International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page:www.iscientific.org/Journal.html

Determination of antimicrobial activity of aqueous extracts of *Azadirachta indica* and Probiotic on periodontal pathogens: An in vitro study

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Abstract

Chlorhexidine (CHX) has intrinsic limitations, hence the search for an anti-plaque agent that is both efficient and potentially safe has given rise to other alternatives. Objectives: To assess the efficiency of Neem, probiotic, and CHX mouthwashes on plaque and gingivitis.The current research was performed on forty-five healthy participants in the age group of 18-25 years. They were categorized into 3 groups with 15 samples in each as-group I-Neem (*Azadirachta indica*) mouth wash, Group II- probiotic mouth rinse, and Group-III- chlorhexidine mouth wash. At baseline and 14 days after each mouth wash, Oral Hygiene Index – Simplified (OHI-S), Gingival Index (GI) and Plaque Index (PI) were noted. The obtained data was evaluated statistically. The effectiveness of neem, probiotic, and CHX mouth rinse on plaque buildup, gingival health, and oral hygiene status were not significantly different. Mouthwashes with neem and probiotics may prove to be less harmful substitutes for CHX.

Keywords: Gingivitis, mouth rinse, neem, Plaque, probiotics

Full length research article **Corresponding Author*, e-mail: n.race@qu.edu.sa

1. Introduction

Dental plaque is the primary cause of the two most prevalent dental illnesses, periodontal disease and dental caries [1,2]. One illness that affects the tissues that support the teeth is periodontal disease. Gingivitis typically stems from poor dental hygiene. The bacteria in dental plaque are one of the main causes of periodontal inflammation. As a result, it's crucial to reduce plaque by stopping the spread of dangerous germs [3]. Methods for mechanically controlling plaque have several inherent drawbacks. As a result, other therapy strategies such as the use of mouth rinse have been recommended as supplemental chemical plaque reduction methods [1]. Brushing your teeth, using dental floss, or utilizing interproximal brushes are mechanical approaches. Dentifrice, mouthwash, and other chemical treatments are used [4]. Mouthwashes have the ability to clean interproximal surfaces and other hard-to-reach places, as well as to slow the development of biofilms on soft tissues [5]. Chemical and herbal mouthwashes are the two main types on the market. A second-generation compound with cationic characteristics is chlorhexidine [6]. CHX is currently thought to be the most efficient anti-plaque agent, but it has some drawbacks, including an unpleasant taste and a tendency to discolour teeth (browning). As a result,

researchers have been looking for a safe, effective alternative to CHX mouthwash. These alternatives include natural herbs, probiotics, ozone water, and others [1,3]. Natural compounds with antioxidant, anti-inflammatory, and antibacterial characteristics include triphala and Nagavalli (P. betel) [7]. Natural herbs are utilised to treat a variety of oral health issues, including triphala, tulsipatra, neem, clove oil, and many more [5]. An effective substitute is herbal mouthwash since it contains time-tested herbal extracts and oils like neem oil, clove oil, and peelu that truly improve oral health [4]. These mouthwashes are also devoid of chemicals and alcohol.The herb neem (*Azadirachta indica*) has long been used to treat skin blemishes, infections, and swelling. Neem functions as an anti-inflammatory by inhibiting prostaglandin E and 5 HT. The term "azadirachtin" is used to describe the antibacterial effect because it is known to damage bacterial cell walls [3]. Another potential technique for inhibiting plaque growth is probiotics, which have been shown to improve dental health [1].

The objective of the current research was to determine how well neem, probiotic, and chlorhexidine mouthwashes worked to reduce plaque and gingivitis.

2. Materials and Methods

Study design: The present study was done in department of Periodontology after obtaining approval from institutional Ethical clearance and informed consent from all the participants. Forty five samples were categarised into 3 groups with 15 samples in each group as; group I: neem mouthwash, Group II- probioic mouth was (Darolac sachets,Aristo Pharmaceuticals Pvt. Ltd) and Group III- 0.2% chlorehexidine gluconate (Hexidine, ICPA Health Products Ltd, India).

Sample size estimation: Sample size of forty five was calculated using Software G Power (Version 3.1.9.2, 2014) considering effect size f=0.51, α=0.05 and 80% power of the study. Subjects between the ages of 18 and 25 who were systemically healthy and willing to participate in the research visits were eligible. Exclusion standards: Subjects with aggressive or chronic periodontitis, misaligned teeth, wearing orthodontic appliances, removable partial dentures, a history of oral prophylaxis within the previous 6 months, tobacco users and smokers, subjects receiving any antibiotic therapy within the previous three months, and subjects with a medical or pharmacological history that might compromise the conduct of the study were excluded. Two investigators with training conducted the study. The random number table method was used to create the random allocation sequence.

Mouth rinse preparation: Commercially available probioic (Darolac sachets,Aristo Pharmaceuticals Pvt. Ltd) and 0.2% chlorehexidine gluconate (Hexidine, ICPA Health Products Ltd, India) were used for the study for mouth wash preparation and neem mouthwash was prepared as per the previous study [3].

Inclusion criteria: Those who willing to engage in the study visits and who were between the ages of 18 and 25 were considered eligible.

Exclusion criteria: Tobacco users and smokers, subjects receiving any antibiotic therapy within the previous three months, subjects receiving aggressive or chronic periodontitis, misaligned teeth, wearing orthodontic appliances, removable partial dentures, a history of oral prophylaxis within the previous six months, and subjects with a medical or pharmacological history that might compromise the conduct of the study were excluded. The investigation was carried out by two trained investigators. The random allocation sequence was made using the random number table method. All study participants received oral prophylaxis to maintain baseline homogeneity. For 14 days, each group was instructed to rinse their mouths with a specific mouthwash for 60 seconds, twice daily, 30 minutes after brushing their teeth. Group II, however, was instructed to rinse with Darolac (probiotic) sachets, which were swallowed after the rinse. To maximise the effects of the mouthwash, the individuals were instructed to fast for the following 30 minutes. Using the Silness and Loe plaque index (PI), Loe and Silness gingival index (GI), and OHI-simplified at baseline and after 14 days following mouthwash, clinical examination was conducted after the trial period. To avoid confounding bias, all individuals were told to clean their teeth twice a day with the provided toothpaste and toothbrush. While the probiotic group individuals received daily freshly produced 15 ml of mouthwash, the cups of herbal (neem) and CHX mouthwash had marks for measurement. The patients were told to stop using mouthwashes and to let researchers know right once if they saw any negative side effects. On the seventh and fourteenth days, all individuals were instructed to appear at the Department of Periodontics in order to measure plaque and gingival index.Statistical evaluation of the obtained data was done using SPSS 22.0. with ANOVA test. The 5% level of significance (p0.05) was used to determine significance.

3. Results and discussion

3.1 Result

Upon intergroup comparison there was no significant difference among all the tested groups for OHI-S, plaque index, and gingival index (Table 1). There was decrease in plaque, gingivitis and OHI-S from baseline to after 14 days after mouth rinse all groups. Inter group comparison was insignificant.

3.2 Discussion

In the current study, three variables—OHI-S, PI, and GI—were used to examine the effectiveness of probiotic, herbal (neem), and CHX mouth rinse on dental health. After 14 days, all three groups in the study experienced a considerable decrease in gingival bleeding, plaque buildup, and dental hygiene, according to the data.

Significant bactericidal effects of stabilised chlorine dioxide have been observed against oral microorganisms linked to gingivitis and periodontitis. Chlorine dioxide has a wide range of biocidal action that is predominantly caused by the oxidative destruction of essential biomolecules [7].

The effects of neem-containing mouthwash on plaque and gingivitis were assessed by Jalaluddin et al. They came to the conclusion that neem mouthwash is an efficient substitute for chlorhexidine mouthwash in decreasing plaque and gingivitis [3]. Probiotic, herbal, and CHX mouthwashes

were tested for their effectiveness on the gingival health of healthy individuals by Deshmukh et al. They came to the conclusion that these mouthwashes can be effective substitutes for CHX with fewer adverse effects [1].

Table 1: Intergroup comparison of changes seen in the OHI-S, plaque index, and gingival index

Variable	Interval	Group I	Group II	Group III	p
OHI-S	Baseline	1.21±0.21	1.21±0.21	1.33±0.21	0.834(NS)
	14 days	0.30±0.13	0.30±0.13	0.33±0.21	0.643(NS)
GI	Baseline	0.77±0.12	0.77±0.12	0.78±0.08	1.753(NS)
	14 days	0.04±0.01	0.04±0.01	0.04±0.02	0.532(NS)
PI	Baseline	0.30±0.06	0.30±0.06	0.30±0.07	0.685(NS)
	14 days	0.07±0.02	0.07±0.02	0.06±0.03	0.063(NS)

GI=Gingival index, PI=Plaque index, OHI-S- oral hygiene index- simplified

In comparison to mouthwash containing chlorine dioxide, Siddeshappa et al. discovered that herbal mouthwash was statistically more effective at preventing plaque and gingivitis [7]. According to Jaidka et al's evaluation of water, xylitol mouthwash, chlorhexidine mouthwash, and herbal mouthwash on plaque and gingival inflammation, the herbal mouthwash had the greatest anti-plaque, anti-gingivitis, and anti-microbial impact [4]. Khobragade et al. discovered that using an indigenous herbal mouthwash (triphala, miswak ginger (*Zingiber officinale*), lemon extract (Citrus limon), and peppermint water) along with 0.2% chlorhexidine gluconate resulted in a statistically significant decrease in both clinical and microbiological parameters [8].Children's plaque inhibition was studied by Vanishree et al. using 0.02 M alum mouthrinse, herbal mouthrinse, and saline. They discovered that the alum group's (group I) constituents were effective at preventing the growth of plaque and might be used as an alternative to antimicrobial mouthwash. It is advised to do additional lengthy research on a sizable population to ascertain the effectiveness of mouthwashes containing alum and herbal (Hi-ora) mouthwash in enhancing oral health status [5]. Himalaya and Namboodiri's herbal mouthwash has been found by Ezhil et al to have significant antibacterial effectiveness against S. mutans [9]. Neem and mango mouthwashes can be used as effective substitutes for CHX in youngsters, according to Dandekar and Winnier [10].According to Nigam et al, the antibacterial and antiseptic properties of *Azadirecta indica* (neem), *Syzgium aromaticum* (clove), *Mentha longifolia* (mint), and *Ocimum sanctum* (tulsi) make them suitable for use as mouthwash [11]. According to Srivastava et al's comparison of the effectiveness of probiotic and chlorhexidine mouthwashes on the amount of plaque buildup and gingival inflammation, both groups experienced an improvement in their oral hygiene over the course of 28 days, but the mean difference was greater in the probiotic group. According to Harini and Anegundi's analysis, probiotic mouthwash significantly decreased plaque buildup and gingival inflammation [13].

By replacing pathogenic germs with non-pathogenic endogenous or commensal bacteria, probiotic treatment is a natural and alternative approach to treating infectious diseases [9]. The most probiotic organisms are found in the Lactobacillus and *Bifidobacterium* genera. A low molecular weight bacteriocin that is produced by lactobacilli inhibits a variety of bacterial species linked to oral disorders. In addition to metabolising lactose and generating lactic ions from lactic acid, *Bifidobacterium* species also synthesise vitamins while producing beneficial short-chain fatty acids [12].Salmonella, Escherichia coli, and Streptococcus are only a few of the Gram-positive and Gram-negative microorganisms that are successfully eradicated by neem mouthwash[3]. As an addition to regular oral hygiene practises, herbal mouthwashes may help reduce plaque and inflammation [14]. *P. granatum* and *S. persica*-containing herbal mouthwash is more effective at lowering the plaque index, gingival index, and oral hygiene index-simplified [6].Numerous bioactive substances, including *azadirachtin*, *nimbin*, and *nimbidin*, have been linked to the beneficial characteristics of neem [10]. Herboral has been shown to be helpful against plaque since it contains a

combination of neem, tulsi, triphala, clove, celery, licorice, oak tree, bakula, katha, and spearmint [15].

Chlorhexidine is widely regarded as the gold standard for reducing dental plaque because of its potent antiplaque and antibacterial properties, but herbal mouthwash was found to be equally effective at doing so without causing tooth discoloration or bad breath [15]. In the current study, neem and probiotic mouthwash is regarded as more cost-effective than mouthwash containing 0.2% chlorhexidine gluconate. Neem extract can therefore be used as a superior mouthwash substitute to 0.2% chlorhexidine gluconate. The reduction in the GI and PI scores between groups I, II, and III post rinsing demonstrated the antigingivitis and plaque-inhibiting capabilities of chlorhexidine, proboitic, and neem mouthwash.

Limitation: The drawbacks of the present study were, smaller sample size and only three products were compared. Further studies are needed to validate the results.

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