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Herbal Mouthwash For Enhanced Oral Health

Savinash Mani Shukla[1] and Jyotsna Mishra[2]

1.Department of Chemistry, School of Sciences, SAM Global University, Bhopal, M.P, India

2.Department of Chemistry, Trinity Institute of Technology and Research, Bhopal, M.P, India

ABSTRACT

It is important to maintain oral health due to the significant impact of plaque formation and increased oral bacteria on oral health. Consumer demand for natural and organic food and personal care products has increased substantially in recent years. People want healthier alternatives and they want to feel more control over what their bodies are exposed to on a daily basis. The beneficial effects of various herbs such as German chamomile, Terminalia barbadensis, aloe vera, green tea, peppermint, turmeric, neem, trifoliate leaves, pomegranate extract, guava extract, propolis, alum, Dalin leaves, Mulleti and others. Which reveals properties such as chlorhexidine. Herbal mouthwashes rich in antibacterial, anti-inflammatory and antioxidant properties have emerged as promising alternatives.

Synergistic mixture of neem, tulsi, guava leaf, turmeric, ginger rhizome and peppermint oil extracts used as mouthwash is better compared to chlorhexidine in terms of gingival index, bleeding index and colony count.

Using green mouthwash is one way to improve your oral hygiene without negative side effects. In conclusion, proper oral care is important for general health, and oral herbs have attracted attention for their beneficial effects. This research article examined the effectiveness of mouthwash compared to chlorhexidine mouthwash.

Key Words – Green mouthwash, chlorhexidine, gingivitis, extracted sample, DCP, DPP.

INTRODUCTION

Oral diseases are common now a days, it is to be a very big issue worldwide (1-2). Oral health is affected the quality of life (3). Since the modernization, advertisement changes the life style; dental caries have been observed to be more frequent. This is related with the alteration in human diet and techniques of food preparation, meals become more starchy, rich sugar, sticky and adherable (4). The more refined food items do not stimulate saliva flow efficiently and also do not ensure self cleaning as their unrefined counter parts, desperate buffering and re mineralization of enamel and cause dental plaque accumulation (5).

Modernization of healthcare and access to food varieties per periodontal diseases (due to Vitamin C deficiency) are decreases but the ineffective removal of dental plaque in association with chronic stress, inherent smoking, dyslipidemia, diabities etc may result in periodontitis affects a large number of peoples worldwide (6). It also generates other problems like tissue degeneration as well as neoplasms and their treatment may result in hypo salivation, immune deficiency, malnutrition with other oral diseases (7). Oral hygiene is must for healthy oral cavity. Brushing and flossing are necessary for it (8).

Mouthwashes play a vital role in oral care, offering therapeutic benefits through gargling and rinsing the mouth. Various oral issues, spanning from bad breath to periodontal diseases, can necessitate the use of mouth rinses (9). They are especially important for managing secondary infections like oral mucositis. Now varieties of mouthwashes are available in the market. They are dominated by the use of many synthetic additives, some of the long-term health effects of these relatively new substances such as sodium lauryl sulfate (SLS) and triclosan etc.

Looking at the importance and significance of the work, the present paper is designed on the concerned topic. A green mouthwash is consisted with pure essential oils which is distilled liquids extracted from flowers, leaves, bark, stems, roots, shrubs and trees and botanicals ingredients that have been known for their medicinal benefits for thousands of years (10). It has antibacterial, antimicrobial, anti-inflammatory etc. properties.

EXPERIMENTAL

Collection of Plant materials

Leaves of *Ocimum tenuiflorum (tulsi), Azadirachta indica* (neem), *Psidium guajava* (guava), rhizome of *Zingiber officinale* (ginger), rhizome of *Curcuma longa* (turmeric) were randomly collected from matured plants from various areas of Bhopal on the basis of geographical availability. All collected plants were cleaned, shade dried for 5 days. After five days leaves were taken and pulverized into moderately coarse powder and stored in airtight container for further use.

Extraction with single solvent by maceration method

In this case Maceration process of extraction was employed. Powdered plant leaves and oils were weighed (20 gm) and packed in citrus juice were added as extraction solvent in the bottles and labeled. Further it is kept for 24 hrs and then filtration was performed to separate the solvent and powdered plant material. The solvent was evaporated to gain pure extract.

Formulation of Green Mouthwash

The mouthwashes is prepared by adding a natural products with spearmint oil in the appropriate amount of solvents. The herbal mouthwashes are prepared by the formula given as-

Green Sample Dissolve the Tulsi extract 2.0 ml, Neem extract 2ml, Ginger extract 3ml, Turmeric extract 2ml and Gauva extract 1.5 ml in a separate container and add spearmint oil 0.2ml and mix it properly then slowly add distilled water to make a volume up to 100ml and obtain a clear solution and well shake it.

Phytochemical screening

Phytochemical examinations were carried out for green sample of the extracts as per the standard methods and results of which are reported in Table 1.

Organoleptic properties of prepared formulations

For the physical evaluation following test are determined-

Physical Appearance - Physical parameter such as colour, odour, taste and consistency were examined by visual examination. The colour of the sample was light brown and taste was good.

pH – To check the pH of the prepared formulations take these formulations and deep the electrodes in it. Standardized digital Elico pH meter model number LR- 108 is used for all the determinations. The pH of sample was 6.56.

Stability Study- Stability studies are done with open and close container. Here, by subjecting the formulisations to room temperature and in refrigerator for specific time period.

Two sets of formulation are taken to check their stability, and put first in the normal temperature for several days and after that put in the refrigerator for some interval of days and after that conclude that formulation are stable at room temperature as well as cool temperature.

Palatability Determination - It is the property of being acceptable to the mouth. The mouthwash are tested separately for that criterion by three members in a blind style. The test of green mouthwash is lightly bitter.

Identification of extracted sample through instrumental Methods

After ascertain the above mentioning group tests, instrumental methods i.e. Thin Layer Chromatography (TLC) Voltammetry and Polorgraphy have been used to confirmation and identification of extracted compounds.

Detection and Calculation of R_f Value

1. Once the chromatogram was developed the R_f Value of the spot was calculated using the formula:

$$\mathbf{Rf} = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

The four basic modes of chromatography adsorption, partition, ion exchange and size exclusion can be applied to the analysis of pharmaceutical system or plant natural products by a number of techniques which differ from each other according to the nature of the stationary and mobile phase and the apparatus used. TLC was performed by using silica gel as stationary phase. Typically, silica gel was applied to plates, activated at 100ºC and then used for the study. The results of TLC studied are discussed in Table- 2. It shows presence of alkaloids, saponins, flavanoids, and phenols.

Voltametric and Polarographic Methods

Apparatus

An Elico (Hydrabad) pulse polarograph model CL-362 coupled with an x-y polarocard (recorder) model LR-108 and an electrode assembly consisting of three electrodes, a dropping mercury electrode used as an indicator electrode, a SCE as reference electrode and a coiled platinum electrode used as an auxiliary electrode.

The pulse polarograph had the following specification-

Voltammetric and Polarographic Study of Green Sample

A known concentration (10ml) of extracted sample was taken in a 0.1M Potassium Chloride and 0.001% gelatine are used as supporting electrolyte for the polarographic analysis of above sample. The pH 4.0 ±0.1 is kept by using solutions sodium hydroxide solution and hydrochloric acid solution. This solution is taken in a polarographic cell and nitrogen gas was bubbled for 5 minutes for deaeration.

Figure 2 is the Differential pulse Polarogram of extracted green mouthwash of sample. It shows well defined polarographic waves with Ep -0.38 and -0.74 V vs. SCE.

For confirmation of the analysis external spiking method is used. The resulting DCP and DPP curves of spiked analyte showed a peak with no change in E ½ and Ep values but peak height increases. Thus confirming the presence of species in extracted sample of and also enabling the use of developed procedure for an accurate qualitative and quantitative analysis of different origin sample especially for plant extracted samples. Thus the developed polarographic could be successfully used for accurate analysis of such type of sample. The standard deviation never exceeded 0.02 confirming the reliability of the analysis.

Antimicrobial Study of green mouthwash

Marketed Chlorhexidine, sample and green sample were tested against various pathogens viz. *Actinobacillus, Actinomycetemcomitans, Prophromonas gingivalis and Prevotella intermedia* respectively by disk diffusion method (11). On the basis of results obtained it could be concluded that green extracted sample was found to be effective antibacterial activity against gram positive and gram negative bacteria and compare this with the standard antibiotic *Penicillin*. The range of concentrations of the samples used for microbial activity against

various micro-organisms is in between 100-500 mg/ml and the 37^0C temperature was selected and time duration was 36 hours. A good number of reproducible results have been observed.

The result of disk antibacterial study of standard antibiotic with gram-positive and gram-negative bacteria of antibacterial study of marketed standard Chlorohexidine and extracted green mouthwash with gram positive *Actinobacillus, Actinomycetemcomitans, Prophromonas gingivalis and Prevotella intermedia* and negative microbes at a concentration of 0.5 mg/ml and 1mg/ml. The results of which are reported in Table 3.

On the basis of the obtained results by antimicrobial activity against mentioned various bacteria it could be concluded that green sample shows very good results like against *Actinobacillus* at 1mg/ml concentration it show 50% inhibition over control antibiotic and 69% over chlorohexidine . It shows 36% inhibition over control antibiotic and 47% over control chlorohexidine against *Actinomycetemcomitans* pathogen. These are remarkable results but it not show effective results with other two microbes i.e. *Prophromonas gingivalis* and *Prevotella intermedia.*

Sample Collection and Microbiological Analysis

Plaque samples were collected and sent for microbiological analysis to estimate colony-forming units. Prior to this analysis, all subjects received a complete oral prophylaxis treatment. Marginal plaque samples were collected along the gingival margin of all teeth. To prevent contamination from saliva, the site was isolated with cotton rolls and gently dried with an airway syringe. Marginal plaque samples were collected using a sterile jaquette scaler and were immediately transported to the laboratory in brain heart infusion broth as the transport medium. Subsequent microbial analysis was conducted (12).

The participants were randomly divided into two groups. Each group comprised of 12 participants. In each sample, participants were instructed to rinse with 10 ml of the assigned mouthwash sample for 30 seconds, twice daily, without revealing the specific type of mouthwash. In addition to the mouthwash usage, all participants were provided with an orthodontic tooth brush for use during the study.

Follow-Up

After the 10th and 23rd days, the participants were recalled for further assessments. During these follow-up visits, we recorded the gingival and bleeding indices. Furthermore, we collected supra gingival plaque samples using sterile jaquette scalers for subsequent microbiological analysis.

Statistical Analysis

The data collected for this study will be presented in terms of mean values and standard deviations to describe the central tendency and variability of the measurements(13).

Inter-group Comparison

To assess differences between the groups, conduct a statistical analysis and employ analysis of variance (ANOVA) with post hoc tests or the Kruskal-Wallis test (14), depending on the distribution of the data. These methods will be used to examine group variations and make detailed group comparisons as necessary.

Intra-group Comparison

To evaluate changes within each group over time, perform pair-wise comparisons between different time points and use paired t-tests or the Wilcoxon signed-rank test, depending on the distribution of the data. These tests will help us identify statistically significant differences within each group at various time intervals.

A significance level of $p<0.05$ will be used to determine the statistical significance of the results. Any p-value below 0.05 will be considered indicative of a statistically significant finding in this study.

Table 4 represents a comparison of the Gingival Index (GI) among four samples on the first day, 10th day, and 23 rd day of the study. Notably, we observed a significant difference in mean GI between the first day and the 10th day ($p<0.05$), while no significant difference was found after the 23rd day.

Furthermore, within each sample, a significant decrease in GI was observed from the first day to the 23rd day, indicating a substantial improvement in gingival health over time. Additionally, we found a significant difference in mean GI within each sample across all three time points. The post hoc test results further confirmed these significant differences.

When bacterial colony counts are compared between the time points within green sample and standard marketed chlorohexidine, it was observed that green sample is almost similar effective in decrease in bacterial count as chlorohexidine (15).

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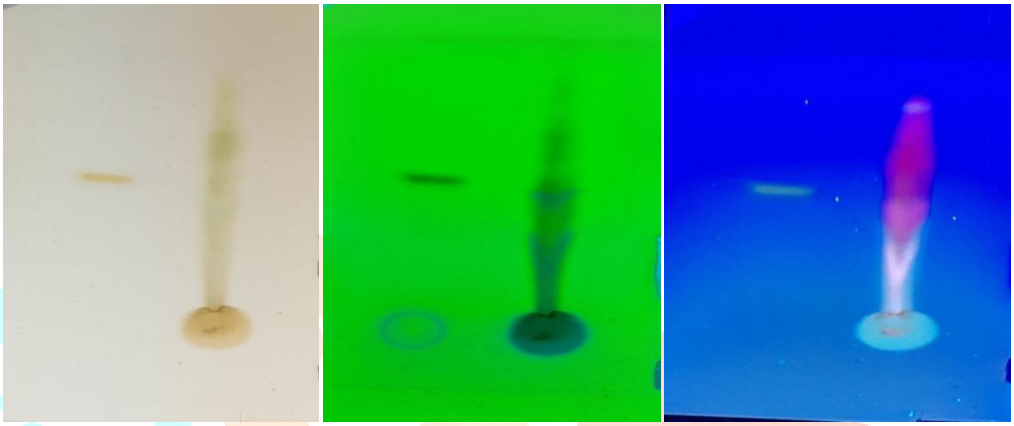

Figure 1 :Results of TLC of Flavonoids

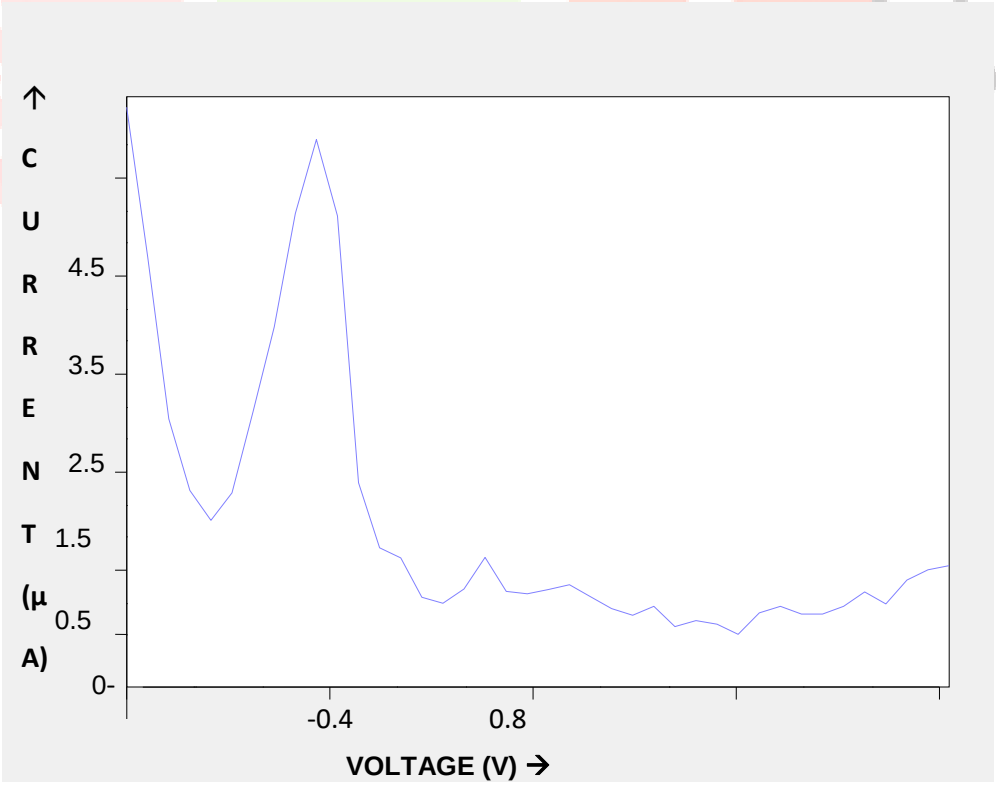

Figure 2. Differential Pulse Poarogram (DPP) of Green Sample of Mouth Wash in 0.01M KCl at pH 4.0±0.1

Table: 1. Preliminary Phytochemical screening results

S. N.	Phytoconstituents	Test Name	Methanolic extract
1.	Alkaloids	A) Wagner's Test B) Hager's test	+ -
2.	Glycosides	Leagel's test	-
3.	Saponins	Foam test	+
4.	Diterpenes	Copper acetate test	-
5.	Phenols	A) Ferric chloride test B) FC reagent	+ +
6.	Carbohydrates	A) Fehling test B) Benedicts test	- -
7.	Flavonoids	A) Lead acetate B) Alkaline reagenttest	 + +
8.	Proteins	Xanthoproteic test	-
9.	Tannin	Gelatin test	-
10.	Sterol	Salkowski test	-

(+) Positive, (-) Negative

Table 2. Results of TLC of Flavonoids

S. N.	Mobile phase	Distance of solute	R_f value
1.	Toluene: Ethyl acetate Formic acid (5:4:1)		
2.	**(*Green Sample*)** Dis. Travelled by mobile phase= 5.0cm No. of spot at long UV= 2 No. of spot at short UV=4 No. of spot at normal light =2	Long- 3.9, 4.1 Short- 2.7, 2.9, 3.3, 4.0 Normal Light-3.2, 2.9	Long –0.78, 0.82 Short – 0.54, 0.58, 0.66, 0.80 Normal light – 0.64, 0.54

Table: 3.Antimicrobial study of Green sample at 1mg/ml concentration

S.N	Test Organism	Inhibition zone (mm)Con. of complex 2mM/10ml (B)	Control antibiotic 1.0 mM/10ml (A)	% Change (A-B/A) ×100	Control Chlorohexidine mM/ml (Y)	% Change (Y-B/Y) ×100
1	*Actino bacillus*	13	26	50	42	69
2	*Actinomycetemc omitans*	12	36	75	23	47
3	*Prophromonas gingivalis*	22	23	4.3	21	-47
4	*Prevotella intermedia*	14	16	12	11	-27

Table 4: Comparison of Gingival Index Associated with the Usage of Mouthwashes in Patients Undergoing Orthodontic Treatment in standard Chlorohexidine and Green Sample

S.N	**Name of Sample**	**First day**		**After 14 days**		**After 21 days**		P value
		Mean	S.D.	Mean	S.D.	Mean	S.D.	
Gingival Index	Standard marketed Chloro hexidine	1.98	0.46	1.32	0.217	1.11	1.87	<0.001
	Green Sample	1.74	0.445	1.24	0.254	1.12	0.219	<0.001
Bleeding Index	**Name of Sample**	**First day**		**10th day**		**23ed day**		P value
		Mean	S.D	Mean	S.D	Mean	S.D	
	Standard marketed Chlorohexidine	2.40	0.520	1.80	0.450	1.10	0.300	0.004
	Green Sample	2.33	0.488	1.53	0.532	1.03	0.002	<0.001
Bacterial Colony Counts	**Name of Sample**	**First day**		**After 14 days**		**After 21 days**		P value
		Mean	S.D	Mean	S.D	Mean	S.D	
	Standard marketed Chlorohexidine	87200	8200	60100	7315	51300	8190	<0.001
	Green Sample	85530	5570	76020	6115	55250	7135	<0.001