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Evaluation of the Stability, pH, Density and Sedimentation of Green Tea and Green Tea Plus Ginger Mouthwash: A Phytochemical Study

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Abstract

Introduction: Dental plaque is the chief pathologic agent for the commencement of gingivitis. Diseased gingiva if left untreated may result in a compromised periodontium. The progression of gingival diseases can be controlled by regular plaque control. The sole use of mechanical aids in controlling plaque does not always suffice. Using chemical plaque control aids along with the conventional mechanical methods have shown better efficiency in elimination of plaque. Hence a need for adjunctive chemical plaque control is advisable for complete removal. Various new chemical formulations have been tried and tested.

Aim: To assess the viability of a Green tea and a new Green tea plus Ginger mouth rinse formulations. The parameters that were assessed were: pH, sedimentation, density, and stability of the formulations.

Methodology: The pH of the mouthwashes were checked using digital pH meter, Sedimentation was evaluated using the centrifugal machine. Organoleptic parameters that is color, odour and consistency was assessed by visual tactile assessment; And density was checked using the dry pycnometer method.

Results: The green tea and green tea plus ginger mouthwash showed stable characteristics, product quality irrespective of experimental time intervals.

Conclusion: Formulation of a mouthwash containing green tea and green tea plus ginger was stable at the end of one month interval. Hence it can be used for further *in vivo* studies.

Keywords: Herbal mouthwash; Green tea; Ginger; Phytocemical Stability

Introduction

Gingivitis is caused mainly by dental plaque which is responsible for its initiation [1]. Diseased gingiva if left untreated may result in a compromised periodontium [2]. This progression towards the breakdown of periodontium can be controlled by the means of plaque control. Mechanical plaque control remains the standard therapy for plaque removal.

However, using just the mechanical means of plaque control like brushing and flossing are not completely efficacious [2]. Moreover, mechanical removal of plaque, the bacteria that are present in the soft tissue can re-colonise as they are not removed [3].

For this purpose the adjunct use of chemical plaque control agents have shown a synergistic effect and better control of plaque and gingival inflammation. A number of chemical formulations have been tested for enhancing oral health [3]. These can be available in several forms such as: chewing gums, gels, chips, dentifrices and mouthwashes.

Mouthwashes can be a safe and effectual delivery system for plaque reduction. However, the alcohol content of these rinses and their unpleasant taste is undesirable to some patients. No rinse amongst them is without any disadvantages, hence the search for an ideal and effective mouth rinse still continues.

India is well heeled with natural herbal agents who have been used for treating several diseases. Sadly, the use is very restricted due to limited product testing [4]. Tea is obtained from the leaves of a plant called the “Camellia sinensis” [4]. There are three

types of tea available presently which is based upon the level of its fermentation. These are: 1. Green tea which is non-fermented, 2. Oolong tea which is partially fermented and lastly 3. Black tea that is completely fermented [5]. Green tea polyphenols restrain the growth of bacteria causing periodontal breakdown and other oral bacteria thereby preventing dental caries, halitosis, gingivitis, and Periodontitis [6].

Likewise the anti-inflammatory properties of ginger have been known and esteemed for quite a long time. Early in 1970s the prostaglandin inhibitory properties of ginger was discovered, which got reaffirmed with passing time. Ginger functions by suppressing prostaglandin synthesis by inhibiting the cyclooxygenase-1 and cyclooxygenase-2.

The present research was conducted with the purpose to combine Green tea and Ginger both which are totally herbal products and observe its effect on plaque progression. The following study was a pilot in vitro study conducted on a smaller scale prior to an in vivo study, as we need to evaluate the stability of these mouthwashes before we utilize them for an in vivo study in children. So an in vitro study was planned to evaluate the stability and various other parameters for a period of one month, which are required for a mouth rinse.

Hence the aim of the present study was the evaluation of the stability (in terms of organoleptic parameters like color, odour, consistency and brightness), sedimentation rate, density and pH of Green tea and Green tea plus Ginger mouthwash.

Materials and Method

Dried green tea leaves and fresh ginger were obtained to prepare the mouthwash. Green tea mouthwash was 100% (w/v) green tea containing, while the green tea plus ginger mouthwash had 50% (w/v) green tea and 50% (w/v) green tea plus ginger.

Initially the Green tea extract was prepared. For this purpose the dried green tea leaves (100gm) were soaked into 500 ml of methanol solution for two days. The solution obtained was then strained by a strainer and shifted to a glass plate. The plates were left at room temperature for a time of three to four days. Scraping out of the crystal powder of extract from the plates was then carried out. Similarly green tea plus ginger mouthwash was prepared in the same way but by adding 50% green tea leaves and 50% ginger in the extract. After extraction, the extract was filtered and taken to the vacuum evaporator to remove the solvent.

The mouth rinse was then prepared.

Mouthwash preparation

The extract obtained for both the mouthwashes was scraped off from the plates and mouthwash was prepared by adding 3 gm of each extract, 0.12 gm sodium saccharin, and 1litre distilled water was added in each mouthwash. Once 3gm extract was added with 1 litre distilled water, the mixture was stirred vigorously until all the particles were dissolved. Thereafter the obtained mixture was filtered. The mouthwashes were then filled in sterilized amber colour bottles of 100 ml each. These are kept stable for 48 hours prior to the testing.

The extracts were stored in along with their lids. Categorization of both the extracts was performed at 3 different storage temperatures and recorded at time laps of baseline (0 day), 7 days, 14 days, 21 days and 28 days. These were:

1. Oven storage at 37 °C
2. Room temperature storage at 24 °C -26 °C and
3. Refrigerator storage at -4.8 °C

The extracts were withdrawn aseptically with the aid of a pipette and the following were assessed: pH, using a digital pH; Sedimentation using a centrifuge machine at 1,500 and 3,000 rpm for 5 minutes, organoleptic parameters that is color, odour and consistency by visual tactile assessment and density, using the dry pycnometer method. The sample distribution was done as depicted in Table 1.

MOUTHRINSE	TOTAL SAMPLE	SAMPLE DESCRIPTION		
Green Tea	1 LITRE	100ml (Room temperature) (24 °C –26 °C)	100ml (Oven) (37 °C)	100ml (Refrigerator) (±4.8 °C)
Green tea plus Ginger	1 LITRE	100ml (Room temperature) (24 °C –26 °C)	100ml (Oven) (37 °C)	100ml (Refrigerator) (±4.8 °C)

Table 1: Sample Description

Results

Characterization of the samples was done at 0, 7, 14, 21 and 28 days, in 3 different storage mediums that were refrigerator, oven and room temperature. Table 2, 3 and 4 shows the pH rates, sedimentation rates, and density rates respectively at 3 different temperatures for 1 month.

pH TESTING		TIME INTERVAL (DAYS)					
STORAGE	MOUTHWASH	0	7	14	21	28	F (p value)
OVEN (37 ˚C)	Green tea	5.02	4.73	4.79	4.65	4.5	38.12 (0.36)
	Green tea + Ginger	4.10	3.89	3.85	3.89	3.5	
REFRIGERATOR (±4.8 ˚C)	Green tea	5.02	5.05	4.65	4.69	4.8	29.98 (0.57)
	Green tea + Ginger	4.10	4.09	3.85	3.85	3.4	
ROOM TEMPERATURE (24 ˚C –26 ˚C)	Green tea	5.02	4.65	4.69	4.65	4.4	198.01 (0.87)
	Green tea + Ginger	4.10	3.86	3.89	3.85	3.7	

Table 2: Determination of pH

SEDIMENTATION		TIME INTERVAL (DAYS)					
STORAGE	MOUTHWASH	0	7	14	21	28	F (p value)
OVEN (37 ˚C)	Green tea	0	0	0	0	1	28.78 (0.67)
	Green tea + Ginger	0	0	0	0	0.2	
REFRIGERATOR (±4.8 ˚C)	Green tea	0	0	0	0	1	172.18 (0.898)
	Green tea + Ginger	0	0	0	0	0.5	
ROOM TEMPERATURE (24 ˚C –26 ˚C)	Green tea	0	0	0	0	1	166.89 (0.455)
	Green tea + Ginger	0	0	0	0	0.5	

Table 3: Sedimentation Rates

DENSITY		TIME INTERVAL (DAYS)					
STORAGE	MOUTHWASH	0	7	14	21	28	F (p value)
OVEN (37 ˚C)	Green tea	0.998	0.997	0.968	0.99	1.001	89.13 (0.97)
	Green tea + Ginger	1.006	0.978	0.938	0.987	1.001	
REFRIGERATOR (±4.8 ˚C)	Green tea	0.998	0.997	0.997	1	0.99	67.90 (0.47)
	Green tea + Ginger	1.006	0.968	0.968	1.001	0.987	
ROOM TEMPERATURE (24 ˚C –26 ˚C)	Green tea	0.998	0. 997	0.978	0.987	1	47.57 (0.56)
	Green tea + Ginger	1.006	0. 938	0.938	0.978	1.002	

Table 4: Density Rates

Organoleptic Parameters

The organoleptic properties of color, brightness, odour, and consistency did not change for a period of 1 month. However a negligible colour change was observed in the mouthwashes stored at room temperature.

Statistical test: ANOVA test was used to test the difference between the different groups for various parameters. As the p value was not significant there was no statistical difference in the various parameters among the two mouth washes.

Discussion

There has been a definite need in dentistry to develop an ideal herbal mouth rinse that has better biocompatibility and lesser side effects. Natural products with antimicrobial activity may be essential agents to prevent caries, periodontal disease, and oral candidiasis [7].

Abundant research has been carried out in the field of dentistry to evaluate the efficacy of phytochemicals in several different ways. The present study was conducted to examine and evaluate the efficacy of green tea and ginger in the form of a mouth rinse as both the products are herbal and its applicability has not yet been tried in Paediatric dentistry.

The results showed no statistically significant difference in pH within the 30 days experimental time interval between the oven and refrigerator groups, but showed a significant difference in the room temperature group. At the end of the study, the pH of the extract was below 5.5, which is considered a critical pH value for dissolution of enamel, and thus must be modified in other formulations.

In the sedimentation test, at all-time intervals and at different rotations, the sediments 0.2 mm, 0.5 mm, and 1 mm were observed. This variation in density was small and independent of experimental time interval or storage location.

Analysing the different density values at the initial and final time intervals, an increase of 0.006 g/L was found in oven storage

conditions, while in the refrigerator and at room temperature, the increase was 0.005 g/L. This variability was observed due to the high molecular weight molecules that got aggregated, water loss from the extract, evaporation of the extract itself. Nevertheless these variations did not hinder in evaluating the other parameter tests for the extract.

Identification and purity of the plant, as well as evaluation of its active ingredients, are essential when trying to identify good quality products [8].

Various studies have been conducted to assess the microbial activities of several bacteria and the effect of phytochemicals over them. They have found affirmative results and phytochemicals did inhibit the bacterial multiplication. Thus plant products can be used as a good alternative in dentistry for several uses like in treating oral infections, dental caries, gingival and periodontal diseases, etc [9].

This study has been done taking a smaller quantity for both the mouthwashes; a future research needs to be conducted to evaluate the microbiological parameter for the combination of green tea and ginger as done for green tea by Deshpande and Amrutya 2017 [9]. Furthermore *in vivo* studies are recommended to check its efficacy against plaque and biofilm.

Conclusion

Based on the results, it can be concluded that the green tea and green tea extract mouthwashes were tested in vitro and satisfactory stability and quality were found, enabling the use of this formulation for further use in treating plaque and gingivitis in children. The following conclusions can be drawn from the present study;

1) Green tea mouth rinse showed best results at refrigerating temperature (±4.8 °C) in terms of pH, density and organoleptic stability until the time period of 28 days.

2) Green tea plus ginger mouth rinse showed best results at refrigerating temperature (±4.8 °C) in terms of density and organoleptic stability, while the pH was most stable at room temperature (24 °C –26 °C), at the end of 28 days.

3) Sedimentation was seen for both the rinses at all the three temperatures, however when compared individually, green tea plus ginger mouth rinse showed lower sedimentation rates as compared to that of green tea mouth rinse.

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