anti-plaque activity.Triphala,Manuka Honey and chlorhexidine showed no adverse effects and hence no soft tissue alterations encountered in any of the group and during any test period. Triphala and chlorhexidine mouthwash shown a comparable reduction i.e.>55% of the average baseline plaque score. Manuka Honey were appreciated by children and it had shown 53%average reduction in baseline Dental plaque score. MIC(minimum inhibitory concentration) study for Triphala, Manuka Honey and chlorhexidine on *Streptococcus mutans*MTCC890 shown comparable result. It promotes more research to be done in this field.

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