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A Review: Anti-Dental Plaque Potential of Selected Malaysian Herbs

Abstract **–** Dental plaque is a biofilm that forms on the teeth. Chlorhexidine (CHX) is the most commonly used anti-plaque agent in mouthwash, and it has been considered the gold standard in dental practice for around three decades. However, CHX does have some disadvantages. These disadvantages have led to the search for medicinal plant based alternative mouthwashes. Herbal products are slowly being recognized as effective, safe, non-toxic, and having fewer side effects on humans. The use of herbs as a source of economic growth in Malaysia has emerged in 2011. This review comprises 11 potential Malaysian herbs noted for their anti-plaque potential namely, *Eurycoma longifolia* Jack, *Morinda citrifolia*, *Centella asiatica*, *Hibiscus sabdariffa* L*, Ficus deltoidea* Jack*, Zingiber officinale* Roscoe, *Andrographis paniculata* (Burm.f.) Nees*, Orthosiphon stamineus*, *Clinacanthus nutans* Lindau, *Melastoma malabathricum* L, and *Moringa oleifera* Lam. Out of these 11 herbs, 8 have been identified by the government of Malaysia that can be commercially developed under first Entry Point Project (EPP1) for the nation's agriculture National Key Economic Area (NKEA). The present review focusses on the traditional use, the valuable phytochemical, anti-plaque potential and the cytotoxicity of the selected herbs. These information will serve as a guide for pharmaceutical companies or cosmetic manufacturers in formulating herbal mouthwashes containing anti-dental plaque properties of the Malaysian herbs. Further investigations into their clinical therapeutic potential may be worthwhile.

Keywords –*Dental plaque, Malaysian herbs, mouthwash*

1 INTRODUCTION

Dental plaque is a soft, sticky, colourless film of bacteria that constantly forms on teeth and gums. It develops as a result of a natural process involving the interaction between the mouth, bacterial metabolism and its waste products, and the food we consume. The most effective approach to prevent dental plaque is by targeting *Streptococcus mutans* (*S. mutans*) as this bacteria is primarily responsible for causing dental plaque. Antimicrobial mouthwashes have been suggested as adjuncts for mechanical plaque control methods. Chlorhexidine (CHX) is an antimicrobial agent that is commonly used in mouthwash to prevent biofilm formation (1). Concentrations of CHX mouthwash vary from 0.12% to 0.2%, depending on the intended use (2). Over the past three decades, it has been considered the gold standard of dental practice, but it does have some disadvantages. There are several disadvantages on the use of CHX mouthwash, such as tooth staining, taste disturbance/alteration, oral mucosa soreness, oral mucosa irritation, mild desquamation and mucosal ulceration/erosions and a general burning sensation and burning tongue (3). It is also related to production of resistant bacteria that impairs long-term use (4).

Considering these drawbacks of CHX mouthwash, the search for alternative products from herbs is considered. Herbs comprise several bioactive compounds that can exhibit antimicrobial and anti-biofilm properties. Also, though different herbs are used for different formulation, the similarity in their properties could be attributed to the shared bioactive compounds like polyphenols, flavonoids and terpenoids, which have been shown to interfere biofilm formation. These compounds are capable of inhibiting bacterial adhesion, disrupt biofilm structure, and inhibit quorum sensing, a communication system used by bacteria to coordinate biofilm development. Thus, despite the differences in the herbs, the presence of similar bioactive compounds could lead to similar preventive effects against biofilm formation.

Malaysia, a country with rich biodiversity, has been gifted with valuable herbs resources in its

tropical rainforest. Global demand for herbal products, which amounted to RM672 billion in 2006, is projected to triple by 2020 (5). The Malaysian government is aware of the potential of the herbal industry as well as the consumer's demand for high-valued herbal products and as such has identified certain species of herbs to be developed commercially in Malaysia (5). All the 11 herbs, *Eurycoma longifolia* Jack, *Morinda citrifolia*, *Centella asiatica*, *Hibiscus sabdariffa* L, *Ficus deltoidea* Jack, *Zingiber officinale* Roscoe, *Andrographis paniculata* (Burm.f.) Nees, *Orthosiphon stamineus*, *Clinacanthus nutans* Lindau, *Melastoma malabathricum* L, and *Moringa oleifera* Lam have a potential as an anti-plaque agent which are native to Malaysia and easily available.

This review focusses on plant extracts or phytochemicals that inhibit the growth of *S. mutans* and biofilm formation and explores the mechanism of inhibitory effects against bacteria. Also discussed are studies evaluating the cytotoxicity effect of these herbs on the gingival fibroblast which can serve as a guide for pharmaceutical companies or cosmetic manufacturers in formulating herbal mouthwashes containing anti-plaque properties derived from indigenous Malaysian plants. The antibacterial mechanism of the Malaysian herbs and their cytotoxicity study on gingival fibroblast cells considered in this review have been presented in **Table 1**.

In general, Malaysian herbs such as *Eurycoma longifolia* Jack, *Morinda citrifolia*, *Centella asiatica*, *Hibiscus sabdariffa* L, *Ficus deltoidea* Jack, *Zingiber officinale* Roscoe, *Andrographis paniculata* (Burm.f.) Nees, *Orthosiphon stamineus*, *Clinacanthus nutans* Lindau, *Melastoma malabathricum* L, and *Moringa oleifera* Lam may be useful in preventing and treating dental plaque and may provide a safe, accessible, and affordable method for treating oral diseases. However, more clinical trials are needed as most of the evidence is based on *in vitro* and animal studies.

2 MATERIAL AND METHODS

Several databases were searched, including PubMed, Google Scholar, Scopus, and Science-Direct using keywords 'Malaysian herb', 'oral pathogen', '*Streptococcus mutans'* combined with the respective plant's scientific name which includes *Eurycoma longifolia* Jack, *Morinda citrifolia*, *Centella asiatica*, *Hibiscus sabdariffa* L, *Ficus deltoidea* Jack, *Zingiber officinale* Roscoe, *Andrographis paniculata* (Burm.f.) Nees, *Orthosiphon stamineus*, *Clinacanthus nutans* Lindau, *Melastoma malabathricum* L, and *Moringa oleifera* Lam. The data covered here comes from literature retrieved from 2010 to the present. This review though reflects on the potential of Malaysian herbs, however, articles published by authors outside Malaysia relating to these herbs have also been included. Out of 87 articles retrieved from PubMed, Google Scholar, Scopus, and Science-Direct, 10 were rejected because full articles could not be obtained.

3 *EURYCOMA LONGIFOLIA* JACK

3.1 Traditional Uses

The plant *Eurycoma longifolia* Jack (Genus: Eurycoma; Family, Simaroubaceae), is one of the most popular tropical herbs found in South-East Asian countries such as Malaysia, Indonesia, and Vietnam. This plant can also be found in certain regions of Cambodia, Myanmar, and Thailand (6). Locally, this plant is called "Tongkat Ali", with the name referring to its long-twisted roots which are referred to as walking sticks. Extracts of the plant, especially the roots, have traditionally been used by men to increase testosterone levels. There are many benefits associated with the plant extract, particularly the roots in indigenous traditional medicine, including anti-malarial, antipyretic, anti-ulcer, cytotoxic, and aphrodisiac effects (6).

3.2 Phytochemical Compounds

A variety of spectral techniques and chemical reactions have been used to identify and isolate the phytochemical constituents in *E. longifolia* jack roots, stems, leaves, and bark. Phytochemical screening of roots and stems extracts revealed the presence of phenolic compounds, flavonoids, terpenoids, alkaloids, protein and cardiac glycosides (7). Also, the chromatographies and spectral analysis of *E. longifolia* jack roots have isolated 16 compounds such as eurylophenolosides A, eurylophenolosides B, hispidol B, piscidinol A, 24-epipiscidinol A, bourjotinolone B, 3-episapeline A, bourjotinolone A, 3-methoxy-4-hydroxybenzoic acid, syringaldehyde, 3-chloro-4-hydroxybenzoic acid, 3-chloro-4-hydroxyl benzoic acid-4-O-β-d-glucopyranoside, isotachioside, and scopoletin (8). Study of *E. longifolia* jack leaves revealed the presence of phenolic compounds such as gallic acid, chlorogenic acid, epigallocatechin gallate, vanillic acid, and epicatechin gallate (9).

Table 1. Malaysia herbs, mechanism of antibacterial activity and their safety profile

Scientific Name	Local Name	Mechanism of Antibacterial Activity	Cytotoxicity Study	Reference(s)
Eurycoma longifolia Jack	Tongkat ali	May result from cell wall damage and enhanced permeability of the membrane, as indicated by increased leakage of alkaline phosphatase, electrolytes, nucleic acids, and proteins	Non-toxic to human primary gingival fibroblasts at a concentration of less than 118.5 g/ml	[11, 12]
Morinda citrifolia	Mengkudu	Inhibitory mechanism of *M. citrifolia* against *S. mutans* has not been studied	No study was carried out on the cytotoxicity involving gingival fibroblasts. Animal study showed chronic intake of *M. citrifolia* fruit aqueous extract caused liver damage, while chronic intake of leaf extract at the same concentration (2 mg/ml) had no toxicity effects	[19, 20]
Centella asiatica	Pegaga	The inhibitory mechanism against bacteria might occur by inhibiting quorum sensing, a cell density-dependent mechanism in which genes are expressed	Based on MTT assay, assessment of cytotoxicity of water extract of *C. asiatica* against oral keratinocyte cell line showed that the cell viability was 90 % at 100 µg/ml concentration	[24, 25]
Hibiscus sabdariffa L	Roselle	The mechanism of antibacterial activity of hibiscus acid that obtained from *H. sabdariffa* calyces acetone extract is by increasing the permeability of the bacterial membrane	Cytotoxicity study showed that *H. sabdariffa* extract is non-toxic to the oral cells. Viability of human gingival fibroblast cells are 82.9% after treated with *H. sabdariffa* extract at concentration of 144 mg/ml	[31, 34]
Ficus deltoidea Jack	Mas cotek	The antibacterial activity of *F. deltoidea* essential oil is by disrupting the bacterial membrane structure or cell wall	Lack of cytotoxicity study of *F. deltoidea* on gingival fibroblast cell. However, neither acute nor subchronic oral administration of ethanol extracts of *F. deltoidea* leaves at a concentration of 2000 mg/kg caused clinical symptoms of toxicity or mortality in male mice	[39, 40]

Zingiber officinale Roscoe	Halia	A mechanism of antibacterial inhibition of red ginger revealed the mechanism of antibacterial inhibition is by disrupting of bacterial cell wall	The cytotoxicity study of *Z. officinale* rhizomes on human gingival fibroblast was measured by MTT cell viability assay revealed non-significant differences compared to the control at a concentration of 100 µg/ml	[47, 48]
Andrographis paniculata (Burm.f.) Nees	Hempedu bumi	Mechanism of antibacterial effects was by reducing total superoxide dismutases (SODs) activity through downregulation of *sodA* and *sodM* expression	No cytotoxic effect on human gingival fibroblast at a concentration less than 12.5%	[54, 55]
Orthosiphon stamineus	Misai kucing	Mechanism of antibacterial effects was by disturbing of membrane structure of cell wall of the bacteria	No cytotoxicity studies of *O. stamineus* on gingival fibroblast cell. Not toxic for oral consumption at a dose of up to 5 g/kg	[39, 57]
Clinacanthus nutans Lindau	Belalai gajah	Inhibitory mechanism of *C. nutans* Lindau against *S. mutans* has not been studied	MTT assay showed that ethanol extract was non-toxic to human gingival fibroblast at a concentration less than 1099 µg/ml, while water extract is non-toxic at a concentration less than 2627 µg/ml	[63]
Melastoma malabathricum L	Senduduk	Mechanism of antibacterial effects was by disrupting the bacteria cell membrane	No study of *M. malabathricum* extract on gingival fibroblast cell has been performed. However, animal study revealed oral consumption of *M. malabathricum* leaves are safe for subacute and subchronic at a concentration up to 500 mg/kg daily	[68, 69]
Moringa oleifera Lam	Monggai	Inhibitory mechanism of *M. oleifera* against *S. mutans* has not been studied	*In vitro* cytotoxicity study revealed only 33.31% of the fibroblast cells survived after exposure to the 5% ethanol extracts of *Moringa oleifera* leaves	[77]

3.3 Anti-plaque Potential

A previous clinical study revealed that an ethanolic extract of *E. longifolia* jack roots inhibited *S. mutans* at a concentration of 62.5 mg/ml in saliva of adults at high-risk for caries (10). The author also found that ethanolic extract of its roots inhibited *Lactobacillus* sp. at 125 mg/ml and *Candida albicans* at 31.3 mg/ml. The mechanism of antibacterial effect of *E. longifolia* jack extract may result from cell wall damage and enhanced permeability of the membrane, as indicated by increased leakage of alkaline phosphatase, electrolytes, nucleic acids, and proteins (11).

3.4 Cytotoxicity Study on *E. longifolia* Jack

A cytotoxicity study revealed that *E. longifolia* jack extract hydrogel was non-toxic to human primary gingival fibroblasts at a concentration of 118.5 g/mL (12).

4 MORINDA CITRIFOLIA

4.1 Traditional Use

Morinda citrifolia (*M. citrifolia*) also known as "Noni" or "Indian mulberry," is a popular medicinal plant in Southeast Asia. In Malaysia, this herb is known as "Mengkudu" and has traditionally been used to treat menstrual cramps, bowel irregularities, urinary tract infections, infantile diarrhoea, and nasal congestion (13). Ripe fruits can be eaten and used to make juice and are popular as a supplement for obesity, senility, diabetes, inflammation, arthritis, hypertension, and atherosclerosis (14).

4.2 Phytochemical Compounds

M. citrifolia L. leaves contain phenolic acids mainly gallic acid, orientin, rutin and ferulic acid (15). While *M. citrifolia* L. fruit extract was found to have anthocyanins, yellow flavonoids, carotenoids and vitamin C (16).

4.3 Anti-plaque Potential

It was found that an aqueous extract prepared from fresh, ripe fruits of *M. citrifolia* had antibacterial activity against *S. mutans* at a concentration of 125 µg/ml (17). Also, a study of silver nanoparticles coated with *M. citrifolia* extract had inhibition effect against *S. mutans* biofilm. The inhibitory activity increases with increase in concentration of the extract (18). To date, the inhibitory mechanism of *M. citrifolia* has not been studied.

4.4 Cytotoxicity Study on *M. citrifolia*

Even though there is no study on the cytotoxicity involving gingival fibroblasts, the aqueous extract of *M. citrifolia* leaves did not show any cytotoxic effects on human periodontal ligament at concentrations ranging from 0.015-1.00% (w/v %) (19). In contrast, in a female mice model study, chronic intake (after 6 months) of *M. citrifolia* fruit aqueous extract at the dose of 2 mg/ml caused liver damage, while chronic intake of leaf extract at the same concentration had no toxicity effects (20).

5 *CENTELLA ASIATICA*

5.1 Traditional Use

Centella asiatica (*C. asiatica*) is one of the most used medicinal plants in Malaysia. In Malaysia, it is known as "Pegaga", and in Europe and America as Indian pennywort, or as gotu kola (21). This herb belongs to the plant family Apiaceae (Umbelliferae) and is found almost everywhere. It is used as food, beverage, and medicine. *C. asiatica* has traditionally been used in Malaysia for treating skin irritations, mental weakness, and hypertension (13). Apart from being used for traditional and alternative medicine, *C. asiatica* is also commonly consumed as a vegetable.

5.2 Phytochemical Compounds

C. asiatica rich in triterpenes and phenolic compounds that contribute to its pharmacological properties. The important bioactive compounds in *C. asiatica* are asiaticoside, madecassoside, asiatic acid, madecassic acid, chlorgenic acid, rutin, quercetin and kaempferol (22).

5.3 Anti-plaque Potential

An *in vitro* study of fresh leaf of *C. asiatica* water extract showed no activity against Streptococci isolated from human saliva but had the antibacterial effect against Lactobacilli and *S. aureus* at MIC values of 4 mg/ml and 8 mg/ml; respectively (23). Also, the interventional study showed that there was no statistically significant difference of *S. mutans* count after 3 months used of water extract of *C. asiatica* mouthwash in patients with type II diabetes mellitus (24). The mechanism of bacterial inhibition of *C. asiatica* extract might occur by inhibiting quorum sensing, a cell density-dependent mechanism in which genes are expressed (25).

5.4 Cytotoxicity Study on *C. asiatica*

Based on MTT assay, assessment of cytotoxicity of water extract of *C. asiatica* against oral keratinocyte cell line showed that the cell viability was 90 % at 100 µg/ml concentration (24).

6 HIBISCUS SABDARIFFA L

6.1 Traditional Use

Originating from India, *Hibiscus sabdariffa* L *(H. sabdariffa)* or roselle is commonly cultivated in Malaysia and was introduced to Africa at a very early date. The plant is also grown in Sudan, Egypt, Nigeria, Mexico, Saudi Arabia, Taiwan, the West Indies, and Central America (26). *H. sabdariffa* belongs to the Malvaceae family. It has been used in traditional medicine to treat colds, toothaches, urinary tract infections, urinary stones and gynaecological problems after delivery (26). This plant also has cardiovascular protective effect (27), anti-diabetic effect (28), nephroprotective and hepatoprotective effects (29).

6.2 Phytochemical Compound

A previous study of ethanol extract of *H. sabdariffa* showed presence of saponins, tannins, phenolic, flavonoids, triterpenoids, glycosides and other ingredients such as fatty acids, furans group, organosulfur, hydrazine, alkyl halide, alcohol, aminophenol, benzothiazoles, lactone and dicarboxylic acid ester (30).

6.3 Anti-plaque Potential

The ethanol extract of *H. sabdariffa* was investigated *in vitro* and *in vivo* for its potential usage in dentistry. A previous *in vitro* study showed that the MIC and MBC of *H. sabdariffa* against *S. mutans* were 7.2 mg/ml and 57.6 mg/ml; respectively (31). A part of that, recent *in vitro* antibacterial activity of *H. sabdariffa* against *S. mutans* showed promising results. *H. sabdariffa* extract at concentration above 75 mg/ml showed significant greater inhibitory zone compared to 0.2% chlorhexidine (32). On top of that, study of roselle effervescent tablet 10% showed no significant difference with sodium perborate effervescent tablet in inhibiting *S. mutans* on acrylic resin plates (33). The mechanism of antibacterial activity of hibiscus acid that obtained from *H. sabdariffa* calyces acetone extract is by increasing the permeability of the bacterial membrane (34).

6.4 Cytotoxicity Study on *H. sabdariffa*

Cytotoxicity study showed that *H. sabdariffa* extract is non-toxic to the oral cells. Viability of human gingival fibroblast cells are 82.9% after treated with *H. sabdariffa* extract at concentration of 144 mg/ml (31).

7 FICUS DELTOIDEA

7.1 Traditional Uses

Ficus deltoidea Jack (*F. deltoidea*) is commonly known as “Mas Cotek, Telinga Beruk, and Serapat Angin” in Peninsular Malaysia; as “Sempit-Sempit and Agoluran” in Sabah and Sarawak (35). *F. deltoidea* belongs to the family Moraceae and is widely distributed in Malaysia, Thailand, and Indonesia. A wide variety of medicinal uses have been reported for this plant among Malays, including after-birth tonics, menstrual cycle disorder, leucorrhoea, anti-diabetic medications, treat sores, wounds, and rheumatism (35,36). The roots of *F. deltoidea* extract possess antioxidant, anti-melanogenic and cytotoxic potential (37).

7.2 Phytochemical Compounds

Different parts of *F. deltoidea* contain different types of phytochemical constituents. A major component of the plant is the leaves, which contain mainly polyphenols, triterpenoids, saponins, and tannins, but very little alkaloids. Fruits are primarily composed of triterpenoids, alkaloids, and flavonoids, whereas stems are primarily composed of flavonoids, saponins, and alkaloids (38).

7.3 Anti-plaque Potential

In vitro study of *F. deltoidea* essential oil showed moderate inhibition against *S. mutans* with the MIC and MBC value were 1.25 mg/ml and 2.5 mg/ml; respectively (39). After exposure to the oils, a field emission scanning electron microscope demonstrated alterations in the Gram-negative bacteria's cell structure, as they became pleomorphic and lysed. This suggests that the antibacterial activity of *F. deltoidea* essential oil occurs by disrupting the bacterial membrane structure or cell wall (39).

7.4 Cytotoxicity Study on *F. deltoidea*

To date, there is no cytotoxicity study of *F. deltoidea* on gingival fibroblast cell. However, neither acute nor subchronic oral administration of ethanol extracts of *F. deltoidea* leaves at a

concentration of 2000 mg/kg caused clinical symptoms of toxicity or mortality in male mice (40).

8 ZINGIBER OFFICINALE

8.1 Traditional Use

Zingiber officinale Roscoe (*Z. officinale*) or ginger, an herbal plant from the Zingiberaceae family. There are two varieties of Malaysian ginger, namely Halia Bentong and Halia Bara (red ginger) (41). Bentong ginger is the most popular variety of *Z. officinale* in Malaysia and has been used as spice and medicine for treating cancer, cardiovascular disease, diabetes, and several other illnesses such as cold, nausea, asthma, and cough (42). Red ginger or its scientific name *Zingiber officinale* var. rubrum is widely used in traditional medicine in Asia. Unlike other gingers, it is not used as a spice in cuisines. In traditional medicine, it is used for treating headaches, indigestion, nausea, vomiting, and cancer. In addition, it is widely used to treat autoimmune diseases, hypertension, hypercholesteremia, hyperuricemia, bacterial infections, and cancer (43).

8.2 Phytochemical Compounds

A previous phytochemical analysis of essential oils distilled from the rhizomes of Malaysian *Z. officinale* found that the mainly compounds were monoterpene hydrocarbons (19.7–25.5%), oxygenated monoterpenes (23.6–33.7%), sesquiterpene hydrocarbons (21.3–35.6%), oxygenated sesquiterpenes (1.5–3.9%), and other minor classes of compounds (0.7–2.7%) (44). The chemical composition of red ginger includes monoterpenes, sesquiterpenes, diterpenes, vanilloids, flavonoids, amino acids, vitamins, and trace elements such as iron, copper, manganese, zinc, chromium, nickel, and strontium (43).

8.3 Anti-plaque Potential

Z. officinale extract showed promising antibacterial activity against *S. mutans*. The MIC and MBC of *Z. officinale* extract against this cariogenic bacterium was 0.02 mg/mL and 0.04 mg/ml; respectively (45). On the other hand, crude extract and methanolic fraction of *Z. officinale* strongly inhibited a variety of virulence properties which are critical for its pathogenesis (46). Also, the authors found that *in vivo* study showed the excellent reduction of caries development in treated group of rats as compared to the untreated group (46). A mechanism of antibacterial inhibition of red ginger revealed the mechanism of antibacterial inhibition is by disrupting of bacterial cell wall (47).

8.4 Cytotoxicity Study on *Z. officinale*

The cytotoxicity study of *Z. officinale* rhizomes on human gingival fibroblast was measured by MTT cell viability assay revealed non-significant differences compared to the control at a concentration of 100 µg/ml indicate that this herb is non-toxic to the human gingival fibroblast (48).

9 ANDROGRAPHIS PANICULATA

9.1 Traditional Use

Andrographis paniculata (Burm.f.) Nees (*A. paniculata*) is a plant in the Acanthaceae family commonly known as the "King of Bitters" or locally known as “Hempedu bumi”. There is a wildly widespread distribution of *A. paniculata* in Southern Asia. *A. paniculata* has been used as a medicinal food for centuries. Traditionally, the leaves and roots of this plant are used for a variety of medicinal purposes in Asia and Europe (49). *A. paniculata* has been used to treat snake bites and poisonous insect stings, fevers (such as malaria and dengue), diarrhoea, flu, skin diseases, and upper respiratory infections for centuries (50). This plant is extensively used in traditional medicine in Malaysia and helps against hypertension, diabetes, and bacterial and viral infections (13).

9.2 Phytochemical Compounds

Its aerial parts mainly leaves and stems contain active phytochemicals such as diterpenoids and 2′-oxygenated flavonoids, including andrographolide, neoandrographolide, 14-deoxy-11, 12-didehydroandrographolide, 14-deoxyandrographolide, isoandrographolide, 14-deoxyandrographolide-19-β-D-glucoside, homoandrographolide, andrographolidegraphan, andrographolidegraphosterin, and stigmasterol (51).

9.3 Anti-plaque Potential

A recent study has shown that water extract of *A. paniculata* has antibacterial activity against *S. mutans* (52). Furthermore, the leaves of *A. paniculata* were used to synthesize calcium hydroxide-based silver nanoparticles which demonstrated antimicrobial activity against oral pathogen including *S. mutans* (53). There is a possibility that it inhibits bacteria by reducing total superoxide dismutases (SODs) activity through

downregulation of *sodA* and *sodM* expressions (54).

9.4 Cytotoxicity Study on *A. paniculata*

A. paniculata extract at a concentration less than 12.5% did not show any cytotoxic effect on human gingival fibroblast (55).

10 *ORTHOSIPHON STAMINEUS*

10.1 Traditional Use

Orthosiphon stamineus (*O. stamineus*) is also known as “Misai kucing” and widely grown in Southeast Asia and the tropical countries. In Southeast Asia and European countries, the leaves of this plant are commonly used to make herbal tea, known as Java tea. There are several other names for *O. stamineus*, including *Orthosiphon aristatus*, *Orthosiphon spicatus*, *Orthosiphon blaetter*, *Indischer nierentee*, and Feuilles de Barbiflore. The Orthosiphon species comes in two varieties: the white variety (white flower) and the purple variety (purple flower). Compared to white varieties, purple varieties contain a greater number of bioactive compounds. Medicinal properties are usually found in the leaves and stem tips. This plant has been used traditionally for treating diabetes and chronic renal failure throughout southern China, Malaysia, and Thailand (56).

10.2 Phytochemical Compounds

Study of essential oil of *O. stamineus* revealed β-caryophyllene as the main compound. Other major compounds present in *O. stamineus* oil were α-humulene, eugenol, bicyclogermacrene, α-copaene, caryophyllene oxide and methyl eugenol (39).

10.3 Anti-plaque Potential

The essential oil of *O. stamineus* showed moderate inhibition against *S. mutans* with MIC and MBC values ranging 1.25–2.5 mg/ml (39). However, there was no inhibition on biofilms. The inhibitory mechanism may be due to disturbances of membrane structure or cell wall of the bacteria since the field emission scanning electron microscope study showed significant alterations of Gram-negative bacteria cells as they became pleomorphic and lysed after exposure to the oils (39).

10.4 Cytotoxicity Study on *O. stamineus*

There were no cytotoxicity studies of *O. stamineus* on gingival fibroblast cell. However, several studies investigated the possible toxic effects of orally administered *O. stamineus* extract in rats. No lethality, adverse manifestations, or delayed toxic effects were seen at a dose of up to 5 g/kg (57).

11 *CLINACANTHUS NUTANS LINDAU*

11.1 Traditional Use

Clinacanthus nutans Lindau (*C. nutans*), a member of the Acanthaceae family, is a very well-known traditional herb and vegetable in Southeast Asian countries. It is commonly known as "belalai gajah" or "pokok stawa ular" by Malay, "you dun cao," by Chinese, "payayor" or "slaed pang pon," by Thai, and as "daun dandang gendis" or "kitajam" by Javanese (58). *C. nutans* leaves are used by these communities for a wide variety of medicinal purposes, including treating fever, diabetes, and cancer (59). Traditionally, the plant's leaf was decocted with water for oral consumption or soaked in alcohol for a week for topical application (58).

11.2 Phytochemical Compounds

The study of its root demonstrated the presence of lupeol, lup-20(29)-en-3-one, lup-20(29)-en-ol acetate, stigmasterol, sitosterol, β-amyrin, betulin, campesterol, squalene, vitamin E and oleic acid in ethyl acetate extract. In contrast, the methanol root extract contained lupeol, betulin, stigmasterol, sitosterol, myrin, vitamin E and campesterol but not squalene, oleic acid or other lupeol derivatives (60).

11.3 Anti-plaque Potential

A previous study of chloroform extract of *C. nutans* leaves and its pure isolated compound, purpurin-18 phytol ester significantly reduced *S. mutans* biofilm formation below 50% at 250 µg/ml (61). The *C. nutans* extracts had been used for synthesizing silver nanoparticles. The use of *C. nutans* extracts in synthesizing silver nanoparticles has shown antibacterial activities against *S. mutans*. The silver nanoparticles-*C. nutans* has been shown to be effective against *S. mutans* at a concentration of 2.5 mg/ml (62).

11.4 Cytotoxicity Study on *C. nutans*

Based on MTT assay, ethanol extract of *C. nutans* is non-toxic to human gingival fibroblast (HGF-1) at a concentration less than 1099 µg/ml, while water extract is non-toxic at a concentration less than 2627 µg/ml (63).

12 MELASTOMA MALABATHRICUM

12.1 Traditional Use

Known as "Senduduk", *Melastoma malabathricum* L is a member of the Melastomataceae family that is widely distributed in the south-east Asian region including Malaysia. Over 4,000 species of Melastomataceae can be found worldwide. Malaysia has at least 12 species of Melastoma, most of which are used as traditional medicine by the Indigenous people. This plant is commonly found along the side of the road, abandoned areas, and open land. Traditionally, it has been used to treat diabetes, diarrhoea, dysentery, and high blood pressure (64). When consumed as herbal medicine, the leaves are typically boiled in water and cooled at room temperature.

12.2 Phytochemical Compounds

The methanol extract of its leaves showed the presence of 5-hydroxymethylfurfural, pyrogallol, phytol, palmitic acid methyl ester, palmitic acid, 8,11-octadecadienoic acid methyl ester, phytol, stearic acid methyl ester, trans-squalene, and tocopherol (65). While the water extract of its leaves contained gallic acid, 4-O-caffeoylquinic acid, 3-O-trans-coumaroylqinic acid, arteamisinin l, quercimeritrin, quercetin, norberginin, and digiprolactone (66). The study of its stem barks demonstrated the presence of α-amyrin, β-sitosterol, hexadecenoic acid, stearic acid and hexacosanoic acid in its acetone extract (67).

12.3 Anti-plaque Potential

The study of acetone extract of its stem bark revealed some of the compounds including α-amyrin, β-sitosterol, hexadecenoic acid, stearic acid and hexacosanoic acid have antibacterial properties against *S. mutans* (67). Other than its stem bark, *M. malabathricum* leaves also had moderate antibacterial activity against *S. mutans*. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of the acetone extracts of *M. malabathricum* leaves were 6.25 mg/ml and 25 mg/ml, respectively. The mechanism of antibacterial effects was by disrupting the bacteria cell membrane (68).

12.4 Cytotoxicity Study on *M. malabathricum*

To date, no study of *M. malabathricum* extract on gingival fibroblast cell has been performed. However, animal study revealed oral consumption of *M. malabathricum* leaves are safe for subacute and subchronic at a concentration up to 500 mg/kg daily (69).

13 MORINGA OLEIFERA LAM

13.1 Traditional Use

In Malaysia, *Moringa oleifera* Lam (*M. oleifera*), also known as "Monggai", "Gemunggal", "Kelor", or "Merunggai" has a long history of use as a traditional medicinal plant. This plant belongs to the Moringaceae family and is native to the sub-Himalayan regions of India, Pakistan, Bangladesh, Sri Lanka, Afghanistan, East and West Africa, the Arabian Peninsula, Southern Asia, and Southern Florida (70). Various parts of *M. oleifera* including roots, leaves and flowers are used in traditional medicine to treat diarrhoea and hypertension. It is also beneficial for pregnant and lactating mothers as they improve milk production and are prescribed for anaemia (71).

13.2 Phytochemical Compounds

Different methods of extraction resulted in varying amounts of chemical compounds in *M. oleifera.* Methanolic leaf extracts of *M. oleifera* revealed the presence of alkaloids, carbohydrates, coumarins, flavonoids, glycosides, phenol, proteins, quinones, saponins, steroids, tannins and terpenoids (72). In contrast, 5-nonanol-dibutylcarbinol, 5-hydroxyl-2-(hydroxyl methyl)-4H-pyran-4-one, 2-octenoic acid, 1-hydroxyl-2,2,6,6-tetramethyl-3-(4-nitroso-1-piperazinylmethyl)piperidine-4-one, tetradecanoic acid, pentadecanoic acid, 1, 2-epoxyhexadecane (oxirane), hexadecanoic acid, 6-octadecenoic acid, 1, 2-benzene dicarboxylic and 11-bromoundecanoic acid were found in aqueous and ethanol leaf extracts of *M. oleifera* (73).

13.3 Anti-plaque Potential

A previous study showed that *M. oleifera* leaves extract has antibacterial effect against *S. mutans* (74). Another study of distilled water and ethyl alcohol extracts of *M. oleifera* leaves showed

excellent antimicrobial activities against *S. mutans* and inhibited formation of cariogenic biofilm (75).

The study demonstrated that the distilled water and ethyl alcohol extracts of *M. oleifera* leaves reduced the growth of *S. mutans* at a concentration of 25 µg/ml and 6.25 µg/ml, respectively. Also, ethyl alcohol extracts of *M. oleifera* leaves had stronger anti-biofilm activity against cariogenic biofilm than distilled water extracts. Other than *in vitro* studies, the use of *M. oleifera* in oral care has been clinically investigated. A clinical study of the moringa-based dentifrice was effective in reducing plaque accumulation (76). The authors compared the use of moringa toothpaste with miswak toothpaste. Comparing moringa toothpaste to miswak toothpaste, the author found that plaque index scores had statistically significant decreases. Additionally, moringa toothpaste also reduced gingival inflammation.

13.4 Cytotoxicity Study on *M. oleifera*

In vitro cytotoxicity study revealed 5% ethanol extracts of *M. oleifera* leaves were toxic to fibroblast cells. After exposure to the tested solution, only 33.31% of the fibroblast cells survived. This percentage, however, was greater than the percentage of fibroblast cells that survived after exposure to 2.5% NaOCl and 2% chlorhexidine, which was 28.89% and 39.37%, respectively (77).

14 CONCLUSIONS

The disadvantages of synthetic compounds for combating oral plaque have led to the search for other plant-based alternatives. The use of herbal extracts for the control of oral plaque is considered a fascinating alternative to chlorhexidine. This review has focussed on the use of Malaysian herbs under NKEA that have anti-plaque potential such as *Eurycoma longifolia* Jack, *Morinda citrifolia*, *Centella asiatica*, *Hibiscus sabdariffa* L, *Andrographis paniculata* (Burm.f.) Nees, *Zingiber officinale* Roscoe, *Ficus deltoidea* Jack and *Orthosiphon stamineus*. While *Clinacanthus nutans* Lindau, *Melastoma malabathricum* L, and *Moringa oleifera* Lam are also included since these indigenous herbs were also have potential to be used as an anti-plaque agent in future. All the eleven plant extracts have antibacterial effects against *S. mutans*, a pathogen responsible for dental plaque formation, while some of them showed no cytotoxicity on the gingival fibroblast cells. Since these plants can be easily obtained, it could be a cost-effective plant-based alternative that can be exploited for the benefit of humans.

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