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## 2.0 REVIEW OF LITERATURE

Oral health has always been considered an essential part of general health since it is closely associated with general well-being (Baiju et al. 2017). Poor oral health can negatively impact the quality of life and mental well-being, affecting daily life in both direct and indirect ways (Antoniadou & Varzakas, 2024). Dental caries, or tooth decay, is a common disease caused by the interaction of cariogenic bacteria with fermentable carbohydrates in the diet, leading to the demineralization of tooth enamel and dentin (Awadh Al-Shahrani, 2019). If left untreated, tooth decay can progress, causing significant damage to the tooth structure, resulting in pain, infection, and even tooth loss. Practicing good oral hygiene and making healthy dietary modifications are essential in preventing and reducing the risk of dental caries (Sharma & Bhandary, 2024).

Tooth decay is influenced by a combination of environmental and genetic factors. Consuming sugar-rich diets, poor oral hygiene, dental plaque buildup, high levels of cariogenic bacteria, and inadequate salivary flow, have been reported as risk factors for caries (Selwitz et al. 2007). In addition, genetic factors play a significant role in determining the risk of tooth decay, accounting for approximately 49.1 to 62.7% of the variation in caries risk scores (Haworth et al. 2020). The World Health Organization (WHO), reports that approximately 3.5 billion people worldwide suffer from oral diseases, predominantly affecting those in middle-income countries. Globally, dental caries pose a significant health concern, affecting approximately 2 billion people with permanent dentition and 514 million children with primary dentition (Global Oral Health Status Report WHO, 2022).

To maintain oral hygiene and treat oral disorders, various chemical and synthetic agents are employed, offering unique approaches for prevention and treatment. Unfortunately, these chemical and synthetic agents have notable disadvantages, including disrupting the oral microbiota balance, causing tooth discoloration, being expensive, and contributing to the development of antibiotic-resistant pathogenic bacteria (Taheri et al. 2011). Despite continuous advancements in the field of dental care, dental caries remain a prevalent issue,

highlighting the urgent need for innovative and preventive strategies to complement existing methods and effectively address this persistent public health concern (Fejerskov, 2004; Oral Health in America, 2024). Herbal extracts and their derivatives offer a promising alternative to synthetic drugs for treating oral issues, providing an affordable, safe, and biocompatible solution (Refaey et al. 2024). The World Health Organization, notes that over 75% of people worldwide rely on herbal plants for their main healthcare due to their remarkable physicochemical and medicinal properties. In the treatment of oral disorders, herbal therapy can be utilized as an antibacterial, anti-inflammatory, astringent, anesthetic, and anti-cariogenic to combat infections, reduce swelling, tighten tissues, alleviate pain, and prevent tooth decay (Martinez et al. 2017).

## **2.1 Medicinal Plants**

- 2.1.1 *Achyranthes aspera* L.
- 2.1.2 *Acalypha indica* L.
- 2.1.3 *Abrus precatorius* L.
- 2.1.4 *Azadirachta indica* A. Juss.
- 2.1.5 *Barleria cuspidata* F. Heyne ex Nees.
- 2.1.6 *Euphorbia hirta* L.
- 2.1.7 *Ficus benghalensis* L. var. *benghalensis*
- 2.1.8 *Piper betle* L.
- 2.1.9 *Psidium guajava* L.
- 2.1.10 *Pongamia pinnata* (L.) Pierre.
- 2.1.11 *Tridax procumbens* L.
- 2.1.12 *Solanum virginianum* L.

## **2.2 *In silico* study to Identify Potential Inhibitors of *Streptococcus mutans* Glucosyltransferases**

- 2.2.1 Molecular Docking Studies
- 2.2.2 Molecular Dynamic Simulations
- 2.2.3 Glucosyltransferases of *S. mutans*

## **2.3 Optimization of Polyherbal Formulation**

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## **2.5 Virulence Factors of *Streptococcus mutans***

- 2.5.1 Inhibition of Acid Production of *S. mutans*
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2.5.3 Inhibition of Preformed Biofilm of *S. mutans*

2.5.4 SEM Analysis of *Streptococcus mutans* Biofilm Eradication

## 2.6 Network pharmacology

2.6.1 Protein-Protein Interaction Network

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## 2.1 Medicinal Plants

Medicinal plants are a vital source of traditional medicine, offering bioactive compounds and therapeutic agents. This review explores 12 selected species, detailing their Linnaeus taxonomical classification, botanical description, and potential medicinal properties.

### 2.1.1 *Achyranthes aspera* (L.)

#### Taxonomical classification

Kingdom : Plantae

Phylum : Angiosperms

Class : Eudicots

Order : Caryophyllales

Family : Amaranthaceae

Genus : *Achyranthes*

Species : *aspera*



Plate 2.1 *Achyranthes aspera* L. Root

#### Botanical Description

*Achyranthes aspera*, commonly known as chaff or prickly chaff-flower, is a significant plant belonging to the Amaranthaceae family. It is a wild, perennial, and erect herb that grows 1-2 meters tall with a tap root system and herbaceous stem covered with hairs (Srivastav et al. 2011). The plant has simple, petiolate leaves arranged in an opposite decussate manner; its flowers are sessile, complete, actinomorphic, and hypogynous with 5 tepals in the perianth. The male reproductive structure of flowers consists of 10 stamens, out of which 5 are fertile. The female reproductive structure is bi-carpellary with a single style and capitate stigma (Lakshmi et al. 2018). This botanical description is well supported by various studies, highlighting the plant's distinct features and its classification within the family. The widespread presence and distinct morphology make this plant a notable subject of study in botanical and pharmacological research.

### 2.1.2 *Acalypha indica* L.

#### Taxonomical classification

Kingdom : Plantae  
 Phylum : Angiosperms  
 Class : Eudicots  
 Order : Malvales  
 Family : Euphorbiaceae  
 Genus : *Acalypha*  
 Species : *indica*



Plate 2.2 *Acalypha indica* L. Leaf

#### Botanical Description

*Acalypha indica* Linn. is an annual, erect herb that grows 30-75 cm tall, featuring numerous, long, and ascending branches with fine hairs. The leaves are ovate or rhombic-ovate in shape, acute or sub-obtuse at the tips, 2.5-7.5 by 2-4.5 cm long with crenate-serrate margins. The petioles are usually longer than the leaf blades and are slender, accompanied by minute stipules. The plant produces clusters of small flowers in axillary spikes, surrounded by large, leafy bracts. The ovary is hispid, and the small capsule is quite concealed by the bract. The capsule contains only one seed, which is ovoid, smooth, pale brown, and measures 1-2 mm in length (Male et al. 2021).

### 2.1.3 *Abrus precatorius* L.

#### Taxonomical classification

Kingdom : Plantae  
 Phylum : Angiosperms  
 Class : Eudicots  
 Order : Fabales  
 Family : Fabaceae  
 Genus : *Abrus*  
 Species : *precatorius*



Plate 2.3 *Abrus precatorius* L. Habit

#### Botanical Description

*Abrus precatorius*, commonly known as rosary pea or jequirity bean, features compound leaves with 5 to 9 pairs of elliptic to oblong leaflets, each

measuring 1 to 4 cm in length and giving them a glossy appearance (Kumar et al. 2018). The arrangement of leaflets enhances the plant's ability to capture sunlight for photosynthesis while minimizing water loss. The leaf petioles are slender and may exhibit a slight twist, allowing the leaflets to adjust their orientation for optimal light exposure (Mishra et al. 2017). Anatomically, the leaves possess a thick cuticle, well-defined mesophyll layers, and a network of vascular bundles that provide structural support and efficient transport of nutrients (Pulipati et al. 2016). Notably, the leaves and seeds contain toxic compounds such as abrin, which have significant implications for both medicinal uses and safety in handling (Amin et al. 2019). Due to its striking red seeds and its use in traditional medicine, *A. precatarius* serves both aesthetic and functional roles in various cultures.

#### **2.1.4 *Azadirachta indica* A.**

##### **Taxonomical classification**

Kingdom : Plantae  
Phylum : Angiosperms  
Class : Eudicots  
Order : Sapindales  
Family : Meliaceae  
Genus : *Azadirachta*  
Species : *indica*



**Plate 2.4 *Azadirachta indica* A. Leaf**

##### **Botanical Description**

*Azadirachta indica* also known as the neem tree grows up to 40-50 feet tall with a straight trunk, spreading branches, and a broad, round crown. Its bark is rough and dark brown, while its compound leaves have 5-15 leaflets and alternate with each other. The tree bears many-flowered panicles, mostly in the leaf axils, with ovate sepals about 1cm long and sweet-scented white oblanceolate petals. It produces yellow, ellipsoid, glabrous drupes that are 12-20mm long with a garlic-like odor. Fresh leaves and flowers appear in March-April, and the fruits mature between April and August, depending on the locality (Hashmat et al. 2012).

### 2.1.5 *Barleria cuspidata*

#### Taxonomical classification

Kingdom : Plantae  
 Phylum : Angiosperms  
 Class : Eudicots  
 Order : Lamiales  
 Family : Acanthaceae  
 Genus : *Barleria*  
 Species : *cuspidata*



**Plate 2.5 *Barleria cuspidata*  
F.Heyneex Nees**

#### Botanical Description

This perennial shrub reaches up to 1 meter in height with terete or four-angled stems and bristled nodes. It has 2-4 axillary thorns up to 2cm long. The opposite leaves are 6-14.5cm long and 2-6cm wide, ovate-elliptic, and acuminate at the apex. Flowers are yellow, solitary, or clustered, with a bi-lipped corolla followed by ovoid capsules containing 2 orbicular, hairy seeds. Flowering and fruiting occur from August to November (Indian Biodiversity Portal).

### 2.1.6 *Euphorbia hirta*

#### Taxonomical classification

Kingdom : Plantae  
 Phylum : Angiosperms  
 Class : Eudicots  
 Order : Malpighiales  
 Family : Euphorbiaceae  
 Genus : *Euphorbia*  
 Species : *hirta*



**Plate 2.6 *Euphorbia hirta* L. Habit**

#### Botanical Description

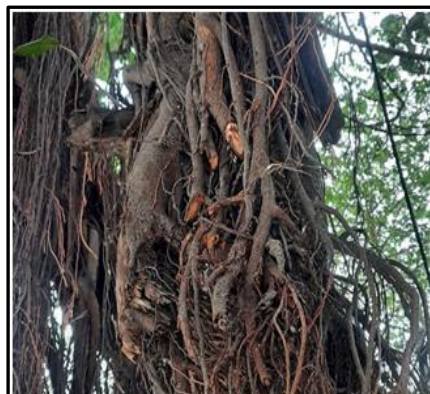
This small, annual herb grows up to 50 cm tall, with hairy stems and opposite, elliptical, oblong, or oblong-lanceolate leaves, featuring faintly toothed margins and a darker upper surface. The flowers are tiny, numerous, and tightly clustered in dense cymes about 1 cm in diameter. The fruits are yellow, three-celled, hairy, and keeled capsules, each

containing three brown, four-sided, angular, and wrinkled seeds (Ghosh et al. 2019).

### 2.1.7 *Ficus benghalensis*

#### Taxonomical classification

Kingdom : Plantae  
 Phylum : Angiosperms  
 Class : Eudicots  
 Order : Rosales  
 Family : Moraceae  
 Genus : *Ficus*  
 Species : *benghalensis*



**Plate 2.7 *Ficus benghalensis* L.  
 var. *benghalensis*  
 Proproot**

#### Botanical Description

*Ficus benghalensis*, commonly known as the banyan tree, is a massive tree that can reach about 100 feet in height, with branches spreading across a wide area supported by prop roots. Its smooth bark turns greyish-white with age and the wood is soft, and porous, and exudes milky, sticky latex. The tree has glossy, leathery ovate leaves with 3-7 basal nerves and 4-6 pairs of secondary nerves, measuring 10-20cm in length with 2-5cm long petioles. The tree produces very small, separate male and female flowers. The male flowers crowd near the mouth of the receptacle, while female flowers have a shorter perianth and a long style, both in the same receptacle. The globose fruits are sessile in axillary pairs, dark red, and turn to dark purple when ripe, measuring 1.5-2.0cm in diameter, with a fleshy pericarp and tiny seeds. The fruit is not edible for humans but is eaten by birds and monkeys (Gopukumar & Praseetha, 2015).

### 2.1.8 *Piper betle*

#### Taxonomical classification

Kingdom : Plantae  
 Phylum : Angiosperms  
 Class : Eudicots  
 Order : Piperales  
 Family : Piperaceae  
 Genus : *Piper*  
 Species : *betle*



**Plate 2.8 *Piper betle* L. Leaf**

### **Botanical Description**

*Piper betle* is a dioecious root climber that grows 3 to 10 meters high with support. It has lateral branches, swollen and articulate stems with raised whitish streaks and pinkish stripes, and internodes about 12cm long. The simple, alternate, spiral leaves are greenish to yellowish, broadly ovate, 7-8.5cm wide, 9-11cm long, with a cordate base and acuminate apex. The inflorescence is an axillary spike up to 5.5cm long. Male inflorescences are cylindrical catkins about 10cm long and 2cm in diameter, while female spikes are 2.5-4cm long and 0.5cm in diameter. Flowers are very small, unisexual, and aromatic when mature. Fruiting spikes are 3-5cm long, orange, and drooping (Biswas et al. 2022).

#### **2.1.9 *Psidium guajava***

##### **Taxonomical classification**

Kingdom : Plantae  
 Phylum : Angiosperms  
 Class : Eudicots  
 Order : Myrtales  
 Family : Myrtaceae  
 Genus : *Psidium*  
 Species : *guajava*



**Plate 2.9 *Psidium guajava* L.**

**Leaf**

##### **Botanical Description**

*Psidium guajava*, tree is a small to medium-sized tree or shrub with multiple branches, 2-7m tall with smooth, thin, and copper-colored, shedding off easily to reveal a greenish skin underneath the bark. Leaves are characterized by their opposite phyllotaxy, leathery texture, and elliptical to oval shape. They typically measure 7-15cm in length and 3-5cm in width. The leaves have prominent parallel veins that originate from the midrib and extend towards the edges. This unique structure and appearance make them easily recognizable. The flowers either singly or in groups of two or three at leaf junctions on new growth. Flowering branches are short with four to five leaf pairs, and when a flower sets, the terminal bud stops growing until the next season. Both main and side shoots can bear flowers, though buds appear sporadically along the

branches. The flowering period lasts 25 to 45 days, depending on the cultivar, season, and location. Guava fruit is generally round, ovoid or pear-shaped, with a diameter of 5–10cm and a weight of 50–200g, with a thin and light yellow, often with a hint of pink outer layer (Dhirendra et al. 2024).

### 2.1.10 *Pongamia pinnata*

#### **Taxonomical classification**

Kingdom : Plantae  
Phylum : Angiosperms  
Class : Eudicots  
Order : Fabales  
Family : Fabaceae  
Genus : *Pongamia*  
Species : *pinnata*



**Plate 2.10 *Pongamia pinnata* (L.) Pierre Leaf**

#### **Botanical Description**

*Pongamia pinnata*, commonly known as karanj or pongam tree, is a medium-sized deciduous tree with high drought tolerance and moderate aerosol salt tolerance. Its leaves are alternate, pinnately compound, 2-4 inches long, evergreen, and hairless. The flowers are lavender, pink, or white, 2-4 together, short-stalked, and pea-shaped, measuring 15-18mm long. The pods are 3-6cm long, 2-3cm wide, smooth, brown, thick-walled, hard, and indehiscent, containing 1-2 seeds. The seeds are compressed, ovoid or elliptical, bean-like, 10-15cm long, dark brown, and oily. The root system includes a thick and long taproot with numerous, well-developed lateral roots. The bark is thin, gray to grayish brown and yellow on the inside. It's important to note that all parts of the plant are toxic and can induce nausea and vomiting if ingested (Sangwan et al. 2010). *Pongamia pinnata* is noted for its ecological benefits, including its ability to improve soil fertility through nitrogen fixation and its use in reforestation and agroforestry practices (Kumar et al. 2022). Additionally, the tree has traditional medicinal uses, particularly in treating skin ailments and inflammatory conditions (Farhana et al. 2023). Overall, *Pongamia pinnata* plays a significant role in sustainable agriculture and environmental conservation.

### 2.1.11 *Tridax procumbens*

#### Taxonomical classification

Kingdom : Plantae  
 Phylum : Angiosperms  
 Class : Eudicots  
 Order : Asterales  
 Family : Asteraceae  
 Genus : *Tridax*  
 Species : *procumbens*



Plate 2.11 *Tridax procumbens* L.

#### Leaf

*Tridax procumbens* is a perennial herb with taproot and a semi-prostrate growth habit, reaching 15-40cm in height. It has cylindrical, often purplish stems covered with long, white hairs. Its elongated, opposite leaves are ovate with serrated margins and feature hairs on both sides. The inflorescence is a capitulum with three-toothed white female ray florets and yellow tubular bisexual disc flowers. Seedlings have 1-7mm long hypocotyls, glandular-hairy cotyledons, and first true leaves with distinct midribs. The plant can be cross- or self-pollinated, attracting thrips, beetles, bees, and butterflies, and a single plant can produce 500 to 2500 seeds (Dattaray, 2022). *Tridax procumbens* is often recognized for its medicinal properties, including its use in traditional medicine to treat wounds, fevers, and gastrointestinal disorders, attributed to its anti-inflammatory and antimicrobial effects (Bhat et al. 2019). Additionally, the plant plays a significant role in soil conservation and as a cover crop due to its ability to suppress weeds and improve soil health (Singh & Gupta, 2022). Overall, *Tridax procumbens* serves both ecological and medicinal purposes, making it a valuable plant in various contexts.

### 2.1.12 *Solanum virginianum*

#### Taxonomical classification

Kingdom : Plantae  
 Phylum : Angiosperms  
 Class : Eudicots  
 Order : Solanales  
 Family : Solanaceae  
 Genus : *Solanum*  
 Species : *virginianum*



Plate 2.12 *Solanum virginianum* L.  
Habit

### **Botanical Description**

*Solanum virginianum* is a spiny, dark green perennial herb with a woody base, growing 2-3m tall. Found across India, often in wastelands and along roadsides, it produces globular, glabrous berries. The plant's greenish grey stems are covered in numerous pricks and dense tomentose and stellate hairs when young. The spines, which are 1-3cm long, glabrous, shiny, and straight, add to its unique appearance. Leaves have sharp, yellow prickles along the midrib, are hairy on both sides, and vary in shape from obtuse or subacute to elliptic or ovate. Petioles are slightly hairy and spiked. Flowers may appear singly or in cymes, accompanied by a spiky, hairy calyx tube. The corolla lobes are acute, deltoid, and purple with outer hairs. *Solanum virginianum* boasts beautiful blue flowers and produces globular, edible berries measuring 1.25-2cm in diameter. The immature berries feature white and green stripes, turning yellow as they mature. Its distinctive morphology and ecological roles make it an interesting subject for further research in botany and pharmacology (Swain & Bhatnagar, 2023).

Table 2.1 Review of the selected medicinal plants used traditionally to treat dental caries

Botanical name	Common name	Vernacular Name (Tamil)	Treatment for oral ailments	References
<i>Achyranthes aspera</i>	Prickly Chaff flower	Nayuruvi	Treats caries, strengthens teeth, relieves toothache, manages halitosis, teeth whitening, maintains strong and healthy gums, and is used as a toothbrush.	Malik et al. 2022
<i>Acalypha indica</i>	Indian nettle	Kuppaimeni	Used to cure gum and tooth disease	Senthilkumar and Rani, 2024
<i>Abrus precatorius</i>	Rosary pea	Kundumani	Raw leaves are chewed to kill worms in teeth	Silambarasan et al., 2023
<i>Azadirachta indica</i>	Neem	Veppa Maram	Prevents cavities, bleeding, and sore gums cures gum disease and oral infections	Lakshmi et al., 2015
<i>Barleria cuspidata</i>	Spiny Barleria	Manchatcemmulli	Treats toothache	
<i>Euphorbia hirta</i>	Asthma plant	Amman pacharisi	Prevents toothache and gum disorders the young stem is used as a toothbrush	Raj and Ayyanar, 2024
<i>Ficus benghalensis</i>	Banyan	Alamaram	Controls caries, periodontal disorder, oral candidiasis, halitosis/ stop bad breath, strengthens gum, raw leaf chewed orally to strengthen teeth, improve body health	Raj and Ayyanar, 2024
<i>Piper betle</i>	Betel vine	Vettilai	Inhibits plaque and periodontal disorder	Silambarasan et al. 2023
<i>Psidium guajava</i>	Guava	Koyya	Root - gums, teeth cleaning	Divyashree and Ravi, 2014
<i>Pongamia pinnata</i>	Indian beech	Pungai	Cure tooth decay and young stems - used as toothbrush for oral hygiene	Fugare et al. 2021
<i>Tridax procumbens</i>	Tridax daisy	Vettukaya poondu	Toothache and mouth ulcers, dental infections	Vinayagam et al. 2024
<i>Solanum virginianum</i>	Indian nightshade	Kandankathiri	Seed smoke is let into affected tooth regions to kill worms in a decayed tooth	Silambarasan et al. 2023

Table 2.2 Review of phytochemical and antioxidant activities of selected plant extracts

Plant	Solvent	Qualitative	Quantitative estimation	DPPH (IC <sub>50</sub> )	References
AAR	Ethanol	Alkaloids, anthraquinone glycosides, saponins, phenols, flavonoids, proteins, and diterpenes.	TPC: 240.7±8.19 GAE/g; TFC:0.011±0.005 RE/g; TTC: 26.53±3.03 RE/g;	68.92	Kumar and Jat, 2017 Kumar et al. 2018
AIL	Methanol	Phenols, flavonoids, alkaloids, tannins, terpenoids, cardiac glycosides, saponins, anthraquinones, anthocyanins, coumarins, and reducing sugars.	TPC:111.321 GAE/g TFC:29.896 RE/g	28.330	Poojar et al. 2017
APL	Methanol	Phenols, flavonoids, steroids, terpenoids, alkaloids, sugar, proteins, sterols, cardiac glycosides, coumarins, tannins, saponins, and quinones.	TPC:34.67±2.52 GAE/g TFC:27.55±1.84 RE/g	62.86	Refilda et al. 2021 Farhana et al. 2023
AZL	Methanol	Alkaloids, flavonoids, saponins, terpenoids, glycosides, polyphenols, steroids, tannins, and coumarins.	TPC:5.53 GAE/g; TFC:11.15 RE/g TAC:10.67±0.46; TTPC:13.13±0.5%	70.29	Sutrisno et al. 2024
BCL	Methanol	Alkaloids, cardiac glycosides, tannins, flavonoids, and phenols	-	41.6	Devi and Nisha, 2022 Devi and Nisha, 2024
EHL	Methanol	Saponins, flavonoids, tannins, phenolics, glycosides, alkaloids, and steroids.	TPC: 8.59 mg/ml; TFC: 4.04%; TTC: 9.58%; TAC: 4.58%	72.96	Anarado et al. 2020 Basma et al. 2011
FBP	Methanol	Alkaloids, flavonoids, saponins, steroids, cardiac glycosides, tannins, phenols, and triterpenoids.	TPC:5.395 GAE/g TFC:55.326 RE/g	71.47	Mazumder et al. 2018 Etratkhah et al. 2019
PBL	Methanol	Alkaloids, phenols, flavonoids, tannins, saponins, glycosides, terpenoids, and steroids.	TPC:136.33±1.02 GAE/g; TFC:52.16±0.61 RE/g;	16.33	Syahidah et al. 2017
PGL	Methanol	Tannins, flavonoids, alkaloids, saponins, glycosides, and terpenoids.	TPC: 8.00±0.1 GAE/g; TFC:5.03±0.49 RE/g	78.83 42.33	Venkatachalam et al. 2012
PPL	Methanol	Flavonoids, carbohydrates, glycosides, saponins, tannins, and steroids	TPC:0.031 GAE/g	116.88	Arote et al. 2009
TPL	Methanol	Phenols, flavonoids, tannins, saponins, alkaloids, and steroids.	TFC:55.43±0.58 RE/g	96.28	Kushwaha et al. 2019 Pooja Singh et al. 2017
SVF	Ethanol	Alkaloids, flavonoids, terpenoids, steroids, phenols, tannins, glycosides, saponins, and carbohydrates.	TPC: 268.4±0.42 GAE/g; TFC:192.88±0.27 RE/g	92.52	Saraswathi et al. 2021

AAR-Achyranthes aspera Root, AIL-Acalypha indica Leaf, AZL-Azadirachta indica Leaf, APL-Abrus precatorius Leaf, BCL-Barleria cuspidat Leaf, EHL-Euphorbia hirta Leaf, FBP-Ficus benghalensis Prop root, PBL-Piper betle Leaf, PGL-Psidium guajava Leaf, PPL-Pongamia pinnata Leaf, TPL-Tridax procumbens Leaf, SVF-Solanum virginianum Fruit. TPC-Total Phenolic Content, TFC-Total Flavonoid Content, TAC-Total Alkaloid Content, TTC-Total Tannin Content, TTPC-Total Terpenoid Content.

Table 2.3 Review on the antimicrobial activity of selected plant extracts against cariogenic microbes.

Plants	Extract	Oral pathogens	Conc. (mg/ml)	IZD (mm)	MIC	MBC	Biofilm Inhibition (%)	Important bioactive Compounds	References
<b>AAR</b>	Aqueous	<i>S. mutans</i>	10%/250 µl	21	NA	NA	≤94%	Ecdysone, oleanolic acid, betaine, ecdysterone, proanthocyanidin, corrosolic acid, ursolic acid, stachysterone, 22-acenotide, 25s-ionokesterone, stigmaterol	Yadav et al. 2016; Jebashree et al. 2011
	Methanol	<i>S. mutans</i> <i>C. albicans</i>	2.5/5 mg/disc	10/ 10 13/12					
<b>AIL</b>	Methanol	<i>S. mutans</i>		NA	500	-	214.6	Tricosene, phytol, dihydroactinidiolide, loliolide, docosanol, 1-eicosanol, 1-triacontanol, 3,7,11,15 tetramethyl -2-hexadecen-1-ol, octacosanol.	Batubara et al. 2016; Jannat et al. 2019
	n-hexane Chloroform				500 -	500 -	196.9 149.8		
<b>APL</b>	Aqueous	<i>L. rhamnosus</i> <i>S. mutans</i> <i>S. epidermidis</i> <i>E. coli</i> <i>S. aureus</i> <i>B. subtilis</i>	200mg/ml	7 2.5 4 5 5 -	NA	NA	NA	Glycosides (abrusoside AD and abrusgenin), flavonoids and saponins (glycerin) abrine, trigonelline, abruslactone A, hemiphloin, abrusoside A, abrusoside B, abrusoside C, abrusoside D, choline, hypaphorine, precatorine, glycyrrhizin, montanyl alcohol, inositol, D monomethyl ether, pinitol.	Diwan, 2023 Andika et al. 2023
	EtAc	<i>A. viscosus</i> <i>S. mutans</i>	50% w/v	- 12.2	>12.5	25			
<b>AZL</b>	Methanol	<i>S. mutans</i> <i>E. faecalis</i> <i>S. aureus</i> <i>C. albicans</i>	3mg/ml	25 18 18 -	125µg 500µg 250µg -	250 1mg 500 -		Azadirachtin, nimbin, quercetin, nimbidin, sodium nimbinate, nimbolide, gedunin, salanin	Mistry et al. 2014
<b>BCL</b>	Methanol	<i>S. mutans</i>	50µg/ml	23	14.72	20	12	β-sitosterol-β-D-glucoside, luteolin-7-O-glucoside, Fumaric acid, hexadecyl 3-methylbut-3-enyl ester, Naphthalene, Pyrogallol, Vitamin E, beta-sitosterol, Cycloeucaenol, Palmitic acid, Linolenic acid, Phytol.	Devi and Nisha, 2024
		<i>S. aureus</i>	30µl	22	0.20	20	12		
		<i>K. pneumoniae</i>		22	2.6	20	12		
		<i>C. tructae</i>		30	21.8	20	12		
		<i>C. albicans</i>		10		-	-		
		<i>L. acidophilus</i>		-		-	-		
<i>S. cerevisiae</i>		-		-	-				

Plants	Extract	Oral pathogens	Conc. (mg/ml)	IZD (mm)	MIC	MBC	Biofilm Inhibition (%)	Important bioactive Compounds	References
<b>EHL</b>	Ethanol	<i>S. mutans</i> <i>L. acidophilus</i> <i>E. coli</i> , <i>C. albicans</i>	100µl	11 10 13 14	NA	NA	NA	Azulene, 2-propenoic acid,3-phenyl, phytol Bis(2-ethylhexyl)	Murugappan et al. 2022 Shanmugam et al. 2017
<b>FBP</b>	Aqueous	<i>S. mutans</i> <i>L. acidophilus</i>	250	6 8	NA	7% 10%	NA	Globulol, phytol, friedelanol, lanosterol, lupenyl acetate, lupeol, epifriedelanol, cyclolaudenol Myristic acid, palmitic acid, linoleic acid, phthalic acid, stearic acid, triacontanol, dioctyl ester, dihydrobrassicasterol, stigmasterol, sitosterol, ergosterol acetate, furostano, protodioscin, benganoic acid, and bengalensinone.	Manikandan et al. 2017 Verma et al. 2015
<b>PBL</b>	Ethanol Aqueous	<i>S. mutans</i>	1:30g/ml	20.6 18.3	5 10	NA	NA	Eugenol, 4 chromanol Phenol-2-methoxy4-(2-propenyl)-acetate 2,5-dimethoxy-4-ethylamphetamine Squalene, gamma-tocopherol	Deshpande & Kadam, 2013
<b>PGL</b>	Methanol	<i>S. mutans</i> <i>C. albicans</i>	5mg /disc	14 15	<0.076 0.625	NA	NA	Oleanolic acid, limonene, guavanoic acid, guava coumaric acid, jacoumaric acid, goreishic acid, Guavin (A, B, Cand D), strictinin, guajjaverin, ursolic acid, procyanidin, leucocyandin, stachyurin, and cyanidol.	Jebashree et al. 2011
<b>PPL</b>	Ethanol	<i>S. mutans</i>	12.5 mg/ml	NA	No activity	NA	NA	Ovalichromene B, isopongaflavone, pongaglabroni karanjin, pongapin, pinnatin, pongachromene, kanjoni glabrachromene I, isopongaglabol, pongaglabol, gamatin	Bodiba et al. 2018
<b>TPL</b>	Ethanol	<i>C. albicans</i> . <i>S. mutans</i> <i>E. feacalis</i>	60%	10 15 12	10 10	20 40	NA	Akuammidine, kaempferol, lutein, ferulic acid, galgravin, caffeic acid, tannic acid, lignans, epicatechin.	Arma et al. 2022 Ikewuchi et al. 2015
<b>SVF</b>	Ethanol	<i>S. mutans</i> <i>L. acidophilus</i> <i>P. aeruginosa</i> <i>S. oralis</i> <i>S. mitis</i>	10g/100ml	28 16 14 21 22	NA	NA	NA	Solasonine, solasodine, solamargine, byzantioside B, citroside A, matenoside A, lupeol, diosgenine, caffeic acid, esculentin, carpesterol, campesterol, daucosterol, stigmasterol, cyclocatanol, sitosterol, B-solamargine, linolenic acid, oleic acid, stearic acid, palimitic acid.	Perumal, 2023

## **2.2 *In silico* study to Identify Potential Inhibitors of *Streptococcus mutans* Glucosyltransferases**

### **2.2.1 Molecular Docking Studies**

Molecular docking, a computational technique predicting the binding affinity of ligands to receptor proteins, has emerged as a powerful tool in drug development. Nutraceuticals, bioactive substances found in food sources, can be utilized for disease management by targeting specific molecular pathways (Agu et al. 2023). When a plant's crude extract exhibits significant biological activity, isolating and characterizing its active compounds enable researchers to use *in-silico* studies, such as molecular docking, to investigate potential drug targets. This approach involves leveraging known structures from databases to identify new targets, including docking phytochemicals from a specific plant onto drug targets for emerging diseases. Correlating the biological activity of medicinal plants with their molecular targets enables researchers to discover novel, disease-specific therapies, revealing the potential of medicinal plants to combat a range of diseases (Asiamah et al. 2023).

### **2.2.2 Molecular Dynamic Simulations**

Molecular docking can be combined with molecular dynamic simulations to study the dynamic behaviour of protein-ligand complexes. These simulations can help in understanding the conformational changes that occur upon ligand binding and the stability of the complex (Dhanik et al. 2013). The main advantage of this approach is its ability to explicitly account for structural flexibility and entropic effects, enabling a more accurate estimate of the thermodynamics and kinetics associated with drug–target recognition and binding (De Vivo et al. 2016).

### **2.2.3 Glucosyltransferases of *S. mutans***

*Streptococcus* species include many gram-positive bacteria found in the oral cavity or alimentary tract, some of which are pathogenic and can significantly impact human health (Coykendall, 1989; Kohler, 2007). Phylogenetic analyses divide the genus *Streptococcus* into eight monophyletic groups: Mitis, Sanguinis, Anginosus, Pyogenic, Bovis, Mutans, Downei, and Salivarius (Shao et al. 2013; Richards et al. 2014). Among these groups, certain streptococcal species produce glucosyltransferases (Gtfs), enzymes that catalyze the synthesis of

glucans (Polysaccharides) from sucrose. Glucans play an important role in the development of oral biofilm. In particular, the glucan biofilms produced by the Gtfs from *S. mutans* are essential for the formation of dental plaque on the tooth surface (Hoshino & Fujiwara, 2022). As a result, many researchers in the field of dentistry have focused on studying streptococcal Gtfs to identify potential inhibitors for preventing dental caries.

Based on the thorough virtual screening of 100 quinic acid derivatives, five compounds demonstrated favorable drug-relevant properties and a good fit with low binding energy. These compounds were 1,3,4,5-tetra hydroxy cyclohexane-2,4-diene-1-carboxylic acid, (1R)-1,3,4-trihydroxy-5-methylcyclo-hexane carboxylic acid, (1S,3R)-1,3,5-trihydroxy cyclohexane carboxylic acid, 1R,3S,4R,5S)-1,3,4-trihydroxy-5- methylcyclohexane carboxylic acid, and 1-hydroxy- 3-methyl cyclohexane-1-carboxylic acid. Among the identified compounds, (1R)-1,3,4-trihydroxy-5-methylcyclohexane carboxylic acid, a novel quinic acid derivative, exhibited high binding energy as a potential inhibitor for GTF-SI (Kannan et al. 2023). Propolis has emerged as a promising natural source for inhibiting *S. mutans* activity. Among the 93 compounds identified in propolis through LC/MS-MS, diosmetin, cosmosiin, genistin, and 3'-methoxy-5'-hydroxy soflavone-7-O- $\beta$ -D-glucoside showed high inhibiting activity against glucosyltransferase-C and antigen I/II, and exhibited high docking stability with conserved amino acid residues and low dynamics fluctuation (Jauhar et al. 2023).

A screening process identified antioxidant and anti-inflammatory molecules that inhibit the crystal structure of Gtf-C. Notably,  $\beta$ -Eudesmol,  $\beta$ -Humelene, and  $\beta$ -Caryophyllene exhibited a greater binding affinity of -9.9, -9.4 and -9.0Kcal/mol, respectively. The top-hit  $\beta$ -Eudesmol complex demonstrated superior stability (-1500KJ/mol), compared to the reference complex (G43, -1100KJ/mol), suggesting that B-Eudesmol inactivation of GtfC can prevent dental caries (N et al. 2021). Similarly, the triterpenoid compound, ursolic acid showed strong hydrogen (Tyr430, and Asp909) and hydrophobic interactions with the novel active sites of Gtf-SI (Trp517, Leu433, Leu434, and Phe907), which play a key role in the formation of biofilm. Ursolic acid effectively disrupted the adhesion and aggregation of mature biofilms formed by *S. mutans*,

demonstrating its potential to prevent biofilm-related oral diseases (Liu et al. 2021).

GC-MS analysis of *P. guajava* revealed seven compounds, including corynan-17-ol, 18,19-didehydro-10-methoxy-acetate, copaene, 3-bicyclo (5.2.0) nonane, 2-methylene-4,8,8-trimethyl-4-vinyl, azulene,  $\alpha$ -caryophyllene, alloaromadendrene oxide-(1), and androstane-17-one, 3-ethyl-3-hydroxy-(5a) which exhibited strong interactions with glucosyltransferase (GTF), indicating significant anti-cariogenic potential (Bhagavathy et al. 2019). A virtual screening of 1,50,000 commercially available compounds, identified a promising quinoxaline derivative, specifically 2-(4-methoxyphenyl) -N- (3- [[2- (4-methoxyphenyl ethyl] imino}-1,4 - dihydro -2- quinoxalinylidene) ethanamine, as a potential Gtf inhibitor. This discovery paves the way for new strategies to control biofilm-based dental caries (Ren et al. 2016). Molecular docking techniques enable the screening of potential compounds from numerous plants for their anti-cariogenic effects, specifically targeting glucosyltransferases. This approach holds promise for the development of novel drugs aimed at oral infections, with the Gtfs site serving as a key drug target. Consequently, inhibiting GTF activity has emerged as an important mechanism in the pursuit of new therapeutic agents.

### **2.3 Optimization of Polyherbal Formulation**

Traditional medicinal plants have been widely incorporated into oral care products due to their natural therapeutic properties. These plants offer numerous benefits, including anti-inflammatory, antibacterial, and anti-plaque effects, making them valuable additions to toothpaste, mouthwashes, chewing gums, and other oral hygiene products. This helps prevent and treat oral diseases such as dental caries, gingivitis, and periodontitis (Tiwari et al. 2024). To develop effective oral care products, optimizing extract concentration is important. Response Surface Methodology (RSM) is a statistical and mathematical technique that helps in optimizing processes by evaluating the effects of multiple variables and their interactions.

According to (Said and Amin, 2016), RSM is particularly ideal for optimizing extraction processes in food and herbal plants, as it can identify

interrelationships between variables. This approach ensures the optimal presence of active ingredients, thereby enhancing therapeutic properties while maintaining cost-effectiveness and batch-to-batch consistency. Additionally, RSM fosters innovation by exploring new combinations and formulations, ultimately leading to safer and more efficient oral care solutions. The integration of traditional knowledge with modern techniques and dental care practices can lead to sustainable and effective solutions, benefiting both individual health and the environment (Kumar, 2014) This systematic approach enabled us to evaluate interactions between different herbal extracts, accelerating the development of novel antimicrobial agents with improved efficacy, reduced toxicity, and cost-effectiveness. Similarly, (Mapeka et al. 2024), screened individual extracts and essential oils of 17 culinary herbs and spices in a 1:1 ratio using the design of experiments (DOE) approach in MODDE 9.1 software to identify the best combination for antimicrobial effect using micro broth dilution assay against six common foodborne pathogens. The combination of *Rosmarinus officinalis* with either *Syzygium aromaticum* or *Salvia officinalis* methanol extracts showed synergistic antimicrobial effects against *B. cereus* with  $\Sigma$ FIC values of 0.25mg/ml and 0.31mg/ml, respectively. The DOE predicted an optimal combination of 59.5% *R. officinalis*, 40% *S. officinalis*, and 0.5% *S. aromaticum*, producing a MIC of 0.17mg/ml, which was experimentally confirmed.

This combination showed a strong correlation between predicted and experimental MIC values ( $r = 0.73$ ). An effective antimicrobial combination was identified as 56% *R. officinalis* and 44% *S. officinalis* (MIC = 0.19mg/ml), recommended for future studies. This study highlights the potential of combining culinary herbs and spices for enhanced antimicrobial effects, which could be valuable for both food safety and medicinal applications. It also underscores the importance of further research to explore the interactive effects of herbs and spices in antimicrobial treatments.

Tanisha et al (2024) demonstrated the potent synergistic effect of combining seed-aqueous extracts of *Nelumbo nucifera*, *Chenopodium quinoa*, and *Salvia hispanica* in an equivalent ratio (1.5mg/ml). The data collected for the antimicrobial activity of the extract's combinations were interpreted to be one of the following categories: synergy; indifferent; additive; or antagonistic. The

interpretation was made based on the fractional inhibitory concentration index (FICI) results. FICI of  $\leq 0.5$  indicates synergism,  $>0.5-1$  indicates additive effects,  $>1$  to  $\leq 4$  indifference, and  $>4$  is considered to be antagonism. Compared with the data of the individual extracts, the MIC values of the extract-extract combinations against all strains of the tested microorganisms were significantly lower, ranging from 0.97 to 1.17, 0.97 to 4.69, 0.50 to 1.17, 1.17 to 3.12 and 2.34 to 4.69 mg/mL for *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Candida albicans* respectively. *L. bateri* aqueous - *S. alata* ethanol extracts and *S. alata* aqueous *R. cummunis* ethanol extract combinations showed a synergy effect against all the test microorganisms. The combination exhibited significantly the highest activity in reducing obesity and diabetes in diet-induced obese diabetic mice with notably lower toxicity.

Numerous plants are highly effective in the treatment of dental caries and oral hygiene. However, the combination of specific plants could increase the antibacterial activity due to synergistic interactions. Instead of combining extracts randomly, the DOE study provides an interesting and promising cost-effective approach whereby varied herb extracts can be combined at optimum proportions for the best biological effect. The DOE therefore created an innovative opportunity for the future development of the most effective antimicrobial agents for the pharmaceutical and food industries.

## **2.4 Composition of Ingredients Used in the Dentifrice Formulation**

Dentifrices, such as toothpaste and toothpowder, are essential for maintaining oral hygiene by removing food debris, plaque, and stains, thus preventing cavities and promoting overall dental health. Herbal tooth powders, used for centuries, are considered essential for dental hygiene. Recently, natural products have been investigated for their potential to prevent plaque-related diseases like dental caries, due to the adverse effects of chemicals in many marketed products (Saliasi et al. 2018). Conversely, synthetic dentifrice products often contain triclosan and chlorhexidine, which can cause gum irritation, canker sores, burning, tooth staining, altered taste, and inflammation. Poor oral hygiene practices can lead to periodontal disorders,

plaque accumulation, and gingivitis, ultimately resulting in tooth loss (Lertpimonchai et al. 2017; George et al. 2009).

In the context of the growing consumer demand for natural and safe oral care products, many researchers are developing herbal-based tooth powders that offer enhanced antimicrobial properties. These formulations utilize the synergistic effects of multiple herbs, preventing antimicrobial resistance, and making them a more beneficial choice for oral hygiene (Chaiwaree et al. 2023). Gaykar and Tambe, (2023) identified a unique formulation with the blending of herbal ingredients like tulsi, neem, clove, cinnamon, ginger, amla, triphala, mint, black salt, sodium lauryl sulfate, and calcium carbonate. This blend possesses antibacterial, antiseptic, and cooling properties, which create an effective tooth powder that keeps the mouth fresh and helps prevent tooth decay.

Bharathi et al (2020) formulated and evaluated a tooth powder made of traditional ingredients like neem stem and bark, acacia stem, mentha leaves, and ginger was found to be effective against *E. coli* and *S. aureus* suggesting a viable option for dental hygiene. The combined powder of clove, cinnamon, pepper, neem, kala namak, babul sal, arjuna, madhunashini, licorice, ritha, triphala (amla, baheda, hirda), lodhara, vavding, pudina, and tulsi met all the required standards of organoleptic and physical characteristics like color, odor, taste, stability, foamability, and abrasiveness of the toothpowder evaluated by (Dakhurkar et al. 2019). Prince & Gopinath (2022) designed, formulated, and evaluated 200 mg effervescent toothpaste tablets using a direct compression method. The F5 formulation is small, chewable, and transformed into a paste when chewed and meets all the organoleptic and physicochemical properties, which offers a promising alternative to traditional toothpaste for improving oral hygiene.

Thus, the usage of herbal ingredients in polyherbal toothpowder offers a natural and effective alternative to conventional toothpaste. These ingredients, such as neem, tulsi, and clove, possess antibacterial, anti-inflammatory, and refreshing properties that promote oral hygiene and prevent dental issues like tooth decay and gum disease. By leveraging the therapeutic benefits of these natural compounds, polyherbal toothpowder not only enhances dental health but

also minimizes the exposure to synthetic chemicals found in commercial dental products. This makes it a safer and more sustainable option for maintaining oral health.

## 2.5 Virulence Factors of *Streptococcus mutans*

Dental caries closely linked to *S. mutans* bacteria, is one of the most common health problems (Patel, 2020; Krzyściak et al. 2014). *S. mutans* is a gram-positive facultative anaerobic bacterium that forms short chains (Lemos et al. 2019). These strains are found in higher quantities in carious lesions compared to caries-free teeth. Normally, *S. mutans* is naturally present in the mouth, but high sugar intake and poor oral hygiene can lead to caries. The bacterium metabolizes sucrose, producing dextran, a sticky polysaccharide that forms plaque on the gums (Kasvala & Monpara, 2020; Jakubovics et al. 2021). To cause disease, *S. mutans* must adhere to the tooth surface and form a biofilm. Once the biofilm is established, dietary carbohydrates produce acid, initiating caries progression. The main virulence factors of *S. mutans* include its ability to adhere to the tooth surface, form biofilms, and acidogenic, and aciduric microbes (Ghilan et al. 2023). Several natural herbs used to combat virulence factors of *S. mutans* have been scientifically proven to be non-toxic. The herbs administered in standardized doses effectively treat periodontitis, halitosis, mouth ulcers, and cancers.

### 2.5.1 Inhibition of Acid Production of *S. mutans*

*Origanum vulgare* L. (OEO1) and *Origanum heracleoticum* L. (OEO2) at MIC concentrations effectively inhibited organic acid production by *S. mutans* and maintained the initial pH when compared to the positive control (Atazhanova et al. 2024). Sanpinit et al (2022) in their study reported that the anti-acidogenic effect of 35 Thai herbal formulations (THF) against *S. mutans*, demonstrated promising anti-quorum sensing activity by significantly inhibiting the acidogenesis of *S. mutans*. Among them, THF-DC 02 (*Acmella oleraceae*<sup>Wp</sup>, *Avicennia marina*<sup>L</sup>), THF-DC 04 (*Albizia myriophylla*<sup>Sb</sup>, *Alpinia galanga*<sup>Rh</sup>, *Avicennia marina*<sup>L</sup>, *Ocimum tenuiflorum*<sup>L</sup>), THF-DC 20 (*Euphorbia neriifolia*<sup>L</sup>, *Terminalia bellirica*<sup>F</sup>, *Terminalia chebula*<sup>F</sup>), and THF-DC 29 (*Acmella oleraceae*<sup>Wp</sup>, *Avicennia marina*<sup>L</sup>, *Piper betle*<sup>L</sup>, *Streblus asper*<sup>Sb</sup>, *Syzygium aromaticum*<sup>Fl</sup>) produce promising anti-acidogenic or anti-QS activities. According to Rudin et al (2023)

the natural flavonoid, phloretin(400 µg/ml) effectively inhibited the lactic acid production of *S. mutans* preventing early and late biofilm formation, and reducing up to 80% of lactic acid production.

### 2.5.2 Inhibition of Hydrophobic Nature of *S. mutans*

Karnjana et al (2023) during their investigation observed that the ethanolic extracts of *Streblus asper*, *Cymbopogon citratus*, and *Syzygium aromaticum* modified the hydrophobic adhesion of bacterial cell *S. mutans* with a hydrophobicity index greater than 60%, exhibited a lower level of hydrophobicity than the untreated cells. It acted in a concentration-dependent manner from 1/4 to 2MIC against *S. mutans* ATCC 25,175 and both CSM06 and CSM25 isolates. According to Yuan et al (2023) *Origanum vulgare* L. (OEO1) and *Origanum heracleoticum* L. (OEO2) at MIC concentrations effectively inhibited hydrophobic nature of *S. mutans* when compared to the positive control. The bacterial adhesion gets weaker with the lowest hydrophobicity index exhibited by the OEOs which breaks the hydrophobic adhesion of *S. mutans* to n-hexadecane hydrocarbons. The presence of carvacrol,  $\gamma$ -terpinene, and *p*-cymene in OEO massy help regulate the virulence properties of *S. mutans*. *Thymus vulgaris* demonstrated the greatest inhibition (21.66%) on the hydrophobicity of *S. mutans*, followed by *Thymus zygis* (42.21%), and *Thymus vulgaris* (Carvacrol) (56.72%) at 1/2 MIC level (Park et al. 2023).

### 2.5.3 Inhibition of Preformed Biofilm of *S. mutans*

A biofilm is a structured community of sessile cells attached to the surface. The virulence of cariogenic biofilms is associated with the spatial organization of *S. mutans* and exopolysaccharides on teeth. In the oral cavity, *S. mutans* interact with other microbes to form complex cariogenic biofilms, comprising various microbes and extracellular matrices (Cai & Kim, 2023). Natural products, resulting from secondary metabolism, yield chemicals with significant pharmaceutical effects on various microorganisms. As a result, numerous natural products and their main compounds have been screened all over the world, indicating the herb's potential to improve oral health.

Plant extracts or phytochemicals are reported to strongly inhibit the growth of oral pathogens, reduce the development of biofilms and dental plaque.

Molecular targeting of *S. mutans* gtf enzyme can help combat virulent biofilm formation and overcome caries issues. Rudin et al (2023) analysed the effect of Phloretin on *S. mutans* viability and biofilm formation and reported that the MIC and MBC tested on planktonic *S. mutans* culture was 400µg/ml. At sub-inhibitory concentrations, phloretin demonstrated a dose-dependent inhibitory effect, gradually reducing cell viability and growth. This effect was also observed in biofilm formation, with significant inhibition at 200µg/ml and 400µg/ml concentrations in 4h and 24h biofilm assays, respectively.

The study of Mehdipour et al (2022) demonstrated that the herbal extract of *Carum copticum* exhibited better antibiofilm and antimicrobial potential of various herbal extracts against *S. mutans* compared to *Phlomis bruguieri* and *Marrubium parviflorum*. Zayed et al (2021) was observed strong antibiofilm activity of alcoholic green tea extracts (3.12mg/ml) against *S. mutans* isolates. Hickl et al (2018) stated that the potent effect of Mediterranean methanol extracts of *Rosmarinus officinalis* and *Salvia sclarea* had the most significant antimicrobial effects against all tested oral pathogens, while *Cistus spp.* extracts exhibited the highest antibiofilm activity against *S. mutans*. Furthermore, combinations of these plant extracts could serve as main antimicrobial components in alternative antibacterial formulations facilitating the prevention of biofilm-related oral diseases such as caries or periodontitis. A licorice which is an important traditional Chinese herbal medicine, was observed to have antibacterial properties at a concentration of 25µg/ml against *S. mutans* (Chen et al. 2019).

According to Ham and Kim (2018) the methanol extracts of nine plants, *Melonis Pedicellus*, *Agastachis Herba*, *Mori Cortex Radicis*, *Diospyros kaki* leaves, *Agrimoniae Herba*, *Polygoni Multiflori Radix*, *Lycopi Herba*, *Elsholtziae Herba*, and *Schizonepetae Spica*, inhibited biofilm formation by *S. mutans*. Yadav et al (2016) reported the biofilm inhibitory rate of *Achyranthes aspera* root aqueous extract to be ≤94% against *S. mutans*. A study by Gomashe et al (2014) reported that the methanol extracts of *P. guajava* were found to significantly inhibit the biofilm formation by *S. mutans*.

Several plants exhibit significant potential in combating the virulence factors of *Streptococcus mutans*, a primary contributor to dental caries. Natural

compounds found in plants have shown promising antimicrobial, antibiofilm, and enzyme-inhibitory properties. These plant extracts offer a natural and effective approach to preventing and treating dental caries by targeting the key mechanisms of *S. mutans* virulence. Integrating these botanical solutions into oral care products provides a safer, more sustainable alternative to synthetic chemicals, promoting overall dental health and reducing the risk of tooth decay.

#### **2.5.4 SEM analysis of *Streptococcus mutans* Biofilm Eradication**

For ultrastructural characterization of biofilm matrix and embedded bacterial cells, customized scanning electron microscopy protocols (e.g., osmium tetroxide (OsO<sub>4</sub>), ruthenium red (RR), tannic acid (TA), and ionic liquid (IL)) are preferred due to their superior image quality, magnification, resolution, minimal sample loss, and preservation of sample structure. Producing images that accurately reflect sample structure is the first step in assessing the effects of pharmacological treatments on clinical biofilms. Extracting quantitative parameters from these images, possibly using specific software, can elevate scanning electron microscopy from an accessory technique to a comprehensive quantitative method.

*Streptococcus mutans* is an important oral pathogen for dental caries, a widespread oral infectious disease. *S. mutans* synthesize exopolysaccharide (EPS) using glycosyltransferases (Gtfs), resulting in biofilm formation on the tooth surface. The bacterial cells within these biofilms develop strong resistance to harsh environments, such as antibiotics and host defense mechanisms, making biofilm-based infections challenging to eradicate. Discovering novel antibiofilm agents, especially from natural products, helps to develop effective strategies against this kind of disease (Zhang et al. 2021).

The SEM images of the biofilm treated with Epigallocatechin gallate depicted multilayers of cells arranged in short chains of microorganisms adhered to an unstructured matrix, which was not continuous and did not enmesh or protect the microorganisms entirely. Importantly, confocal images demonstrated that treatment with EGCG affected the 3D structure and organization of *S. mutans* biofilm, which presented a biofilm matrix more confined to the location of the microcolonies (Aragão et al. 2024).

Rudin et al (2023) investigated the effect of Phloretin (200µg/ml) on biofilm formation on saliva-coated hydroxyapatite (sHA) surfaces, simulating the human oral environment. After 24 h, SEM analysis revealed a drastic reduction in biofilm formation, with only a scarce number of surface-associated cells detected. Furthermore, these cells underwent significant morphological changes, transitioning from a symmetric ellipsoidal shape to elongated forms with multiple dysmorphic or swollen cells and asymmetric division signs. According to Ham et al (2022) SEM analysis revealed that raffinose can effectively control *S. mutans* biofilm formation on hydroxyapatite discs under conditions similar to those of the human oral environment. Biofilm formation after 1,000 µM raffinose treatment was reduced significantly, compared to the control (no raffinose treatment), which ultimately targeted the glucosyl transferase enzyme).

Zhang et al (2021) their study reported that shikimic acid (SA) compound derived from *Illicium verum* extract was found to reduce the EPS synthesis by *S. mutans* and modulate the transcription of biofilm formation-related genes, leading to fewer bacterial cells in its biofilm. Additionally, Shikimic acid interacted with cell membranes and membrane proteins, causing damage to bacterial cells. *Ex vivo* testing on bovine teeth demonstrated that significantly SA decreased the number of *S. mutans* cells and EPS accumulation on dental enamel surfaces. Moreover, SA exhibited negligible toxicity to human oral cells, as evaluated by an *in vitro* biocompatibility assay. In conclusion, shikimic acid displayed remarkable antibiofilm activity against *S. mutans* showcasing as a novel anticaries agent. Relucenti et al (2021) during their investigation reported that bacterial biofilms are a significant concern, as they can lead to infections resistant to antibiotics.

According Li et al (2018) SEM images at 2000x, 5000x, and 10,000x magnification confirmed alterations in the exopolysaccharide of *S. mutans*. Treatment with curcumin or chlorhexidine significantly reduced the overall quantity of *S. mutans* EPS compared to the untreated control. Furthermore, long-term treatment (24h) resulted in a more obvious reduction than short-term treatment.

Scanning Electron Microscopy (SEM) analysis is a powerful tool used to study the eradication of *Streptococcus mutans* biofilms by plant extracts. SEM provides high-resolution images that reveal the detailed surface morphology

of biofilms, allowing researchers to observe the structural changes and effectiveness of plant extracts in disrupting and removing biofilms. By comparing SEM images before and after treatment with plant extracts, scientists can visually confirm the reduction or eradication of biofilms, providing valuable insights into the antibiofilm properties of these natural compounds.

## **2.6 Network Pharmacology**

Network pharmacology is an emerging pioneering approach in the drug delivery process through system biology and network analysis using various bioinformatic tools and databases (Saima et al. 2024). Network pharmacology has been a promising method for advancing drug discovery and illuminating the underlying mechanism of multi-targeted compounds (Wang et al. 2022; Li et al. 2021). Network pharmacology developed by Hopkins enhances a high rate of clinical success with fewer side effects, and around 40% of the current drug discoveries were contributed by this approach (Chen et al. 2022). Emerging studies have highlighted the application of network pharmacology in various fields, particularly in the identifying mechanisms of herbal compounds and ayurvedic formulations in the management of various diseases (Saima et al. 2024).

Caries dental is a complex disease; most drugs are highly ineffective, and the success rate of drug discovery is in constant decline. Systems, network medicine, and network pharmacology are revolutionizing disease definition, diagnosis, treatment, and cure (Nogales et al. 2022). By applying network pharmacology to dental caries research, scientists can identify key targets and pathways involved in the disease. This method helps in understanding how various bioactive compounds interact within the biological networks of dental caries. For instance, natural compounds from herbs may inhibit bacterial adhesion, reduce acid production, and enhance remineralization. Network pharmacology can optimize these effects, developing holistic and effective treatments. Together, dental caries research and network pharmacology offer a comprehensive understanding of the disease, aiming to develop innovative strategies to address its multifaceted nature.

The results obtained from network pharmacology are validated by *in vivo*, *in vitro*, or even *in silico* experiments. One of the most commonly used *in*

*silico* techniques is molecular docking. Molecular docking is a technique capable of predicting the interactions between a ligand and a target at a molecular level; a degree of affinity is obtained according to the binding energy expressed in  $\Delta G$  (Pinzi & Rastelli, 2019). With a pharmacological network strategy, the molecular mechanism by which compounds affect caries and *Streptococcus mutans* can be identified.

### **2.6.1 Protein-Protein Interaction Network**

Protein-protein interaction (PPI) network analysis is a crucial aspect of understanding cellular processes and disease mechanisms underlying dental caries. By mapping the protein interactions, researchers can identify key pathways and potential therapeutic targets and develop more effective treatments. PPI networks provide a comprehensive view of biological systems, facilitating a holistic understanding of cellular mechanisms and driving advances in biomedical research and novel therapeutic strategies (Vidal et al. 2011; Keskin et al. 2016).

Wang et al (2013) in their study utilized PPI network analysis to identify candidate genes for dental caries, revealing three key clusters: cytokine network-related genes, matrix metalloproteinases (MMPs) family, and transforming growth factor-beta (TGF- $\beta$ ) family. These clusters play important roles in tooth development and carious lesions, providing new insights into dental caries molecular mechanisms.

### **2.8.2 Gene Ontology Enrichment Analysis**

Gene Ontology (GO) enrichment analysis is a powerful tool used to identify the biological functions, cellular components, and processes associated with dental caries genes. A study by Wang et al (2013) applied GO enrichment analysis to a genome-wide association study (GWAS) dataset, revealing key GO terms related to sphingoid metabolism, ubiquitous protein ligase activity, cytokine secretion regulation, and ceramide metabolism. These findings provide valuable information into the molecular mechanisms underlying dental caries and highlight the importance of polygenic interactions in the disease.

Guzmán-Flores et al (2024) reported the molecular mechanism of curcumin in preventing dental caries and inhibiting *Streptococcus mutans*. They

found that curcumin interacts with 134 therapeutic targets mainly involved in apoptosis and inflammation. Seven key proteins (MAPK1, BCL2, KRAS, CXCL8, TGFB1, MMP9, and IL1B) bind curcumin and it affects metabolic pathways related to lipid, purine, and pyrimidine metabolism in *Streptococcus mutans*. Zhao et al (2024) investigated the potential mechanisms of shikonin in treating periodontitis. They found that shikonin interacts with 22 targets, involved in cellular response to hypoxia, xenobiotic stimulus and apoptotic process regulation. Key pathways included human cytomegalovirus infection and PI3K-Akt signaling. Key genes identified were AKT1, CCL5, CXCR4, PPARG, PTEN, PTGS2, and TP53. Molecular docking and MD results confirmed shikonin stable binding to these targets, providing potential therapeutic targets for periodontitis.

Network Pharmacological analysis provides In-depth knowledge of the pharmacological mechanisms and interactions enabling the therapeutic use of complex plant extracts. Traditional systems like Ayurveda and Traditional Chinese medicines benefit from the potential synergistic effects of multi-component, multi-channel, and multi-targets approaches which seem to be a better proposition for the treatment of chronic, complex, multifactorial diseases (Bhatia et al. 2022). Network pharmacology aims to enhance efficacy and safety of existing drugs and explore new therapeutic possibilities, rationalizing the use of complex formulations in traditional medicine.