A Comparative Antimicrobial Potential Of Camellia Sinensis And Vachellia Nilotica Formulation Based Mouthwash And Commercial Mouthwash

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Abstract

Background: Camellia sinensis (Green tea) has good antioxidant, antimicrobial activity and it is used for evasion of periodontal disease development. Microbial dysbiosis of oral microflora containing gram-positive and gram-negative anaerobic microorganisms and these organisms can bring about the advancement of cariogenic dental plaque on the teeth and commencement of dental caries. Green tea leaves contain a high convergence of fluorides, which contribute towards its cariostatic activity alongside different parts present in the tea. Vachellia nilotica (Babul) contains rich content of fiber and proteins. It has good antimicrobial activity. It is very helpful for treating mouth ulcers.

Aims: To compare antimicrobial potential of Camellia sinensis and Vachellia nilotica herbal formulated mouthwash and commercial mouthwash.

Materials and Methods: The plant extract was prepared with 1g dried green tea leaves and 1g powder of Babul mixed with 100ml distilled water and boiled for 10 minutes, at 60-80°C on a heating mantle. The extract was filtered using Whatman No.1 filter paper. Further the herbal formulation of Green tea and Babul and commercial mouthwash was subjected to determine the antimicrobial activity.
**Results:** Antimicrobial activity of green tea extract and Babul formulated mouthwash has good Antibacterial activity as there is a high zone of inhibition in Enterococcus faecalis and Streptococcus aureus. Commercial mouthwash has good Antibacterial activity as there is a high zone of inhibition in Streptococcus mutans.

**Conclusions:** Green tea and Babul herbal formulated mouthwash has good antimicrobial activity when compared to commercial mouthwash.

**Key:** Antimicrobial, Babul, Green tea, Herbal formulation, Mouthwash, Periodontitis

**INTRODUCTION:**
The oral environment gives ideal circumstances to the proliferation of microorganisms, where microbe disease might cause dental caries by establishing an acidic environment and biofilm around teeth and gums(1). Oral cleanliness measures have been rehearsed by various populations and societies all over the world since vestige(2). Dental caries is quite common of the most widely recognized human illnesses that influence by far most people. Inhibiting the production of the Streptococcus mutans in the dental cavity would tend to improve teeth and gums(3).

The predominance of microbes that cause dental caries can be effectively decreased through effective systems, for example, tooth-brushing, interdental cleaning, and the utilization of commercial antimicrobial mouthwashes(4). Everyday utilization of mouthwashes is suggested for appropriate dental hygiene(5).

Camellia sinensis(Green tea) is rich in polyphenolic compounds, essentially flavonoids like catechins, epicatechin gallate (ECG), epicatechin (EC), epigallocatechin (EGC) and epigallocatechin gallate (EGCG)(6). Flavonoids have been found to have antioxidant and antimicrobial activity. Green tea has been represented to be useful for evasion of periodontal disease development(7)(8). The advancement of porphyromonas gingivalis, prevotella intermedia and prevotella nigrescens on human buccal epithelial cells is ruined by the green tea part EGCG. These microscopic organisms are vigorously engaged with the obliteration of periodontal tissues and can prompt a decrease in periodontal disease(9). Likewise, the release of catechins into the periodontal tissues has been found to repress the development of harmful end metabolites of P. gingivalis(10)(11). Overproduction of free radicals harms gingival tissues, periodontal tissues, and alveolar bone in periodontal disease pathogenesis(12)(13). Green tea leaves contain a high convergence of fluorides, which contribute towards its cariostatic activity alongside different parts present in the tea(14). Microbial dysbiosis of oral microflora containing gram-positive and gram-negative anaerobic microorganisms and these organisms can bring about the advancement of cariogenic dental plaque on the teeth and commencement of dental caries(15)(16). Streptococcus mutans and Lactobacillus play a significant part in the commencement and movement of dental caries because of their capacity to endure lower pH, with higher metabolic action at pH 6–6.5(17)(18).

Vachellia nilotica(Babul) contains rich content of fiber and proteins. Its units contain more fiber and have a lesser measure of protein when contrasted with the leaves. It has good antimicrobial activity. It is very helpful for treating mouth ulcers(19). It could be helpful to clean the mouth, relieve pain, stop the bleeding of the gums, and could likewise assist with fixing the teeth(20)(21). Babul extracts showed antimicrobial activity against Streptococcus mutans(22). Applying a paste of Babul leaf powder relieves oral issues, for example, plaque development and gum disease because of its antibacterial property(23)(24).

Individual properties of these compounds have been established earlier, but there is no study till date which evaluates the antimicrobial potential of a combination of two plant extracts. Our study aims to explore the antimicrobial potential of Camellia sinensis and Vachellia nilotica formulated mouthwash in comparison with commercial mouthwash.

**MATERIAL AND METHODS :**
HERBAL FORMULATION PREPARATION:

Green tea and Babul extract was commercially obtained, 1 mg of dried Green tea and 1 mg of Babul powder was dispersed in 100 mL of distilled water and boiled for 10 minutes at 60-80°C. The extract was filtered using Whatman No.1 filter paper. Then again the filtered solution was reheated in a heating mantle for 20-30 minutes to condense the 100ml solution into a 5ml solution of Green tea and Babul herbal solution.

![Image showing Green synthesis of green tea extract and Babul](image)

Figure 1: Images showing Green synthesis of green tea extract and Babul

ANTIMICROBIAL ACTIVITY

Streptococcus mutans, Streptococcus faecalis, Streptococcus aureus and Candida albicans are the bacteria and fungi used in this activity. Organisms will be cultured using standard methodology. Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) will be studied using a well diffusion method.

**Antibacterial activity:**
The agar well diffusion method was used in antibacterial activity of Babul and green tea mouthwash and commercial mouthwash. Different concentrations of Babul and green tea mouthwash and commercial mouthwash were tested against Streptococcus mutans(gram +), Streptococcus faecalis, and Streptococcus aureus. The fresh bacterial suspension was dispersed on the surface of Muller Hinton agar plates. Different concentrations of nanoparticles (25,50&100μL) were incorporated into the wells and the plates were incubated at 37°C for 24 h. The antibiotics were used as positive control. Zone of inhibition was recorded in each plate.

**Antifungal activity:**
The Candida albicans fungal was isolated using dilution method. The agar well diffusion method and disc diffusion methods were used to determine the antifungal activity of Babul and green tea mouthwash and commercial mouthwash. Different concentrations of Babul and green tea mouthwash and commercial mouthwash was tested against the oral pathogenic Candida albicans. The fresh fungal suspension was dispersed on the surface of Rose Bengal agar plates. Different concentrations of nanoparticles (25,50 &100μL) were incorporated into the wells and the plates and discs were incubated at 37°C for 48 h. The antibiotics were used as positive control. Zone of inhibition was recorded in each plate.

MOUTHWASH PREPARATION:
The mouthwash was prepared using green tea and Babul herbal formulation, ethanol, distilled water, sucrose, sodium benzoate, clove oil, sodium dodecyl phosphate with 0.1% in concentration. Green tea and Babul are the main constituents, ethanol acts as a solvent to solubilise the ingredients. Sodium benzoate acts as a preservative and clove oil acts as a flavoring agent.

RESULTS AND DISCUSSION:

Zone of inhibitions were measured from the edge of the punched hole (ditch) to the outer border of bacterial inhibition (translucent area) at four different randomly selected perpendicular places. These zones of inhibitions were measured after 24hrs.
Figure 2: Culture plates showing Antimicrobial activity of green tea extract and Babul herbal formulated mouthwash

Figure 2 depicts that, when compared to all organisms Enterococcus faecalis has more zone of inhibition in 100μL of 31mm, followed by Streptococcus aureus has 21mm of zone of inhibition in 100μL and Streptococcus mutans has 20mm of zone of inhibition in 100μL concentration. It has good antibacterial activity when compared to antifungal activity.

<table>
<thead>
<tr>
<th>Green tea and Babul mouthwash</th>
<th>25μL</th>
<th>50μL</th>
<th>100μL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococcus mutans</td>
<td>14</td>
<td>16</td>
<td>20</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>17</td>
<td>23</td>
<td>31</td>
</tr>
<tr>
<td>Streptococcus aureus</td>
<td>15</td>
<td>19</td>
<td>21</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>13</td>
<td>15</td>
<td>19</td>
</tr>
</tbody>
</table>

Table 1: Zone of inhibition and Antimicrobial activity of green tea extract and Babul herbal formulated mouthwash

Table 1 shows, green tea and babul formulated mouthwash under Streptococcus mutans has 14mm zone of inhibition in 25μL concentration, 16mm in 50μL concentration and 20mm in 100μL concentration. Enterococcus faecalis has 17mm zone of inhibition in 25μL concentration, 23mm in 50μL concentration and 31mm in 100μL concentration. Streptococcus aureus has 15mm zone of inhibition in 25μL concentration, 19mm in 50μL concentration and 21mm in 100μL concentration. When comparing all antibacterial organisms, Enterococcus faecalis has good results in green tea and babul formulated mouthwash. Candida albicans has 13mm zone of inhibition in 25μL concentration, 15mm in 50μL concentration and 19mm in 100μL concentration.

By comparing fig 2 and table 1, it shows that Antimicrobial activity of green tea extract and Babul formulated mouthwash has good Antibacterial activity as there is a high zone of inhibition in Enterococcus faecalis and Streptococcus aureus. There is a moderate antifungal activity against Candida albicans as there is less zone of inhibition. As the concentration of extract increases, the zone of inhibition also increases.
Figure 3 shows, when comparing all organisms Streptococcus mutans has more zone of inhibition of 23mm in 100μL concentration, followed by Streptococcus aureus has 21mm of zone of inhibition in 100μL concentration and Enterococcus faecalis has 19mm of zone of inhibition in 100μL concentration.

<table>
<thead>
<tr>
<th>Commercial mouthwash</th>
<th>25μL</th>
<th>50μL</th>
<th>100μL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococcus mutans</td>
<td>20</td>
<td>22</td>
<td>23</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>15</td>
<td>18</td>
<td>19</td>
</tr>
<tr>
<td>Streptococcus aureus</td>
<td>19</td>
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<td>21</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>19</td>
<td>21</td>
<td>22</td>
</tr>
</tbody>
</table>

Table 2 shows commercial mouthwash under Streptococcus mutans has 20mm zone of inhibition in 25μL concentration, 22mm in 50μL concentration and 23mm in 100μL concentration. Enterococcus faecalis has 15mm zone of inhibition in 25μL concentration, 22mm in 50μL concentration and 19mm in 100μL concentration. Streptococcus aureus has 19mm zone of inhibition in 25μL concentration, 20mm in 50μL concentration and 21mm in 100μL concentration. When comparing all antibacterial organisms, Streptococcus mutans have good results in commercial mouthwash. Candida albicans has 19mm zone of inhibition in 25μL concentration, 21mm in 50μL concentration and 22mm in 100μL concentration.

By comparing fig 3 and table 2, it shows that Antimicrobial activity of commercial mouthwash has good Antibacterial activity as there is a high zone of inhibition in Streptococcus mutans. There is a moderate antifungal activity against Candida albicans as there is less zone of inhibition.

Few studies have shown that the green tea and Babul plays a vital part in the counteraction of damage to gingival tissues, periodontal ligament, and alveolar bone in periodontal disease pathogenesis(25). Daily utilization of green tea and Babul mouthwash can decrease gingival bleeding, depth of the pocket and advance periodontal healing(26)(27). Periodontal therapy might be credited to the presence of polyphenols by a reduction in expression of matrix metalloproteins (MMP 9)(28).
The explanations behind antimicrobial activity of Babul and green tea might incorporate hydrophilic mixtures, for example, polyphenols, gums (poly-saccharides) and tannins (29). There is expanding proof to help that the plants of family Acacia are somewhat high in bioactive auxiliary mixtures and are consequently prone to hold guarantee for drug disclosure (30)(31). Optional mixtures in Acacia are significant for different capabilities, chief among these are Anti-cancer (triterpenoid and saponins), diuretic (glucosides), natriuretic (glucosides), significant nutraceutical (poly-saccaride and gum) anti-digestive disorder (saponins, tannins and flavonoids), anti oxidant (polyphenols), antiplasmodial (tryptamine, tannins, natural acids and saponins) (32).

Studies have recommended that green tea and Babul herbal mouthwash can really decrease the microbial microorganisms, can be utilized as an assistant to periodontal treatment in constant periodontitis and, alongside scaling and root planing a time of two weeks, could work on the scores of different periodontal parameters (33). Furthermore, no proof of toxic impacts, for example, irritation, burn, vesicle or mucous disturbance influence was noticed, and regardless of comparable antibacterial impacts of green tea and Babul mouthwash with the chemical chlorhexidine, they showed that the utilization of natural green tea mouthwash was more secure and more affordable (34)(35). From the results of our study the mouthwash formulations with a combination of Babul and green tea proves a significant antimicrobial activity. In another clinical preliminary, it was reasoned that green tea mouthwash could work on the gingival health of patients with marginal gingivitis (36).

CONCLUSION:
By comparing the antimicrobial activity of green tea and Babul herbal formulation based mouthwash and commercial mouthwash, it can be concluded that green tea and Babul herbal formulated mouthwash has good antimicrobial activity when compared to commercial mouthwash. As the concentration of extract increases, the zone of inhibition also increases.

CONFLICTS OF INTEREST:
The authors declare no conflict of interest.

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