Republic of Iraq
Ministry of Higher Education and Scientific Research
University of Baghdad
College of Dentistry

Efficacy of propolis-based mouthwashes on dental plaque and gingival inflammation

A Project Submitted to

The College of Dentistry, University of Baghdad, Department of Periodontics in Partial Fulfillment for the Bachelor of Dental Surgery

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2023 A.D. **1444 A.H.**

بسم الله الرحمن الرحيم

﴿وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ﴾

صدق الله العظيم

سورة يوسف (اية 76)

Certification of the Supervisor

I certify that this project entitled **"Efficacy of propolis-based mouthwashes on dental plaque and gingival inflammation" was prepared by (Afnan Yousif , Afnan Haider)** under my supervision at the College of Dentistry/University of Baghdad in partial fulfilment of the graduation requirements for the Bachelor degree in dentistry.

Assistant Prof. Nada Kadhim Imran

The supervisor

Dedication

We dedicate this project to God Almighty our creator, our strong pillar, our source of inspiration, wisdom, knowledge and understanding.

To my beloved and my idol …. My father

To the one who enlighten my life with her love and patience my mother….

To one who her encouragement brightened our way our supervisor….

Acknowledgement

First of all, we would like to thank almighty "ALLAH" for inspiring us the energy, patience and strength to accomplish this work. A special peace to our messenger Mohammed (peace be upon him). Our sincere appreciation to **Prof. Dr. Raghad Al. hashimy,** Dean of the College of Dentistry, University of Baghdad, for continuously supporting the students. Great thanks to **Dr. Maha Shukri** (Head of Department Of Periodontics) for her high ethics and for standing help. Great thanks to our supervisor **Dr. Nada K. Imran,** we would like to express gratitude to the scientific care and to the encouragement especially her advices which light our way Thank everyone who helped us in the completion of the search for scientific truth

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Abbreviation	Scientific name
AAP	American Academy of Periodontology
EFP	European Federation of Periodontology
CHX	Chlorhexidine
DM	Diabetes Mellitus
PD	Pocket Depth
CAL	Clinical Attachment Level
PI	Plaque Index
GI	Gingival Index
PPD	Probing Pocket Depth
CFU	Colony-Forming Unit
EEP	Ethanolic Extract of Propolis
MIC	Minimum Inhibitory Concentration
MBC	Minimum Bactericidal Concentration
PBS	Papillary Bleeding Score

Abstract

Background: phytotherapy is the usage of herbal species with medicinal properties for the management of various diseases. Periodontal diseases are involve in the role of both the bacteria and the host immune response. Tooth brushing with dentifrice are effective in reducing the level of dental plaque. Over the years, various researches have shown the importance of herbal products in the management of periodontal diseases. Propolis is a resinous substance obtained from the bee hives that has antioxidant, anti-bacterial, anti-virus, antifungal, anti-tumor and anti- inflammatory effect .

Aim of the study: to evaluate the efficacy of propolis-based mouthwash on dental plaque and gingival inflammation and to assess the effect of Propolis mouthwash as an adjunctive treatment to periodontal therapy.

Conclusion: the superior efficacy of propolis-based mouthwashes in reducing gingival inflammation can be attributed to the propolis' potent anti-inflammatory properties. In addition, Propolis has shown more long-lasting effect than chlorhexidine. The results suggest that propolis-based mouthwashes have potential benefits in reducing plaque and gingival inflammation. However, methodological limitations along with small sample sizes in some of the included studies weaken the strength of the evidence. Therefore, further well-designed clinical trials with large sample sizes and adequate follow-up period are recommended to discern the efficacy of propolis mouthwash on plaque and gingivitis.

Keywords: Propolis mouthwash, Chlorhexidine, Efficacy, Plaque, Gingivitis.

Aim of the study

1. To evaluate the efficacy of propolis-based mouthwash on dental plaque and gingival inflammation.
2. To assess the effect of Propolis mouthwash as an adjunctive treatment to periodontal therapy.

Chapter One
Review of literature

1.1. Periodontal disease:

1.1.1. Introduction:

Periodontal diseases comprise a wide range of inflammatory conditions that affect the supporting structures of the teeth (the gingiva, bone and periodontal ligament), which could lead to tooth loss and contribute to systemic inflammation. Periodontal disease initiation and propagation is through a dysbiosis of the commensal oral microbiota (dental plaque), which then interacts with the immune defense of the host, leading to inflammation and disease. This pathophysiological situation persists through bouts of activity and quiescence, until the affected tooth is extracted or the microbial biofilm is therapeutically removed and the inflammation subsides.(1)

Dental plaque is a dynamic complex oral biofilm consisting of bacterial toxin and carbohydrate matrices which adhere to each other and to dental surfaces. Destruction of the gingival tissues is caused by interaction of an inflammatory process in the periodontal tissue and microorganisms in the dental plaque(2).

Adequate and proper plaque control measures have to be applied-regularly .Nevertheless, mechanical methods might not be feasible and/or sufficient. Hence, chemical preparations such as antimicrobial mouthwashes have been suggested as an adjunctive for mechanical plaque control.(3)

1.1.2 . Classification of periodontal diseases:

A classification scheme for periodontal and peri-implant diseases and conditions is necessary for clinicians to properly diagnose and treat patients as well as for scientists to investigate etiology, pathogenesis, natural history, and treatment of the diseases and conditions. The workshop was co-sponsored by the American Academy of Periodontology (AAP) and the European Federation of Periodontology (EFP) and included expert participants from all over the world.

Planning for the conference, which was held in Chicago on November 9 to 11, 2017 and began in early 2015. [4]

Workshop classified gingival diseases into [5] :

- Periodontal health and gingival health
- Gingivitis: dental biofilm-induced
- Gingival diseases: non-dental biofilm-induced

A new classification for peri-implant diseases and conditions was developed by the workshop. An effort was made to review all aspects of peri-implant health, diseases, and relevant aspects of implant site conditions and deformities to achieve a consensus for this classification that could be accepted worldwide. Case definitions were developed for use by clinicians for individual case management and also for population studies.

❖ This classification is divided into four groups [6]:

- Peri-implant health.
- Peri-implant mucositis.
- Peri-implantitis.
- Peri-implant soft and hard tissue deficiencies.

1.1.3. Pathogenesis of periodontal disease:

When the *P. gingivalis* is found in the sub gingival sulcus of the human oral cavity, it relies on the fermentation of amino acids for energy production, a property required for its survival in deep periodontal pocket, where sugar availability is low. Being an obligate anaerobe, *P. gingivalis* serves as the secondary colonizer of dental plaque, often adhering to primary colonizers such as *Streptococcus gordonii* and *P. intermedia.*[7]

There is a strong relationship between the bone destruction in periodontal diseases and the causative subgingival colonies of bacteria. The inflammatory

process of periodontal diseases is highly characterized by leucocytes infiltrations functioning to prevent more bacterial invasion. There are a lot of factors related to the recruitment of leucocytes such as cytokines, chemokines, bacterial products, lipid mediators, complements, and the cross-reaction between the two arms of human immune response: innate immune response and adaptive immune response. [8] Both innate and adaptive immunity normally work to help each other to maintain healthy state and prevent diseases. Damage in periodontitis can occur directly and indirectly.[9] The direct damage occurs as a result of the released pathogenic chemicals, while the indirect one occurs due to the host immune response to the bacterial pathogenic chemicals. The pathogenic substances released by bacteria are various and include lipopolysaccharide, lipoteichoic acid, extracellular vesicles, enzymes such as hyaluronidase, proteinase and collagenous, toxins such as leukotoxins, and metabolites such as hydrogen sulfide.[10]

1.2. Mouthwashes:

1.2.1. Introduction:

A mouthwash is a flavored, usually antiseptic solution used for cleaning the mouth and freshen the breath. Mouthwash or mouth rinse is an antiseptic solution used as an effective home care system by the patient to enhance oral hygiene. Some manufacturers of mouthwash claim that antiseptic and anti-plaque mouth rinse kill the bacterial plaque causing cavities, gingivitis, and bad breath.[11]

Mouthwashes are usually a mixture of antimicrobial agents with water or alcohol and contain-additional ingredients, such as surface-active agents-and flavor compounds. The most frequently used antimicrobial agents include chlorhexidine (CHX), Cetylpyridinium Chloride (CPC), Delmopinol, Hexe-Tidine, metal ions Cu2+, Sn2+, and Zn+, triclosan, xyl-itol, and essential oils (OE) .[12]Mouthwashes is a complement to mechanical plaque control and cannot be considered as

substitute.A mouthwash may be recommended to treat infections, reduce inflammation, relieve pain and reduce halitosis or to deliver fluoride locally for caries prevention. The preventive use of mouthwash is mainly to control dental caries and the therapeutic use is to inhibit or reduce plaque associated bacteria**.**[13]

Oral hygiene play a very important role in preventing number of diseases. Improper oral hygiene can cause various oral diseases such as periodontal diseases and systemic diseases. Hence, everyone should be educated about the importance of oral hygiene.[14]

1.2.2. Types of Mouthwash:

Mouth rinses are generally classified as either:

A- Cosmetic.

B- Alcohol & free alcohol.

C- Therapeutic.

A- Cosmetic rinses: are commercial over-the-counter products that help to remove oral debris before or after brushing, temporary suppress bad breath, diminish bacteria in the mouth and refresh the mouth with a pleasant taste (e.g. listerine)

- **Listerine:** Mouth wash or gargles contains several essential oil or their components: thymol, menthol, and eucalyptol. that have essential effect to treat throat infection and help reduce radiation-induced oral mucositis, Gargles containing essential oils can also be very effective in treating candidiasis in AIDS or a simple sore throat.[15]

B- Alchoholic & free alchoholic mouth rinses:

Most mouthwashes you see in drug stores contain an alcohol (specifically ethanol) which cause initial burning sensation, and also bring an unpleasant taste and dryness of the mouth.[16] Aside from burning sensations, alcohol in mouthwash also destroys almost all the bacteria in your mouth – both the bad and

good bacteria. This means that unless you're consistently using mouthwash each and every day, there are a lot of opportunities for bad breath to actually build up and an imbalance of bacteria to occur. (17)

Alcohol-free mouthwash may not completely wipe your mouth clean, but it does target more bad bacteria than good, creating a favorable balance to avoid further complications or bad breath. People who experience xerostomia (dry mouth), an otherwise low saliva flow due to certain medicinal side effects, radiation therapies or systemic diseases such as Sjogren's syndrome or diabetes, can all benefit from using alcohol free mouthwashes, also is particularly beneficial for people who have a history of alcohol abuse as well (18)

A mouthwash should never replace-mechanical plaque control Rather, mouthwashes should compliment your regular dental care routine to improve your oral health. (19)

C. Therapeutic rinses: often have the benefits of their cosmetic counterparts, but also contain an added active ingredient, ex. fluoride or chlorhexidine, that help protect against some oral diseases such as chlorhexidin.(20)

1.3 Chlorhexidine Mouthwash:

1.3.1. Introduction:

Chlorhexidine is an antiseptic and disinfectant. It works against a wide range of bacteria by stopping them growing and spreading. It also works against some fungi and viruses, particularly the ones found in your mouth . CHX helps to prevent build-up of plaque for up to 12 hours by forming a protective coating over your gums and teeth. This helps prevent or treat mouth infections and gum disease. It also help in reduce symptoms if you have a mouth ulcer or sore throat.(21)

The usual dose used is 10 ml , twice a day (pour 10 ml into the measuring cup rinse the mouthwash around your mouth for about 1 minute, spit out the

mouthwash and do not swallow it and then wait at least 30 to 60 minutes before having something to eat or drink).[22]

Chlorhexidene mouthwashes used in dentistry function for both prophylactic and therapeutic purposes via chemomechanical action. For these applications, CHX is used at a concentration (0.12 or ,0.2) in order to obtain an ideal dosage of 18-20mg per application to maximize efficacy, while minimizing adverse drug reactions as seen in (Table 1-1). [23]

Table(1-1): Chlorhexidine mouthwash and their uses in dentistry.

Clinical condition	Recommended Treatment	Active Ingredient(s)	Commercial mouthwash examples	Instructions for use
Oral mucositis (OM) **Oral mucositis grade 3-4**	Antimicrobial, as an adjunct to oral hygiene protocol or in replacement of oral hygiene protocol for grade 3 and 4 OM	0.12% CHX	Peridex, Periogard	15 mL rinse for 30 seconds, 3 times a day for 3 weeks 15 mL rinse for 30 seconds, 6 times a day for patients with grade 3 OM and 8 times a day for patients with grade 4 OM for 15 days (no mechanical hygiene should be completed during this time)
Periodontal Disease	Antimicrobial, as an adjunct to oral hygiene	0.12% CHX	Peridex, Periogard	15 mL rinse for 30 seconds, 2 times a day (mornings and evenings) following tooth brushing and/or SRP for 15-30 days, with a maximum of 6 months before re-evaluation, if deemed necessary by the practitioner
Prophylactic in periodontal, implant, and extraction surgeries (pre-surgical and post-surgical)	Antimicrobial, as an adjunct to proper surgical technique	0.1-0.2% CHX	Peridex, Periogard	0.2% CHX 10 mL rinse for 60 seconds immediately before surgery, and 0.12% CHX 15mL rinse for 30-60 seconds twice a day following surgery for 2-3 weeks depending on procedure performed.

It is considered the most potent chemotherapeutic mouthwash and gold standard for reducing *S. mutans* and dental plaque. Although, it appears that a CHX mouthwash concentration of 0.2% is significantly more effective in reducing supra gingival plaque.[24]Mouthwash at 0.12% is most effective as a prophylaxis in

preventing the development of gingivitis if the patient's teeth were professionally scaled prior to its application to ensure a plaque-free dental surface.

CHX mouthwash also acts as a prophylactic in periodontal and implant procedures. It may be used prior to oral surgery as well as afterwards, when mechanical maintenance may become difficult or even impossible due to pain and discomfort.Oral biofilm is the main etiologic factor for the development of periodontitis and peri-implantitis, and adversely affect the process of wound healing in the oral cavity [25,26] .

1.3.2. Side effects of chlorhexidine:

There are multiple side effects of CHX mouthwashes: [27]

A. **Staining**: CHX might cause staining of tooth surfaces, restorations, and the tongue. Often, a thorough cleaning can remove any stains. But if you have a lot of anterior white fillings, your dentist might not prescribe CHX.
B. **Alteration in taste**: a lot of people experience an alteration in taste during treatment. In rare instances, permanent taste alteration is experienced after the treatment has run its course.
C. **Tartar formation**: you may have an increase in tartar formation. [28]

1.3.3. Mechanism of action of chlorhexidine:

Chlorhexidine's broad-spectrum antimicrobial effects are due to its ability to disrupt microbial cell membranes. The positively charged CHX molecule reacts with negatively charged phosphate groups on microbial cell surfaces - this reaction both destroys the integrity of the cell and result in leakage of intracellular material, and allows CHX to enter the cell, causing precipitation of cytoplasmic components and ultimately cell death.The specific means of cell death is dependent on the concentration of CHX , lower concentrations are bacteriostatic and result in leakage of intracellular substances such as potassium and phosphorous, whereas

higher concentrations are bactericidal and cause cytoplasmic precipitation. Also Chlorhexidine prevents sticking of bacteria to the teeth and oral mucosa and causes damage to bacteria by increasing the permeability of bacterial cell walls and changing osmotic balance. [29,30]

1.3.4. Basic instructions of mouthwash use:

It bears repeating that mouthwash isn't a replacement for-mechanical plaque control . It's also not necessary to use mouthwash in order to keep your mouth clean. Most mouthwash products recommend that you use them twice per day, after brushing and flossing.[31]

A. Brush your teeth first: start by thoroughly brushing and flossing your teeth. If you're brushing with fluoride toothpaste, wait a while before using mouthwash. The mouthwash can wash away the concentrated fluoride in the toothpaste.

B. How much mouthwash to use: pour your oral rinse of choice (about 10ml used only) into a plastic measuring cup provided with the product as much mouthwash as the product instructs you to use.

C. Ready, set, rinse: during rinsing never swallow it Mouthwash isn't meant for ingesting, and it won't work if you drink it. While you're rinsing, gargle for 1minutes.You may want to set a watch or try to count to 60 second in your head.

D. Spit the mouthwash out into the sink.[32]

1.3.5. Precautions when using chlorhexidine mouthwash:

- Use prescription mouth rinses as directed (i.e., dose, frequency, duration). If a dose is missed, use the rinse as soon as possible; doubling the dose will have no therapeutic effect.
- With over-the-counter products, look for mouthrinses that have the American Dental Association Seal of Acceptance. The Seal shows that a company has

provided data demonstrating that a product is safe and effective for the purpose claimed.

- Mouth rinses does not replace the mechanical plaque control. Mouth rinses may offer additional benefit in terms of reducing the risk of bad breath, cavities, or gum disease; or for relief of dry mouth or pain that results from oral sores.
- Children younger than the age of 6 should not use mouth rinse, unless directed by a dentist, because they may swallow large amounts of the liquid inadvertently. [33]

1.4. Propolis mouth wash:

14.1. Introduction:

The word Propolis originates from Greek: «pro» = in front, «polis» = city. The meaning, in front of the city suits well the protecting role of propolis for the bee colony. The Greek world Propolis means also to glue and describes also the role of Propolis to cement openings of the bee hive. Another name of Propolis is bee glue. Propolis was already known in ancient Egypt, where it was probably used as an adhesive .

In nature, honeybees use Propolis for structural sealing of the hive. Although this product has gained acceptance in folk medicine for a thousand years, it has been recently rediscovered by researchers. The chemical composition of Propolis varies depending on regional, seasonal, and vegetational changes in plant sources from which it is collected by the bees.[34]

Propolis was mentioned by the Greek philosopher Aristoteles. In his Historia animalium it was referred to a substance which the bees smeared at the hive entrance and used as cure for bruises and sores as seen in figure(1-1) [35]

Figure (1-1):Propolis.

1.4.2. Propolis origin:

The Propolis origin is determined by comparative chemical analysis of Propolis and the glue from the botanical source. Some researchers use pollen analysis to determine Propolis origin, however this method is not acceptable as pollen collected by the bees for their nutrition needs can be mixed with Propolis in the hiv.[38] **Spectrophotometry**, especially the Folin–Ciocalteu method, is the most widely used for the routine determination of total content of phenols and certain groups of flavonoids in Propolis in addition to **NMR** metabolic profilling itself could give more detailed information especially when isomeric compounds should be identified for the Propolis [36]

1.4.3. Propolis composition:

Propolis is composed mainly by the plant resins and exudates that bees gather. Bees add wax, and also some secretions and pollen to it. The composition of Propolis depends on its botanical and thus also on its geographical origin. Several hundred different compounds have been characterized in the different

Propolis types. The typical components of Propolis are the phenolics: flavonoid aglycones, (flavones and flavanones), phenolic acids and their esters, as the volatiles. The typical compounds of Brazilian Propolis are prenylated derivatives of p-coumaric acidand of acetophenone, as well as diterpenes and lignans. The major components of Propolis can be summarized in table (1-2) [37]

Table (1-2):The major compounds of Propolis as analyzed in recent publications.

Class of components	Group of components	References
Resins	45 to 55 % flavonoids	Pápay et al., 1987 - Hungary Bankova et al., 1987 - Bulgaria Nagy et al., 1989 - Czechoslovakia Omar, 1989 - Egypt Greenaway et al., 1990a - UK Greenaway et al., 1990b - Austria, Ecuador, Germany, Israel, UK, USA Wang and Zhang, 1988 - China Mizuno et al., 1987 - Japan
	phenolic acids and esters	Nagy et al., 1985 - Hungary Wollenweber et al., 1987 - West Germany Bankova et al., 1992 - Bulgaria, Mongolia
Waxes and fatty acids	25 to 35 % most are usually from beeswax, but many are of plant origin	Pápay et al., 1987 - Hungary
Essential oils	10 % volatiles	Petri et al., 1988 - Hungary
Pollen	5 % proteins probably from pollen; free amino acids (AA): 16 AA's at more than 1 % of total AA's of which arginine and proline together make up 45.8 %, 8 AA's occur in traces	Gabrys et al., 1986 - Poland
Other organics and minerals	5 % 14 trace minerals of which Fe & Zn are most common, others e.g.: Au, Ag, Cs, Hg, La, Sb;	Scheller et al., 1989 - Poland
	ketones	Bankova et al., 1987 - Bulgaria
	lactones	Cuellar and Rojas, 1987 - Cuba
	quinones	Cuellar and Rojas, 1987 - Cuba
	steroids	Cuellar and Rojas, 1987 - Cuba
	benzoic acid and esters	Greenaway et al., 1987 - UK
	vitamins, only B_3	Greenaway et al., 1987 - UK
	sugars	Greenaway et al., 1987 - UK
General review		Walker and Crane, 1987 - World Asis, 1989 - World Crane, 1990 - World Inoue, 1988 - Japan

1.4.4. Propolis Uses:

There are multiple uses of Propolis, which can be summarized in the following points: [38,39]

- Propolis is mostly used in natural medicine as a health enhancing use as :-
- Food supplement, in medicine.
- Dental and veterinary medicine.
- In cosmetics uses.
- For food preservative or antioxidant .
- Use as a phytoinhibitor in agriculture.
- Used as air disinfectant.
- Propolis possesses interesting physical properties that make it a suitable component used in wood preservatives or varnishes and it is claimed that the famous violins of stradivarius contained propolis.
- The Propolis of the stingless bees called geopropolis was used by Amazon Indians as arrow cement.
- Because of its acaricide and antibiotic activity Propolis been proposed to be used against varroa24 or American foul brood.

1.4.5. Mechanism of action of Propolis:

The antimicrobial action of Propolis not completely understood, seems to be complex and may vary according to its composition. The compounds known to have antimicrobial action are mainly the flavonoids and cinamic acids. As an anti-infammatory agent, propolis is shown to inhibit synthesis of prostaglandins, activate the thymus gland, support the immune system by promoting phagocytic activity, stimulate cellular immunity, and augment healing effects on epithelial tissues. Additionally, Propolis contains elements, such as iron and zinc that are important for the synthesis of collagen. [40]

Propolis exhibits effective bactericidal activity against Gram-positive bacteria and limited action to Gram-negative bacteria.such as *Staphylococcus aureus (S. aureus)* and Gram negative bacteria like *Salmonella.*[41]

The efficacy of Propolis for inhibition of the activity of glycosyltransferase enzyme of *Streptococcus circuits, Streptococcus mutant* and *Streptococcus sobrinus* has been confirmed in vivo and in vitro.[42] Propolis induces the synthesis of insoluble glycan and inhibits the activity of glycosyltransferase enzyme [43]

Researchers evaluated the antibacterial activity of Propolis against some anaerobic oral pathogens and confirmed that its effectiveness against *Lactobacillus acidophilus, Actinomyces naeslundii, Prevotella oralis, Prevotella melaninogenica, Porphyromonas gingivalis, Fusobacterium nucleatum* and *Veillonella parvula,* mainly due to the presence of flavonoids and aromatic compounds such as caffeic acid in its composition. [44]

The antimicrobial effect of Propolis against periodontal pathogens has been studied in vivo and in vitro and reported significant improvements in clinical attachment level(CAL) and propoing pocket depth (ppd) along side a trend towards a reduction of *Porphyromonas gingivalis* (a pathogen that plays a key role in periodontal disease) in gingival cervical fluid (GCF)in patients treated with Propolis solutions. Similarly, proved the effectiveness of isolated Propolis products such as artepillin C, baccharin and ursolic acid as antimicrobial compounds against *P.gingivalis*; hence artepillin C and bacchatin are bacteriostatics and ursolic acid is a powerful destructor of the bacterial membrane, probably because of its highly lipophilic nature.[45]

1.4.6. Propolis storage:

Storage In general, Propolis is fairly stable, but proper storage is important. Propolis and its extracts should be stored in airtight containers in a dark place, away from excessive and direct heat. Over 12 months of proper storage, propolis will lose very little or none of its antibacterial activities. It was shown that propolis ethanol extracts exhibited unchanged antimicrobial activity after 15 years of storage. [46]This stability is probably due to the stability of the phenolic substances, mainly responsible for the antibacterial properties of Propolis. [47]

Analysis of Brazilian Propolis which was frozen for 15 years revealed the same composition, showing that Propolis can be stored as frozen without change for a long time. On the other hand, Propolis contains aromatic and heterocyclic compounds and flavones and anthraquinones that are sensible to photo.Thus Propolis should be stored in the dark, ethanol solutions should be made out of brown glass[48] The freshness of Propolis can be determined by measuring the activity of alpha-glucosidase, the activity of which decreases exponentially at room temperatures.[49]

1.4.7. Propolis and Periodontal Diseases:

It can be concluded that Propolis is safe to use and can enhance the results of periodontal and treatment option in periodontal diseases and during supportive periodontal therapy. Nonetheless, for these conclusions to be definitely confirmed, further well-designed research, with broader samples, standardized protocols and long-term follow-up to ensure reliable results, is required.[50] Reduction of insoluble polysaccharide by Propolis may not only reduce the bulk of plaque but also affect the cariogenic potential of plaque [51]

In rat models, Propolis when administered systemically reduced alveolar bone loss when assessed by morphologic and histologic parameters . Sub-gingival

irrigation with Propolis extract as an adjuvant to periodontal treatment was found to be more effective than conventional treatment according to both microbiological and clinical parameters [(52)]Hence, Propolis may be recommended in cases of gingivitis and periodontitis. However, Propolis may have a limited role when compared to CHX as an anti-plaque agent. [(53)](hidka *et al*…2008a) study the effectiveness of a Propolis- containing mouth rinse in the inhibition of de novo plaque formation and the result was that CHX mouth rinse significantly better than the others in plaque inhibition. (Agel *et al* 2004) also proved the beneficial effect of Propolis on saliva antioxidants.(54)

Another study by (el-sharkawy et al 2006) was the only one where Propolis was used as a dietary supplement. It provided a comparison of the use of a placebo and the ingestion of 400 mg of Propolis in patients with long-standing Diabetes Mellitus (DM) associated with periodontitis, reporting a significant reduction in periodontal parameters. The group treated with Propolis showed a greater reduction in pocket depth (PD) and an increase in (CAL) as compared with the control group, probably because of the anti-inflammatory, antimicrobial and antioxidant activities of Propolis.(55)

1.4.8 The Comparative Evaluation between Propolis and Chlorhexidine mouth rinses in periodontal diseases:

A number of clinical trials have investigated the efficacy of Propolis and CHX on dental plaque and gingivitis and compared between them. Some of these studies show better efficacy for Propolis in reducing gingival inflammation[(56),(57)]. (Surbhi *et al* ,2018) compared the effect of 0.2% chlorhexidine gluconate, raw Propolis on dental plaque and gingival inflammation. Thirty subjects in the age group of 20-40 years were enrolled in the study, PI and Modified Gingival Index (MgI)were recorded at baseline and oral prophylaxis was performed. Subjects were

then randomly divided into three groups and were asked to rinse with 10ml mouthwash twice daily for 15 days. Group A received 0.2% chlorhexidine gluconate mouth wash, Group B received raw Propolis diluted with distilled water (1:1). Subjects were recalled on 7 day and 28 day for reevaluation and recording plaque index and modified gingival index. The results showed that all the mouthwashes were effective in reducing plaque and gingival inflammation. over a period of 28 days[56].

(Kumar *et al*,2019) evaluated the effect of propolis mouthwash on plaque accumulation and gingivitis by comparing the plaque and gingival indices at baseline and 5-day interval, and the mouthwash was compared with both positive and negative controls. Propolis showed 68%, and chlorhexidine showed a 16% increase in plaque index on the 5th day. Propolis showed 7%, and chlorhexidine showed a 9% increase in the gingival index on the 5th day. The result showed that CHX is better than Propolis in reducing plaque formation, but Propolis is better for reduction of gingival inflammation[57]. The superior efficacy of Propolis-based mouthwashes in reducing gingival inflammation can be attributed to the Propolis' potent anti-inflammatory properties. Propolis inhibits prostaglandin production through inhibiting lipoxygenase and cyclooxygenase enzymes, resulting in a rapid and potent reduction in pain and tissue inflammation[58].

Another study found that Propolis could be used as ulternative to CHX with less side effects. Triple blind study was done by (Dehghani *et al*,2019) to evaluate the effect of Propolis and chlorhexidine mouthwashes on plaque and gingival indices in patients who are undergoing orthodontic treatment. In total, 37 patients aged from 15 to 35 years those who have been undergoing fixed orthodontic treatment were studied, after that, one of the mouthwashes that containing either Propolis or CHX was randomly prescribed to patients. The

patients were asked to use mouthwashes twice a day after brushing their teeth for three weeks consecutively. PI, GI and CPI were determined on Ramford teeth at the beginning and at the end of three weeks for each patient. The difference between the values of plaque index (P<0.001), gingival index (P=0.006) and periodontal index (P= 0.005) before and after administration of Propolis were statistically significant. The difference was also statistically significant for all three indexes of plaque (P<0.001), gingival (P=0.001) and periodontal (P=0.003) before and after chlorhexidine mouthwash usage. The indices after using mouthwashes were not statistically significant different between two mouthwash groups. It seems that Propolis mouthwash can be used as a suitable alternative in patients with fixed orthodontic treatment without the side effects of CHX[59].

(Ashok Seth *et al*,2022) investigate the effectiveness of subgingival irrigation with Propolis extract compared to chlorhexidine as an adjunct to mechanical debridement to bring out the restoration of periodontal health in patients with chronic periodontitis. Twenty subjects were selected and randomly assigned into two groups of ten subjects each, which received subgingival irrigation with 0.2% chlorhexidine (control group) and propolis extract (test group) after initial scaling and root planning on the 7^{th} day and 15^{th} day as an adjunctive treatment. PI, GI, and PPD were assessed at baseline, on the 15^{th} day, and on the 30^{th} day. Microbial analysis for the colony-forming unit (CFU) was done at baseline and on the 30^{th} day. Significant reduction was observed in PI, GI, PPD, and CFU counts from baseline to 30 days in both the groups. On intergroup comparison, the group received irrigation with chlorhexidine showed slightly better results compared to the group that received irrigation with Propolis.[60]

The application of Propolis against a broad spectrum of oral bacteria may be beneficial for improving oral health. In addition, current opinion is that the use

of standardized preparations of Propolis is safe and less toxic than many other synthetic drugs. (Champakesan *et al* ,2019) evaluate the antimicrobial efficacy of herbal and 0.2% chlorhexidine gluconate mouth rinse against *Candida albicans*. The minimum inhibitory concentration and antimicrobial effectiveness (zone of inhibition) of a herbal mouth rinse and 0.2% CHX mouth rinse were determined by broth macro-dilution and agar well diffusion method, respectively. The zone of inhibition of *C. albicans* was 26 mm for the 0.2% chlorhexidine mouth rinse, whereas it was 12 mm for the A rowash liquid mouth rinse. CHX mouth rinse (0.2%) has a better antimicrobial efficacy against the *C. albicans* when compared to herbal mouth rinses.[61]

(Akca *et al*,2016) made a comparison between the antimicrobial effect of ethanolic extract of Propolis (EEP) and chlorhexidine gluconate (CHX) on *P. gingivalis , Aggregatibacter actinomycetemcomitans(A.a), Gram-positive bacteria, Gramnegative, C. albicans ,P. intermedia.* 0.2% of CHX oral rinse solution and the ethanolic extract of propolis (EEP) were applied on the strains of the species. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) for both antimicrobial agents were determined by conducting agar dilution and broth microdilution test methods. The EEP solution inhibited the growth of all planktonic species as much as CHX except *P. gingivalis* and *A. a*. The effect of EEP was not related to 80% of ethanol since it did not affect the growth of any bacteria and the fungus. In addition, EEP was more effective against Gram-positive bacteria and *C. albicans* than Gram negative bacteria. Among the anaerobic bacteria, EEP seemed to be more effective on *P. intermedia* than CHX. This study suggested that EEP could be as effective as CHX on oral microorganisms in their biofilm state.[62]

Although CHX bonds to oral structures and slowly releases in the oral environment and has a long-lasting effect, (Anauate-Netto *et al*,2014) showed Propolis mouthwash to have more long-lasting effects and a higher efficacy compared to CHX and suggested that 2% Propolis mouthwash is stronger than 0.12% CHX and has a 45-day lasting effect. The purpose of his study was to compare the effects of typified Propolis and chlorhexidine mouth rinses on gingival health in a randomized double-blind placebo-controlled clinical trial. Sixty participants were randomized to 3 mouth rinses study groups: 1) 2% typified Propolis (n = 20); 2) 0.12% chlorhexidine (n = 20), and 3) placebo (n = 20). Participants rinsed unsupervised twice a day for 28 days. The Papillary Bleeding Score (PBS) was measured on the mesio-buccal surfaces of all teeth at baseline and 28 days thereafter. Co-variance analysis was employed to compare PBS average values and the number of sites with PBS $\geq$ 2 among study groups. Sub-group analysis was further applied to participants who were < 40 years-old. The results show efficacy of Propolis mouth rinse when comparing before and after treatment protocols significantly for a reduction of mean PBS scores. For younger participants Propolis mouth rinse was superior to all groups in reducing mean PBS scores and significant when compared to 0.12% chlorhexidine mouth rinse [(63,64)]

1.5. Conclusion: the chlorhexidine is more efficient in reducing plaque accumulation but Propolis more efficacious in reducing gingival inflammation. The superior efficacy of Propolis-based mouthwashes in reducing gingival inflammation can be attributed to the long-lasting effect than chlorhexidine.

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