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RESEARCH ARTICLE
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Anticariogenic Anti Inflammatory and Cytotoxic Effects of Mouthwash Prepared Using Oolong Tea and Triphala Formulation

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ABSTRACT

Background: Oolong tea has remarkable health benefits despite it not being the most well-liked supplement. It can help with blood sugar management and weight loss, as well as prevent cancer, cardiovascular disease, and age-related cognitive decline. It could play a part in preventing cancer. Since ancient times, triphala has been utilized in traditional Ayurvedic medicine as a multipurpose remedy for symptoms ranging from stomach aches to tooth decay.

Aim: To assess the anticariogenic, anti-inflammatory and cytotoxic effects of mouthwash prepared using Oolong tea and Triphala formulation.

Materials and methods: An in vitro study was conducted comparing standard mouthwash with the mouthwash prepared using oolong tea and triphala formulation.The anti-inflammatory activity of the mouthwash was determined by EA assay and BSA assay and anti- cariogenic property was determined by zone of inhibition. The cytotoxicity was determined by brine shrimp lethality assay.

Results: The results showed that as the concentration of the mouthwash increases the percentage of inhibition. The cytotoxicity was determined by testing the mouthwash on brine shrimps.The various concentrations were 5,10,20,40 and 80 micrometers.The values noted were 8,7,7,6 and 5 respectively. The number of live nauplii present were calculated by using the following formula. Number of dead nauplii/number of dead nauplii+number of live nauplii x100.

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Conclusion: It can be concluded that the mouthwash prepared using oolong tea and triphala show strong anti cariogenic, anti inflammatory and cytotoxic effects.

Keywords: *Oolong tea, Triphala, Mouthwash, anti inflammatory, anti cariogenic, cytotoxic*

INTRODUCTION

Herbal mouthwash is a natural alternative to conventional mouthwashes that is gaining popularity due to its potential health benefits and lack of harsh chemicals. Herbal mouthwashes are made from natural ingredients such as herbs, essential oils, and plant extracts, which have been traditionally used in various cultures for oral hygiene and medicinal purposes. These ingredients are believed to have anti-bacterial, anti-inflammatory, and antioxidant properties that may promote healthy gums and teeth, freshen breath, and prevent oral diseases. Despite the increasing use of herbal mouthwashes, there is limited scientific evidence to support their efficacy and safety. Therefore, there is a need for research to evaluate the effectiveness, safety, and mechanism of action of herbal mouthwashes to provide evidence-based recommendations for their use. This study aims to investigate the efficacy of herbal mouthwash in improving oral health outcomes compared to conventional mouthwashes and to identify the active ingredients and mechanisms of action of herbal mouthwashes. The findings of this study may have significant implications for improving oral health and reducing the use of chemicals in oral hygiene products.

Numerous species and endogenously wild tea trees have been found in primitive woodlands in Yunnan province, supporting the theory that the tea plant, Camellia sinensis or Camellia assamica, originated in the hilly region of southern China((GREEN TEA, BLACK TEA AND SEMI-FERMENT...)). Oolong tea, a semi-fermented tea with its own distinct scent and flavor among the six basic types of tea (white, green, oolong, yellow, black, and dark teas) from *Camellia sinensis*, has become a popular beverage as proven by the increasing production. Chinese oolong tea leaves have a very high amount of polymerized polyphenols.((Hosoda et al. 2000)). Oolong tea is a plant that is native Fujian province in southeast China.((Mendez 2016)).Modern pharmacological research demonstrates how oolong tea excels in preventing obesity and managing diabetes in terms of health benefits.

In oolong tea, Epigallocatechin-3-gallate inhibits the growth of malignant cells. It is also helpful in reducing obesity.(Ng et al. 2018)). This plant can grow upto 1900 meters in height.According to studies, oolong tea has anti-obesity and hypolipidemic properties.(Ng et al. 2018; Han et al. 1999)

Globally, the number of degenerative physiological changes connected to aging is rising. Nearly all essential body systems are affected by these geriatric disorders. In the Ayurvedic system of Indian medicine, the rejuvenating and preventative therapy known as Rasayana therapy deals with the prevention, amelioration, and treatment of geriatric illnesses by raising bodily immunity generally, combating infections and antigens, and preventing carcinogenic mutations. Triphala is a plant that is native to India. It has been used in traditional ayurvedic medicine since ancient times as a multi purpose treatment for symptoms ranging from dental caries to stomach ailments.*Emblica officinalis* (Amalaki), *Terminalia bellerica* (Bibhitaki), and *Terminalia chebula* (Haritaki) fruit are the main ingredients of triphala, a well-known and very effective polyherbal Ayurvedic medicine. Triphala is used to treat digestive and rejuvenative issues. (Analytical determination of triphala ...)

Triphala is also believed to promote longevity and promote overall health.Additionally, research has revealed that triphala has a similar impact to chlorhexidine in reducing dental caries. Many of the ethnomedical claims have been supported by scientific research conducted in the last two decades, and studies have revealed that Triphala has anti-inflammatory, antipyretic, analgesic, antibacterial, wound-healing, anticariogenic, antistress, hypoglycaemic, anticancer, chemoprotective, and chemopreventive properties.

Thus this study aims to find out the anti cariogenic, anti inflammatory and cytotoxic

effects of mouthwash prepared using Oolong tea and Triphala formulation.

Our team has extensive knowledge and research experience that has translated into high quality publications(Vishnu Prasad et al. 2018; Ramesh Kumar et al. 2011; Ganapathy et al. 2022; Arumugam et al. 2021; Mohanavel et al. 2020; Muthukrishnan 2021; Chellapa et al. 2020; Markov et al. 2021; Felicita 2017; Uthrakumar et al. 2010)

MATERIALS AND METHODS

Preparation of herbal extract of oolong and triphala

Oolong tea and triphala(a blend of three fruits - amla, haritaki, and bibhitaki) were obtained and dried. 2 grams of oolong tea and 1 gram of triphala was taken for the preparation of mouthwash. 100 ml of distilled water was taken and to this the oolong tea and triphala was added. This was next heated in a heating mantle for 15 to 20 minutes.After the heating process was done it was then filtered and extract was obtained.

The oolong tea and triphala extract that was obtained was then heated again. This time it was condensed from 100 ml to 5ml.

Formulation of herbal mouthwash

For the preparation of the mouthwash, initially 0.3 gm of sucrose and 0.01gm of sodium lauryl sulfate and then 0.001gm of sodium benzoate was all mixed in 8 ml of distilled water and 2ml of the oolong tea and triphala extract. The standard that was used to compare the final results was chlorhexidine gluconate.

Evaluation of cytotoxicity using Brine Shrimp Lethality Test

At the end of 24 h, wells of the microtiter plates were inoculated with oolong-triphala extract of 0µL, 5µL, 10µL, 20µL, 30µL, 50µL concentrations. Mouthwash at concentrations 0µL, 10µL, 20µL, 30µL, 40µL, 50µL was added in another microtiter plate. In one of the wells in both plates, a sterile salt solution was used as control. A. nauplii were collected in a petri dish from the cylindrical jar. 10 hatched nauplii were collected using a dropper and added in each well in both plates. They were counted using a magnifying glass. This was cross checked by another researcher to avoid any error. At the end of 24 h, nauplii in each well in each of the microtiter plates were counted.(Solanki et al. 2021)

Anti inflammatory activity

Egg albumin assay

The approach provided by (Bhattacharya et al. 2012) was modified to investigate the compounds' in vitro anti-inflammatory activity. Fresh hen eggs were used to extract the egg albumin. By serial dilution, different concentrations of the separated chemicals and diclofenac) were created at concentrations (3.9-500 g/mL). The test tubes were filled with 5 mL of the reagent combination, which included 2 mL of compound/standard medication reconstituted with 2% tween 80 in double-distilled water, 0.2 mL of egg albumin, 2.8 mL of phosphate buffered saline (PBS, pH 6.4), and 5 mL of the reagent mixture. Water that had been twice-distilled served as the control (blank). The mixtures were progressively combined by shaking the test tubes, then incubated at 37 C for 15 min., followed by 5 min. in a water bath heated to 70 C. After cooling the mixture, 200 L of it was pipetted into each well of a 96-well plate, and the absorbance at 660 nm was measured. The % inhibition of protein denaturation was evaluated by calculating denaturation in control as 100 % and calculated by using the formula:

% inhibition= 1⁄4 100 (At/Ac - 1)

(At 1⁄4 absorbance of the test sample, Ac 1⁄4 absorbance of the control sample.) (Analytical determination of triphala ...; Anokwah et al. 2022)

The various concentrations were 10µl, 20µl, 30µl,40µl and 50µl.

Bovine Serum Albumin assay

The oolong-triphala mouthwash extract was subjected to bovine serum assay. It was set to five different concentrations of 10 mL, 20 mL, 30 mL, 40 mL, and 50 mL in a solution containing 1 mL of BSA. The solution was then kept at room temperature for 10 minutes, boiled for 10-15 minutes at 55 °C, and then spectrophotometry

was used to determine the inhibitory level(Mohapatra et al. 2020) .When heated, BSA denatures and starts to show antigens connected to Type III hypersensitivity reaction, which are linked to conditions like rheumatoid arthritis, glomerulonephritis, serum sickness, and systemic lupus erythematosus. 400 l of plant crude extract were combined with 2 mL of 1% bovine albumin at a pH of 6.8 and 1N HCl, and the mixture was then heated in a water bath for 20 minutes at 55°C before being cooled to room temperature. The absorbance value was measured at 660 nm. Dimethyl sulfoxide was used in place of an equivalent number of ZrO2 nanoparticles reinforced with clove and cardamom as a control. As benchmarks, various diclofenac sodium concentrations were utilized.(Mohapatra et al. 2020; Selvaraj et al. 2021)

Assessment of Antioxidant activity using DPPH assay

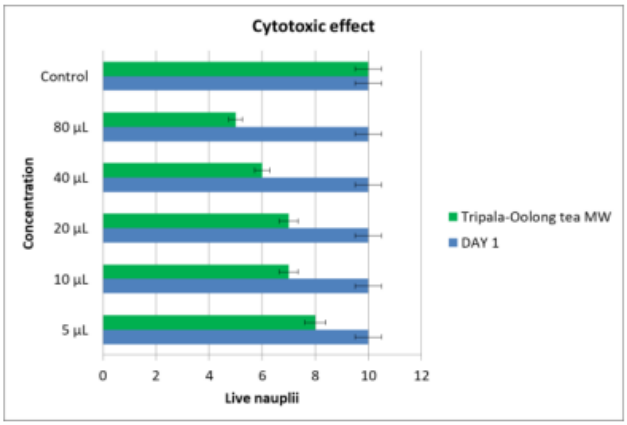
The antioxidant capacity of the extracts and solvent fractions was analyzed by measuring their free radical scavenging activity, using the DPPH radical scavenging assay.

The antioxidant capacity of the mouthwash containing oolong-triphala extract was examined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Various concentrations of mouthwash containing oolong-triphala extract (10 ml, 20 ml, 30 ml, 40 ml, and 50 l) were combined with 450 ml of 50 mMTrisHCl buffer (pH 7.4) and incubated for 30 minutes. The number of DPPH free radicals was reduced after incubation, and this was determined using the wavelength of 517 nm.((Jibu et al. 2021))

Statistical analysis

Perform statistical analysis using one-way ANOVA followed by Tukey's post hoc test. Consider $p<0.05$ as statistically significant.

RESULTS



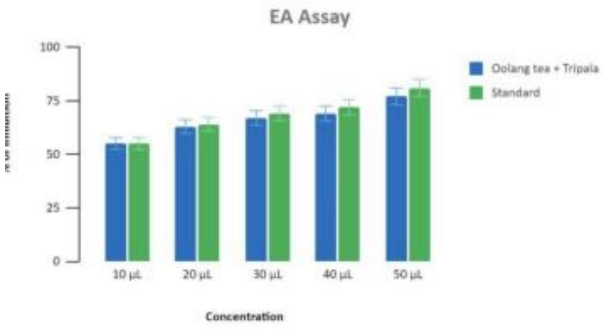


FIGURE 1: The cytotoxic effects can be measured using live nauplii. The concentrations are then compared with the number of live and dead brine shrimps.

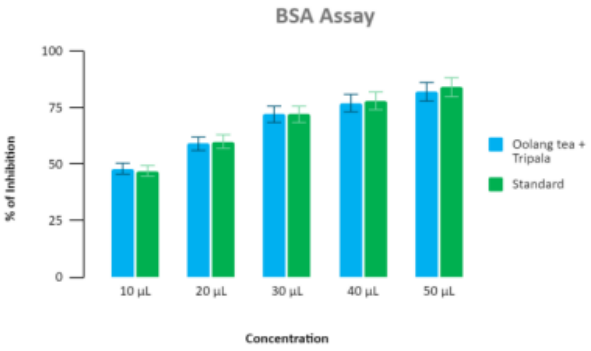


FIG 2: The bovine serum assay shows the percentage of inhibition against the various concentrations of oolong-triphala mouthwash extract taken. This shows that at various concentrations the anti-inflammatory property of the extract prepared has shown significantly good results.

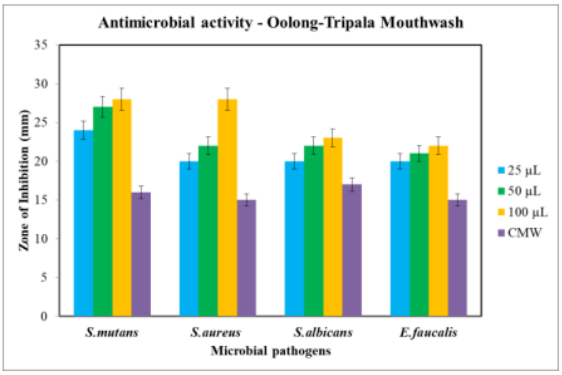


FIG 3: This graph shows the antimicrobial activity and the results were compared with the standard chlorhexidine gluconate. The zone of inhibition was seen to be 25 microlitre, 50 microlitre and 100 microlitre. It was 24,27 and 28 for S. mutans respectively. For S. aureus it was 20,22 and 28 respectively. 20,22 and 23 in the case of S.albicans and 20,21 and 22 for S. faecalis

DISCUSSION

Oolong tea has been demonstrated to have antibacterial characteristics that can help decrease plaque and prevent tooth decay. It also includes high quantities of antioxidants. Oolong tea can be used as a mouthwash to help decrease oral bacteria and support healthier teeth and gums. Triphala is a herbal remedy that has been utilized for ages in conventional Ayurvedic treatment. The three fruits used to make it are bibhitaki, haritaki, and amla. Triphala's anti-inflammatory and antibacterial effects have been demonstrated to help reduce oral irritation and guard against gum disease. Triphala mouthwash may also aid in breath freshening.

In the present study, we have obtained results that show the percentage of inhibition vs concentration in which the concentration of oolong tea and triphala mouthwash was considered.They were taken for various concentrations. 10µl, 20µl, 30µl, 40µl and 50µl. The percentage of inhibition increases when the concentration of the mouthwash increases. Oolong tea and Triphala are natural ingredients that have been used for centuries in traditional medicine for various health benefits. There is some evidence to suggest that mouthwash prepared using Oolong tea and Triphala may have anti-cariogenic, anti-inflammatory, and cytotoxic effects.

Cytotoxic Effect: Oolong tea and Triphala have been found to have cytotoxic effects on various cancer cell lines in laboratory studies. These compounds may have the potential to inhibit the growth and spread of cancer cells in the oral cavity. However, more research is needed to confirm these findings. Evaluation of acute toxicity of triphala herbal formulation research conducted by (Srivastava et al. 2018) This study investigated the acute toxicity of a triphala herbal formulation in rats. The results showed that the formulation was safe and did not cause any significant toxicity or adverse effects.

Anti-cariogenic Effect: Studies have shown that Oolong tea and Triphala contain compounds that can inhibit the growth of *Streptococcus mutans*, a bacterium that is a major cause of tooth decay. A mouthwash prepared using these ingredients may help prevent the formation of dental caries and promote good oral health. A previous research conducted and evaluated the efficacy of a triphala mouthwash in reducing dental caries in school children. The results showed that the triphala mouthwash significantly reduced the number of caries in the treatment group compared to the control group.(Tandon et al. 2010)

Anti-inflammatory Effect: Oolong tea and Triphala contain polyphenols and flavonoids that possess anti-inflammatory properties. These compounds may help reduce inflammation in the oral cavity and prevent the development of gum disease. A mouthwash prepared using these ingredients may help soothe inflamed gums and promote overall oral health. This study investigated the efficacy of a triphala mouthwash in reducing inflammation and improving gum health in patients undergoing scaling and root planing(Chowdhury et al. 2016) The results showed that the triphala mouthwash significantly reduced inflammation and improved periodontal health compared to a control group. The results of another research showed that oolong tea reduced inflammation and oxidative stress in the lungs, suggesting that it may have potential as an anti-inflammatory agent.(Huang et al. 2021)

Overall, a mouthwash prepared using Oolong tea and Triphala may have potential benefits for oral health. However, more research is needed to fully understand the effects of these ingredients. The objective of the study was to ascertain the effects of a mouthwash prepared with triphala and

oolong tea on dental plaque, gingival inflammation, and microbial growth and compare it with the commercially available mouthwash. Similar results were obtained in our study, which proved that there are strong anti-inflammatory compounds present in both oolong tea and triphala.

This is one of the first researches in which oolong-triphala combination herbal extract is subjected to brine shrimp lethality test to check its cytotoxicity. However, the limitations of this research cannot be expected that the outcomes of cytotoxicity, anti-inflammatory activity, and antibacterial activity might be converted into therapeutic effectiveness because the study was done in vitro. To confirm the effectiveness of this herbal mouthwash, more research should be done.

CONCLUSION

This study concludes that the mouthwash prepared using oolong tea and triphala has anti carcinogenic, anti inflammatory and cytotoxic effects. Its properties can be used to treat various periodontal diseases.

FUTURE SCOPE

The results from this study show that oolong tea and triphala combination mouthwash has potential use in treating oral diseases.Oolong tea and Triphala are known for their anti-inflammatory and antioxidant properties. Incorporating these properties into a mouthwash formulation could help reduce oral inflammation and oxidative stress, which are associated with various oral health conditions.

Inflammatory oral conditions: Oolong tea and Triphala have demonstrated anti-inflammatory properties individually. Investigating the combined effects of these two ingredients in managing inflammatory oral conditions such as gingivitis, periodontitis, or oral mucositis could provide valuable insights into their synergistic anti-inflammatory potential.

Inflammation plays a crucial role in oral wound healing. The combination of Oolong tea and Triphala's anti-inflammatory effects could be explored for their potential in enhancing oral wound healing processes, including post-surgical or traumatic oral tissue injuries.

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