BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, June 2024. Vol. **21**(2), p. 371-389

Potential Role of Medicinal Plants and their Phytochemicals against Plaque forming Oral Microbiota

Yashashree Pradhan[1], Hari Prasad Devkota[2], Ahmad Ali[1*]

[1]Department of Life Sciences, University of Mumbai, Mumbai-400098, Maharashtra, India.
[2]Graduate School of Pharmaceutical Sciences, Kumamoto University, 5-1 Oe-honmachi, Chuo-ku, Kumamoto City, Kumamoto, 862-0973, Japan.

http://dx.doi.org/10.13005/bbra/3232

(Received: 04 December 2023; accepted: 15 April 2024)

Dental plaque, a type of biofilm formed on the tooth surface, is one of the most common dental problems suffered by many individuals all over the world. Various mechanical methods are used to remove plaque and certain chemical agents are used for prevention from dental plaque formation. Though these agents have fast action, long term use of synthetic agents may cause certain side effects. People around the world have been using medicinal plants for oral health care from hundreds of years. Some of such plants that are used in the prevention of dental plaques are *Ocimum basilicum*, *Azadirachta indica*, *Syzygium aromaticum*, *Acacia nilotica* and *Achyranthes aspera*. The essential oils and extracts of these plants contain many bioactive compounds like linalool, estragole, methyl-cinnamate, eugenol, nerol, Betulin, 3,12-oleandione, 1-Hexadecanol, Phytol, Cinnamaldehyde, â-caryophyllene, nimbin, azadirachtin, catechin and quercetin which act against dental plaque forming organisms. When these essential oils and extracts are tested in vitro as well as on some patients it showed major activities against major plaque forming organisms equivalent to the chemical agents used for prevention from plaque. Hence, in long term use the products containing bioactive compounds of *Ocimum basilicum*, *Azadirachta indica*, *Syzygium aromaticum*, *Acacia nilotica* and *Achyranthes aspera* may prove more effective as well as safe. This review deals with the mechanism of plaque formation, its treatment and role of *Ocimum basilicum*, *Azadirachta indica*, *Syzygium aromaticum*, *Acacia nilotica* and *Achyranthes aspera* and their major compounds in the prevention of plaque formation.

Keywords: *Acacia nilotica*, *Achyranthes aspera*, Anti-plaque agents, *Azadirachta indica*, *Ocimum basilicum*, *Syzygium aromaticum*.

Background

The wide variety of microbiota like aerobes, obligate anaerobes, fastidious organisms, and slow growing organisms such as *Actinomyces*, *Bacteroides*, *Bifidobacterium*, *Lactobacillus*, *Candida*, *Aspergillus*, *Fusarium*, *Streptococcus* species are present in the oral cavity. Some of them require a surface for adhesion and growth which further forms the sticky matrix of polymerised glucose[1]. Dental plaque is the gelatinous mass of bacteria adhering to the tooth surface. First step of plaque formation is pellicle formation which is followed by the formation of sticky film known as plaque which leads to dental caries if not treated. The plaque forming bacteria like *Streptococcus mutans*, *Actinomyces viscosus* and

*Corresponding author E-mail: ahmadali@mu.ac.in

Lactobacillus spp. are capable of utilizing the refined carbohydrates for the energy production and generate organic acids as the by-product of the metabolism (Fig 1). Formation of dental plaques follows various mechanisms such as quorum sensing, metabolic communication, co-aggregation[2].

Dental plaque is one of the most common dental health issues in whole world. This can cause dental caries as well as periodontal diseases if not treated. These effects can further lead to loss of affected teeth. Nearly 20-50% of global population suffer from periodontal diseases which are caused due to chronic plaque formation[3]. Approximately 13.2% children and 25.9% adults among global population are affected by dental caries which is the major consequence of dental plaque[4]. Mainly children are very prone to dental plaques due to consumption of sweetened products which contains more amount of sugar. Greater amount of dental plaque can be seen in people with biting abnormalities as well as people with improper oral care[5]. Periodontal diseases can lead to various systemic consequences like cardiovascular system, pulmonary system, skeletal system and digestive system. Due to discontinuities in the oral tissues microbiota present in dental plaque may enter in blood stream which leads to various diseases in body. These microbiota can stimulate acute phase proteins as well as pro-inflammatory cytokines which increases the intensity of diseases like diabetes and atherosclerosis. Spread of bacteria to mucosal linings of gut can cause gastric ulcers as well as spread to lungs can cause pneumonia. It can also cause pregnancy complications like low birthweight of infant[6].

There are various methods used for prevention of formation of plaque like mechanical and chemical methods[7]. The most common and primary treatments include brushing, flossing and cleaning the teeth. Scaling is done for the patients who have accumulated a layer of plaque or tartar on their teeth[8]. Chemical methods like use of anti-adhesive chemicals like fluoride, chlorhexidine, perfluorosulphonamidoalkyl ester, octapinol, delmopinol are effective against *S. sanguis* and *S. mutans*[7]. Anti-microbial agents such as antibiotics, enzymes, bisbiguanide antiseptics, phenols, quaternary ammonium compounds, metal ions, natural products, fluorides, oxygenating agents and other antiseptics are used against plaque formation. The most effective plaque controlling agent is dicationic bisbiguanide antiseptic chlorhexidine. The pre-existing plaque can be removed by the enzyme such as dextranase which loosens the plaque adhered to the dental surface[9]. Some biological methods include use of a mutated strain of *S. mutans* which lacks lactate dehydrogenase gene. Another method specific against *S. mutans* is by targeted peptides or vaccines which shows bactericidal activity as well as inhibits recolonization of *S. mutans* to form biofilm[2].

These chemical agents can show adverse effects on the body like discolouration of teeth and tongue, poor taste, nausea. Besides their side effects, antibiotics also gives rise to the antibiotic resistant bacteria[9]. Various studies are carried out for the search of herbal products for prevention and treatment of plaque for reducing these side effects[10]. Herbal medicine shows preventive action against plaque accumulation and it acts on the predisposing factors of oral microorganisms from ancient times due to presence of bioactive components. Herbal mouthwashes are used for prevention for plaque accumulation and have the similar effect as the synthetic and chemical mouthwashes[11].

Ocimum basilicum, *Azadirachta indica*, *Syzygium aromaticum*, *Acacia nilotica* and *Achyranthes aspera* are some medicinal plants containing essential oils which contains bioactive compounds, showing effect against the plaque forming organisms. These bioactive components show various activities such as anti-microbial and antifungal. These activities play a major role as antiplaque agents[12]. This review deals with the mechanism of plaque formation, treatment and role of *Ocimum basilicum*, *Azadirachta indica*, *Syzygium aromaticum*, *Acacia nilotica* and *Achyranthes aspera* in the prevention of plaque formation.

Main text

Plaque Formation and its mechanism

Dental plaque is the sticky matrix of polymerised sugars, bacteria and salivary proteins. The organic matrix of plaque contains protein-polysaccharide complexes produced by microbes from carbohydrates such as fructans, dextrans, rhamnose, galactose. Small amount of lipids is also present in dental plaque. It also

includes the inorganic components such as calcium, phosphorus, magnesium, sodium and potassium[13]. Formation of dental plaques and dental biofilms follows various mechanisms such as quorum sensing, genetic exchange, metabolic communication, co-aggregation[2]. The majorly present organisms in dental plaque are Bacteria such as *Actinomyces, Bacteroides, Bifidobacterium, Lactobacillus, Streptococcus* species, fungi like Candida and Spirochetes and Mycoplasmas are present in dental biofilm. The first phase of plaque formation is an induction of linking film or conditioning film which is known as pellicle followed by accumulation that is adhesion and growth of bacteria by the mechanism of quorum sensing and then existence phase in which parallel growth and erosion of biofilm takes place. *Tannerella forsythus, Treponema denticola*, *F. nucleatum* and *streptococci* are mainly present organisms in existence phase[13].

There are three main mechanisms of plaque formation as follows:

Quorum sensing

Many organisms use quorum sensing mechanism for the formation of biofilm. It is also known as biofilm signalling. It is cell to cell communication mechanism observed in prokaryotes. This mechanism is facilitated by the exchange of small molecules known as signals or signalling molecules. It can regulate the genes and operons in the cells[14].

Quorum sensing in bacteria is divided into three classes: viz. 1) LuxI/LuxR-type quorum sensing present in Gram-negative bacteria, which uses acyl-homoserine lactones (AHL) as signalling molecules, 2) oligopeptide-two-component-type quorum sensing present in Gram-positive bacteria, which uses small peptides as signalling molecules and 3) LuxS-encoded autoinducer (AI)-2 quorum sensing is present in Gram-positive as well as Gram-negative bacteria[14].

Co-aggregation

A specific cell-to-cell reaction occurring between different bacterial cells is known as co-aggregation. It is one of the most important mechanisms used by oral bacteria for colonization and formation of dental biofilm. The planktonic bacteria which cannot colonize directly on the dental surface by adhesion can attach to the surface by attaching to specific receptors of other bacteria adhered already to the surface of teeth[15]. The early colonizing bacteria of dental biofilm attaches to complementary pellicle receptors via adhesins and the secondary bacteria further attaches to these early colonizers. One of the main bacteria which shows co-aggregation in dental biofilm formation is *Fusobacterium nucleatum* which co-aggregates with *Streptococci* and many obligate anaerobes present in oral cavity and hence play an important role as bridging organism between early and late colonizing bacteria[15]. The co-aggregation between *F. nucleatum* and Gram-negative bacteria are facilitated by lectin-carbohydrate[16]. Whereas co-aggregation between *F. nucleatum* and Gram-positive bacteria is rarely inhibited by sugars. Co-aggregation among oral bacteria contribute to bacterial colonization through physico-chemical mechanisms, as well as to genetic exchange and metabolic communication[17].

Metabolic communication

Metabolic communication is the mechanism in which the by-product of one microorganism can be the energy source of another organism[15]. Short-chain fatty acids produced by oral bacteria are an essential carbon source for some other oral bacteria. Symbiosis is observed in oral *Streptococcus* and *Veillonella* species as *Veillonella* use the short chain acids like lactates produced by Streptococci for the production of energy. Similarly *P. gingivalis* and *T. denticola* also show metabolic communication by the utilization of succinic acid produced by *T. denticola* for the production of phospholipids of cell envelope of *P. gingivalis*[18].

Medicinal plants that prevent plaque formation

***Ocimum basilicum*:**

Sweet basil is one of the plants which is considered for the studies in alternative medicine. *Ocimum basilicum* L. *(O. basilicum)* (Fig. 3). It is 20-60 cm long, aromatic, herbaceous, autogamous plant with white-purple flowers. It is an annual and perennial plant. It is cultivated in many countries but the origins of sweet basil are India and other parts of Africa, South America, Philippines, France, Portugal, Italy, Spain, Greece, Malta, Cyprus. In Mediterranean diets such as soup, cheese and pasta dishes Sweet basil is used. In Iran it is used as vegetable and food flavouring[19].

Traditional use of sweet basil is for the treatment of cough, cold, fevers,

inflammation, digestive illnesses, bug stings, pain during menstruation, anxiety, headaches, sinusitis, migraines, nerve pain, and variety of neurodegenerative ailments, diabetes, cardiovascular diseases, hypertension. Arial parts of sweet basil are rich in essential oils[20]. Essential oil of sweet basil also contains anti-microbial, antiviral, antifungal, nematocidal and insecticidal, antioxidant and anti-obesity, carminative, galactogogue, stomachic and antispasmodic properties[19]. Sweet basil also contains anticonvulsant, anti-acetylcholinesterase activities which are essential for cure of epilepsy and Alzheimer's disease as well as helps in anti-aging[21]. Basil is also used as gargles for reduction of bad odour of oral cavity. The essential oils extracted from sweet basil are used in dental and oral hygiene products. There are many bioactive components present in essential oil of sweet basil which shows anti-plaque effect as well as anti-caries effect on teeth[22].

O. basilicum is used for extraction of essential oils which are used in various fields like culinary, medicine, cosmetics. These essential oils contain a variety of chemicals which act as therapeutic agents in different diseases. The main chemical components like, phenols, esters, alcohols, oxides. The peculiar aroma of basil plants is due to linalool, methyl cinnamate, 1,8-cineole and estragole[23]. The extraction of essential oils is mainly done by gas chromatography and these components of essential oils are identified by mass spectrometry.

Main components in essential oil of the aerial parts of *O. basilicum* are linalool, eugenol, (Z)-cinnamic acid methyl ester, cyclohexene, alpha-cadinol, 2,4 diisopropenyl-1- methyl-1-vinylcyclohexane, 3,5-pyridine-dicarboxylic acid, 2,6-dimethyl-diethyl ester, beta-cubebene, guaia-1(10),11-diene, cadinene, (E)-cinnamic acid methyl ester, beta-guaiene, estragole and nerol. Many components from them acts as anti-microbial agents. These components act against mainly *S. mutans*, *C. albicans*, *S. sobrinus*, *L. casei*, *L. monocytogenes, P. gingivalis*[24].

Activity of *O. basilicum* against dental plaque

Sweet basil contains essential oils which acts against various diseases as well as various microbes. The bioactive compounds present in essential oil acts against dental plaque forming organisms. When essential oil extract of *O. basilicum* was administered to the patients suffering from dental plaque and purulent gingival diseases in dose of 250mg/ day for 3 weeks it showed reduction of dental plaque and gingival diseases[22]. Essential oil extract of sweet basil acts against *S. mutans* when tested using disc diffusion test and MIC in comparison with norfloxacin and ketoconazole. The zone of inhibition observed as 11mm and the MIC was 250ìg/ml. When extract is tested against *C. albicans* it showed zone of inhibition of 12mm and MIC of 500ìg/ml[24]. When essential oil of sweet basil was tested against *C. albicans* inoculated in brain heart infusion broth it showed MIC of 0.87mg/ml where as a nano emulsion containing sweet basil essential oil showed MIC of 0.41mg/ml which suggests that the nano emulsion is more effective against dental plaque in less quantity[25]. When essential oil of sweet basil is tested in vitro against *S. mutans* and *L. casei* biofilms using chlorohexidine as standard it showed MIC and MBC of 0.31ìl/ml and 1.25ìl/ml respectively[26]. Micro emulsion of sweet basil essential oil containing mouthwash inhibits the biofilm formation of *S. mutans* as well as it also reduces the adherence of biofilm[27]. *O. basilicum* essential oils showed zone of inhibition between 4.0 to 4.8 cm when tested against *S. mutans* and *S. sorbinus*[28].

Azadirachta indica

Azadirachta indica (Fig. 4) i.e., Neem or Margosa is one of the trees used in traditional medicine for cure of many ailments. It belongs to Meliaceae family. It is tree ranging 40-50 feet in height and straight trunk containing dark brown bark. It contains 5-15 leaflets per leaf. Flowers are small and white in colour. *A. indica* produces green coloured fruits which turns yellow after ripening. *A. indica* is native to East India and Burma and also cultivated in Tropical Africa, South and Central America, Singapore, Malaysia, Philippines, Saudi Arabia[29].

In traditional medicine *A. indica* is used for treatment for many diseases. Extracts of *A.* indica leaves and bark are used for treatment of leprosy, helminthiesis, rheumatism, respiratory disorders, skin ulcers, anorexia, diabetes, urinary disorders[30]. Leaves, bark and fruits of *A. indica* contains essential oils which shows various properties like anti-bacterial, anti-fungal, larvicidal,

anti-diabetic, anti-ulcer, anti-inflammatory, immunomodulator, anti-malarial, anti-HIV, anti-tumor, anti-hypertensive, antioxidant[29]. These essential oils also show anti-dental caries property. Due to this property *A. indica* oils are used in toothpastes and dental care products as well as in skin care products[31].

A. indica is one of the medicinal plant used in pharma as well as cosmetic industries. It contains essential oils which shows presence of various compounds. Major compounds present in aqueous extract of *A. indica* are alkaloids, esters, reducing sugars, carbohydrates, flavanoides, tannins, terpenes, phenolic compounds, isoprenoids, saponins. Ethanolic extract of *Azadirachta indica* showed presence of alkaloids, reducing sugars, carbohydrates, flavonoids, tannins, phenolic compounds, saponins, glycosides. Apart from these compounds GC-MS analysis of ethanolic extract showed presence of phytol, acetic acid, 4-Cycloocten-1-ol, 8,8'-(iminodi-2,1-phenylene), Hydroxy pivalic acid, 1,3-Diphenyl-2-azafluorene, Germanicol, acetate and Lup-20 (29)-2n-3-ol. These all compounds are extracted and studied by GC-MS[32]. From all these compounds Nimbin, Azadirachtin, Catechin and Quercetin are major bioactive compounds present in *A. indica* which acts against dental plaque.

These compounds show anti-microbial activity against various gram-positive and gram-negative microbes. Bioactive compounds present in *A. indica* acts against *S. mutans*, *S. sobrinus*[29]. These compounds also show effect against fungal pathogens such as *C. albicans* causing dental diseases[33].

Activity of *Azadirachta indica* against dental plaque

A. indica contains various compounds in its extracts as well as in essential oils. These compounds acts against various ailments. One of its activity is against dental plaque forming organisms. When n- hexane extract of *A. indica* was tested against *C. albicans* with ditchwell diffusion method it showed zone of inhibition of 28mm[34]. Ethanolic extract of *A. indica* leaves when tested in vitro by agar diffusion method it showed similar zone of inhibition as 2% sodium hypochlorite against *C. albicans* and *E. faecalis*[35]. When ethanolic extract of neem leaves tested in vivo and further tested for reduction of microbial load in dental plaque sample as well as significant decrease in colony forming units of *C. albicans* and *E. faecalis*[36]. When *A. indica* extract containing gel was given to patients suffering from baseline plaque for 3-6 weeks of treatment it showed significant decrease in amount of S. *mutans* and *Lactobacilli* when compared with treatment using chlorhexidine gluconate[37]. When aqueous extract of neem was tested using ditch plate method against *S. mutans*, *S. salivarius*, *S. sanguis* and *S. mitis* showed zone of inhibition of 3.8cm, 2.9cm, 3.4cm and 2.7cm respectively for 50% concentration and 48hr of incubation[38]. Acetone extract of neem showed zone of inhibition of 22mm against *S. mutans* whereas for *S. salivarius* chloroform extract showed zone of inhibition of 18mm. Ethanolic and aqueous extract of neem leaves showed effective inhibition of 16mm against *F. nucleatum* when tested using disk diffusion method[39]. Ethanolic extract of *A. indica* leaves showed minimum bactericidal concentration of 250ìg/ml and 500ìg/ml for *S. mutans* and *S. mitis* respectively whereas for *S. salivarius* and *S. sanguis* it is 5mg/ml and 1mg/ml respectively[40].

Syzygium aromaticum

Syzygium aromaticum (Fig. 5) or clove is an aromatic plant which is used in various industries. It is a member of Myrtaceae family. It has height of about 8-12 m. leaves of this tree are quadrangle in shape and flowers are arranged in clusters. Flower buds are pale in colour initially which turns green and further to red which is an indication that they are ready for harvest[41]. Clove is native to Maluka islands in East Indonesia. Major producers of clove are India, Sri-Lanka, Indonesia, Tanzania, Brazil, Pakistan, Madagascar and Malaysia[42].

S. aromaticum is used traditionally in many industries for flavouring, fragrance, and also in medicine. In many cuisines clove is used as flavoring agent. Clove contains essential oil which is rich in many compounds which shows therapeutic actions against various diseases. Essential oils from clove shows effects like antioxidant, antibacterial, antifungal, antiviral, hepatoprotective, cytotoxic, anesthetic, analgesic, antinociceptive, anti-inflammatory, larvicidal[42]. These essential oils also show effects against dental pathogens which causes dental plaque and

Table 1. List of plaque forming microorganisms inhibited by bioactive compound of *Ocimum basilicum*, *Azadirachta indica*, *Syzygium aromaticum*, *Acacia nilotica* and *Achyranthes aspera*

Compound	Plants	Bacteria (Bactericidal activity)		Fungi (Fungicidal activity)	References
		Gram-positive	Gram-negative		
Linalool	*O. basilicum* *S. aromaticum*	*S. mutans* *S. sobrinus* *Lactobacilli*			Shirazi *et al*., 2014
Eugenol	*O. basilicum* *S. aromaticum* *A. nilotica*	*S. mutans*	*L. monocytogenes* *P. gingivalis*	*Candida*	Marchese *et al*., 2017
Nerol	*O. basilicum* *S. aromaticum* *A. nilotica*	*S. mutans*			Astuti*et al*., 2016
Phytol	*A. nilotica A. aspera*			*C. albicans*	Islam *et al*., 2018
Estragole	*O. basilicum*			*C. albicans*	Sakkas And Papadopoulou., 2017
Methyl-cinnamate	*O. basilicum*	*S. mutans*	*L. acidophillus*	*Candida*	Shirazi *et al*., 2014
1-Hexadecanol	*A. aspera*	*S. mutans*		*C. albicans*	Madhumathi and Vijayakumar , 2014
Betulin	*A. aspera*	*S. mutans*		*C. albicans*	Viszwapriya *et al*., 2017
Cinnamaldehyde	*A. nilotica*	*S. mutansS. mitis*	*L. monocytogenes*	*C. albicans*	Worreth *et al*., 2022
â-caryophyllene	*S. aromaticum*	*S. mutans* *L. monocytogenes*	*L. acidophillus* *P. gingivalis*	*Candida*	Jardón-Romero *et al*., 2022
Nimbin	*A. indica*	*S. mutans* *S. sobrinus* *S. salivarius*	*P. intermedia*	*C. albicans*	Lakshmi*et al*., 2015
Azadirachtin	*A. indica*	*S. mutans* *S. sobrinus* *S. salivarius*	*L. acidophilus*	*C. albicans*	Chatterjee*et al*., 2011
Catechin	*A. indica*	*S. mutans* *S. sobrinus* *S. salivarius* *P. gingivalis*	*A. faecalis* *L. acidophilus*	*C. albicans*	Gupta*et al*., 2017
Quercetin	*A. indica*	*P. gingivalis*	*A. faecalis*	*C. albicans*	Lahiri*et al*., 2021

periodontal diseases. Due to this property clove oil is used in many dental care products as well as it is also used in traditional medicine[43].

S. aromaticum is a very effective plant used in many industries like food and medicine. The essential oils of clove contain many bioactive compounds which are helpful in treating many diseases. Chemical composition of clove essential oil was studied by using GC-MS analysis. Major groups present in clove essential oils are flavonoids, hydroxybenzoic acids, hydroxycinnamic acids, phenolic acids and tannins[41]. The essential oils of clove contain â-caryophyllene, eugenol, á-humulene, eugenol acetate, 2-heptanone, ethyl hexanoate, humulenol, calacorene, calamenene, Methyl salicylate, á-pinene, limonene, p-cymene, 2-Heptyl cetate, linalool, (E)-â-Ocimene and many more compounds are present in small amounts. These compounds show potential benefits against various diseases like gastrointestinal diseases, skin infections and also respiratory infections[43].

Major components present in *Syzygium aromaticum* are linalool, eugenol, â-caryophyllene. Bioactive compounds present in clove acts against *S. mutans*, *C. albicans*, *S. sobrinus*, *Lactobacillus* spp.[24]. These compounds also show effect against fungal pathogens such as *C. albicans* causing dental diseases[33].

Activity of *Syzygium aromaticum* against dental plaque

S. aromaticum is one of the herbs used for treatment of dental plaques. It contains essential oils and many phytochemicals in its extracts. Aqueous extract of *S. aromaticum* shows MIC of 31.25 mg/ml against *S. mutans*[45]. Essential oil extracted from *S. aromaticum* showed zone of inhibition of 19mm against *Lactobacillus* spp., 13mm against *S. mutans* and 9mm against *S. salivarius*. *S. aromaticum* extract shows growth inhibition activity against *P. gingivalis*, *S. mutans*, *P. intermedia* and *A. viscosus*[43]. *S. aromaticum* oil shows MIC of 0.2 mg/ml and MBC of 0.8mg/ml

Fig. 1. The overall process of the dental caries

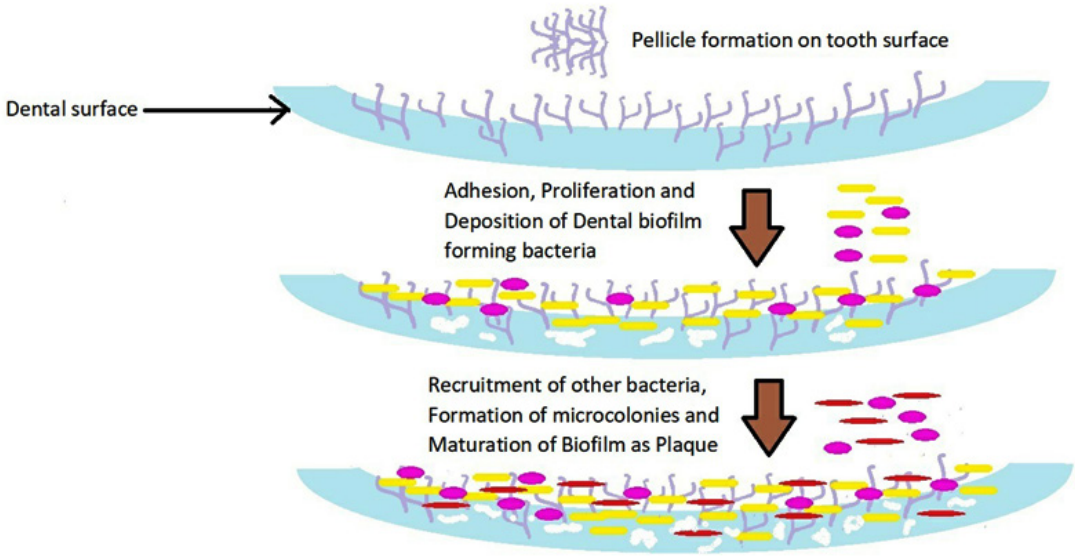

Fig. 2. Diagrammatic representation of Dental Biofilm formation

against *S. mutans* and *S. sobrinus* whereas for *S. ratti*, *S. anginosus* and *A. actinomycetemcomitans* MIC is 0.8mg/ml and MBC 1.6mg/ml. For *S. sanguinis* and *S. criceti* MIC is 0.4mg/ml and MBC 1.6mg/ml. apart from these *S. gordonii*, *F. nucleatum*, *P. intermedia* and *P. gingivalis* are inhibited at MIC of 0.1mg/ml and MBC of 0.2 mg/ml in comparison with ampicillin and gentamycin as standard drugs[46]. Essential oil extracted from *S. aromaticum* shows MIC of 6.25ìg/ml and MBC of 25ìg/ml against *P. gingivalis*[47].

Acacia nilotica

Acacia nilotica (Fig. 6) commonly known as babool is one of the medicinal plants which is used in traditional medicine. It belongs to Mimosaceae family. It is perennial tree approximately 20 m in height[48]. It has black coloured stem. Flowers are golden yellow in colour and are 1.2-1.5 cm in diameter. It has straight or slightly curved, hairy, thick, gray and are of 5-15 cm in length. Leaves are 30-40 mm long and bipinnate[49]. It is native to India, Pakistan, Iran, Israel, Zambia, Egypt, Kanya, Nepal, Ethiopia[48].

A. nilotica is used in traditional medicine for treatment of various diseases. Due to its medicinal properties it shows various activities like antibacterial, antioxidant, anti-hypertensive, antifungal, anti-plasmodium, anti-spasmodic, anti-diabetic, anti-acetylcholinesterase, anti-mutagenic[49]. Due to presence of phytochemicals in the extracts and essential oils it also shows activity against dental plaque and dental caries. Babool is also used in many commercial products used for dental care and oral hygiene[50].

A. nilotica is a very effective plant used in many medicines. The essential oils of *A. nilotica* contain many bioactive compounds which are helpful in treating many diseases. Chemical composition of A. *nilotica* essential oil was studied by using GC-MS analysis. Major groups present in *A. nilotica* essential oils are flavonoids, hydroxybenzoic acids, hydroxycinnamic acids, polyphenols, saponins and tannins[49].

Major components present in *A. nilotica* are cinnamaldehyde, eugenol, nerol and phytol. Bioactive compounds present in *A. nilotica* acts against *S. mutans*, *S. sobrinus*[51]. These compounds

Fig. 3. *Ocimum basilicum*

Fig. 4. *Azadirachta indica*

Fig. 5. *Syzygium aromaticum*

also show effect against fungal pathogens such as *C. albicans* causing dental diseases[52].

Activity of *Acacia nilotica* against dental plaque

A. nilotica contains essential oils and bioactive compounds in extracts which shows activity against dental plaque. When oil extracted from *A. nilotica* tested against *S. mutans* and *C. albicans* it shows MIC of 9.75ìg/ml[53]. Ethanolic extract of *A. nilotica* showed MIC of 5mg/ml and zone of inhibition of 31mm against *S. mutans* whereas petroleum ether extract showed MIC of 10mg/ml and zone of inhibition of 17.5mm[54]. Methanolic extract of *A. nilotica* twig extract shows zone of inhibition of 40.12 cm and MIC of 0.19mg/ml against *S. mutans* and zone of inhibition of 42.07cm and MIC of 0.19mg/ml for *C. albicans*[51]. Ethyl acetate extract of *A. nilotica* shows inhibition zone of 14.67cm against *C. albicans* whereas methanolic extract showed inhibition zone of 27cm when tested using chlorohexidine diacetate as standard[55]. Aqueous extract of *A. nilotica* shows zone of inhibition of 15.66mm against *S. mutans* when incubated for 48hr[56].

Fig. 6. *Acacia nilotica*

Achyranthes aspera

Achyranthes aspera (Fig. 7) or Devil's horse whip is an annual or perennial herb used in traditional medicine. It belongs to amaranthaceae family. It is about 1-2 meter in height and have woody base. Leaves are thick, rounded and 6-20 cm in length. Flowers are greenish white and present at axillary or terminal position and are 75 cm long. Its seeds are subcylindrical are reddish brown in colour[57]. *Achyranthes aspera* is found in India, America, Australia, South Andaman Islands, Baluchistan, Ceylon. It is reported as alien invasive species in northern Bangladesh[58].

A. aspera is traditionally used in many medicines for cure of various ailments. The essential oils present in it are used as therapeutic agents in traditional medicine for treatment of piles,

Fig. 7. *Achyranthes aspera*

oedema, snake bites, pneumonia, rheumatism, scabies and many other skin diseases[57]. Volatile compounds present in *A. aspera* essential oil also show various activities like anti-microbial, antiviral, antifungal, antifertility, anti-inflammatory, antiarthritic, antiparasitic, anticarcinogenic, antioxidant, antiallergic, anti-obesity, antidandruff, antiulcerogenic, antidiarrheal, analgesic, bronchoprotective, antidepressant[58]. *A. aspera* also shows antiplaque activity and prevents dental problems by inhibiting dental pathogens[59].

A. aspera is an effective plant against many diseases due to presence of essential oils. These essential oils contain many bioactive compounds which shows therapeutic effects. Chemical composition of *A. aspera* oil was studied by GC-MS analysis[60]. Major groups present in *A. aspera* oil are saponins, flavonoids, terpenoids, alkaloids, long chain compounds, aliphatic alcohols, ketones, phenols[58].

Major bioactive compounds present in *A. aspera* are Betulin, nerol, eugenol, 1-Hexadecanol and Phytol which are present in essential oils[57]. These compounds show activity against dental plaque forming bacteria. Major activity is seen against *S. mutans*, *C. albicans*, *L. acidophilus*, *P. gingivalis*[29].

Activity of *Achyranthes aspera* against dental plaque

Anti-plaque activity of *A. aspera* is due to presence of bioactive phytochemicals present in its extracts. Methanolic extract of *A. aspera* leaves shows antibacterial activity against *S. mutans*, *L. acidophilus*, *S. salivarius* and *S. sanguis*[61]. Zone of inhibition against *S. mutans* of Aqueous extract of *A. aspera* was 13mm whereas it is 18mm, 16mm and 23mm for benzene, petroleum and methanolic extract respectively. MIC of aqueous extract against dental pathogens was 100mg/ml whereas MIC of methanolic extract was 50mg/ml. When

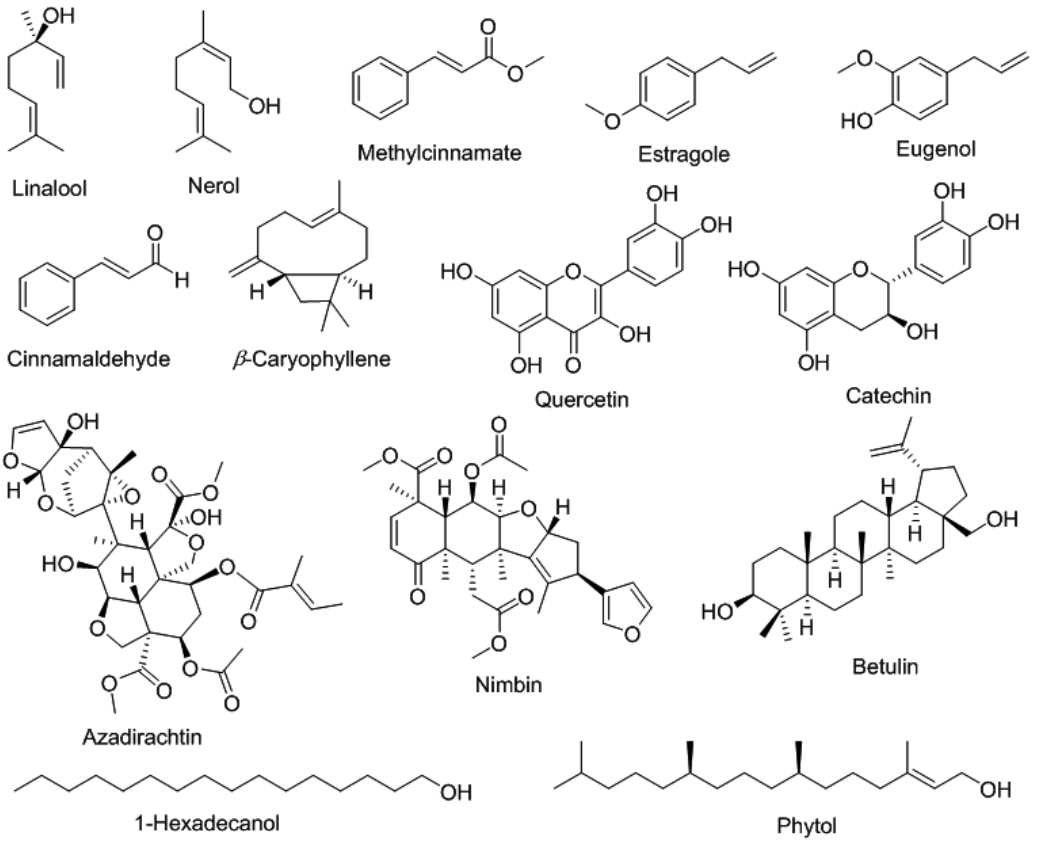

Fig. 8. Structures of bioactive compounds

aqueous extract is tested against dental pathogen using disk diffusion method it showed zone of inhibition of 7mm and for methanolic extract it was 4mm. Erythromycin and chloramphenicol are used as standard drugs for determining of MIC and zone of inhibition[62]. Zone of inhibition of 21mm against *S. mutans* was observed by using Aqueous extract of *A. aspera* roots and stem and chlorohexidine as standard[63]. MIC of *A. aspera* aqueous extract against *C. albicans* was 1.56mg/ml and 0.78mg/ml for acetone extract[64]. Methanolic extract shows MIC of 125ìg/ml and 62.5ìg/ml for essential oils of *A. aspera* against *S. mutans*. Whereas MBC for essential oils of *A. aspera* against *S. mutans* is 125ìg/ml[65].

Bioactive compounds of *Ocimum basilicum*, *Azadirachta indica*, *Syzygium aromaticum*, *Acacia nilotica* and *Achyranthes aspera* and its mechanism of action *against* Dental Plaque

Essential oils, alcoholic and aqueous extracts of *O. basilicum*, *A. indica*, *S. aromaticum*, *A. nilotica* and *A. aspera* contains bioactive components that are linalool, estragole, methyl-cinnamate, eugenol, nerol, Betulin, 3,12-oleandione, 1-Hexadecanol, Phytol, Cinnamaldehyde, â-caryophyllene, nimbin, azadirachtin, catechin and quercetin which acts against many plaque and biofilm forming organisms.

Linalool (Fig. 8) is mainly found in Lamiaceae, Lauraceae, Apiaceae families. Linalool have various properties such as antibacterial, antifungal and mosquito repellent action. It is found in *O. basilicum* and *S. aromaticum*. Antimicrobial activity of linalool is observed by the techniques such as MIC and disc diffusion method. It is also effective against dental plaque forming organisms like *S. mutans*, *Lactobacilli* and *S. sobrinus*[66]. These are the main plaque forming organisms found in dental biofilm. Linalool has the ability to degrade biofilm by penetrating in the extracellular polysaccharides or the slime layer produced by these organisms which results in the killing of bacteria. It also further inhibits the biofilm formation by *S. mutans*[27].

Eugenol (Fig. 8) is one of the bioactive compounds present in *O. basilicum*, *S. aromaticum* and *A. nilotica*. MIC and disc diffusion methods are used for determining the antibacterial and antifungal activity of eugenol. It gives antibacterial activity against *P. gingivilis*, *S. mutans*, *L. monocytogenes* which are major plaque forming microorganisms[46]. Eugenol also shows antifungal activity against *Candida* spp. which is one of the oral microorganisms. Eugenol alters fatty acids of cell membrane as well as it changes morphology of cell membrane which leads to disruption of cell membrane. These changes further lead to increase in non-specific permeability of cell membranes. This permeability also affects the transport of ATP and ions in the cell which is followed by cell death. Eugenol also produces intracellular reactive oxygen species which induces cell death and inhibition of *Candida* spp. as well as inhibition of *S. mutans*, *P. gingivalis*, and *L. monocytogenes*[67].

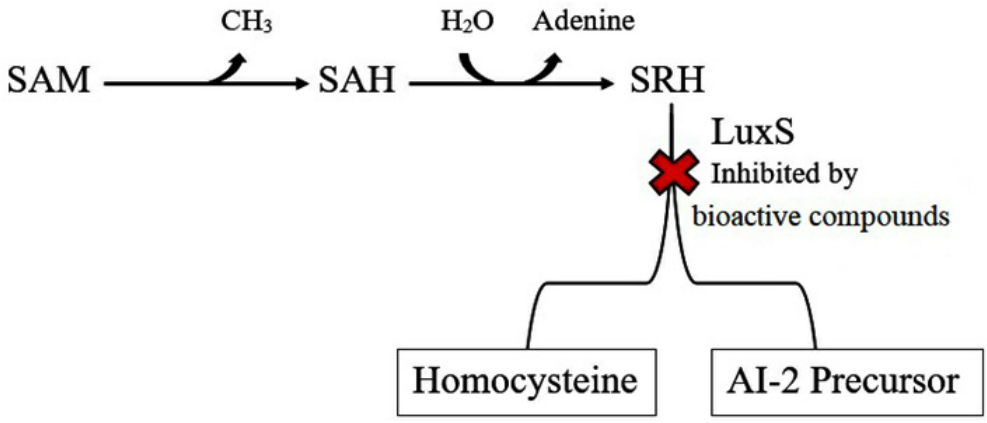

Fig. 9. : Mechanism of action of bioactive compounds from *Ocimum basilicum*, *Azadirachta indica, Syzygium aromaticum, Acacia nilotica* and *Achyranthes aspera*

Nerol (Fig. 8) is one of the components in the *O. basilicum*, *A. nilotica* and *S. aromaticum*[24]. Anti-microbial activity is observed by techniques such as MIC and disc diffusion method. It shows the effect against *S. mutans* which is the major plaque forming microorganism in the oral cavity. Nerol works by a similar mechanism like linalool i.e., degradation of extracellular polysaccharide of dental biofilm which leads to disruption of the plaque[27].

Phytol (Fig. 8) is one of the compounds presents in *A. nilotica* and *Achyranthes aspera*. It shows anti-microbial activity against *C. albicans*[68]. MIC was carried out against these organisms and it was found to effective at concentration of 62.5ìg/ml of phytol. It inhibits biofilm formation by *C. albicans* by inhibiting proteins required for biofilm synthesis. This further reduces the microbial growth and plaque formation[69].

Estragole (Fig. 8) is also known as methyl chavicol. It is the major component of essential oils of *O. basilicum*. Anti-microbial and antifungal activities of estragole is observed by the technique of disc diffusion method. It has restricted spectrum of action. It shows antifungal effect against *C. albicans*. As *C. albicans* is one of the oral pathogens which can act as a biofilm forming organism, estragole inhibits its growth and hence it is effective for the prevention against dental plaque[70].

Methyl-cinnamate (Fig. 8) is present in the essential oil of *O. basilicum*. Anti-microbial activity of methyl-cinnamate is observed by the techniques such as MIC and disc diffusion method. It shows antifungal activity against *Candida* spp. It give antibacterial activity against *S. mutans* and *L. acidophilus* which are main organisms of dental plaque formation and eventually dental caries[24].

1-Hexadecanol (Fig. 8) from *A. aspera* shows antibacterial as well as antifungal effect. It acts against *S. mutans* and *C. albicans* which are main organisms in plaque formation. Effect of 1-Hexadecanol on these organisms was studied using molecular docking. 1-Hexadecanol inhibits the SAP protein in *C. albicans* which is responsible for virulence and pathogenesis. It also inhibits spaP gene in *S. mutans* which is responsible in biofilm and plaque formation. Due to these inhibitions, reduction of growth and further reduction in plaque can be seen[71].

Betulin (Fig. 8) present in *A. aspera* essential oil. It shows anti-microbial effect against *S. mutans*, *C. albicans*. These organisms are major plaque forming organisms present in oral cavity. Betulin is tested against these pathogens by BIC i.e. biofilm inhibiting concentration and it was observed that it inhibits the pathogens at concentration of 240ìg/ml. Betulin inhibits the growth of these bacteria by inhibiting the adherence. It targets and inhibits vicRK and gtf genes which are responsible for formation of biofilm. This results in the inhibition of pathogens and further reduction of dental plaque[72].

Cinnamaldehyde (Fig. 8) is one of the bioactive compounds presents in *A. nilotica*. These activities are studied by using MIC technique. It shows antibacterial activity against *P. gingivilis*, *S. mutans*, *S. mitis*, *L. monocytogenes* which are major plaque forming microorganisms[73]. Cinnamaldehyde also shows antifungal activity against *C. albicans*. Cinnamaldehyde inhibits the adhesion of the organisms to dental surface which leads to reduction of biofilm formation and plaque formation.

â-caryophyllene (Fig. 8) is present in *S. aromaticum* essential oil â-caryophyllene shows many properties such as anti-microbial, anti-inflammatory, anaesthetic, anti-cancer. Its anti-microbial activity is observed by the techniques of MIC and disc diffusion method. It gives antibacterial activity against *P. gingivilis*, *A. viscosus*, *S. mutans*, *L. monocytogenes*, *P. intermedia*[46]. It also shows activity against *Candida* spp. It reduces cell adhesion ability and biofilm formation which further leads to reduction in plaque formation[43].

Nimbin (Fig. 8) from *A. indica* shows antibacterial as well as antifungal activity against dental pathogens. It acts against *S. mutans*, *S. salivarius*[74]. Bactericidal activity of nimbin was seen against *S. sobrinus* with the MIC of 240 ìg/ml[75]. In these dental pathogens nimbin inhibits glucan synthesis which further leads to reduction in aggregation of streptococci and their colonization for formation of dental plaque[76]. It also inhibited *C. albicans* which forms dental biofilm causing plaque. It shows anti-adhesive effect by increasing hydrophobicity of cell surface which reduces the colonization of pathogens[77].

Azadirachtin (Fig. 8) shows effect against dental plaque forming organisms as well as gingivitis causing organisms. It shows effect against *S. mutans*, *S. sobrinus*, *C. albicans*, *L. acidophilus*, *S. salivarius* with MIC of 250 ìg/ml in comparison with chlorohexidine mouthwash[78]. Azadirachtin inhibits the cell adhesion to the dental surface and reduces the biofilm formation which further results in inhibition of dental plaque formation[76].

Catechin (Fig. 8) is one of the bioactive compounds presents in *A. indica*. It shows anti-plaque activity as well as anti-biofilm forming activity. It shows effect against *S. mutans, L. acidophilus, P. gingivalis, A. faecalis, C. albicans, S. sanguis* and *S. salivarius*[79]. Catechin shows anti-microbial effect with concentration of 180ìg/ml when studied using MIC. It shows antiplaque activity by inhibiting the biofilm formation by reducing the quorum sensing protein LuxS. It also alters the expression of AHL molecule required for quorum sensing. Catechin also reduces genomic DNA which leads to anti-microbial activity[75].

One of the bioactive compounds which acts as anti-plaque and anti-quorum sensing agent present in *A. indica* is quercetin (Fig. 8). It shows inhibitory effect against *P. gingivalis, A. faecalis, C. albicans*. When tested by disk diffusion method quercetin showed inhibition at concentration of 10mg/ml[80]. It shows MIC 190ìg/ml. It inhibits biofilm formation by inhibition of LuxS and AHL in bacteria[75]. In *C. albicans* quercetin reduces cell adhesion ability and biofilm formation which further leads to reduction in plaque formation[81].

These bioactive compounds present in *O. basilicum*, *A. indica*, *S. aromaticum*, *A. nilotica* and *A. aspera* interfere in AMC and signalling. In all the methylation reactions *S-adenosylmethionine* (SAM) is a major methyl group donor. Autoinducer-2 (AI-2) which is a product of LuxS gene is an important molecule for interspecies communication in gram-positive as well as gram-negative bacteria. However, this is complex due to the dual role of LuxS in signalling and activated methyl cycle (AMC) which is a crucial metabolic pathway[82]. AI-2 is derived from SAM through a sequence of enzymatic reactions. Toxic intermediate *S*-adenosyl homocysteine (SAH) is formed after donation of a methyl group from SAM. This *S*-adenosyl homocysteine (SAH) is then hydrolysed to *S*-ribosyl homocysteine (SRH). LuxS cleaves SRH to produce homocysteine and AI-2 precursors[82].These compounds lower expression of the LuxS gene. This further leads to reduction in levels of homocysteine and AI-2 precursor as well as accumulation of toxic SRH molecules in cells (Fig. 9). This further acts on disruption as well as prevention of production of biofilm.

70% of cell membrane of *C. albicans* contains polyunsaturated lipids. Some bioactive compounds induces lipid peroxidation in cell membranes of *C. albicans*. This further leads to incorporation of lipid peroxides in cell membrane and disruption and deformation[83].

DISCUSSION

As an effect of modern lifestyle almost, every individual carries risk of getting affected by dental plaque and then eventually with dental caries which are caused by the microorganisms present as microbiota of the oral cavity. *Actinomyces*, *Bacteroides*, *Bifidobacterium*, *Lactobacillus*, *Streptococcus species*, *Candida* and *Spirochetes* and *Mycoplasmas* are some of the main organisms present in the oral cavity[1]. These organisms are able to form oral biofilm by the specialized mechanism of quorum sensing, co-aggregation and metabolic communication.

To avoid such effects of dental plaque various techniques like physical, chemical and biological methods are used[2]. Besides these effects it shows side effects like discolouration of teeth, development of resistance against anti-microbials. To avoid these side effects new strategies such as pharmaceutical products containing herbal extracts which are effective as equal as chemical agents are under research[12]. Some of the traditional medicinal plants which are used for the treatment of plaque are *O. basilicum*, *A. indica*, *S. aromaticum*, *A. nilotica* and *A. aspera*. Various researchers have studied effects of bioactive compounds from these plants and provided data which shows the efficiency of these compounds over chemical agents.

O. basilicum, *A. indica*, *S. aromaticum*, *A. nilotica* and *A. aspera* has many effects such as anti-microbial, antiviral, antifungal, nematocidal, insecticidal, anti-diabetic, anti-ulcer, anti-inflammatory, immunomodulator, anti-

malarial, anti-HIV, anti-tumor, anti-hypertensive, antioxidant, hepatoprotective, cytotoxic, anesthetic, analgesic, antinociceptive, anti-spasmodic, anti-acetylcholinesterase, anti-mutagenic, antifertility, antiarthritic, antiparasitic, anticarcinogenic, antiallergic, anti-obesity, antidandruff, antiulcerogenic, antidiarrheal, bronchoprotective, antidepressant [20,29,42,49,58]. Essential oils from these plants are used in culinary, traditional medicine as well as in formulations of dental care products.

Essential oils of *O. basilicum* contain linalool, methyl-cinnamate, estragole, eugenol and nerol[19]. Whereas nimbin, azadirachtin, catechin, quercetin from *A. indica*[37]. linalool, eugenol, â-caryophyllene from *S. aromaticum*[43]. cinnamaldehyde, eugenol, nerol, phytol from *A. nilotica*[51]. Betulin, nerol, 1-Hexadecanol, Phytol from *A. aspera* are the main bioactive components acting against dental plaque forming organisms[60].

The methods which are used in observing anti-microbial activity of these bioactive components are mainly MIC and disc diffusion methods. Linalool from *O. basilicum* and *S. aromaticum* acts against *S. mutans*, *S. sobrinus* and *Lactobacilli*[66]. 1-Hexadecanol and Betulin from *A. aspera* are effective against *S. mutans* and *C. albicans*[71,72]. Nimbin and Azadirachtin from *A. indica* is effective against *S. mutans, S. sobrinus, S. salivarius, L. acidophilus* and *C. albicans*[76]. These compounds act by interrupting the formation of the slime layer and eventually leads to removal of dental biofilm[27, 66]. Eugenol from *O. basilicum, S. aromaticum* and *A. nilotica* shows good effect against *S. mutans*, *P. gingivalis*, and *L. monocytogenes* as well as *C. albicans*[47]. Similarly Phytol from *A. nilotica* and *A. aspera* as well as Estragole from *O. basilicum* are also effective against *C.* albicans[54]. Quercetin from *A. indica* shows effect against only *P. gingivalis, A. faecalis* and *C. albicans*[75].these compounds alter the cell membrane and form reactive oxygen species which interrupts in bacterial growth[46,67,70]. Nerol from *O. basilicum, S. aromaticum* and *A. nilotica* also acts against *S. mutans*[27]. Methyl-cinnamate from *O. basilicum* acts against *C. albicans*, *L. acidophilus*, *S. mutans*[66]. Cinnamaldehyde from *A. nilotica* acts against *C. albicans*, *L. acidophilus*, *S. mutans* and *S. mitis*[73]. â-caryophyllene from *S. aromaticum S. mutans, L. monocytogenes, L. acidophillus, P. gingivalis, P. intermedia* and Candida species[45]. Catechin from *A. indica* acts against *S. mutans, S. sobrinus, S. salivarius, P. gingivalis, A. faecalis, L. acidophilus* and *C. albicans*[79]. These compounds degrade the biofilm resulting reduction in the plaque[24,27,66].

CONCLUSION

O. basilicum, *A. indica*, *S. aromaticum*, *A. nilotica* and *A. aspera* contains essential oils contain bioactive compounds such as linalool, estragole, methyl-cinnamate, nerol, eugenol, nimbin, catechin, azadirachtin, Quercetin, â-caryophyllene, cinnamaldehyde, phytol, Betulin and1-Hexadecanol. Main targets of these compounds are *S. mutans*, *C. albicans, L. acidophilus*, *S. sobrinus*, *P. gingivalis*, *A. faecalis*, *S.* mutans and *L. monocytogenes* which are majorly found microorganisms in dental plaque. Theses bioactive components have similar effects as the chemical anti-plaque agents without any side effects and hence can be used for long term treatment against dental plaque. Hence the herbal products containing bioactive compounds of *O. basilicum*, *A. indica*, *S. aromaticum*, *A. nilotica* and *A. aspera* may prove to be more effective in the long run.

ACKNOWLEDGMENT

We acknowledge the funding received from Research Society for the Study of Diabetes in India (RSSDI/HQ/Grants/2017/342).

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

Funding source

Research Society for the Study of Diabetes in India (RSSDI/HQ/Grants/2017/342)

Authors' Contribution

Conceptualization, Y.P., H.P..D. and A.A.; writing—original draft preparation, Y.P., H.P.D. and A.A.; writing—review and editing, Y.P., H.P.D. and A.A. ; visualization, Y.P., H.P.D. and A.A. .; supervision, A.A. ; project administration, A.A.; funding acquisition, A.A.. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement

Not Applicable.

Ethics Approval Statement

Not Applicable.

REFERENCES

1. R. Ananthanarayan, C. Paniker, (10th ed.) Textbook of Microbiology, Universities Press: Hyderabad (2017)
2. Colombo A.P., do Souto R.M., da Silva-Boghossian C.M., Miranda R., Lourenço T.G. Microbiology of oral biofilm-dependent diseases: have we made significant progress to understand and treat these diseases ?. Curr Oral Health Rep. 2015;(2):37-47. https://doi.org/10.1007/s40496-014-0041-8
3. Nazir M.A. Prevalence of periodontal disease, its association with systemic diseases and prevention. Int J Health Sci. 2017;11(2):72.
4. https://www.cdc.gov/nchs/fastats/dental.htm (2022)
5. Davidovich E., Grender J., Zini A. Factors associated with dental plaque, gingivitis, and caries in a pediatric population: A records-based cross-sectional study. IJERPH. 2020;17(22):8595. https://doi.org/10.3390/ijerph17228595
6. Baghaie H., Kisely S., Forbes M., Sawyer E., Siskind DJ. A systematic review and meta analysis of the association between poor oral health and substance abuse. Addiction. 2017;112(5):765-79. https://doi.org/10.1111/add.13754
7. Dimitrova S., Dimitrov D., Boyadzhieva E., Simeonova E. Study of the Ph of Saliva and Dental Plaque Using Dental Preparations Containing Basil and Eucalyptus Essential Oils. IOSR JDMS.2017;16(3):98-102. 10.9790/0853-16030998102
8. S. Nanda, A. Nanda, R. Khan, Cosmetics and Technology. 7th ed. Birla Publications (2018).
9. Deng T., Feng Y., Xu L., Tian X., Lai X., Lyu M., Wang S. Expression, purification and characterization of a cold-adapted dextranase from marine bacteria and its ability to remove dental plaque. Protein Expression and Purification. 2020. 1;174:105678. https://doi.org/10.1016/j.pep.2020.105678
10. Ali A. Herbs that heal: The philanthropic behavior of nature. Ann Phytomedicine. 2020;9(1):7-17. http://dx.doi.org/10.21276/ap.2020.9.1.2
11. Megalaa N., Kayalvizhi G., Silas A.J., Sajeev R., Saravana Kumar M.S. Role of herbal leaf extracts in caries prevention. Int J Cont Med Res. 2014;1(2):71-8.
12. Dhama K., Karthik K., Khandia R., Munjal A., Tiwari R., Rana R., Khurana SK., Ullah S., Khan RU., Alagawany M., Farag MR. Medicinal and therapeutic potential of herbs and plant metabolites/extracts countering viral pathogens-current knowledge and future prospects. Curr. Drug Metab. 2018; 1;19(3):236-63. https://doi.org/10.2174/1389200219666180129145252
13. Reddy S. Essentials of Clinical Periodontology and Periodontics, 6rd ed. Jaypee: New Delhi (2022).
14. Basavaraju M., Sisnity V.S., Palaparthy R., Addanki P.K. Quorum quenching: signal jamming in dental plaque biofilms. J Dent Sci. 2016;11(4):349-52. https://doi.org/10.1016/j.jds.2016.02.002
15. Vila T, Sultan AS, Montelongo-Jauregui D, Jabra-Rizk MA. Oral candidiasis: a disease of opportunity. Journal of Fungi. 2020;16;6(1):15. https://doi.org/10.3390/jof6010015
16. Xu W., Zhou W., Wang H., Liang S. Roles of Porphyromonas gingivalis and its virulence factors in periodontitis. Adv Protein Chem Struct Biol. 2020; 1;120:45-84.https://doi.org/10.1016/bs.apcsb.2019.12.001
17. Marsh PD., Zaura E. Dental biofilm: ecological interactions in health and disease. J. Clin. Periodontol. 2017;44:S12-22. https://doi.org/10.1111/jcpe.12679
18. He J., Li Y., Cao Y., Xue J., Zhou X. The oral microbiome diversity and its relation to human diseases. Folia microbiologica. 2015;60:69-80. https://doi.org/10.1007/s12223-014-0342-2
19. Joshi R.K. Chemical composition and antimicrobial activity of the essential oil of Ocimum basilicum L.(sweet basil) from Western Ghats of North West Karnataka, India. Anc Sci Life. 2014;33(3):151. 10.4103/0257-7941.144618
20. Ahmed AF., Attia FA., Liu Z., Li C., Wei J., Kang W. Antioxidant activity and total phenolic content of essential oils and extracts of sweet basil (Ocimum basilicum L.) plants. Food Science and Human Wellness. 2019; 1;8(3):299-305. https://doi.org/10.1016/j.fshw.2019.07.004
21. Yaldiz G., Camlica M., Pradhan Y., Ali A. Chemical Characterization, Biological Activities, and Some Medicinal Uses of Different Sweet Basil (Ocimum basilicum L.) Genotypes. InNatural Product Experiments in Drug Discovery 2022; 41-61. 10.1007/978-1-0716-2683-2_3
22. Singletary K.W. Basil: A brief summary of potential health benefits. Nutr Today. 2018;53(2):92-7.
23. Avetisyan A., Markosian A., Petrosyan M.,

Sahakyan N., Babayan A., Aloyan S., Trchounian A. Chemical composition and some biological activities of the essential oils from basil Ocimum different cultivars. BMC Complement, Alt Med. 2017;17(1):1-8. https://doi.org/10.1186/s12906-017-1587-5

24. Padalia R.C., Verma R.S., Chauhan A., Goswami P., Singh V.R., Verma S.K., Darokar M.P., Singh N., Saikia D., Chanotiya C.S. Essential Oil Composition and Antimicrobial Activity of Methyl cinnamate-Linalool Chemovariant of Ocimum basilicum L. Rec Nat Prod. 2017;11(2):193. https://doi.org/10.1016/j.indcrop.2012.12.005
25. da Silva Gündel S., Velho M.C., Diefenthaler M.K., Favarin F.R., Copetti P.M., de Oliveira Fogaça A., Klein B., Wagner R., Gündel A., Sagrillo M.R., Ourique A.F. Basil oil-nanoemulsions: Development, cytotoxicity and evaluation of antioxidant and antimicrobial potential. J Drug Deliv Sci Technol. 2018;46:378-83. https://doi.org/10.1016/j.jddst.2018.05.038
26. Wiwattanarattanabut K., Choonharuangdej S., Srithavaj T. In vitro anti-cariogenic plaque effects of essential oils extracted from culinary herbs. JCDR. 2017;11(9):DC30. 10.7860/JCDR/2017/28327.10668
27. Astuti P., Saifullah T.N., Wulanjati M.P., Yosephine A.D., Ardianti D. Basil essential oil (*Ocimum basilicum* L.) activities on *Streptococcus mutans* Growth, biofilm formation and degradation and its stability in micro-emulsion mouthwash formula. Int J of Pharm and Clin Res. 2016;8(1):26-32.
28. Choi O., Cho S.K., Kim J., Park C.G., Kim J. In vitro antibacterial activity and major bioactive components of Cinnamomum verum essential oils against cariogenic bacteria, Streptococcus mutans and Streptococcus sobrinus. Asian Pac J Trop Biomed. 2016;6(4):308-14. https://doi.org/10.1016/j.apjtb.2016.01.007
29. Hashmat I., Azad H., Ahmed A. Neem (*Azadirachta indica* A. Juss)-A nature's drugstore: an overview. Int Res J Biol Sci. 2012;1(6):76-9.
30. Alzohairy MA. Therapeutics role of Azadirachta indica (Neem) and their active constituents in diseases prevention and treatment. eCAM. 2016;2016. https://doi.org/10.1155/2016/7382506
31. Tripathi P., Singh A. *Acacia nilotica*, *Albizia saman*, *Azadirachta indica*: Ethanobotany and Medicinal Uses. Modern Research in Botany. 2020;1:96-103. doi: 10.5897/AJB2015.14834
32. Islas JF., Acosta E., Zuca G., Delgado-Gallegos JL., Moreno-Treviño MG., Escalante B., Moreno-Cuevas JE. An overview of Neem (Azadirachta indica) and its potential impact on health. J. Funct. Foods. 2020; 1;(74):104171. https://doi.org/10.1016/j.jff.2020.104171
33. Suttiarporn P., Choommongkol V. Microwave-assisted improved extraction and purification of anticancer nimbolide from *Azadirachta indica* (Neem) leaves. Molecules. 2020;25(12):2913. 10.3390/molecules25122913
34. Srivastava S.K., Agrawal B., Kumar A., Pandey A. Phytochemicals of *Azadirachta indica* source of active medicinal constituent used for cure of various diseases: A Review. J Sci Res. 2020;64(1):385-90. http://dx.doi.org/10.37398/JSR.2020.640153
35. Singh H., Kaur M., Dhillon J.S., Batra M., Khurana J. Neem: a magical herb in endodontics. Stomatological Dis Sci. 2017;1:50-4. 10.20517/2573-0002.2016.10
36. Dutta A., Kundabala M. Comparative anti-microbial efficacy of *Azadirachta indica* irrigant with standard endodontic irrigants: A preliminary study. JCD. 2014;17(2):133.doi: 10.4103/0972-0707.128047
37. Saleem S., Muhammad G., Hussain MA., Bukhari SN. A comprehensive review of phytochemical profile, bioactives for pharmaceuticals, and pharmacological attributes of Azadirachta indica. Phytother. Res. 2018;32(7):1241-72. https://doi.org/10.1002/ptr.6076
38. Patra JK., Das G., Lee S., Kang SS., Shin HS. Selected commercial plants: A review of extraction and isolation of bioactive compounds and their pharmacological market value. Trends in Food Science & Technology. 2018;1;(82):89-109. https://doi.org/10.1016/j.tifs.2018.10.001
39. Moghadam ET., Yazdanian M., Tahmasebi E., Tebyanian H., Ranjbar R., Yazdanian A., Seifalian A., Tafazoli A. Current herbal medicine as an alternative treatment in dentistry: In vitro, in vivo and clinical studies. Eur. J. Pharmacol. 2020;15;(889):173665. https://doi.org/10.1016/j.ejphar.2020.173665
40. Arumugam B., Subramaniam A., Alagaraj P. A Review on Impact of Medicinal Plants on the Treatment of Oral and Dental Diseases. Cardiovascular & Hematological Agents in Medicinal Chemistry. 2020; 1;18(2):79-93. https://doi.org/10.2174/1871525718666200219140729
41. Cortés-Rojas D.F., de Souza C.R., Oliveira W.P. Clove (*Syzygium aromaticum*): a precious spice. Asian Pac J Trop Biomed. 2014;4(2):90-6. https://doi.org/10.1016/S2221-1691(14)60215-X
42. Hussain S., Rahman R., Mushtaq A., Zerey-Belaskri A.E. Clove: A review of a precious

species with multiple uses. IJCBS. 2017;11:129-33.

43. Pulikottil SJ, Nath S. Potential of clove of Syzygium aromaticum in development of a therapeutic agent for periodontal disease.: a review. S Afr Dent J. 2015 Apr 1;70(3):108-15.
44. iNaturalist contributors, iNaturalist. iNaturalist Research-grade Observations. iNaturalist.org. Occurrence dataset (2022) https://doi.org/10.15468/ab3s5x accessed via GBIF.org on 2022-10-03. https://www.gbif.org/occurrence/3859949331
45. Jardón-Romero EA., Lara-Carrillo E., González-Pedroza MG., Sánchez-Mendieta V., Salmerón-Valdés EN., Toral-Rizo VH., Olea-Mejía OF., López-González S., Morales-Luckie RA. Antimicrobial activity of biogenic silver nanoparticles from syzygium aromaticum against the five most common microorganisms in the oral cavity. Antibiotics. 2022;21;11(7):834. https://doi.org/10.3390/antibiotics11070834
46. Chouhan S., Sharma K., Guleria S. Antimicrobial activity of some essential oils—present status and future perspectives. Medicines. 2017; 8;4(3):58. https://doi.org/10.3390/medicines4030058
47. Zhang Y., Wang Y., Zhu X., Cao P., Wei S., Lu Y. Antibacterial and antibiofilm activities of eugenol from essential oil of *Syzygium aromaticum* (L.) Merr. & LM Perry (clove) leaf against periodontal pathogen *Porphyromonas gingivalis*. Microb Pathog. 2017;113:396-402. https://doi.org/10.1016/j.micpath.2017.10.054
48. Kumar M., Prakash S., Radha, Kumari N., Pundir A., Punia S., Saurabh V., Choudhary P., Changan S., Dhumal S., Pradhan PC. Beneficial role of antioxidant secondary metabolites from medicinal plants in maintaining oral health. Antioxidants. 2021; 30;10(7):1061. https://doi.org/10.3390/antiox10071061
49. Zubair M., Azeem M., Mumtaz R., Younas M., Adrees M., Zubair E., Khalid A., Hafeez F., Rizwan M., Ali S. Green synthesis and characterization of silver nanoparticles from Acacia nilotica and their anticancer, antidiabetic and antioxidant efficacy. Environmental Pollution. 2022;1;(304):119249. https://doi.org/10.1016/j.envpol.2022.119249
50. Patel R.J., Suthar J.V., Patel R., Gajjar K.J., Brahmbhatt J.G. Antimicrobial activity of plant bark used as natural chewing stick against mouth flora and laboratory bacteria. Asian J Microbiol Biotechnol. 2018;3(1):15-23.
51. Kumari R., Mishra R.C., Sheoran R., Yadav J.P. Fractionation of antimicrobial compounds from *Acacia nilotica* twig extract against oral pathogens. Biointerface Res. Appl. Chem. 2020;10:7097-105. https://doi.org/10.33263/BRIAC106.70977105
52. Gupta R.C., Doss R.B., Lall R., Sinha A., Srivastava A., Malik J.K. Babool (*Acacia nilotica*). Nutraceuticals in Veterinary Medicine. 2019:103-11. 10.1007/978-3-030-04624-8_8
53. El Gendy AE., Essa AF., El-Rashedy AA., Elgamal AM., Khalaf DD., Hassan EM., Abd-ElGawad AM., Elgorban AM., Zaghloul NS., Alamery SF., Elshamy AI. Antiviral potentialities of chemical characterized essential oils of Acacia nilotica bark and fruits against hepatitis A and herpes simplex viruses: In vitro, in silico, and molecular dynamics studies. Plants. 2022;28;11(21):2889. https://doi.org/10.3390/plants11212889
54. Muddathir AM., Mohieldin EA., Mitsunaga T. In vitro activities of Acacia nilotica (L.) Delile bark fractions against Oral Bacteria, Glucosyltransferase and as antioxidant. BMC complement. med. ther. 2020;20(1):1-9. https://doi.org/10.1186/s12906-020-03147-4
55. Kumari R., Mishra R.C., Yadav A., Yadav J.P. Screening of traditionally used medicinal plants for their antimicrobial efficacy against oral pathogens and GC-MS analysis of *Acacia nilotica* extract. IJTK, 18(1) (2019) 162-168.
56. Sahni A., Chandak M.G., Shrivastava S., Chandak R. An in vitro comparative evaluation of effect of *Magnifera indica* (Mango), *Azadirachta indica* (Neem) and *Acacia nilotica* (Babool) on *Streptococcus mutans*. JAMDSR. 2016;4(1):1.
57. Bhattacharya K., Chandra G. Bioactivity of Acyranthes aspera (Amaranthaceae) foliage against the Japanese encephalitis vector Culex vishnui group. J. Mosq. Res. 2013;5;3(1). https://doi.org/10.5376/jmr.2013.03.0013
58. Sharma V., Chaudhary U. An overview on indigenous knowledge of *Achyranthes aspera*. J Crit Rev. 2015;2(1):7-19.
59. Carrol DH., Chassagne F., Dettweiler M., Quave CL. Antibacterial activity of plant species used for oral health against Porphyromonas gingivalis. PLoS One. 2020;8;15(10):e0239316. https://doi.org/10.1371/journal.pone.0239316
60. Mary P.A., Giri R.S. GC-MS Analysis of Bioactive Compounds of *Acryranthes aspera*. WJPR. 2017;7(1):1015-56. https://doi.org/10.20959/wjpr20181-10540
61. Singh S., Singh A., Navneet S. 'Ethnobotanical and Pharmacological Benefits of *Achyranthes aspera* Linn. An overview. Int. J. Pharm. Sci. Rev. Res. 2018;48(2):1-7.
62. Panda A., Sabat G., Mohanty B.K. Study of antibacterial activity with stem extracts of

Achyranthes aspera, L. Int J Adv Sci Res Manag. 2018; 3(10):219-223.

63. Yadav R., Rai R., Yadav A., Pahuja M., Solanki S., Yadav H. Evaluation of antibacterial activity of *Achyranthes aspera* extract against Streptococcus mutans: An in vitro study. J Adv Pharm Technol. 2016;7(4):149.
64. Ndhlala A.R., Ghebrehiwot H.M., Ncube B., Aremu A.O., Gruz J., Šubrtová M., Doležal K., du Plooy C.P., Abdelgadir H.A., Van Staden J. Antimicrobial, anthelmintic activities and characterisation of functional phenolic acids of *Achyranthes aspera* Linn.: a medicinal plant used for the treatment of wounds and ringworm in east Africa. Front Pharmacol. 2015;6:274. https://doi.org/10.22270/jddt.v12i3.5468
65. Milho C., Silva J., Guimarães R., Ferreira I.C., Barros L., Alves M.J. Antimicrobials from medicinal plants: An emergent strategy to control oral biofilms. Appl Sci. 2021;11(9):4020. https://doi.org/10.3390/app11094020
66. Shirazi M.T., Gholami H., Kavoosi G., Rowshan V., Tafsiry A. Chemical composition, antioxidant, antimicrobial and cytotoxic activities of *Tagetes minuta* and *Ocimum basilicum* essential oils. Food Sci Nutr. 2014;2(2):146-55. https://doi.org/10.1002/fsn3.85
67. Marchese A., Barbieri R., Coppo E., Orhan I.E., Daglia M., Nabavi S.F., Izadi M., Abdollahi M., Nabavi S.M., Ajami M. Antimicrobial activity of eugenol and essential oils containing eugenol: A mechanistic viewpoint. Crit Rev Microbiol. 2017;43(6):668-89. https://doi.org/10.1080/1040841X.2017.1295225
68. Islam M.T., Ali E.S., Uddin S.J., Shaw S., Islam M.A., Ahmed M.I., Shill M.C., Karmakar U.K., Yarla N.S., Khan I.N., Billah M.M. Phytol: A review of biomedical activities. Food Chem Toxicol. 2018;121:82-94. https://doi.org/10.1016/j.fct.2018.08.032
69. Ghaneian M.T., Ehrampoush M.H., Jebali A., Hekmatimoghaddam S., Mahmoudi M. Antimicrobial activity, toxicity and stability of phytol as a novel surface disinfectant. Environ Health Eng Manag J. 2015;2(1):13-6.
70. Sakkas H., Papadopoulou C. Antimicrobial activity of basil, oregano, and thyme essential oils. J Microbiol Biotechnol. 2017; 27(3):429–438. doi: 10.4014/jmb.1608.08024
71. Madhumathi V., Vijayakumar S. Identification of novel cyanobacterial compounds for oral disease through in vitro and insilico approach. Biomed Aging Pathol. 2014;4(3):223-8. https://doi.org/10.1016/j.biomag.2014.01.009
72. Viszwapriya D., Subramenium G.A., Radhika S., Pandian S.K. Betulin inhibits cariogenic properties of Streptococcus mutans by targeting vicRK and gtf genes. Antonie Van Leeuwenhoek. 2017;110:153-65. https://doi.org/10.1007/s10482-016-0785-3
73. Worreth S., Bieger V., Rohr N., Astasov Frauenhoffer M., Töpper T., Osmani B., Braissant O. Cinnamaldehyde as antimicrobial in cellulose based dental appliances. J Appl Microbiol. 2022;132(2):1018-24. https://doi.org/10.1111/jam.15283
74. Benelli G, Canale A, Toniolo C, Higuchi A, Murugan K, Pavela R, Nicoletti M. Neem (Azadirachta indica): towards the ideal insecticide?. Nat. prod. res. 2017; 16;31(4):369-86. https://doi.org/10.1080/14786419.2016.1214834
75. Lahiri D., Nag M., Dutta B., Mukherjee I., Ghosh S., Dey A., Banerjee R., Ray R.R. Catechin as the most efficient bioactive compound from *Azadirachta indica* with antibiofilm and anti-quorum sensing activities against dental biofilm: An in vitro and in silico study. Appl Biochem Biotechnol. 2021;193:1617-30.https://doi.org/10.1007/s12010-021-03797-1
76. Lakshmi T., Krishnan V., Rajendran R., Madhusudhanan N. *Azadirachta indica*: A herbal panacea in dentistry–An update. Pharmacogn Rev. 2015;9(17):41. https://doi.org/10.4103/0973-7847.156337
77. Martin H., Kavanagh K., Velasco-Torrijos T. Targeting adhesion in fungal pathogen Candida albicans. Fut. Med. Chem. 2021;13(03):313-34. https://doi.org/10.4155/fmc-2020-0052
78. Safiaghdam H., Oveissi V., Bahramsoltani R., Farzaei MH., Rahimi R. Medicinal plants for gingivitis: a review of clinical trials. Iran J Basic Med Sci . 2018;21(10):978.https://doi.org/10.22038/IJBMS.2018.31997.7690
79. Gupta A., Verma U., Lal N., Ojha S. Evolution and exploration of *Azadirachta indica* in dentistry: an update. J Adv Med Res. 2017;21(8):1-5. https://doi.org/10.9734/BJMMR/2017/33538
80. Gutiérrez-Venegas G., Gómez-Mora J.A., Meraz-Rodríguez M.A., Flores-Sánchez M.A., Ortiz-Miranda L.F. Effect of flavonoids on antimicrobial activity of microorganisms present in dental plaque. Heliyon. 2019;5(12). https://doi.org/10.1016/j.heliyon.2019.e03013
81. Gao M., Wang H., Zhu L. Quercetin assists fluconazole to inhibit biofilm formations of fluconazole-resistant Candida albicans in in vitro and in vivo antifungal managements of vulvovaginal candidiasis. Cell Physiol Biochem. 2016;40(3-4):727-42. https://doi.org/10.1159/000453134
82. Virmani R., Hasija Y., Singh Y. Effect of

homocysteine on biofilm formation by mycobacteria. Indian J Microbiol. 2018;58:287-93. 10.1007/s12088-018-0739-8

83. Al Aboody MS., Mickymaray S. Anti-fungal efficacy and mechanisms of flavonoids. Antibiotics. 2020; 26;9(2):45. https://doi.org/10.3390/antibiotics9020045