Hindawi Publishing Corporation
Journal of Chemistry
Volume 2013, Article ID 345059, 6 pages
http://dx.doi.org/10.1155/2013/345059

Research Article

Effect of *Achyranthes aspera* Root and *Terminalia arjuna* Bark on Aerotolerant Responses in *Streptococcus mutans*

C. Mahima and V. Hazeena Begum

Department of Siddha Medicine, Faculty of Sciences, Tamil University, Thanjavur 613007, India

Correspondence should be addressed to V. Hazeena Begum; drvhazeena@gmail.com

Received 29 November 2011; Revised 7 July 2012; Accepted 8 July 2012

Academic Editor: Lorenzo Cerretani

In the present study, the effect of hydroalcoholic extract of *Achyranthes aspera* root (AAR) and *Terminalia arjuna* bark (TAB) on the aerotolerant capacities of *Streptococcus mutans* was examined. AAR and TAB extracts were found to inhibit the prime antioxidant enzymes like manganese superoxide dismutase (MnSOD), NADH oxidases, and glutathione peroxidase (GPx), altering the aerotolerant responses in *S. mutans*. Consequently, *S. mutans* are unable to withstand the oxidative stress and thus are made more susceptible to the antimicrobial activity.

1. Introduction

Streptococcus mutans, the main pathogen involved in the development of dental caries, is an aerotolerant microorganism. *S. mutans* cannot synthesize heme and therefore lacks catalase and cytochrome oxidases required for energy-linked oxygen metabolism. However, *S. mutans* can grow in the presence of oxygen and even consume molecular oxygen through the action of nicotinamide adenine dinucleotide (NADH) oxidase and manganese superoxide dismutase (MnSOD) [1, 2]. These oxidative enzymes prevent the bacterial cell from oxidative stress, thereby preventing the generation of highly toxic radicals and conferring oxygen tolerance [3, 4]. In view of the defense against oxygen toxicity, the lack of catalase in lactic acid bacteria is inconsistent with their aerotolerance [5]. Thus, aerotolerance provides a mechanism for *S. mutans* to protect them from oxidative damage [6].

Superoxide dismutase is the primary line of defense against the toxicity of oxygen radicals to oxygen and hydroperoxide, through the involvement of metal prosthetic group. MnSOD is found in protecting *S. mutans* against exogenous superoxide [7]. Therefore, modulation of superoxide anion generation is an early response as part of the defensive process against pathogen invasion. Superoxide promotes hydroxyl-radical formation and consequent DNA damage in cells of all types [8]. NADH oxidases have been produced as a response of *S. mutans* to oxidative stress. Therefore, reductions of NADH oxidases directly sensitize the *S. mutans* to oxidative stress and no longer give protection. Glutathione peroxidase (GPx) catalyzes the reduction of hydroperoxides at the expense of GSH. It catalyses the reduction of H_2O_2 and organic hydroperoxides including those derived from unsaturated lipids to alcohol. It also protects the biomembrane from oxidative attack. Induction of protective systems after exposure to free radicals or ROS may be an important adaptive response to nonlethal insults by these reactive species. The main objective of our study was to examine the effect of *Achyranthes aspera* root (AAR) and *Terminalia arjuna bark* (TAB) on the aerotolerant properties in *S. mutans*. The two plant materials were mentioned in siddha literature to give relief from tooth ache. Based on Siddhar's claims, anticariogenic activity of AAR and TAB extracts could be well pronounced by interfering with the defense system of *S. mutans* and making them more susceptible for the drug action. A preliminary phytochemical evaluation of the AAR and TAB extracts was performed.

2. Materials and Methods

2.1. Bacterial Strain and Growth Conditions. *Streptococcus mutans* A 32-2 strain (serotype c) is used in our study. *S.*

mutans A 32-2 strain is obtained as gift from Dr. Richard L.Gregory, Ph.D., Professor of Oral biology and Pathology, School of Dentistry and medicine, Indiana University, USA.

S. mutans was maintained in our laboratory with weekly subculture on Todd Hewitt agar or Brain heart infusion agar or Trypticase soy agar plates, all supplemented with 1% glucose and long-term storage in the specified medium at −70°C in 50% glycerol solution. *S. mutans* A 32-2 was incubated at 37°C at 10% CO_2 in Candle jar.

2.2. Collection of Plant Materials & Extract Preparation. The roots of *Achyranthes aspera* and *Terminalia arjuna* bark were collected from the Tamil University campus, Thanjavur, India. The collected roots and barks were shade dried and powdered. The dried powders of AAR and TAB were separately extracted with 50% ethanol at 60°C by continuous hot percolation using Soxhlet apparatus and used for the study.

2.3. Preliminary Phytochemical Testing. 500 mg of the dried ethanolic extract was reconstituted in 10 mL of ethanol and it was subjected to preliminary phytochemical testing for the presence of alkaloids, flavonoids, tannins, saponins, terpenoids and other different chemicals groups of compounds by standard methods [9, 10]. The color intensity or the precipitate formation was used as analytical responses to the tests.

2.4. Preparation of Bacterial Cell Lysate. Overnight culture of *S. mutans* A 32-2 grown in Todd-Hewitt broth was harvested and incubated with 100 mg/L and 500 mg/L of AAR and TAB extracts, respectively. Cells devoid of extracts served as control. The samples were incubated at 37°C for 3 hours. After incubation, the cells were removed, homogenized, and centrifuged to remove the cell debris. The cell lysate was used as the enzyme source.

2.4.1. Role of AAR and TAB on Superoxide Dismutase in S. mutans Assay of Superoxide Dismutase (SOD). Superoxide dismutase activity was determined by the procedure of Kakkar et al. [11] with slight modifications.

Bacterial cell lysate was used as the enzyme source. Cell lysate included both the treated (AAR/TAB) and untreated samples.

0.5 mL of sample was diluted to 1 mL with water. Then 2.5 mL of ice-cooled ethanol and 1.5 mL ice-cooled chloroform were added. This mixture was shaken for 1 minute at 4°C and then centrifuged. The enzyme activity in the supernatant was determined. The assay mixture contained 1.2 mL of sodium pyrophosphate buffer (0.025 M, pH 8.3); 0.01 mL of phenazine methosulphate, 0.3 mL of nitro blue tetrazolium (NBT); 0.2 mL of NADH (780 M), appropriately diluted enzyme preparation and water in a total volume of 3 mL. Reaction was initiated by the addition of NADH. After incubation at 30°C for 90 seconds, the reaction was stopped by the addition of 1 mL glacial acetic acid. The reaction mixture was stirred vigorously and shaken with 4 mL of n-butanol. The intensity of the chromogen in the n-butanol layer was measured at 560 nm against butanol blank. A system devoid of enzyme served as control. Enzyme activity was expressed as 50% of NBT reduction/min/mg protein.

2.4.2. Role of AAR and TAB on NADH Oxidase in S. mutans NADH Oxidase Assay. NADH oxidase was assayed by the method of Steyn-Parve and Benert [12] with slight modification.

NADH oxidase activity was assayed at 30°C by monitoring the oxidation of NADH in the reaction mixture (1 mL) at absorbance, 340 nm. The reaction mixture contained 40 mM potassium phosphate buffer (PPB), 0.2 mM EDTA, 0.17 mM-NADH, and cell lysate containing enzyme. One unit of NADH oxidase was defined as the amount of enzyme (mg protein) that catalyzed the oxidation of 1.0 μmol NADH min^{-1}.

2.4.3. Role of AAR and TAB on GPX Assay of Glutathione Peroxidase (GPX) (EC.1.11.1.9). The activity of glutathione peroxidase was assayed by the method of Rotruck et al. [13].

The reaction mixture consisted of 0.2 mL each of 0.8 mM EDTA, 10 mM sodium azide, 2.5 mM H_2O_2, 0.4 mL of phosphate buffer (0.32 M; pH 7.0), and 0.1 mL of sample was incubated at 37°C at different time intervals. The reaction was arrested by the addition of 0.5 mL of 10% TCA and the tubes were centrifuged at 2000 rpm. To 0.5 mL of supernatant, 4 mL of 0.3 M disodium hydrogen phosphate and 0.5 mL DTNB (40 mg of DTNB in 100 mL of 1% sodium citrate) were added and the color developed was read at 420 nm immediately. The activity of GPX was expressed as μmoles of glutathione-oxidized/min/mg protein.

2.4.4. Protein Estimation. Total proteins were assayed by the method of Lowry et al. [14].

2.5. Statistical Analysis. All the experiments were done in triplicates and the results were presented as the mean ± SD. One-way analysis of variance (ANOVA) followed by the Tukey's test for multiple comparison values of $P < 0.001$ was considered to be significant. Statistical Package for Social Studies (SPSS) 7.5 version was used for this analysis.

3. Results and Discussion

In the present study, preliminary phytochemical testing shows (Table 1) the presence of high amount of glycosides, alkaloids, tannins, phenolics and other all the principal secondary metabolites in hydroalcoholic extracts of AAR and TAB. The result obtained in the present experiment is well supported by previous studies. Phytochemical studies on *Achyranthes aspera* revealed the presence of glycosides of oleanolic acid, peroxidase, saponins, indole acetic acid, polyphenol, ecdysome, and flavanoids in various parts of the plant. Betaine and achyranthine are the principal alkaloids identified from the whole plant [15]. The various constituents of the dried bark extract of *Terminalia arjuna* (IPC-53) include acids such as arjunic acid, gallic acid, ellagic acid, arjunin and terminic acid, glycosides such as arjunetin, arjunosides I–IV, the flavone arjunolone, tannins, oligomeric

Table 1: Preliminary phytochemical screening of hydroalcoholic extract AAR and TAB.

S. no	Phytoconstituents	Inference	
		Hydroalcoholic extract of AAR	Hydroalcoholic extract of TAB
1	Alkaloids	++	+
2	Terpenoids	+	+
3	Carbohydrates	+	+
4	Glycosides	++	+
5	Protein and amino acids	+	+
6	Fixed oils and fats	–	–
7	Tannins	++	++
8	Saponins	++	+
9	Steroids (phytosterols)	+	++
10	Flavonoids	–	+
11	Phenols	+	++
12	Volatile oil	–	–

++ means abundant; + denotes average; – denotes absent.

Table 2: MnSOD assay.

Sample	Drug dosage	MnSOD (50% NBT reduction/min/mg protein)
Control	—	25.56 ± 1.18
AAR	100 mg/L	18.13 ± 1.35**
AAR	500 mg/L	18.78 ± 1.16**
TAB	100 mg/L	17.18 ± 1.28***
TAB	500 mg/L	28.48 ± 1.78*

The results are mean ± SD of three independent experiments.
Statistical significance: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, t-test compared to control.

proanthocyanidins (OPCs), coloring matter, and essential oils and minerals such as calcium, magnesium, zinc, and copper [16].

In our experiment, Table 2 showed the activity of MnSOD in control and AAR/TAB treated cells. AAR at 100 mg/L and 500 mg/L concentration showed significant reduction in the MnSOD activity when compared to the control cells. In case of TAB treated cells, TAB at 100 mg/L concentration showed significant reduction, while at 500 mg/L concentration showed enhanced MnSOD activity when compared to the control cells. Therefore, TAB at 500 mg/L concentration is not able to cause oxygen toxicity.

Interestingly, the lowest concentrations of TAB (100 mg/L) resulted in decreases in MnSOD activity in *S. mutans*. An understanding of the optimal concentrations of TAB extract may require careful titration to determine predictable and dependable therapeutic strategies against adverse effects. Previous report suggests that *T. arjuna* extract-induced oxidative stress is upstream of signaling events that might alter the pro- and antiapoptotic balance in HepG2 cells. Phenolic compounds are generally known to show not only their antioxidant effects but also prooxidant actions under *in vitro* conditions. Hence, it is possible that treatment of HepG2 cells with *T. arjuna* extract can deplete the GSH levels and promote oxidation induction, which switches the mode of death via apoptosis. Therefore,

Table 3: NADH oxidase assay.

Sample	Drug dosage	NADH oxidase (μmol of NADH oxidized/ min/mg protein)
Control	—	3.699 ± 0.25
AAR	100 mg/L	2.266 ± 0.15***
AAR	500 mg/L	2.466 ± 0.18***
TAB	100 mg/L	0.933 ± 0.14***
TAB	500 mg/L	2.133 ± 0.10***

The results are mean ± SD of three independent experiments.
Statistical significance: $^{***}P < 0.001$, t-test compared to control.

Table 4: Glutathione peroxidase (GPx) assay.

Sample	Drug dosage	GPX (μmol GSH utilized/min/mg protein)
Control	—	9.58 ± 0.22
AAR	100 mg/L	6.38 ± 0.26***
AAR	500 mg/L	4.86 ± 0.12***
TAB	100 mg/L	4.31 ± 0.24***
TAB	500 mg/L	5.79 ± 0.19***

The results are mean ± SD of three independent experiments.
Statistical significance: $^{***}P < 0.001$, t-test compared to control.

the cytotoxic action of this drug may be attributed to its prooxidant action on the cells. This may be able to account for the discrepancy between *in vitro* cytotoxicity and *in vivo* antitumor activities of *T. arjuna* extract [17].

It has been reported that reduction in SOD activity increases the sensitivity of *S. mutans* to oxygen tolerance, through the enhancement of Fenton. It increases the amount of hydroxyl radical leading to cell death [18]. Since SOD is proven to play role in aerotolerance in *S. mutans* [19], decrease in SOD activity by AAR and TAB might benefit them as anticaries.

Iron ions stimulate the generation of highly reactive and toxic oxygen species such as hydroxyl radicals. *In vitro* experiments have shown that Fe^{2+} catalyzes non-enzymatic hydroxyl radical synthesis from H_2O_2 via the Fenton reaction. But, H_2O_2 remained intact in the absence of iron ions at physiological pH [20]. Therefore, tight regulation of the intracellular free iron ion concentration is believed to be significant factor in organism survival under aerobic conditions. One of the important characteristics that distinguish *S. mutans* from other oral streptococci is its ability to ferment mannitol and sorbitol [21]. According to Yamamoto et al., A new antioxidant gene, Dpr, in the chromosomal DNA of *S. mutans* was identified [5]. Dpr was found to have iron-binding ability. Thus, a possible protective role of Dpr might be to sequester iron, thereby protecting cells from peroxides and conferring oxygen tolerance. Dpr did not directly react with oxygen and reactive oxygen species. The iron-binding ability of Dpr might indirectly contribute to oxygen tolerance in *S. mutans*.

Although Streptococci have a preference for anaerobiosis, O_2 affected the growth on mannitol with a variation dependent on strain [3]. The growth response to O_2 was correlated with the ability of strains to induce NADH oxidase

and superoxide dismutase (SOD) under aerobic conditions [4]. These findings suggested that NADH oxidase plays an important role in the regulation of aerobic metabolism through the regulation of NAD from the additional NADH derived from the oxidation of mannitol-1-PO_4 to fructose-6-PO_4.

In our study, the NADH oxidase activity of control and AAR/TAB treated cells was estimated under aerobic condition, as evidenced in Table 3. AAR and TAB treated cells at concentration of 100 mg/L and 500 mg/L showed significant reduction in the NADH oxidase activity compared to the control cells. TAB at 100 mg/L concentration showed maximum inhibition of 74.77%. AAR and TAB imposes an oxygen dependent toxicity, apparently both by inhibition of growth and by loss of viability. Decrease in NADH oxidases in treated cells relates to the susceptibility of the organism to oxidative challenge.

Reduced transition metal cations commonly enhance oxidative damage to cells caused by hydroperoxides formed as a result of oxygen metabolism or added externally. As expected, the cations Fe^{2+} and Cu^{+} enhanced killing of *Streptococcus mutans* GS-5 by hydroperoxides [22]. Iron and copper present in AAR and TAB substantiate their role on increased oxidative damages caused due to the reduction in the antioxidant enzymes like SOD and NADH oxidases in *S. mutans*.

Polyphenols as catechin act on different bacterial strains belonging to different species (*Escherichia coli*, *Bordetella bronchiseptica*, *Serratia marcescens*, *Klebsiella pneumonaie*, *Salmonella choleraesuis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus subtilis*) by generating hydrogen peroxide [23] and by altering the permeability of the microbial membrane [24]. Polyphenolic compounds which have anticariogenic property [25] also reported to exhibit prooxidant properties under appropriate conditions [26]. Thus, AAR and TAB, rich in polyphenols might act as pro oxidant, providing free radicals at higher rate during the treatment of *S. mutans* cells with AAR and TAB. As a consequence, SOD and NADH oxides, the major antioxidant enzymes present in *S. mutans*, could no longer able to compete and scavenge the overcrowding free radical formation and ultimately lead to the deterioration of the SOD and NADH activities.

Table 4 showed the GPx activity of the control and AAR/TAB-treated cells (100 mg/L and 500 mg/L). The GPx activity was significantly reduced in AAR- and TAB-treated cells when compared to the control cells. The reduction in GPx activity in the both extracts may be attributed to the presence of terpenoids. The present study shows the presence of terpenoids in AAR and TAB extracts. The mechanism of action of terpenes is speculated to involve membrane disruption by the lipophilic compounds. Accordingly, Mendoza et al. [27] found that increasing the hydrophilicity of kaurene diterpenoids by addition of a methyl group drastically reduced their antimicrobial activity. Food scientists have found the terpenoids present in essential oils of plants to be useful in the control of *Listeria monocytogenes* [28].

Another possible mechanism of GPx inhibitory activity might be favored by flavonoids present in AAR and TAB. Activity of flavonoids are probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls, as described above for quinones. More lipophilic flavonoids may also disrupt microbial membranes [29]. Both terpenoids, and flavonoids present in AAR and TAB were found to disturb the membrane of the bacteria. According to the finding of Thomas, GSH, the substrate for GPX activity, was normally imported from external source into the *S. mutans* cells [30]. Thus, when the bacterial cell membrane is being disturbed by flavoniods and terpinoids present in AAR and TAB, *S. mutans* no longer able to import GSH and ultimately, no substrate is available for carrying out GPx activity. AAR and TAB were found to reduce the enzyme activity, thereby reducing the tolerance response. This results in the accumulation of potent cytotoxic agents like free radicals, peroxides in *S. mutans* that cause lethality.

Ellagic acid and quercetin were found were to be potent inhibitors of GPx [31]. Our results show the presence of polyphenolic compounds in hydroalcoholic extract of AAR and TAB and these results are consistent with earlier studies that reported the presence of ellagic acid TAB [32].

The GSH-dependent reduction system is responsible for maintaining a reduced environment and plays an important role in survival in the presence of oxidative stress in *Escherichia coli* and *Saccharomyces cerevisiae* [33]. Sherrill and Fahey found that cellular GSH protected *S. mutans* ATCC 33402 against growth inhibition by the thiol oxidizing agent diamide [34]. Now, presence of AAR/TAB makes *S. mutans* incapable of being protected from thiol oxidizing agents. Therefore, reduction in the glutathione-related enzymes ultimately makes *S. mutans* unable to withstand the oxidative stress and, hence, made it more liable to the antimicrobial activity.

4. Conclusion

The anticariogenic activity of AAR and TAB extracts is well pronounced by interfering with the defense system of *S. mutans* and, thus, making the organism more susceptible for the drug action. Our results suggest that the inability of *S. mutans* to consume oxygen has a negative impact on its capability to infect and cause disease. The disruption of the oxygen metabolizing pathways by AAR and TAB extracts may lead to novel drug targets and a way to control the growth of the organism and diminish the incidence of dental caries. The extracts can be subjected to isolation of the active compound to carry out further pharmacological evaluation and molecular mechanisms of their actions against *S. mutans*. This will surely complement to the previously known therapeutic values of AAR and TAB and improve the popularization of the plants.

References

[1] R. Cannio, G. Fiorentino, A. Morana, M. Rossi, and S. Bartolucci, "Oxygen: friend or foe? Archaeal superoxide dismutases in the protection of intra- and extracellular oxidative stress," *Frontiers in Bioscience*, vol. 5, pp. D768–D779, 2000.

[2] J. C. Wilkins, K. A. Homer, and D. Beighton, "Analysis of *Streptococcus mutans* proteins modulated by culture under acidic conditions," *Applied and Environmental Microbiology*, vol. 68, no. 5, pp. 2382–2390, 2002.

[3] M. Higuchi, "The effect of oxygen on the growth and mannitol fermentation of *Streptococcus mutans*," *Journal of General Microbiology*, vol. 130, no. 7, pp. 1819–1826, 1984.

[4] M. Higuchi, "Reduced nicotinamide adenine dinucleotide oxidase involvement in defense against oxygen toxicity of *Streptococcus mutans*," *Oral Microbiology Immunology*, vol. 7, no. 5, pp. 309–314, 1992.

[5] Y. Yamamoto, M. Higuchi, L. B. Poole, and Y. Kamio, "Role of the dpr product in oxygen tolerance in *Streptococcus mutans*," *Journal of Bacteriology*, vol. 182, no. 13, pp. 3740–3747, 2000.

[6] J. M. Bruno-Bárcena, M. A. Azcárate-Peril, and H. M. Hassan, "Role of antioxidant enzymes in bacterial resistance to organic acids," *Applied and Environmental Microbiology*, vol. 76, no. 9, pp. 2747–2753, 2010.

[7] D. Gerlach, W. Reichardt, and S. Vettermann, "Extracellular superoxide dismutase from *Streptococcus pyogenes* type 12 strain is manganese-dependent," *FEMS Microbiology Letters*, vol. 160, no. 2, pp. 217–224, 1998.

[8] A. De Vendittis, M. Amato, A. Mickniewicz et al., "Regulation of the properties of superoxide dismutase from the dental pathogenic microorganism *Streptococcus mutans* by iron- and manganese-bound co-factor," *Molecular BioSystems*, vol. 6, no. 10, pp. 1973–1982, 2010.

[9] K. R. Khandelwal, *Practical Pharmacognasy Techniques and Experiments*, Nirali Prakashan, Pune, India, 2nd edition, 2000.

[10] H. K. Sandhar, M. Kaur, P. Tiwari et al., "Chemistry and medicinal properties of *Holoptelea integrifolia*," *International Journal of Drug Development and Research*, vol. 3, no. 1, pp. 6–11, 2011.

[11] P. Kakkar, B. Das, and P. N. Viswanathan, "A modified spectrophotometric assay of superoxide dismutase," *Indian Journal of Biochemistry and Biophysics*, vol. 21, no. 2, pp. 130–132, 1984.

[12] E. P. Steyn-Parve and H. Benert, "On the mechanism of dehydrogenation of fatty acyl derivatives of coenzyme A. VI. Isolation and properties of stable enzyme-substrate complexes," *The Journal of Biological Chemistry*, vol. 233, no. 4, pp. 843–852, 1958.

[13] J. T. Rotruck, A. L. Pope, H. E. Ganther, A. B. Swanson, D. G. Hafeman, and W. G. Hoekstra, "Selenium: biochemical role as a component of glatathione peroxidase," *Science*, vol. 179, no. 4073, pp. 588–590, 1973.

[14] O. H. Lowry, N. J. Rosenbrough, A. L. Farr, and R. J. Randall, "Protein measurement with the Folin phenol reagent," *The Journal of Biological Chemistry*, vol. 193, no. 1, pp. 265–275, 1951.

[15] Y. C. Kong, S. Y. Hu, and F. K. Lau, "Potential anti fertility plants from Chinese medicine," *American Journal of Chinese Medicine*, vol. 4, no. 2, pp. 105–128, 1976.

[16] Y. S. Prabhakar and D. S. Kumar, "The chemistry of *Terminalia arjuna* (Roxb.) Wight and Arnot with reference to its medicinal uses," *Plantes Medicinales et Phytotherapie*, vol. 22, no. 1, pp. 30–39, 1988.

[17] S. Sivalokanathan, M. R. Vijayababu, and M. P. Balasubramanian, "Effects of *Terminalia arjuna* bark extract on apoptosis of human hepatoma cell line HepG2," *World Journal of Gastroenterology*, vol. 12, no. 7, pp. 1018–1024, 2006.

[18] K. Keyer and J. A. Imlay, "Superoxide accelerates DNA damage by elevating free-iron levels," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 93, no. 24, pp. 13635–13640, 1996.

[19] K. Nakayama and J. Bact, "Nucleotide sequence of *Streptococcus mutans* superoxide dismutase gene and isolation of insertion mutants," *Journal of Bacteriology*, vol. 174, no. 15, pp. 4928–4934, 1992.

[20] B. Halliwell and J. M. C. Gutteridge, "Oxygen toxicity, oxygen radicals, transition metals and disease," *Biochemical Journal*, vol. 219, no. 1, pp. 1–14, 1984.

[21] S. Hamada and H. D. Slade, "Biology, immunology, and cariogenicity of *Streptococcus mutans*," *Microbiological Reviews*, vol. 44, no. 2, pp. 311–384, 1980.

[22] J. C. Dunning, Y. Ma, and R. E. Marquis, "Anaerobic killing of oral Streptococci by reduced, transition metal cations," *Applied and Environmental Microbiology*, vol. 64, no. 1, pp. 27–33, 1998.

[23] E. Haslam, T. H. Lilley, E. Warminski et al., "Polyphenol complexation—a study in molecular recognition," *ACS Symposium Series*, vol. 506, pp. 8–50, 1992.

[24] M. Hattori, I. T. Kusumoto, T. Namba, T. Ishigami, and Y. Hara, "Effect of tea polyphenols on glucan synthesis by glucosyltransferase from *Streptococcus mutans*," *Chemical and Pharmaceutical Bulletin*, vol. 38, no. 3, pp. 717–720, 1990.

[25] G. F. Ferrazzano, I. Amato, A. Ingenito, A. Zarrelli, G. Pinto, and A. Pollio, "Plant polyphenols and their anti-cariogenic properties: a review," *Molecules*, vol. 16, no. 2, pp. 1486–1507, 2011.

[26] S. M. Hadi, S. Azam, A. Malik, and N. Hadi, "Pro-oxidant activity of plant- derived polyphenolic compounds: Implication of anticaries properties," in *Proceedings of the International Conference on Role of Free Radicals and Antioxidants in Health and Disease and the 2nd Annual Conference of Society for Free Radical Research*, pp. 10–12, India, February 2003.

[27] L. Mendoza, M. Wilkens, and A. Urzúa, "Antimicrobial study of the resinous exudates and of diterpenoids and flavonoids isolated from some Chilean Pseudognaphalium (Asteraceae)," *Journal of Ethnopharmacology*, vol. 58, no. 2, pp. 85–88, 1997.

[28] P. Aureli, A. Costantini, and S. Zolea, "Antimicrobial activity of some plant essential oils against *Listeria monocytogenes*," *Journal of Food Protection*, vol. 55, pp. 344–348, 1992.

[29] H. Tsuchiya, M. Sato, T. Miyazaki et al., "Comparative study on the antibacterial activity of phytochemical flavanones against methicillin-resistant *Staphylococcus aureus*," *Journal of Ethnopharmacology*, vol. 50, no. 1, pp. 27–34, 1996.

[30] E. L. Thomas, "Disulfide reduction and sulfydryl uptake by Streptococcus mutans," *Journal of Bacteriology*, vol. 264, pp. 719–725, 1984.

[31] M. Kurata, M. Suzuki, and K. Takeda, "Effects of phenol compounds, glutathione analogues and a diuretic drug on glutathione S-transferase, glutathione reductase and glutathione peroxidase from canine erythrocytes," *Comparative Biochemistry and Physiology B*, vol. 103, no. 4, pp. 863–867, 1992.

[32] P. M. Paarakh, "*Terminalia arjuna* (Roxb.) wt. and am.: a review," *International Journal of Pharmacology*, vol. 6, no. 5, pp. 515–534, 2010.

[33] O. Carmel-Harel and G. Storz, "Roles of the glutathione- and thioredoxin-dependent reduction systems in the *Escherichia coli* and *Saccharomyces cerevisiae* responses to oxidative stress," *Annual Review of Microbiology*, vol. 54, pp. 439–461, 2000.

[34] C. Sherrill and R. C. Fahey, "Import and metabolism of glutathione by *Streptococcus mutans*," *Journal of Bacteriology*, vol. 180, no. 6, pp. 1454–1459, 1998.