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Methodology

A preliminary pilot scale analysis of anti-cariogenic activity of green tea powder extract flavoured with Ginger, Cloves and Mint against clinical oral pathogens

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SUMMARY

Green tea is a popular drink in Asian countries although its popularity continues to spread across the globe. Fairly, recent researchers have begun to look at the possibilities of using green tea in antimicrobial therapy, and their potential prevention of infections. The properties of green tea have shown potential for antimicrobial activity very effectively. The aim of present work is a preliminary pilot scale analysis to study the effect of anti-cariogenic efficacy of green tea extracts flavored with selective traditional medicinal plant extracts against dental carries. The green tea extracts which are rich in its anti-oxidant effect were flavored with different concentrations (1:1, 1:2, 1:3) of certain special ingredients like *ginger*, *cloves* and *mint* to check for its effectiveness against oral pathogens that causes caries. The results of the extractions against the isolated test oral pathogens have shown that there is a potential activity against dental pathogens. The results of this study clearly demonstrates that the minimum inhibitory concentration (MIC) ranges upon treating the test pathogens with green tea extract flavored with *ginger*, *cloves* and *mint* have a significant reduction of caries. In conclusion the *invitro* potentiality of the extracts in combination were determined to be effective in inhibiting the pathogens growth very effectively which

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can prevent caries and tooth decay by acting as a good anti-cariogenic and antimicrobial agent and also in future it can be taken for pilot scale level of product development with these extracts. Hence, the MIC effectiveness of the extracts against the oral pathogens were analyzed statistically using Student 't' test which shows a significant result revealing that the extracts produced 1% significance and ≥0.5% significances against dental pathogens to reduce caries and other secondary infections associated by them to maintain a healthy oral environment.

1. Introduction

Green tea has long been believed to be beneficial to one's health and has a long history of widespread consumption. Evidence shows that green tea was consumed as early as the third century AD, yet multiple stories suggest it was brewed much earlier. Since the third century, green tea has been used for medicinal purposes, such as depression, stomach problems, and anxiety. Green tea is a beverage made from the evergreen plant *Camellia sinensis* and has been enjoyed for thousands of years [1]. Green tea is non-fermented tea. The tea is an infusion of leaves that has been consumed for centuries as a beverage and is valued for its medicinal properties [2]. Tea leaves are known for its antimicrobial activity against many microorganisms and in green tea the leaves are steamed after they are dried [3]. Green tea's numerous health benefits are the result of the large percentage of polyphenols found within the tea, even though the polyphenol content varies due to environmental factors like rainfall and season. This also showed that moderate daily consumption of green tea killed *Staphylococcus aureus* and other harmful bacteria [4]. Recent reports however indicate the tea's antibacterial and bactericidal properties on various bacterial strains isolated from patients with infected root canal. Subsequently, several studies on the antimicrobial properties of Japanese tea have been reported. The antibacterial activity of Turkish tea against *Campylobacter* sp., and the protective activity of tea against infection by *Vibrio cholera 01* have also been reported [2]. Green tea catechins are known for their antioxidant properties. Green tea catechins' effects on reduction of gingival oxidative stress [10]. Green tea can kill bacteria and inhibit viruses like the influenza virus, which lowers the risk of having infections. *Streptococcus mutans* is the primary harmful bacteria in the mouth. It causes plaque formation and is a leading contributor to cavities and tooth decay. Studies show that the catechins in green tea can inhibit the growth of streptococcus mutans. Green tea consumption is associated with improved dental health and a lower risk of caries. Catechins are found to be inhibitory against *Streptococcus mutans* and *Streptococcus sobrin* at minimum inhibitory concentration (MIC) ranging between 50 and 1000 μg/ml [11]. Dental caries is a multifactorial infectious disease in which nutrition, microbiological infection, and host response play important roles. The present preliminary study were done to check the antimicrobial efficacy of green tea extract flavored with natural ingredients against the clinical dental samples from patients. The extracts enhanced with the selected ingredients like *Ginger*, *Cloves*, *Mint* – were tested for its efficacy of treating the oral infections and its use as anti-cariogenic agents effectively.

2. Methodology

2.1. Sample collection

Dental caries samples were collected using sterile cotton swabs from 50 patients between 35 and 55 years old age groups residing in Coimbatore were chosen as our sample size having infected with caries

from Pearls' Denta Care, India. A written informed consent was obtained from all subjects. Tetley Green Tea bags were purchased in packets of 2 g x 100 bags. Powders of *Ginger*, *Cloves* and *Mint* were prepared under sterile *invitro* condition after proper shade drying.

2.2. Green tea extract preparation

The tea bags were taken under sterile condition and it was aseptically cut opened using a sterile scissor to take the green tea powder and the sample extraction was prepared by a method as described in Toda et al., [4]. The extracts were prepared using sterile cold phosphate buffered line. The extracts were kept at room temperature for 4 h and then it was centrifuged at 1500 $\times$ *g* for 15 min.

2.3. Medicinal plant extract enrichment

50 ml of green tea extract prepared were taken. *Ginger* powder extracts was prepared using ethanolic extraction. To 10 ml of green tea extract added three different varying concentrations namely 1:1, 1:2; 1:3 (g/g) ratios for preparation of *ginger* extracts by using ethanol as the solvent. It is followed the same for other two selected ingredients *cloves* and *mint*, respectively. Here the volume of green tea extract remains constant and the added ingredient extract volumes namely *ginger*, *cloves*, *mint* changes accordingly to identify their minimum inhibitory concentration efficacy and their anti-cariogenic activity in each varying concentrations against the test pathogens under *invitro* conditions.

2.4. Test organisms isolated

The major organisms isolated from clinical samples were *Pseudomonas putida, Enterobacter cloacae, Stenotrophomonas maltophilia, S. aureus, Granulicatella adiacens, Pseudomonas oryzihabitans.* It was characterized using gram staining, biochemical assays and the results was further confirmed to their genus and species level with the help of Bioline Laboratory, Coimbatore, TN, India.

2.5. Antibiotic sensitivity test – Kirby–Bauer assay

The Kirby–Bauer assay is a standardized assay used to determine the susceptibility of bacteria to various antibiotics. The zone of inhibition is determined using the zone of clearance surrounding the well and its distance is measured for MIC values. Here 0.1 ml of the sample cultures were taken and plated on to a Mueller-Hinton Agar by swabbing method and the wells created in the plates were filled with 0.1 ml of the prepared green tea ethanolic extracts which are flavoured with natural ingredients like *Ginger*, *Mint* and *Cloves* in triplets. It was then incubated at 37 °C for 24 h. The determination of Zone of Inhibition was interpreted after incubation and is compared with a Standard Table of Antibiotics Susceptibility Datas. It was measured underside of the plate in two planes with a calibrated ruler in millimeters.

2.6. Statistical analysis

Datas were presented as Mean $\pm$ SD and the mean zone of inhibition efficacy analysis were performed with Student 't' test. The analysis was done in SPPS 16.0 statistical package program for Windows 7. The 't' values (0.5%–1%) and the significance value was declared to differ significantly according to different extract ratios.

3. Results

3.1. Invitro *characterization of clinical samples*

The collected dental samples from the subjects were grown under sterile condition and incubated at 37 °C for 24 h. After incubation, the bacterial colonies were observed. *Invitro* characterization using Gram staining and biochemical assays were performed to identify the pathogens from the dental

samples and the results were confirmed with the help of Bioline Laboratory, Coimbatore, TN, India. The characterized test pathogens for this preliminary study from the dental isolates were, *P. putida, E. cloacae, S. maltophilia, S. aureus, G. adiacens, P. oryzihabitans*, respectively.

3.2. Antimicrobial/anti-cariogenic activity

The Minimum Inhibitory Concentration (MIC) range on MHA plates was determined in triplicates for all the three different flavoured extracts for three different ratios (1:1, 1:2,1:3) with control after incubation at 37 °C for 24 h to determine the antimicrobial and anticariogenic efficacy of Green–Tea Extract with medicinal plant extract enrichments which are clearly explained in (graphs-1,2,3). By Comparing the results for all the three extracts, their minimum inhibitory concentration (MIC) were calculated and their anti-cariogenic activity against the selected oral pathogens was found to be effective and hence, tabulated (Tables 1,2,3). From Table 1 it is clear that the zone of inhibition indicates how effective the green tea extract is stopping the growth of bacteria that varies accordingly for different organisms. The zone of inhibition of green tea extract flavoured with *ginger* resulted in the effective MIC range at 1:1 ratio for all test pathogens and *P. oryzihabitans* shows the maximum zone of clearance whereas *G. adiacens* shows the minimum zone of clearance at 1:1 ratio concentration of the extracts(graph-1). Table 2 exhibits the MIC range in 1:1 concentration all the test pathogens. Among which *S. maltophilia* shows the maximum zone of clearance in 1:1 ratio and *S. aureus* shows the lowest zone of clearance range. Thus all other test pathogens shows that there is a significant difference in the zone of inhibition rate according to the different ratios of green tea extract flavoured with *cloves* effectively. From Table 3, it is evident that the two organisms *G. adiacens* shows the minimum zone of inhibition range with all the ratios. *E. cloacae*, *S. aureus, P. oryzihabitans* are showing almost similar in inhibition range values where they produced larger zone of inhibition with green tea extract flavoured with *mint*. *S. maltophilia* and *P. putida* also significantly shows clear zone of inhibition range effectively with the extract used. In comparison of the three tables the observed results for zone of inhibition range measurements were determined using a statistical analysis using SPSS 16.0 version software for Windows. It is calculated that the mean zone of inhibition for the green tea extract flavoured with *ginger*, *cloves* and *mint* were properly identified using Mean ± SD, where the significant ranges were performed and analyzed using Student 't' test (Table 4). Table 4 with statistical analysis indicates the student 't' test value, significance ranges and the mean differences on zone of inhibition for green tea extract flavoured with *ginger* (G), *cloves* (C) and *mint* (M) to determine the anti-cariogenic effect of different flavoured green tea extract for treating oral pathogens and to prevent caries infections effectively. The findings revealed that significant difference on mean Zone of Inhibition was observed between Green tea extract flavoured with *ginger* sample G1 (1:1)and sample G2 (1:2) at 1% level ($t = 7.906^{**}$), between sample G1 (1:1) and sample G3 (1:3) at <0.5% level ($t = 3.796^{*}$), between sample G2 (1:2) and sample G3 (1:3) at <0.5% level ($t = 2.015$). The significant difference on Zone of Inhibition observed between green tea extract flavoured with *cloves* are sample C1 (1:1) and sample C2 (1:2) at <0.5% level ($t = 3.796^{*}$), between sample C1 (1:1) and sample C3 (1:3) at 1% level ($t = 6.635^{**}$), between sample C2 (1:2) and sample C3 (1:3) at ≤0.5% level ($t = 2.712^{*}$). The significant difference on zone of Inhibition was observed between Green tea extract flavoured with *mint* was sample M1 (1:1) and sample M2 (1:2) at ≤0.5% level ($t = 2.739^{*}$), between sample M1 (1:1) and sample M3 (1:3) at <0.5% level ($t = 3.796$), between sample M2 (1:2) and sample M3 (1:3) at ≥0.5% level ($t = 2.390^{*}$), respectively. Hence, the minimal inhibitory concentration effectiveness of the extracts against the oral pathogens shows a significant result which reveals that, green tea extracts flavouring with *ginger*, *cloves* and *mint* produced 1% significance and ≥0.5% significances for using these extracts effectively against dental pathogens to reduce caries and other infections caused by them. From the mean differences we can understand that green tea extract flavoured with *ginger* (G1 = 15.1667, G2 = 16.8333, G3 = 18.6667), *Cloves* (C1 = 15.8333, C2 = 18.1667, C3 = 19.0000) and *Mint* (M1 = 15.8333, 16.8333, 18.1667) are very effective at 1:3 ratio of the extracts. Hence, this study reveals that the flavoured extracts with 1:3 ratio concentration was found to be much effective in controlling the growth of the oral pathogens studied here and it can be used in many oral applications for preventing caries and other oral infections caused by dental pathogens.

4. Discussion

Recent human studies suggest that green tea may contribute to a reduction in the risk of cardiovascular disease and some forms of cancer, as well as to the promotion of oral health and other physiological functions, such as antioxidant and antibacterial activities, antihypertensive effect and body weight control [5]. Oral pathologies such as dental caries, periodontal diseases and teeth loss can greatly influence human health. Amongst those, dental caries is caused as a result of infectious diseases caused by numerous reasons related to nutrition and bacterial infections. There are reports that tea consumption may decrease dental caries in humans and laboratory animals. A study found that mouth rinsing with green tea extract (0.61%) protected from erosion and abrasion of the tooth dentine similarly to mouth rinsing with fluoride extract (250 ppm) or chlorhexidine extract as found in oral hygiene products [6–9]. Green tea has many well-known effects and benefits. It has anti-diabetic, hypocholesterolemic, anti-inflammatory, anti-carcinogenic, anti-cavity, thermogenic, probiotic, antimicrobial and antiviral properties [14]. Green tea's antimicrobial properties are effective against a variety of microbes, which include *Helicobacter pylori* (gastric malignancy), *S. aureus* (MRSA), *Oral streptococci* (dental caries), *Mycobacterium tuberculosis* (tuberculosis), *Bacillus cereus* (food poisoning), *Escherichia coli O157* (severe diarrhea and kidney failure), *Legionella pneumophila* (pneumonia), *Candida albicans* (candidiasis), and *Chlamydia trachomatis* (chlamydia) [15]. Starting in China, the green tea craze has expanded worldwide to become the second most consumed drink after water. This increase in popularity is in part due to the increasing awareness of green tea's many health benefits [14]. Green tea is widely available and is often processed in a way that it is preserved for a great length of time. By making green tea more available there is a potential for a cascading effect to occur resulting in many positive changes, such the reduced reliance on commercial drugs and the reduced incidence and prevalence rates in high cholesterol, diabetes, cancer, cavities, and infections. A case report conducted by Molinari et al. [15] revealed that a 44-year-old female of European descent suffering from worsening acute liver failure was participating in a weight-loss program for 6 months that recommended increased physical activity. The intake of dietary supplements containing green tea extract with a concentration of 720 mg/day which resulted in a gradual weight loss and removal of toxics from the system due to infections. It was also found that there was no any adverse consequences of drinking green tea, suggesting that it is most likely safe to consume green tea as a beverage with little to no risk. To date there has not been any study of flavouring the green tea extract with our traditional medicinal plant ingredients such as *Ginger*, *Cloves* and *Mint* used in combination with different ratios against oral pathogens to test their inhibition and effectiveness to prevent caries. Hence, the present study shows that the green tea extract flavoured with *Ginger*, *Cloves* and *Mint* inhibits the activity of six specific oral pathogens isolated from the clinical dental samples significantly at different concentrations of the extracts used. The *ginger*, *cloves* and *mint* have better inhibiting capacity at the higher ratio concentration at 1:3 when compared to lower concentrations (ie., at 1:1, 1:2 levels). At a significant value of ($t = 1\%$) the zone of inhibition is found to be more effective than <0.5% significance range for different extracts. Thus, this work proves that Green–tea extract along with the natural ingredients like *ginger*, *cloves* and *mint* can be used effectively for the treatment of Dental Caries in humans and as well as other associated infections with these organisms.

5. Conclusion

Green tea being a beneficiary on oral health and its administration towards oral health problems such as dental cavities, periodontal diseases and halitosis leads to a conclusion with numerous studies worldwide. However, the regular use of green tea in our regular diet not only improves the antioxidant properties of health but also helps in preventing the aforementioned oral infections to reduce the bacterial activities to maintain health oral habit. To summarize our research conducted on the effects of green tea extracts enriching it with natural components like *mint*, *cloves* and *ginger* in combinations to improve the efficacy level of treatments for the oral infections. On the other hand, a benefit of using green tea in our diet helps us to prevent the risk of getting oral cancer but still clinical studies are required to ascertain the benefits of using it in prevention of oral infections effectively. Hence, this pilot

study analysis revealed a significant interpretation with Green tea extract flavored with *Ginger*, *Cloves* and *Mint* for an effective use as an anti-cariogenic agent.

Conflicts of interest

All authors declared that there are no conflicts of interests with regards to this study or in publishing this research article. The article contains no dealings with human or animals subjects for any analysis, only the dental caries samples were collected directly from dentists.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yclnex.2018.12.002.

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