

# FORMULATION AND EVALUATION OF POLYHERBAL GARGLE

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**Abstract :** This study focuses on the formulation and evaluation of polyherbal gargle using alcoholic extract of *Psidium guajava*, *Azadirachta indica* and *Ocimum sanctum*. The selected herbs are well known for their anti-microbial, anti-inflammatory and anti-oxidant properties. The suitable excipients such as ethanol, glycerin, sorbitol, sodium benzoate and peppermint oil were incorporated in the polyherbal gargle. The prepared gargle was evaluated for physicochemical properties such as pH, viscosity, homogeneity and stability. Phytochemical screening confirmed the presence of alkaloids, flavonoids, tannins and saponins. The anti-microbial activity was assessed against *E. coli* and *S. aureus*. The stability studies indicated that the formulation remain stable for 20 days without any change significant changes in physical and chemical properties. The obtained results suggested that the prepared gargle is safe and effective for use.

**Index Terms -***Psidium guajava*, *Azadirachta indica*, *Ocimum sanctum*, polyherbal gargle, anti-microbial activity, phytochemical screening, stability studies, zone of inhibition.

## I. INTRODUCTION

### INTRODUCTION

Oral health is an important aspect in maintaining the quality of life and overcome the condition of oral infections. It is important in the smooth functioning of the essential functions like eating, breathing, and speaking. Various oral conditions such as dental caries, gingivitis, dental plaque, tooth loss, oral cancer, and birth defects in infants almost affected an estimation of 3.5 billion population worldwide and leads to a growing burden, particularly in low and moderate income countries. The oral infections are usually caused by a wide range of pathogens including viruses, fungi, bacteria and parasites. Poor hygiene, underlying medical conditions, trauma, smoking etc. are among the primary risk factors for oral infections.



gargling

Gargles are defined as the aqueous hydroalcoholic solutions used to treat or help throat infections whereas mouthwashes are liquid, aqueous compositions and are mainly intended to relieve and cure oral infections and maintain oral health. The present study focuses on the formulation and evaluation of a polyherbal gargle comprising of *Psidium guajava* (guava), *Azadirachta indica* (neem) and *Ocimum sanctum* (tulsi) with promising anti-bacterial, anti-microbial and anti-oxidant properties. The selected herbs are rich in bioactive compounds such as flavonoids, tannins, alkaloids and tannins that provide effective therapeutic activity.

### NEED OF THE STUDY.

Oral diseases such as gingivitis, plaque buildup, and bad breath, and dental infections are becoming common due to poor oral hygiene, unhealthy food habits, and microbial contamination. Gingivitis, recorded as the initial stage of gum diseases, occurs when bacteria and plaque accumulate on the tooth and gum surfaces, leading to redness, swelling, pain, and bleeding gums. Conventional mouthwashes as well as synthetic oral care products are being widely used for maintenance of oral hygiene. The drawbacks of these mouthwashes include oral irritation, burning sensation, altered taste, dryness of mouth, and development of microbial resistance. In addition to this, many synthetic products are expensive and not suitable for prolonged daily use. As a result of this, there is a growing interest in herbal alternatives for maintenance of oral health. Medicinal plants such as *Psidium guajava*, *Azadirachta indica* and *Ocimum sanctum* are well known for their anti-microbial, anti-inflammatory and healing properties. The present study was undertaken to formulate and evaluate a polyherbal gargle using these medicinal plants as a safe and effective alternative to synthetic gargles in treatment of oral infections and maintenance of oral health.

### 3.1 Botanical Description

**Psidium guajava:** *Psidium guajava* (guava) is a small evergreen tree belonging to the Myrtaceae family. It has simple green aromatic leaves, white flowers, and round fruit with many seeds. The leaves are rich in tannins, flavonoids, possessing strong anti-microbial and anti-inflammatory properties beneficial in maintenance of oral health.

**Azadirachta indica:** *Azadirachta indica* (neem) is a medium sized evergreen tree belonging to the Meliaceae family. It has compound bitter leaves and medicinal barks. Neem is well known for its anti-bacterial, anti-fungal, and anti-inflammatory activities.

**Ocimum sanctum:** *Ocimum sanctum* (tulsi/holy basil) is an aromatic medicinal herb belonging to the family Lamiaceae. It has hairy stems, aromatic green leaves, and small purple or white flowers. It comprises of essential oils and possess strong anti-oxidant and therapeutic properties.

### 3.2 Justification

The selection of *Psidium guajava*, *Azadirachta indica*, and *Ocimum sanctum* for the polyherbal gargle was based on their strong and well known medicinal and oral care properties. Guava leaves possess anti-microbial and anti-inflammatory activities that help reduce gum infections and plaque formation. Neem is widely used for its strong anti-bacterial and healing properties, making it effective in maintenance of oral hygiene and controlling gingivitis. Tulsi contains bioactive compounds like eugenol that provide anti-bacterial and soothing effect. Combination of these herbs produces a synergistic effect that help relive and cure oral infections naturally and provide a safe, effective and affordable alternative to synthetic mouthwashes.

### 3.3 Aim and objective

The aim and objective of this study was to formulate and evaluate polyherbal gargle containing the alcoholic extracts of *Psidium guajava*, *Azadirachta indica* and *Ocimum sanctum* and evaluate their physicochemical, phytochemical and anti-microbial properties and to assess the stability of the prepared formulation.

## MATERIAL AND METHODS

The material and methods outlines the plan about how the gargle was formulated including the selection of excipients. The steps included are as follows:

### 3.1 Extraction of active constituents

**Extraction of active ingredients from Guava leaves:** Fresh guava leaves approximately 4.2 grams were collected from guava plant and sundried for 3-5 days. They were then powdered in an electric grinder to obtain a fine powder. The obtained powder was immersed in 70 ml of 90% ethanol for a duration 3-4 days at room temperature. After this process, the extracts were filtered using Whatman filter paper. The extracts were concentrated by subjecting to heating in a water bath. The concentrated filtrates were then dried in a freeze dryer and stored at room temperature in a closed bottle or suitable container until further analysis.

**Extraction of active ingredients from Neem leaves:** The neem leaves were subjected to sun drying and mashed. The ethanolic extract includes mashed leaves that weighed 2.9 grams immersed in 70 ml of 90% ethanol for 3-4 days. The extracts were filtered using Whatman filter paper and heated to obtain concentrated filtrate. Lastly, the filtrate is stored in a suitable container until further use.

**Extraction of active ingredients from Tulsi:** The dried *Ocimum sanctum* were subjected to size reduction using an electric grinder. Approximately, 2.9 grams of dried leaves powder was immersed in 70 ml of 90% ethanol for 3-4 days to prepare an ethanolic extract. The extract was subjected to occasional stirring for 3-4 days. The extract was filtered using Whatman filter paper. Following this, the alcoholic extract was concentrated using water bath and the obtained filtrate was stored in tightly closed container or vial.



Guava, Neem, Tulsi leaves powder immersed in 90% ethanol & kept for maceration in a closed container

### 3.2 Formulation of Polyherbal Gargle

Table 3.1: Formulation table

Sr.no.	Ingredients	Qty.	Role
1.	Alcoholic extract of <i>Psidium guajava</i> (guava) leaves	2ml	Anti-microbial & anti-inflammatory
2.	Alcoholic extract of <i>Azadirachta indica</i> (neem) leaves	1.5ml	Anti-bacterial
3.	Alcoholic extract of <i>Ocimum sanctum</i> (tulsi) leaves	1.5ml	Anti-oxidant
4.	Ethanol	10ml	Solvent
5.	Glycerin	5ml	Humectant
6.	Sorbitol	10ml	Sweetening agent
7.	Polysorbate -80	1ml	Solubilizer
8.	Peppermint oil	2-3drops	Flavoring agent
9.	Sodium benzoate	0.1gm	Preservative
10.	Distilled water	Q.s.to 100ml	Vehicle

### 3.2 Formulation Procedure

- Preparation of extract phase  
Take a clean and dry beaker. Add measured quantities of alcoholic extracts of *Psidium guajava* (Guava ;2ml), *Azadirachta indica* (Neem; 1.5ml) and *Ocimum sanctum* (Tulsi; 1.5ml). Mix the extracts thoroughly to obtain a uniform herbal mixture.
- Addition of humectant and sweetening agent  
Add glycerin (5ml) to the above mixture and stir well.  
Then add sorbitol solution (10ml) and continue stirring to improve the consistency of the solution.
- Preparation of preservative solution  
Dissolve sodium benzoate(0.1gm) in a small quantity of warm distilled water separately.  
Stir until completely dissolved.  
Add this to the main mixture with continuous stirring.
- Addition of solubilizer and flavouring agent  
Add polysorbate-80 (1ml) as a solubilizing agent.  
Add 2-3 drops of peppermint oil as a flavouring agent.
- Volume makeup and filtration  
Add distilled water gradually and make up the final volume to 100ml.  
Stir the formulation continuously for 10-15 minutes to ensure homogeneity.  
Filter the formulation to remove any particulate matter.
- Filling and storage  
Transfer the prepared gargle in a clean airtight container.  
Store the formulation in a cool and dry place away from direct sunlight.



filtration of the prepared gargle

### 3.4 Evaluation Parameters

The prepared gargle was evaluated to determine its physicochemical and phytochemical properties. Subsequently, the anti-microbial and stability studies were also carried out.

#### 3.4.1 Physical appearance

The prepared gargle was inspected for color, clarity, homogeneity and presence of any particulate matter. A transparent & homogenous gargle was observed without presence of any particulate matter.

#### 3.4.2 pH determination

The pH of the prepared gargle was determined by immersing the pH paper into the prepared formulation.  
 pH test – 5.5 – 6.



pH measured using pH paper and compared against the standard pH scale

#### 3.4.3. Viscosity

The viscosity was measured using Brookfield viscometer with spindle 01 at a speed of 50 rpm for 60 seconds at temperature 26°C. The results are tabulated as follows:

Table 3.4.3: Viscosity measurement readings



Trial	Viscosity
1.	3.2 cp
2.	3.5 cp
3.	3.3 cp
Average	3.3 cp



#### 3.4.4. Homogeneity

The gargle was visually inspected for homogeneity. The formulation was found to be a uniform mixture without any phase separation.

#### 3.4.5. Phytochemical screening

Table 3.4.5: Phytochemical screening observation table

Sr.no	Phytoconstituents	Test performed	Reagent used	Observation	Result	Pictures
1.	Alkaloid	Dragendorff test	Dragendorff reagent + 2ml filtrate	Orange / red precipitate	Present	
2.	Flavonoid	Alkaline reagent test	Sodium hydroxide (NaOH) + 2ml filtrate + 5 drops of 1% HCl	Intense yellow coloration	Present	

3.	Tannins	Ferric chloride test	Ferric chloride + 2 ml filtrate	Blue / black coloration	Present	
4.	Saponins	Foam test	Distilled water	Foam formation	Present	

### 3.4.6. Stability studies

The gargle was subjected to stability studies and parameters such as color, odor, consistency, pH, phase separation & homogeneity were checked periodically. The prepared gargle was found to be stable with no significant changes.

Table 3.4.6: Stability studies observation table

Sr.no.	Parameters	Initial	3 days	10 days	20 days
1.	Color	Light green to yellow	Light green to yellow	Light green to yellow	Light green to yellow
2.	Odor	Characteristic	Characteristic	Characteristic	Characteristic
3.	Consistency	Stable	Stable	Stable	Stable
4.	pH	5.5	5.48	5.48	5.455
5.	Phase separation	No phase separation seen	No phase separation seen	No phase separation seen	No phase separation seen
6.	Homogeneity	Excellent	Excellent	Excellent	Excellent

### 3.4.7. Antimicrobial testing

#### Medium

3.2 gm of Nutrient agar is added in 100 ml distilled water and autoclaved at 121 °C for 15min at 15 lbs and poured in sterile petri plate and allowed to set at ambient temperature.

#### Procedure

Nutrient agar was inoculated with the broth cultures of *Escherichia coli* & *Staphylococcus aureus*. Then well was made using sterile cork borer in each plate. The prepared formulation was poured in separate wells of agar plate.

These agar plates were kept in an incubator for 24 hours. Zone of inhibition was measured in mm.

Table 3.4.7: Observation table of anti-microbial testing of prepared gargle

Sr.no.	Sample	Oral microbes	Zone of inhibition	Activity
1.	Formulation (polyherbal guava gargle)	<i>Escherichia coli</i>	13-16 mm	Moderate to good
2.	Formulation (polyherbal guava gargle)	<i>Staphylococcus aureus</i>	15-18 mm	Moderate



e. coli

*E. coli* shows better sensitivity whereas *S. aureus* shows moderate inhibition, conforming its potential use in controlling oral and microbial infections.

## IV. RESULTS AND DISCUSSION

### 4.1 Results

table 4.1: organoleptic properties

Sr.no.	Parameter	Inference
1.	Color	Light green to yellow
2.	Odor	Pleasant, characteristic smell with peppermint note
3.	Taste	Slightly bitter with refreshing & cooling effect
4.	Appearance	Clear, free from any particulate matter.
5.	Consistency	Low viscosity liquid consistency

table 4.2: result

Sr.no.	Parameter	Observation
1.	Physical appearance	Light yellow to green
2.	pH	5.5
3.	Viscosity	3.3 cp
4.	Homogeneity	Excellent
5.	Anti-microbial activity	Strong anti- microbial activity against E. coli.
6.	Phytochemical screening (test)	Presence of alkaloids, flavonoids, tannins & saponins.
7.	Stability	Stable

The formulated polyherbal gargle containing extracts of *Psidium guajava*, *Azadirachta indica* and *Ocimum sanctum* showed good physicochemical and anti-microbial properties. The formulation was clear, homogenous, stable, free from particulate matter. It had pleasant odour, acceptable pH (5.5-6.0) and low viscosity (3.3Cp), making it suitable to use. Phytochemical screening confirmed the presence of alkaloids, flavonoids, tannins and saponins. The formulation showed effective anti-microbial activity against *E. coli* and *S. aureus*. Stability studies revealed no significant changes in color, odor, pH, or homogeneity during storage. Overall the study indicates the polyherbal gargle is safe, stable and effective in treating and maintaining oral health.

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