Characteristics and Antibacterial Activity of Chitosan and Green Tea Extracts on Mouthwash

Karakteristik dan Aktivitas Antibakteri dari Ekstrak Kitosan dan Teh Hijau pada Obat Kumur

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ABSTRACT

Dental caries and gingivitis can be mitigated through the use of mouthwashes. However, chemical-based mouthwashes are associated with several adverse effects. Safer alternatives to chemical mouthwashes incorporate natural ingredients. Chitosan and green tea have gained prominence in the synthesis (formulation) of mouthwashes, with a focus on characterizing their antibacterial properties. This overview will concentrate on the synthesis methodology, characteristics, and antibacterial efficacy of chitosan and green tea. Chitosan and green tea mouthwashes can be synthesized using the solubilization method. This method entails the creation of a thermodynamically stable, isotropic solution of a substance in water by the addition of a surfactant. Chitosan is distinguished by the presence of glucosamine and N-acetylglucosamine repeating units, both of which contain amino groups. These amino groups exhibit antibacterial properties by interacting with bacterial cell membranes and compromising their structural integrity. In green tea, compounds such as catechins within phenolic groups, including flavanols, flavandiols, and flavonoids, also function as agents by disrupting bacterial cell membranes and cytoplasmic membranes. Both amino groups and catechin compounds demonstrate the ability to inhibit pathogenic oral bacteria. In conclusion, the potentially anti-bacterial activity mouthwashes synthesized from chitosan and green tea, in the future chitosan and green tea extracts to be further used for the preparation of mouthwash.
Keyword: Mouthwash, chitosan, green tea, functional groups, antibacterial activity

ABSTRAK

Karies gigi dan radang gusi dapat dikurangi melalui penggunaan obat kumur. Namun, obat kumur berbahan dasar kimia dikaitkan dengan beberapa efek samping. Alternatif yang lebih aman untuk obat kumur kimiawi adalah dengan menggunakan bahan-bahan alami. Kitosan dan teh hijau telah menjadi perhatian dalam sintesis obat kumur, dengan fokus pada karakterisasi sifat antibakterinya. Penelitian ini bertujuan untuk memberikan gambaran umum tentang metodologi sintesis, karakteristik, dan efektivitas antibakteri kitosan dan teh hijau. Obat kumur kitosan dan teh hijau dapat disintesis dengan menggunakan metode pelarutan. Metode ini memerlukan pembuatan larutan isotropik yang stabil secara termodinamika dari suatu zat dalam air dengan penambahan surfaktan. Kitosan memiliki ciri khas berupa adanya unit pengulangan glukosamin dan N-asetilglukosamin, yang keduanya mengandung gugus amino. Gugus amino ini menunjukkan sifat antibakteri dengan berinteraksi dengan membran sel bakteri dan mengganggu integritas strukturalnya. Senyawa dalam teh hijau, seperti katekin dalam kelompok fenolik, termasuk flavanol, flavandiol, dan flavonoid, juga berfungsi sebagai agen dengan mengganggu membran sel bakteri dan membran sitoplasma. Baik gugus amino maupun senyawa katekin menunjukkan kemampuan untuk menghambat bakteri mulut patogen. Kesimpulannya adalah obat kumur yang disintesis dari kitosan dan teh hijau memiliki gugus fungsi yang dapat bertindak sebagai agen antibakteri yang efektif, sehingga berkontribusi pada penghambatan bakteri mulut.
Kata kunci: Obat kumur, kitosan, teh hijau, gugus fungsi, aktivitas antibakteri

INTRODUCTION

Bacteria in dental plaque can induce dental caries and gingivitis.[1,2] Bacterial growth can be inhibited by using antimicrobial agents such as mouthwash.[3] The use of mouthwash is effective in preventing bacterial growth because it can reach the entire oral cavity.[4,5] Chlorhexidine mouthwash is one of the most effective antimicrobial agents and regarded as gold standard in controlling dental plaque. Chlorhexidine has broad-spectrum antibacterial activity and has been shown to prevent the formation of biofilms that cause caries and gingivitis. However, long-term use of chlorhexidine causes several side effects such as discoloration of the teeth and tongue, taste changes, increased calculus formation, burning sensation, and irritation of the oral mucosa.[3,6,7,8]

The exploration of mouthwash alternatives formulated with natural ingredients has gained significant momentum in recent years.[6] Natural mouthwashes are increasingly in demand due to their effectiveness in combating oral pathogenic bacteria and their minimal side effects compared to synthetic options. For decades, herbal medicines have played an important role in disease therapy, owing to their potent antimicrobial and antifungal properties against pathogenic microorganisms.[9]

Several natural ingredients have demonstrated effective antibacterial properties and are commonly utilized in mouthwash formulations. Examples include chitosan, green tea, ginger, lemongrass, bay leaves, and others. Chitosan and green tea, in particular, have been extensively studied for their antibacterial efficacy against oral pathogens.[3,9,10,11,12] Chitosan has shown significant inhibitory effects on *Streptococcus mutans* and *Lactobacillus brevis*, both of which are key contributors to dental caries.[10] Similarly, green tea exhibits antibacterial properties that effectively reduce oral bacteria, helping to prevent caries, gingivitis, and halitosis.[13] This article review a new exploration about the synthesis of chitosan and green tea-based mouthwashes, along with an analysis of their FTIR characteristics and antibacterial properties.

LITERATURE REVIEW

Chitosan is a linear cationic polysaccharide, composed of a copolymer of 2-amino-2-deoxyglucose (60–100%) and 2-acetylamino-2-deoxyglucose D-glucoside (0-50%).[14] It is the second most abundant natural polymer after cellulose and is derived from the deacetylation of chitin, which is commonly found in the exoskeletons of crustaceans such as shrimp, crabs, and lobsters, as well as in the cell walls of fungi and insects. Due to its biodegradability, biocompatibility, and antimicrobial properties, chitosan has numerous biomedical applications.[15,16,17] These antimicrobial properties make chitosan particularly suitable for incorporation into oral care products such as mouthwash.[18]

The composition and functional groups of a material can be identified through specific vibrational bands corresponding to various functional groups using vibrational spectroscopy in the infrared region.[19] Figure 1 illustrates the characterization of the functional groups and the degree of deacetylation of chitosan. The degree of deacetylation refers to the removal of acetyl groups ($COCH_3$) from chitin. A higher degree of deacetylation results in more active chitosan, as more amine groups replace the acetyl groups. The functional groups in chitosan include OH, CN, NH, amide, and carbonyl groups, appearing at wave numbers 3392 cm^{-1}, 2342 cm^{-1}, 1575 cm^{-1}, 1412 cm^{-1}, and 1076 cm^{-1}, respectively.[20]

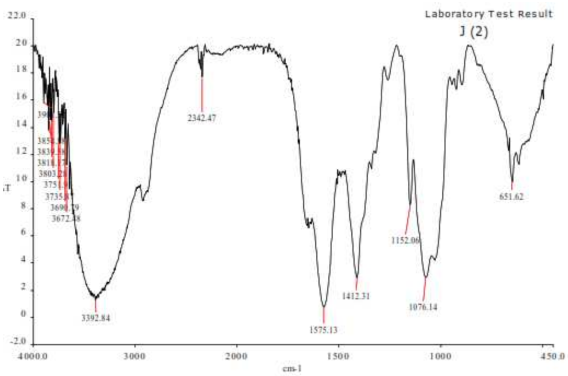

Figure 1. Infrared Spectrum of chitosan[20]

Green tea originates from the leaves of Camellia sinensis which contains polyphenols as much as 45%-90%. Polyphenols in green tea such as flavanols, flavandiols, which the

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flavonoids are phenolic acids. The main flavonoids in green tea are catechins. Green tea catechins are epicatechin (EC), epicatechin-3-galate (ECG), epigallocatechin (EGC), and epigallocatechin-3-gallate (EGCG).[12, 21, 22] Studies have shown that green tea polyphenols can inhibit the growth of oral bacteria and prevent caries, halitosis, gingivitis and periodontitis. These antibacterial properties make green tea very suitable for mouthwash ingredients with the beneficial effects of natural ingredients with lower side effects.[19]

The chemical composition and compounds present in green tea can be identified through infrared spectroscopy. The interpretation of infrared spectra involves correlating vibrational bands with specific chemical compounds in the sample. In the infrared spectrum of green tea, shown in Figure 2, the band at 3394 cm^{-1} is attributed to O-H stretching vibrations in water, alcohols, and phenols, as well as N-H stretching in amines. The C-H stretch in alkanes and the O-H stretch in carboxylic acids appear at 2926 cm^{-1} and 2864 cm^{-1}, respectively. A strong band at 1627 cm^{-1} is associated with the C=C stretch in aromatic rings and C=O stretching in polyphenols. The C-N stretch in the amide-I region of proteins is observed at 1396 cm^{-1}, while C-O-C stretching in polysaccharides is evident at 1741 cm^{-1}. Additionally, C-O stretching in amino acids produces a band at 1037 cm^{-1}. Finally, the weak band at 819 cm^{-1} is the result of out-of-plane C-H bending. Based on these infrared spectra, it is evident that the green tea sample is rich in polyphenols, carboxylic acids, polysaccharides, amino acids, and proteins.[23]

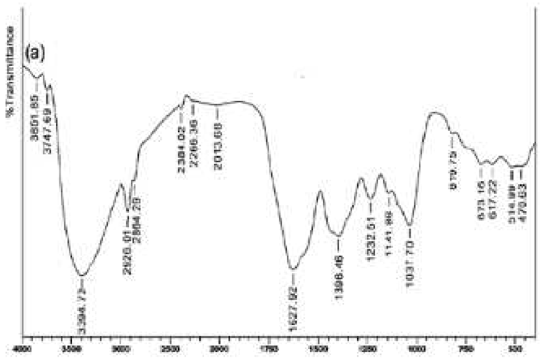

Figure 2. Infrared spectrum of green tea[23]

SYNTHESIS OF CHITOSAN AND GREEN TEA MOUTHWASH

Several researchers have explored how to synthesize chitosan and green tea-based mouthwashes with various concentrations (0.5%-5%) to assess their antibacterial effectiveness. The preparation of chitosan and green tea mouthwash typically involves the solubilization method, which creates a thermodynamically stable isotropic solution by dissolving the active ingredients in water with the addition of a surfactant. Commonly used surfactants include polysorbates 20, 40, and 80, sodium deoxycholate, and sorbitol.[24,25]

In addition to surfactants, mouthwash formulations often contain sweeteners, such as sodium saccharin and mannitol, to impart a sweet taste. Flavouring agents like peppermint oil are also added to provide a fresh taste and sensation upon use. To prevent evaporation of the mouthwash ingredients and enhance the solubility of poorly soluble extracts, humectants such as glycerin are commonly included. Finally, distilled water serves as the solvent for the mouthwash formulation.[8,26,27]

Chitosan and its derivatives exhibit two primary antibacterial mechanisms: the binding of cationic chitosan to sialic acid in phospholipids, which disrupts the movement of microbiological substances, and the penetration of oligomeric chitosan into microbial cells, inhibiting cell growth by preventing the transformation of DNA into RNA.[28,29,30] Additionally, chitosan can chelate essential metal ions, such as calcium and magnesium, which are crucial for bacterial growth and stability. By binding these ions, chitosan prevents bacteria from acquiring the necessary nutrients, thereby inhibiting their replication and growth. Chitosan has amino groups that play an important role in its antibacterial activity, with a high concentration of free amino groups will produce a greater antimicrobial effect.[30]

Research by Guarnieri et al. (2022) showed differences in chitosan interactions with Gram-positive and Gram-negative bacteria due to variations in cell membrane structure. In Gram-negative bacteria, chitosan interacts with anionic structures such as lipopolysaccharides and proteins on the cell surface. In Gram-positive bacteria, chitosan interacts directly with the cell wall layer, which consists of negatively charged peptidoglycan and teichoic acids. [29]

The antibacterial effectiveness of chitosan mouthwash against oral bacteria has been widely reported. Costa et al.[26] found that mouthwash containing chitosan exhibited greater antimicrobial activity compared to commercial mouthwash.[26] The antibacterial effects of chitosan mouthwash against *S. mutans* and *C. albicans*, finding that it reduced bacterial counts to levels comparable to chlorhexidine mouthwash.[7]

Chitosan mouthwash has also been shown to effectively inhibit the growth of cariogenic bacteria. Thongaroon et al.[31] reported that chitosan mouthwash inhibited the growth of *S. mutans* and *L. casei* by more than 3 log CFU/ml within 12 hours of exposure. A reduction of three logs or more is considered highly effective in controlling bacterial growth.[31] Gargling with chitosan mouthwash significantly controls the growth of plaque-causing bacteria. Aparna et al.[32] reported that chitosan mouthwash reduced the growth of *S. mutans* by 50% and *Lactobacillus* by 55%, without disturbing normal flora of oral cavity.

The antibacterial activity of green tea has been attributed to its content of EGCG and other bioactive compounds including catechins, flavonoids, and polyphenols, which damage bacterial cell membranes, inhibit gyrase enzymes, and disrupt the bacterial cytoplasmic membrane.[33,34] The catechins in green tea possess antiplaque and antibacterial properties that help prevent dental caries and gingival enlargement.[19] The antibacterial activity of catechins in green tea occurs through several mechanisms, including disrupting cell walls and membranes, inhibiting intracellular enzymes, causing oxidative stress, damaging DNA, and chelating iron.[35] Instead of catechins, polyphenols contained in green tea can prevent dental caries by inhibiting the growth of Streptococcus mutans in the oral cavity. Another mechanism is through enzyme activity and bacterial growth. Researchers suggest that the effects of green tea on oral microorganisms may help in prevention as well as treatment.[36,37]

Green tea mouthwash has shown antibacterial activity against the primary cariogenic bacterium, *S. mutans*. Kamath et al.[38] reported a significant reduction in the number of *S. mutans* colonies before and after gargling with green tea mouthwash. They also found that a 0.12% chlorhexidine mouthwash and a 0.5% green tea mouthwash were equally effective in reducing *S. mutans* colony counts.[38] In a comparison study, Moghbel et al. found that green tea mouthwash exhibited the same antibacterial effect as chlorhexidine, while also being non-irritating, safer, and more economical.[39] Beyond its effectiveness against cariogenic bacteria, green tea mouthwash has also been shown to reduce the number of colonies of periodontopathogenic bacteria, including *P. gingivalis* and *Aggregatibacter actinomycetemcomitans*.[40]

CONCLUSION

Mouthwash from natural ingredients with minimal side effects can be an alternative to chemical-based mouthwash. Mouthwashes synthesized from chitosan and green tea feature functional groups that can act as effective antibacterial agents, thereby contributing to the inhibition of oral bacteria. The antibacterial effectiveness of chitosan mouthwash and green tea mouthwash is even proven to be equally effective when compared to commercial mouthwash.

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