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FORMULATION AND EVALUATION OF HERBAL MOUTHWASH BY USING LEAF OF GREEN TEA FOR GINGIVITIS

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Abstract: Oral hygiene plays a vital role in order to prevent oral diseases oral. Oral hygiene refers to keep the mouth, teeth, gums, and tongue clean and in good condition. The Mouth wash is an effective way to kill the micro-organisms and various bacteria from the oral cavity. In the recent research, polyherbs shows benefits regarding to oral health. Now-a-days, peoples mostly used mouthwash routinely to kill various micro-organisms from mouth, teeth, gums, tongue, etc. to maintain oral health. In the present study of the mouthwash, the polyherbal mouthwash is prepared from the green tea leaves, tulsi, cinnamon, clove oil, etc. by extracting them from the maceration techniques. The presence of tannins and phenolic compounds helps to indicates chances of activity against gingivitis, plague and antimicrobial property of green tea leaves. The Agar diffusion method by using petri plate was used to determine activity against gingivitis, plague and anti-microbial activity of aqueous green tea leaves extract. Three bacterial cultures namely Streptococcus bacteria (gram positive bacteria) and plague bacteria (gram negative bacteria) and Actinomyces (gram positive bacteria) were used for determination of activity against gingivitis, plague and antimicrobial property of polyherbal mouthwash. Herbal mouth wash was prepared by using green tea leaves extract. Prepared mouthwash was evaluated for various parameters such as color, odor, pH, foaming index, etc. Prepared mouth wash was compared with marketed mouth wash for activity against gingivitis, plague and antimicrobial activity.

KEY WORDS: Green tea, Tulsi, clove oil, cinnamon, honey

INTRODUCTION

The meaning of MOUTHWASH is a usually antiseptic liquid preparation for cleaning the mouth and teeth or freshening the breath. Mouthwashes are often prescribed in dentistry for prevention and treatment of several oral conditions. In the recent times the use of naturally occurring products what is otherwise known as grandmothers remedy are used on a large scale. This has now called for a newer age of mouth washes but is the new age mouth washes at par with the gold standard or even better than them this study investigates. Spices as clove, oregano, mint and cinnamon, have been employed for centuries as food preservatives and as medicinal plants mainly due to its antioxidant and antimicrobial activities. [1]

Usually, mouthwash is an antiseptic solution which is supposed to reduce the microbial load in the oral cavity, although there are other mouthwashes which may be used for other reasons such as for their analgesic, anti- inflammatory or anti – fungal action. Mouthwash is most commonly used at home as part of an oral hygiene anti cavity mouth rinse, which contains fluoride, which protects teeth from decay. However, it is generally agreed that the use of mouthwash dose not eliminate the need for both brushing and flossing. Some natural products are effective as an additive in improving the oral health.[2]

A person's dental hygiene is greatly told by their mouthwash, which also aids in the relief of gingivitis-related symptoms of blown gums. The maturity of dental cases uses mouthwashes to treat sensitive teeth, ulcerated throats, and xerostomia. Before performing oral surgery on cases, dentists always use mouthwash as an antibacterial agent since it helps to sanitize the face of the lit epoxies and teeth, precluding the impurity of any other pathogens. Due to its long-lasting antibacterial and antifungal exertion against mortal pathogens, medicinal shops are essential in the treatment of diseases. Due to their immediate pain relief, capability to combat oral infections, and reduced side goods, herbal mouthwashes are in high demand. Hydrogen peroxide and chlorhexidine, which are set up in chemical mouthwash, incontinent whiten, sterilize, and reduce tooth discomfort. Still, they have the tendency to discolor teeth and may beget adverse goods, despite being nicely priced.[3]

Some natural herbs included such as:

- Green tea leaf
- Tulsi leaf
- Clove oil
- Cinnamon
- Honey

1. Importance of Green tea

Tea is one of the most popular beverages consumed worldwide. Tea, from the plant *Camellia sinensis*, is consumed in different parts of the world as green, black, or Oolong tea. Among all of these, the most significant effects on human health have been observed with the consumption of green tea. [3]

Scientific name: Camellia sinensis
Biological source: It is obtained from leaves and buds of the Camellia sinensis plant.
Family: Theaceae
Genus: Camellia
Chemical constituents: Catechins, gallocatechol, epicatechin, gingerol, shogaol.
Biological activity: Antibacterial, reduce plaque and gingivitis
Uses:

- Reduced low density lipoprotein
- Reduced gingivitis and plague formation
- Reduced risk of developing Endo material cancer
- Reduced High level of cholesterol by small amount

Fig.1. Green tea leaves

2. Importance of Tulsi

Tulsi leaves are quite effective in treating common oral infections. When chewed, Tulsi leaves help in maintaining oral hygiene. Antibacterial agents namely carvacrol and terpene are present in this plant. Sesquiterpene b-caryophyllene also serves the same purpose. This constituent in FDA approved food additive which is naturally present in Tulsi. [4] Tulsi has some antibacterial and antimicrobial activities which helps to reduce plague formation and also helps to reduce discoloration of teeth.

Scientific name: Ocimum tenuiflorum L.
Biological source: It is obtained from the plant of Ocimum sanctum**.**
Family: Lamiaceae
Genus: Ocimum
Chemical constituents: volatile oil, eugenol, linalool.
Biological activity: Anti-microbial
Uses:

- It Reduced gingivitis and plague formation
- Boost immunity power
- Improve oral hygiene

Fig.2. Tulsi leaves

3. Importance of clove oil:

Because of its many possible advantages for dental health, clove oil which is extracted from the flower buds of the clove tree (Syzygium aromaticum) is a common component in herbal mouthwash formulations. Because of its astringent qualities, clove oil is a useful component in herbal mouthwash formulas, providing advantages such homeostatic action, gum tightening, antibacterial support, and calming relief from oral discomfort. It also shows analgesic and anti – inflammatory properties.[5]

Scientific name: Syzygium aromaticum
Biological source: It is obtained from the dried flowers buds of the clove plant.
Family: Myrtaceae
Genus: Syzygium
Chemical constituents: eugenol, eugenyl acetate
Biological activity: Astringent, analgesic
Uses:

- Temporary tooth ache relief
- Used as antibacterial

Fig.3. Clove oil

4. Importance of cinnamon:

Cinnamon, derived from the bark of the Cinnamomum tree, has been valued for centuries for its aromatic flavors and medicinal properties. Incorporating cinnamon into herbal mouthwash formulations offers several potential benefits for oral health.[5]

Scientific name: Cinnamomum verum
Biological source: It is obtained bark and leaf of Cinnamomum plant.
Family: Lauraceae
Genus: Cinnamomum
Chemical constituents: eugenol, cinnamate, cinnamic acid essential oils
Biological activity: Flavouring agent

Uses:

- Use to against oral pathogens
- Relief nerves pain

Fig.4. Cinnamon

5. Importance of honey*:*

Honey is implicated in the treatment of dental and oral diseases. Honey contains about 181 known substances and nutrients such as amino acids, natural carbohydrates and enzymes, trace minerals [calcium, phosphorus and fluoride]. Diastases enzyme is responsible for converting starch to dextrin and sugars, whereas invertase enzyme is responsible for converting sucrose in a nectar source to glucose and fructose. Glucose oxidase catalyzes the oxidation of glucose to hydrogen peroxide and gluconolactone. The hydrogen peroxide is further converted to water and oxygen by another enzyme catalase. The trace minerals are the building blocks of tooth enamel. Honey as a complementary and alternative medicine (termed as "Apitherapy") has been used for centuries to treat dental and oral diseases.[6]

Scientific name: Apis melifera
Biological source: It is obtained nectar of flowers by honey bees
Family: Apidae
Genus: Honey bee
Chemical constituents: glucose, fructose, carbohydrates
Biological activity: Sweetening agent**,** Anti-inflammatory, antioxidants
Uses:

- Reduces bacteria
- Wound healing

Fig.5. Honey

MATERIAL AND METHODS:

- **Collection of plant material**

Commercial tea powder was purchased from the local distributor of Nashik, Maharashtra, India. Ethanol was procured from Sd Fine Chemicals Ltd., Mumbai, India. Soxhlet apparatus (500 mL) and domestic appliances of ultrasonic bath and microwave**.**

❖ Preparation of herbal leaf extract

1. Extraction of green tea:

Maceration –

Firstly, the leaves of the green tea were taken and washed thoroughly with the distilled water and wind dried for 1 week and another way for drying the leaves is drying them in the hot air oven for 10 to 15 min. They were dried until all the moisture from them is removed and they become completely dry and then the dried leaves were taken for the blending in the blender very finely the leaves were blended and then they were passed though the sieve. The sample leaves extraction was done by the maceration technique by using distilled water. The green tea leaves were weighed as much as 500gm and were soaked in 1000ml of ethanol. The extract was left for 2 days for the maceration and the extract was frequently shaken. Then the filtrate was obtained by filtering the extract and was further concentrated by heating the liquid. The liquid was boiled on temperature between 70-80° C until the excess water was evaporated and the thick drug extract was obtained. [7]

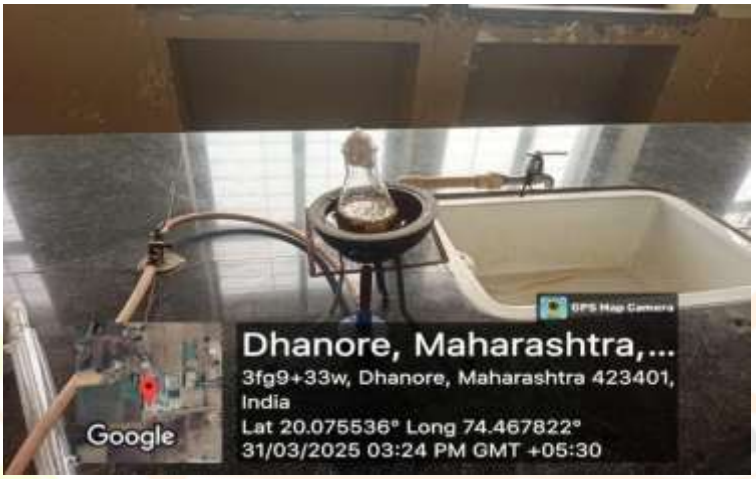

Fig. 6. Extraction of green tea

2. Extraction of Tulsi:

In Tulsi plants, the essential oils such as eugenol, lineal, estragol, linalool, methyl cinamato, limonene, and geraniol make basil oil more of a highly economically valued product. The presence of eugenol in it has been shown to have considerable antioxidant properties and to inhibit lipid peroxidation effectively.[8] Collect some fresh Tulsi leaves about 20 gm, wash them with good water. Dried the leaves by cotton clothes. Crushed them with mortar and pistol, strain the Tulsi juice using muslin cloth.

Fig. 7. Extraction of herbs

Preparation Of Herbal Mouthwash:

Polyherbal mouthwash extract varying strengths were created.

↓

Tulsi, cloves, and cinnamon were the included in the mouthwash's expression.

↓

Honey, and water are the minor ingredients used to the blend.

↓

The minor constituents were put to use in the preservation and flavor improvement processes.

↓

Different attention of the herbal extract was made to examine the mouthwash's implicit antibacterial goods.

↓

The herbal mouthwash factors were pulverized into an oil for use in the extraction.

↓

For 48 hours, 2ml of Tulsi, 5ml of clove oil, and 0.2ml of cinnamon oil were steeped in 100 ml of distilled water at 37 °C.

↓

Herbal extract was filtered after incubation.

↓

Extract was also boiled in its own beaker and set down for cool.

↓

Then honey was added to the distilled water at a rate of 2 ml per 100 ml.

↓

After letting the extract cool, we combined the main and secondary factors according to the table in the following. [9]

Formula:

Sr.no	Ingredient	Roles	Quantity
1.	Green tea	Reduce gingivitis	4ml
2.	Tulsi	Anti-inflammatory	2ml
3.	Clove oil	Analgesic	5ml
4.	Cinnamon	Flavoring agent	0.2ml
5.	Honey	Sweetening agent	2ml
6.	Salt	Preservatives	0.02gm
7.	Water	Vehicle	QS

Table .1

Evaluation of herbal mouthwash:

1. Physical evaluation-

Through visible examination, physical characteristics including odour and color of mouth wash have been checked. [10, 11]

2. pH-

A digital pH meter had been employed for determining exact pH position of the finished herbal mouthwash. A standard buffer result, consisting of around 1 milliliter of mouthwash adulterated in 50 milliliters of distilled water, was used to calibrate pH meter.[12] Also simple pH can also be employed for determining the pH of mouthwash. It can be determined by simple visual detection of color obtained on the pH paper comparing with pH scale.

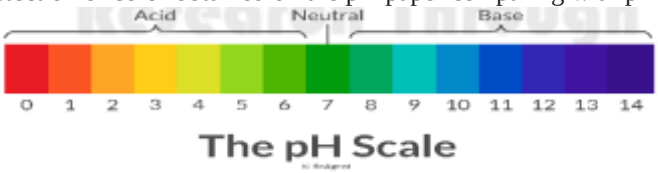

Fig.8.pH scale

3. Taste-

The taste of the herbal mouth wash depends upon the taste of green tea leaves, Tulsi and also on honey.[13]

4. Density-

The internal disunion causes the substance to come thick, sticky and semi-fluid. [14, 15]

5. Foaming Height:

20 mL of distilled water was mixed with the sample and transferred into a measuring cylinder. The mixture was shaken for one minute, and the initial foam height was noted. Allow to settle for 10-15 minutes, the foam height was measured again and noted.[16]

6. Centrifugation test-

The prepared mouthwash filled in test tubes and was centrifuged in the centrifugation machine to examine the phase separation. [17,18]

7. Microbial test-

Using the streak plate technique, the produced mouthwash was inoculated into the agar medium plates with microbes such as Streptococcus, and a sample was then formed. The plates have been placed inside the incubator, where they are being incubated for 24 hours at 37°C. When the incubation length plates have been removed and compared to the control, they have been examined for microbial growth. [19,20]

8. Microscopic test-

A compound microscope with magnification powers of 10 and 40 was used to assess the mouthwash formulation's transparency. [10]

1. Physical evaluation:

From physical evaluation the colour of mouth wash was found to be green and odour was found to be refreshening aromatic odour.

Fig. 9 Herbal mouthwash

2. pH:

The pH of herbal mouth wash was found in between 6-7 which finds yellow color on pH paper.

Fig. 10. pH

3. Taste:

By the tasting of herbal mouth wash, taste was found to be refreshing, and slightly sweet.

4. Density:

The density of herbal mouth wash was found to be 0.876gm/ml.

Fig.11. Density

5. Foaming Height:

The foaming height of the mouthwash was found to be 2.5cm in a measuring cylinder.

Fig .12. Foaming height

6. Microbial test:

The microbial test was found as per following-

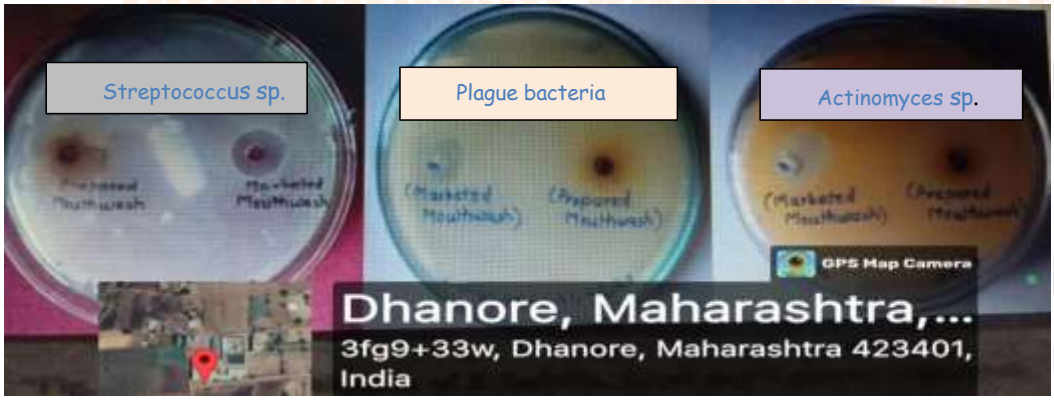

Fig.13.Microbial test

Analysis for Tannins and Phenolic compound:

Sr. No.	Test for Tannins	Observation
1.	Extract + Bromine water	Decolouration of bromine water
2.	Extract + Acetic acid solution	Red colour
3.	Extract + Dilute iodine solution	Transient red colour
4.	Extract + Dilute HNO_3	Reddish to yellow colour
5.	Extract + Dilute NH_4OH and potassium ferricyanide	Red colour
6.	Extract + 5% FeCl solution	Deep blue-black colour

Table 2

Result:

Green tea leaf extract was prepared by maceration technique. Prepared extract was dark green in colour having refreshening aromatic odour. Mouthwash was prepared by using green tea leaf extract & various herbal excipients. The pH of formulated mouthwash was found to be 6.8. Appropriate foam was produced upon application. Microbial study reveals good

antimicrobial activity against streptococcus bacteria, plague bacteria, actinomyces bacteria. Prepared mouthwash was found to be stable for 3 months at room temperature. Prepared mouthwash shows greater zone of inhibition against streptococcus bacteria, plague bacteria, actinomyces bacteria as compared to marketed mouthwash.

Conclusion:

Green tea aqueous extract can be used in mouth wash manufacturing as an antimicrobial agent. Prepared mouth wash can be tested for animal study.

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