Assessment Of Antimicrobial Activity And Cytotoxicity Of Green Tea, Stevia And Fresh Coriander Mouthwash - A Herbal Formulation

Research Article

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Abstract

Introduction: The human mouth is colonised by 700 to 1000 different microbial species. Oral microorganisms are involved in the onset and progression of oral diseases such as dental caries, periodontal disease, and oral cancer. Meanwhile, oral microorganisms can enter the bloodstream via a damaged oral mucosa and cause a rise in systemic antibody levels, increasing the risk of a variety of cardiovascular diseases. Green tea (botanical name *Camellia Sinensis*) from the Theaceae family has been studied in recent years for its beneficial effects on oral health. The use of safer herbal products can aid in the reduction of bacterial activity in the oral cavity, which in turn can aid in the reduction of the aforementioned oral afflictions.

Materials and Methods: The cytotoxicity of mouthwash reinforced with Green tea, stevia and coriander extract was assessed using Brine shrimp assay. 12 well ELISA plates were taken and to each plate 6-8 ml of saltwater was added; followed by adding 10 nauplii to each well. Green tea, stevia and coriander extract was added to each well at different concentrations and was then incubated for 24 h.

Results: The zone of inhibition increased with the concentration of the plant formulation. The highest ZOI was noticed with streptococcus mutans showing 32mm of ZOI at 100µg/ml, while the lowest ZOI for candida albicans showing 20mm at 100µg/ml. The number of live nauplii was highest in 20µL whereas the lowest was noticed in the well where a concentration of 80µL was administered.

Conclusion: The presence of toxic chemicals in mouthwashes tend to irritate the mucosa of the oral cavity in the long run. Substituting these with naturally obtained extract can reduce toxic levels.

Keywords: Herbal Mouthwash; Green tea; Stevia; Coriander; Dentistry.

Introduction

Trillions of microbes exist in the human body, and the oral cavity is one of the most abundant sources of microbes. The human mouth is colonised by 700 to 1000 different microbial species. Oral microorganisms are involved in the onset and progression of oral diseases such as dental caries, periodontal disease, and oral cancer. Meanwhile, oral microorganisms can enter the bloodstream via a damaged oral mucosa and cause a rise in systemic

antibody levels, increasing the risk of a variety of cardiovascular diseases. As per the World Health Organization, cavities directly impact 60 to 90 percent of school-aged children and nearly 100 percent of adults worldwide [1].

Several methods have been used to reduce or eliminate dental biofilms. Mechanical disruption of dental plaque biofilms, such as toothbrushing, is the most commonly used approach worldwide. However, the effectiveness of this is limited. As a general rule,

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chemotherapeutic strategies to disrupt these biofilms, such as the use of mouthwashes, have been considered. These have become commonplace in recent decades, usually following mechanical plaque biofilm control. There is currently a wide variety of over-the-counter mouthwashes available, each containing a different active ingredient for each of these specific indications [2].

Green tea (botanical name *Camellia Sinensis*) from the Theaceae family has been studied in recent years for its beneficial effects on oral health. It is available as a shrub or as an evergreen tree. Polyphenols, the most interesting constituent, have been shown to contribute one-third of the bioactive compounds in green tea. Catechins (flavan-3-ols), also known as tannins, are by far the most popular type of polyphenol. They serve as an astringency constituent [3]. Green tea contains four catechins: epicatechin gallate (ECg), epicatechin, epigallocatechin, and epigallocatechin gallate (EGCG). Carotenoids, tocopherols, ascorbic acid, and minerals such as chromium, magnesium, selenium, and zinc are also found in green tea. The endoplasmic reticulum and mitochondria produce oxygen, which is then converted into hydrogen peroxide, resulting in the formation of reactive oxygen species molecules. These reactive oxygen species molecules can cause DNA and RNA damage, oxidise proteins (enzymes, histones), oxidise lipids, and even cause cell suicide. Green tea consumption can prevent all of these degenerative changes by inhibiting the action of the reactive oxygen species molecule [4, 5].

Coriander (*Coriandrum sativum* L.) is a member of the Apiaceae family. *Coriandrum sativum*, a native of Italy, is now grown in Central and Eastern Europe, Morocco, Malta, Egypt, China, Pakistan, India and Bangladesh [6, 7]. Coriander leaves and seeds are widely used in folk medicine as a cholesterol-lowering agent, digestive stimulant, and anti-hypertensive agent, as well as a seasoning in food preparation. *C. sativum* pharmaceutical applications have also revealed antibacterial, antioxidant, hepatoprotective, and anticonvulsant properties. *C. sativum* essential oil is shown to have a strong antifungal effect against *Candida* species [8].

Stevia rebaudiana is a form of cane sugar. Bertoni is a small perennial shrub native to Paraguay, Brazil, and Argentina in the Asteraceae (Compositae) family. For centuries, native groups have used the leaves of this plant in medicines and to sweeten drinks. It is well documented that sucrose consumption causes a postprandial increase in blood glucose and insulin. In three randomised controlled trials, purified steviol glycosides used in reduced-sugar/calorie meals or as a supplement reduced postprandial blood glucose significantly in both healthy and diabetic subjects. Hence use of stevia by diabetic patients can be a safe alternative [9, 7].

The use of natural and herbal products is a very old concept. Humans have used these products to treat a variety of diseases, and they have grown in popularity as a result. In terms of oral care, the most commonly used herbal product is "Miswak," which has gained prominence in a number of communities as an effective, inexpensive, and efficient method of maintaining oral hygiene. The goal of this publication is to summarise the research on green tea's anti-bacterial effect, coriander's anti-fungal effect, and their cytotoxicity. The use of safer herbal products can aid in the reduction of bacterial activity in the oral cavity, which in turn can aid in the reduction of the aforementioned oral afflictions.

Materials And Methods

Preparation of Green tea and stevia

One gram of Green tea and stevia powder were added to 100ml of double distilled water and placed on a shaker overnight. The mixture was boiled at 70 degree Celsius until bubbles appeared. Phytochemicals present in these powders get activated by this process. The extract was filtered using Whatmann Filter paper 1 and stored at lower temperature for further use.

Preparation of Coriander extract

A total of 25 grams of fresh coriander leaves were added to the green tea, stevia mixture and , boiled at 70 degree Celsius for 15 minutes and filtered. The filtered extract was reduced to 10 ml concentration and stored at low temperatures. Exact measurement of 0.3g of Sodium Lauryl Sulfate and sucrose , 0.001 g of sodium benzoate were added to the final extract.

Antimicrobial activity

Fresh bacterial cultures were prepared, in Hi-Veg broth medium, where 10ul cultures of *C.albicans*, *S. mutans*, *S. aureus* and *E.faecalis* were inoculated, and incubated for 18 h, in a shaker. A nutrient agar medium was prepared and 5 mm wells were made, with different concentrations (25–75 µg/ml) of extract, along with the positive control ampicillin antibiotic disks. The plates were incubated for 18h, at 37 °C and the zones of inhibition were measured.

Cytotoxicity activity

The cytotoxicity of mouthwash reinforced with Green tea, stevia and coriander extract was assessed using Brine shrimp assay. 12 well ELISA plates were taken and to each plate 6-8 ml of saltwater was added; followed by adding 10 nauplii to each well Green tea, stevia and coriander extract. was added to each well at different concentrations (5 µL, 10 µL, 20 µL, 30 µL, 50 µL) and was then incubated for 24 h. After 24 h, the total number of live and dead nauplii was counted and the mortality rate was checked.

Results And Discussion

Antimicrobial activity

The formulated Green tea, Coriander and Stevia extract were evaluated for its antimicrobial activity against oral pathogens; *candida albicans*, *streptococcus mutans*, *staphylococcus aureus* and *enterococcus faecalis*. The antimicrobial activity of Herbal mouthwash was evaluated using the zone of inhibition (ZOI) against microbial cultures. The zone of inhibition increased with the concentration of the plant formulation. The highest ZOI was noticed with *streptococcus mutans* showing 32mm of ZOI at 100µg/ml, while the lowest ZOI for *candida albicans* showing 20mm at 100µg/ml. The zone of inhibition against the evaluated pathogens are summarized in table 1 and depicted in figure 1 and 2.

Table 1. The zone of inhibition against the evaluated pathogens.

Concentration	Zone of inhibition (pathogens)			
	enterococcus faecalis	staphylococcus aureus	streptococcus mutans	candida albicans
25 μ L	24	9	19	9
50 μ L	25	17	26	12
100 μ L	30	26	32	20
Control	27	11	22	14

Figure 1. The herbal mouthwash mediated zone of inhibition against the evaluated pathogens inoculated into 5mm wells.

Figure 2. The graphical representation of herbal mouthwash mediated zone of inhibition against the evaluated pathogens inoculated into 5mm wells.

Figure 3. The graphical representation of Herbal mouthwash mediated Cytotoxicity using Brine Shrimp Lethality Assay.

Cytotoxicity using Brine Shrimp Lethality Assay

The nauplii were exposed to different concentrations (5, 10, 20, 40 and 80 μ L) of Herbal mouthwash and left undisturbed for a time period of 48 hours. The number of live nauplii alive after 24 hours in each well inoculated with various herbal mouthwash was noted for determining the level of cytotoxicity and a chart was formulated (Figure 3). The number of live nauplii was highest in 20 μ L whereas the lowest was noticed in the well where a concen-

tration of 80 μ L was administered.

The present study revealed that the highest ZOI was noticed with Streptococcus mutans showing 32mm of ZOI at 100 μ g/ml, while the lowest ZOI for candida albicans showing 20mm at 100 μ g/ml. A study conducted by Alipour et al; 2018, who assessed the anti-microbial activity for Candida albicans, Lactobacillus acidophilus, Streptococcus mutans, Staphylococcus aureus for Herbal Mouthwash Containing Oak Husk of Quercus brantii and Zataria multiflora, showed significant efficacy in Candida albicans

and *Staphylococcus aureus* than Persica mouthwash while similar efficacy was noted in *Streptococcus mutans* [10]. Another study conducted by Manali Deb Barma et al. demonstrated that silica nanoparticles have a maximum zone of inhibition for *S. aureus* and *E. faecalis* at a concentration of 100 µg/ml [11, 12]. However in the present study, since the formulation for the mouthwash is different using green tea and coriander, the ZOI could have been different. Because each compound has different physical and chemical properties, the antimicrobial activity of different compounds varies with different organisms.

The nauplii alive at 5 µl, 10 µl, 15 µl were 10, which was the highest and the lowest was 2 nauplii alive at 80 µl concentration. The brine shrimp lethality assay is being used to assess the preliminary cytotoxicity of plant extracts based on their ability to kill laboratory cultured larvae (nauplii). It is a precise, low-cost method that necessitates only a small amount of test content. The organism most commonly used in the Brine Shrimp Lethality Assay is *Artemia salina* [13]. During the study period, the nauplii do not receive any food. Malnutrition-inoculated material may be causing the nauplii to die. To ensure that the subject under study has a mortality impact, a control sample containing nauplii but no inoculation of the research material is used. Nauplii can survive for up to 48 hours without food because they still feed on their yolk sac. Brine Shrimp Lethality The number of live Shrimp larvae was lower at higher concentrations than at lower concentrations, and as the concentration of the Herbal Mouthwash increased, so did the cytotoxicity, resulting in a lower number of shrimp larvae still alive after 48 hours.

The majority of mouthwashes on the market contain alcohol as well as other chemicals such as chlorhexidine gluconate and triclosan. These chemicals have a wide range of side effects, from taste disturbance to allergic contact stomatitis. Nontoxic herbal mouthwashes containing various herbs and plant extracts have been introduced to combat such side effects [14]. The increasing use of mouthwashes for extended periods of time has prompted research into the development of effective and safe products, with a greater emphasis on herbal drugs. Plant extracts can be used instead of chlorhexidine digluconate because their polyphenols compounds have antimicrobial properties [15, 16].

Conclusion

The present study is first of its kind to incorporate Green tea, Stevia and Coriander in a mouthwash, with minimal literature evidence. The presence of toxic chemicals in mouthwashes tend to irritate the mucosa of the oral cavity in the long run. Substituting these with naturally obtained extract can reduce toxic levels. The extract was subjected to antimicrobial activity, which yielded good results, proving that use of herbal products can fight against the

oral pathogens warranting more research in this field.

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