Formulation of *Jasminum grandiflorum* leaves mouth wash and evaluating its anti-inflammatory and anti-microbial activity

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Abstract

The present study aims to formulate and evaluate a herbal mouthwash using *Jasminum grandiflorum* leaves extract and compare its antimicrobial and anti-inflammatory activity with commercial mouthwash. The herbal mouthwash was formed from the jasmine leaf powder extraction and combined with sodium lauryl sulphate, sucrose, peppermint oil, and other ingredients. Antimicrobial activity was checked for *Staphylococcus aureus and Escherichia coli*, and anti-inflammatory activity was checked via the inhibition of protein denaturation method. In addition, the physicochemical properties, which were pH, viscosity, turbidity, and surface tension, were assessed to ensure product stability and compatibility. The antimicrobial activity of herbal mouthwash in both bacteria was considerably lower than that of commercial mouthwash. The anti-inflammatory activity was concentration dependent, and both formulations show significant effectiveness in the inhibition of protein denaturation method. The findings highlight the antimicrobial and anti-inflammatory properties and efficacy and quality of herbal mouthwash.

Keywords: Anti-inflammatory, Antimicrobial, *Staphylococcus aureus, Escherichia coli,* Mouthwash

Introduction

Plants have long been used for therapeutic purposes, and herbal medicines have played an important role in healthcare since ancient times. In Africa, where over 80% of the population relies on herbal products and traditional medicine, this habit is strongly engrained. Herbal therapy is increasingly recognized as a beneficial supplement to traditional treatments, even in wealthy nations[1]. *Jasminum* species belong to the family of Oleaceae, and various varieties of this genus show a variety of biological activity[2-4]. *Jasminum grandiflorum* leaves are used to treat diseases of the skin, ulcerative stomatitis, wounds, otorrhea, ulcers, sexual impotency[5], anthelmintics[6], otalgia, strangury, dysmenorrhea, odontalgia, and leprosy[7]. The several species of jasmine exhibit strong cytotoxic and anticancer properties[8,9]. *Jasminum* species are rich in phytoconstituents such as alkaloids, flavonoids, secoiridoid glucosides, triterpenes, iridoids, and lignans components[10].

Bacterial colonization on tooth surfaces and restorations produces dental plaque, a complicated structural entity. It plays a major part in gingival and periodontal diseases[11,12]. Tooth brushing, dental floss, and interdental brushing are popular treatments for periodontal and gingival diseases. Plaque can also be controlled using chemical treatment agents, including mouthwashes, sprays, chewing gum, and varnishes[13, 14]. For oral hygiene, mouthwashes are the most basic tool[15]. Mouthwash is a rinse solution that contains astringents, demulcents, surfactants, and antibacterial agents that freshen and clean the airways[16]. Mouthwashes can be classified into chemical and herbal. Herbal mouthwash contains organic constituents, which are called phytochemicals that consist of anti-inflammatory and anti-microbial properties[17].

Materials and Methods

Preparation of Herbal Mouthwash

The herbal mouthwash was prepared by using *Jasminum grandiflorum* and other pharmaceutical ingredients. Table 1 shows the formulation of herbal mouthwashes. Every ingredient in herbal mouthwashes has a distinct function to improve the quality and efficacy of the product. The main ingredients in this mouthwash were *Jasminum grandiflorum,* sodium lauryl sulphate as a foaming agent, and sodium chloride, which acts as a stability enhancer. Xylitol and clove oil, peppermint oil act as sweetening and flavoring agents that improve the taste and flavor of the mouthwash. As a moisturizing agent, coconut aids protection to the oral tissues.

Table 1 – Ingredients of herbal mouthwash

S.No	Ingredients	Amount in grams
1	Jasmine leaf extract	40ml
2	Sodium Lauryl sulphate	3g
3	Coconut oil	2 ml
4	Sodium chloride	2g
5	Xylitol	2g
6	Peppermint oil	1ml
7	Clove oil	0.5ml
8	Distilled water	Make up to 100ml

The jasmine leaves were washed to remove contaminants and debris, followed by drying out at room temperature to preserve their natural properties. After drying, the dried leaves were converted into powder by using a mortar and pestle and sieved them. 10 grams of jasmine leaf powder were weighed by using a weighing balance and put into a 250 ml conical flask containing 100 ml of distilled water. The conical flask was closed with aluminum foil and left undisturbed for 3 days. After 3 days the extract was filtered by using whatman filter paper grade 01 to get a clear jasmine leaf extract. In this extraction, 40 ml is taken into a conical flask, and 2 ml of coconut oil, 3 g of sodium lauryl sulfate, 2 g of sodium chloride, 0.5ml of clove oil, and 2 g of xylitol are added. Mix the ingredient well and add 1 ml peppermint oil as a flavoring agent and make up to 100 ml using distilled water. The mixture was continuously stirred to ensure even distribution. The resulting mixture was filtered once again to eliminate impurities and undissolved particles. The product was sterilized with an autoclave to improve the safety and stability of mouthwash.

Antimicrobial activity

The antimicrobial activity of mouthwash was evaluated by using conventional microbiological methods against the bacterial strains of *Staphylococcus aureus and Escherichia coli*. In order to make Muller Hinton agar (MHA) medium, 33.8 g of MHA powder was dissolved in 1 L of distilled water and autoclaved for 15 minutes at 121°C at 15 lbs pressure. The autoclaved 20 ml of Muller Hinton agar medium was poured into sterile 100 mm petri dishes with bacterial cultures of *Staphylococcus aureus and Escherichia coli*. Sterile well cutters were used to make wells with a 10mm diameter. The mouthwash samples were added into the wells in different quantities, such as 25 µL, 50 µL, and 100 µL. The plates were incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the zone of inhibition around the wells. This inhibition indicates the potency of the sample. The streptomycin served as a positive control.

Anti-inflammatory activity

The in vitro anti-inflammatory activity of the sample was determined by the inhibition of protein denaturation method. The different concentrations of samples, such as 6.25 µl/mL, 12.5 µl/mL, 25 µl/mL, and 50 µl/mL, were made from stock solution. Bovine serum albumin (BSA) and distilled water were made up of the test control; the test solution consists of 0.45 ml of distilled water and different concentrations of the sample. The diclofenac sodium was used as a standard solution. All the above solutions were adjusted to pH 6.3 using 1N hydrochloric acid. The samples were incubated at 37°C for 20 minutes, and the temperature was increased to 57°C for 3 minutes. After cooling, 2.5 ml of phosphate buffer was added to the solutions. The absorbance was measured using a UV-visible spectrophotometer at 416 nm.

% Denaturation inhibition,

$$= \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control} \times 100$$

Physical appearance and solubility

The physical evaluation of herbal mouthwash was done by sensory and visual examination; it verifies the product's identity and authenticity. The uniformity in appearance ensures the consistent manufacturing process. *Jasminum grandiflorum* powder was dissolved in several solvent types to assess its solubility.

Determination of pH

The digital pH meter is used to calculate the pH of mouthwash. The calibrated pH electrode was dipped into the jasmine herbal mouthwash and tested for pH. A mouthwash with a lower pH can harm gums and teeth.

Determination of foam ability

A foam height measurement test was used to assess the foam ability. In a measuring cylinder, 50 ml of distilled water and 1 ml of herbal mouthwash were combined. After agitating the mixture, the height of the resultant foam was measured. This demonstrates the product quality of herbal mouthwash.

Determination of viscosity

The Ostwald viscometer is used for the measurement of the viscosity of the mouthwash. On an appropriate stand, the viscometer was positioned vertically. The viscometer was filled with mouthwash up to mark A. The amount of time it took for mouthwash to move from mark A to mark B was measured. This test shows the viscosity of herbal mouthwash.

Determination of surface tension

The stalagmometer is a device used to measure the surface tension of herbal mouthwash. A stalagmometer was filled with pure water, and the device was adjusted to make sure that drops formed consistently. The procedure was repeated for the herbal mouthwash after the drop count was noted and the herbal mouthwash's surface tension was calculated.

Determination of turbidity

Turbidity was measured using a nephelometer. The mouthwash sample was put in a nephalometer after being filled in a sample cell and calculates the turbidity and notes its value.

Table 2- Antimicrobial activity in gram negative organism

Sample	Concentration(µg)	Zone of inhibition(mm)
Commercial mouthwash	Streptomycin(100µg)	32
	25 µL	9
	50 µL	12
	100 µL	15

Herbal mouthwash	Streptomycin(100µg)	32
	25 µL	9
	50 µL	11
	100 µL	13

Results

Antimicrobial Activity

Gram negative organism

The antimicrobial activity of herbal mouthwash was tested against gram-negative organism of *Staphylococcus aureus*. Streptomycin (100 µg) was used as a positive control, resulting in a 27 mm zone of inhibition. The commercial mouthwash shows zones of inhibition of 8 mm, 11 mm, and 14 mm at 25 µL, 50 µL, and 100 µL doses, respectively. At the same quantities, the herbal mouthwashes show 7 mm, 9 mm, and 13 mm of zone of inhibition (Fig.1). The commercial mouthwash activity was higher than the prepared herbal mouthwash; these data show both compounds have antimicrobial activity against *Staphylococcus aureus* (Table 2).

Gram positive organism

The antimicrobial activity against gram-positive organism of *Escherichia coli* reveals 32 mm zone of inhibition was created by the positive control streptomycin. The commercial mouthwash at 25 µL, 50 µL, and 100 µL shows the 9 mm, 12 mm, and 15 mm zone of inhibition. Whereas the herbal mouthwash showed 9 mm, 11 mm, and 13 mm zone of inhibition (Fig.2). The commercial mouthwash was more effective at greater doses (Table 3).

Anti-inflammatory activity

The anti-inflammatory activity of both herbal mouthwash and commercial mouthwash was assessed at different concentrations. Both formulations show notable inhibition at lower concentrations and a potent anti-inflammatory effect at the maximum dose (Table 4). The anti-inflammatory activities of mouthwash are dose dependent (Fig 3).

Table 3- Antimicrobial activity in gram-positive organism

Sample	Concentration(µg)	Zone of inhibition(mm)
Commercial mouthwash	Streptomycin(100µg)	27
	25 µL	8
	50 µL	11
	100 µL	14
Herbal mouthwash	Streptomycin(100µg)	27
	25 µL	7
	50 µL	9
	100 µL	13

Physical evaluation and solubility

The herbal mouthwashes are light brown in color and have a pleasant aroma attributed to peppermint oil, which creates a relaxing and energizing scent. Herbal leaf extracts are soluble in water, ethanol, and propylene glycol.

Determination of pH

The formulated mouthwash showed a pH of 6.74, while the permissible range for herbal mouthwashes was 6.0-7.5. This pH determination confirms the safety and non-irritating properties of herbal mouthwashes.

Determination of foam ability

The jasmine leaf mouthwash satisfies the standards for oral care applications, according to the findings of the foam test. The consistent foam structure during mouthwash usage is demonstrated by the exceptional foam stability. Additionally, the foam collapse length suggests that his mouthwash has an appropriate surfactant.

Determination of viscosity

The prepared jasmine leaf herbal mouthwash has a viscosity of 1.146 cps; this limit is the recommended limit for oral care products. This viscosity makes the mouthwash easy to pour, blend, and rinse.

Determination of surface Tension

The usual surface tension range for herbal mouthwashes was 40–55 mN/m. Surface tension enhances the cleansing and spreading qualities of herbal mouthwash. The surface tension value of prepared jasmine mouthwash was 47 mN/m. This number indicates the existence of a surfactant in jasmine herbal mouthwash, which improves spread ability over teeth and gums.

Determination of turbidity

The product's stability during storage, clarity, and appropriateness for oral care products are all guaranteed by the turbidity values. The permissible range for mouthwashes is 5-100 NTU. The turbidity level of produced herbal mouthwash is 85.8 NTU.

Discussion

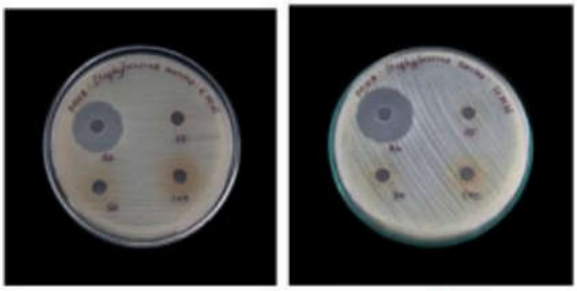
The inclusion of active chemicals in mouthwash has no influence on its efficacy for prevention or medical treatment. It also takes into account aspects including the duration of usage, the user's health, and their level of oral hygiene awareness[18,19]. The mouthwashes chemically contribute to removing the dental plaque, but they cannot completely replace the basic mechanical cleaning technique[20]. Mouthwashes are used by themselves. Their primary target is the outermost layer of bacteria; without tissue damage, they are unable to penetrate the sub gingival area. The most effective strategy to get rid of the plaque is to use both chemical and mechanical treatments. Mouthwash is used as a complement to brushing and has the same effect as rinsing after brushing[21].

Fig.1- Antimicrobial activity of gram-negative organism

Zone of inhibition commercial mouthwash

Zone of inhibition herbal mouthwash

Fig.2- Antimicrobial activity of gram-positive organism



Zone of inhibition commercial mouthwash

Zone of inhibition herbal mouthwash

The previous studies (Galovičová et al., 2022b, and Nagarajappa et al., 2015) show that *Jasminum grandiflorum* demonstrated strong antimicrobial qualities against the microorganisms such as *Staphylococcus aureus* and *Escherichia coli*. The *Jasminum grandiflorum* extract shows a zone of inhibition of 3.67 mm in disc diffusion tests and demonstrated a 54.88% inhibition rate against E. coli at a concentration of 125 µL/L. The extract had inhibition against *Staphylococcus aureus* at greater concentration with a 20 mm zone of inhibition. The results indicate that *Jasminum grandiflorum* has potential antimicrobial properties, especially against Gram-positive bacteria, even if the quantitative data for *S. aureus* was less common than for *E. Coli* [22,23]. The methanolic extract of *Jasminum grandiflorum* shows strong anti-inflammatory activity[24]. During the use of herbal mouthwash, produce mild side effects such as light-headedness and hypogeusia[25]. In both industrialized and developing nations, toothbrushes and toothpaste are generally desired and essential components of total oral hygiene habits [26].

The diverse range of inhibited oral pathogens, coupled with minimal cytotoxic reactions, supports the potential of these formulations as viable alternatives in oral care practices.

Table 4 – Anti-inflammatory activity

S.No	Concentrations(µl/ml)	Percentage of inhibition	
		Commercial Mouthwash	Herbal Mouthwash
1.	6.25	24.0121581	22.7963526
2.	12.5	35.2583587	33.4650456
3.	25	59.5744681	54.1033435
4.	50	71.4285714	70.8206687

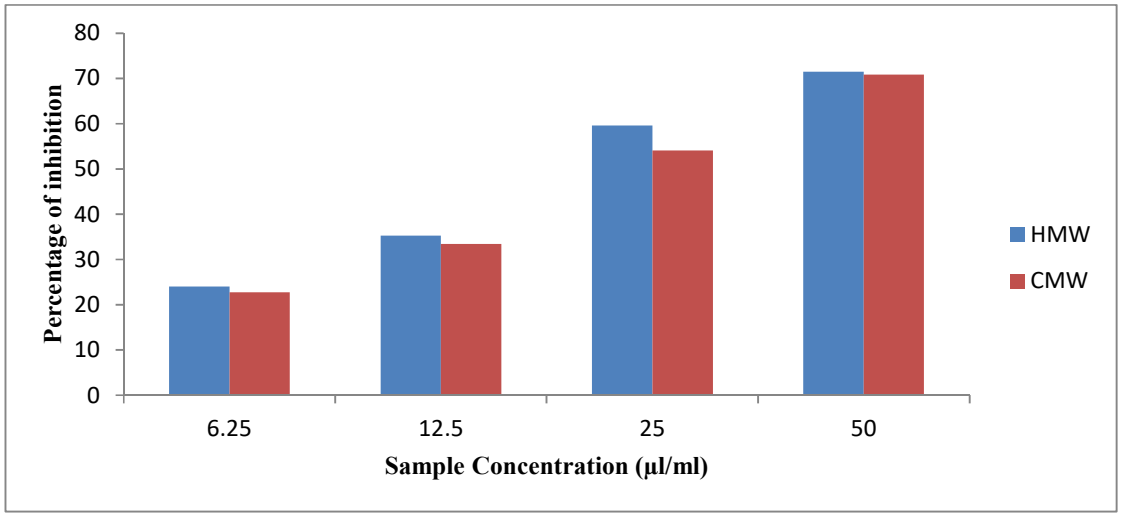


Figure 3: Graphical representation of Protein denaturation of CMW. Along Y axis, Percentage of inhibition (%). Along X axis Concentration of sample(µl/mL)

Conclusion

The present study indicates the herbal mouthwash completes the norms for oral care products, such as pH, viscosity, surface tension, and turbidity. The herbal mouthwash prepared from the jasmine leaf extract was compared to the commercial mouthwash for antimicrobial and anti-inflammatory activity. Both formulations show antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*, although the commercial mouthwash was marginally more effective. This suggests their potential use as an effective mouth cleanser to reduce bacterial load in the mouth and for rinsing teeth.

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