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Formulation and Evaluation of Herbal Gargle against Throat Irritation, Inflammation and Infection

Soumalya Das¹, Tathagata Chowdhury², Sk. Mahbub Alam³, Koushik Das⁴, Soumallya Chakraborty⁵, Somenath Bhattacharya⁶, Dr. Amitava Roy⁷, Dr. Arin Bhattacharjee⁸

^{1, 2, 3, 4} Student, Department of Pharmaceutical Technology, Global College of Pharmaceutical Technology, Nadia, West Bengal, India

^{5, 6} Assistant Professor, Department of Pharmaceutical Chemistry, Global College of Pharmaceutical Technology, Nadia, West Bengal, India

⁷ Professor, Department of Pharmaceutical Chemistry, Global College of Pharmaceutical Technology, Nadia, West Bengal, India

⁸ Principal, Department of Pharmaceutical Technology, Global College of Pharmaceutical Technology, Nadia, West Bengal, India

Abstract: Herbal gargles have gained significant attention as potential alternatives to conventional oral care products due to their perceived natural composition and reported therapeutic benefits. This review aims to provide a comprehensive overview of the efficacy of herbal gargles in promoting oral health and preventing various oral ailments. Our oral cavity is a sweet able place to grow different types of bacterial species either harmless or harmful for human. From ancient age medicinal plants are considered as a store room of different types of biological activity in Ayurveda, Unani and Siddha, and have important role to cleanse tooth and prevent different human pathogens are responsible for unpleasant odour, inflammation of teeth root, dental plaque. The study evaluates a wide range of herbal ingredients commonly found in mouth gargles, including essential oils, plant extracts, and traditional herbal remedies. It explores their antimicrobial properties, antioxidant activity, and potential for reducing plaque formation, gingivitis, and bad breath. Aqueous extract of Tulsi, Turmeric, Clove, Fennel, Betel leaves, Pudina, Ginger, Liquorice shows effective antimicrobial, antifungal activity, anti-inflammatory and anti-plaque properties. In this research work herbal gargle was evaluated depends on various parameter like color, pH, Phase separation, Homogeneity and antibacterial properties. Herbal gargles show potential as adjuncts to conventional oral care, Herbal gargle is suitable for any age group due to less side effect. Healthcare professionals and consumers should approach herbal gargles with caution, taking into consideration individual oral health needs and consulting oral healthcare providers for personalized recommendations.

Keywords: Herbal Gargle, Formulation, Evaluation, Antimicrobial study, E. Coli

I. INTRODUCTION

The first known references to mouth rinsing are described in Ayurveda and Chinese medicine in 2700 BC. Mouth gargle is a chemotherapeutic agent used as effective home care system by the patient. [1]

In the Greek and Roman periods, mouth rinsing became common among the Hippocrates. They recommended a mixture of salt, alum and vinegar.[1]

Gargles are aqueous solutions used to treat the problem related to pharynx and nasopharynx by pushing air from the lungs through the gargle while it is held in the throat. Often, gargles need to be diluted with water before use. Gargles are used to get the medication onto the mucosal surface of the throat.[2,3] The preparations need to have acceptable organoleptic qualities and be quick-acting.

1) *Natural:* Natural gargles which are also known as herbal gargles. eg: liquorice, clove, ginger, salt water.

2) *Chemical:* Gargles, made from chemical compound. eg.: Methyl salicylate, Saccharine sodium.

Such a variety of health advantages come from gargling and rinsing with salt water. It promotes good oral hygiene and dental health with gargling and it supports the postoperative care process. It aids in the recovery of canker sores. Sometimes pregnancy difficulties may be avoided with its aid.





Gargle can be dangerous for children when used orally they didn't even know how to gargling. Due to oral cavity sometimes, gargle might produce sensation and itching in mouth. [4]






II. MATERIALS AND METHODS



A. Collection of Plants

Leaves, stem, buds and dried seeds of Tulsi (*Ocimum sanctum*) [5,6], Clove (*Eugenia caryophyllus*) [7,8], Fennel (*Foeniculum vulgare*) [9,10], Ginger (*Zingiber officinale*) [11,12], Liquorice (*Glycyrrhiza glabra*) [13,14], Turmeric (*Curcuma longa*) [15,16], Fenugreek (*Trigonella foenum-graceum*) [17,18], Cardamom (*Elettaria cardamomum*) [19,20], Betel Leaves (*Piper betel*) [21,22], Pudina (*Mentha piperita* Linn) [23,24], Coriander (*Coriandrum sativum*) [25,26] were randomly collected from mature plants.

TABLE-1 List Of Ingredients With Their Functions

Sl.No	Ingredients	Scientific name	Chemical constituents	Plant part	Functions
1	Tulsi family: <i>Labiatae</i> plant part: fresh leaves	<i>Ocimum sanctum</i>	Eugenol, Estragole, Camphene		Antibacterial, insecticidal and stimulant
2	Clove family: <i>Myrtaceae</i> Plant part: dried flower buds	<i>Eugenia caryophyllus</i>	Eugenol, Caryophylline, Methyl eugenol		Dental analgesic, carminative, stimulant, antioxidant, flavouring agent, an aromatic and antiseptic
3	Fennel family: <i>Umbelliferae</i> Plant part: dried ripe fruit of plant	<i>Foeniculum vulgare</i>	Fenchone, Anethole, Estragole		Carminative, aromatic, stimulant and flavouring agent
4	Ginger family: <i>Zingiberaceae</i> Plant part: rhizomes	<i>Zingiber officinale</i>	Gingerol, Shogaole, Zingiberene		Stomachic, an aromatic, a carminative, stimulant and flavouring agent.


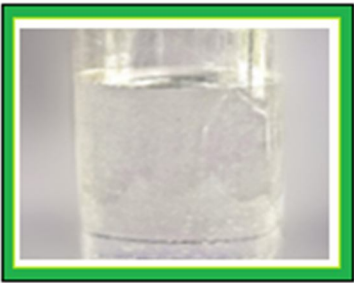
5	<p>Liquorice: family: Leguminoseae Plant part: dried peeled or unpeeled roots and stolons of the plant</p>	<i>Glycyrrhiza glabra</i>	Glycyrrhizin, Chalcone, Enoxolone		Demulcent, mild expectorant. sweetening agent, flavouring agent, antitussive, antispasmodic, anti- inflammatory and antiulcer drug
6	<p>Turmeric: family: Zingiberaceae Plant part: dried, as well as fresh rhizomes of plant</p>	<i>Curcuma longa</i>	Curcumin, Phellandrene, Curcuminoid		Expectorant, a condiment or spice, colouring agent
7	<p>Fenugreek Family: Fabaceae Plant part: Fresh and dried seeds</p>	<i>Trigonella foenum- graceum</i>	Trigonelline, Diogenin, Sotolone		Antioxident, antiulcer, antibacterial, neuroprotective and liver protection propaties
8	<p>Cardamom: Family: Zingiberaceae Plant Part: dried ripe fruits</p>	<i>Elettaria cardamomu m</i>	Terpineol, Eucalyptol, Sabinene		Aromatic, carminative, stimulant and flavouring agent
9	<p>Betel leaves Family: Piperaceae Plant part: Fresh leaves</p>	<i>Piper betel</i>	Arecoline, Methyl euginol, Estragole		Pungent and flavouring agent

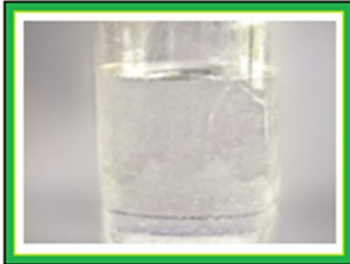
10	<p>Coriander Family: Umelliferae Plant part: fully dried ripe fruits of the plant</p>	<p><i>Coriandrum sativum</i></p>	<p>Dodecanal, p- Cymene, Borneol</p>		<p>Aromatic, carminative, stimulant and flavouring agent</p>
11	<p>Pudina Family: Labiatae Plant part: Fresh leaves</p>	<p><i>Mentha piperita</i></p>	<p>Menthol, Menthone, Eucalyptol</p>		<p>Carminative, stimulant, aromatic, counter- irritant flavouring agent and mild antiseptic</p>

B. Collection of Chemicals

In this formulation Sodium Benzoate is used as preservative, Sorbitol is used for soothing & refreshing and Glycerol is used to treat mouth irritation.

TABLE-2 Chemicals Used In The Preparation Of Gargle

Sl. No.	Ingredients	Raw materials	Functions
1	Sodium Benzoate		Anti-corrosive and preservative
2	Sorbitol		Provide a soothing feeling in mouth , mild sweetness and a refreshing. Resistance to dental caries.

3	Glycerol		Treating sore throat pain, mouth pain and minor mouth irritation
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C. Aqueous Extraction Process of Plant parts-

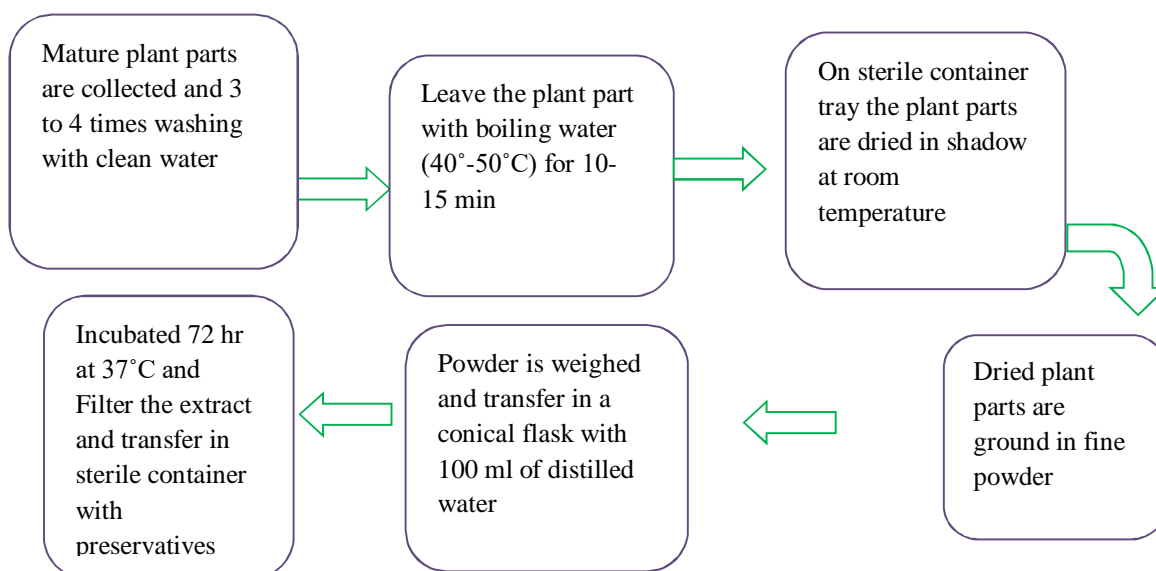


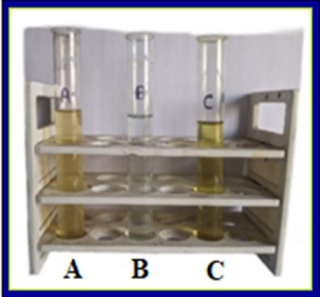
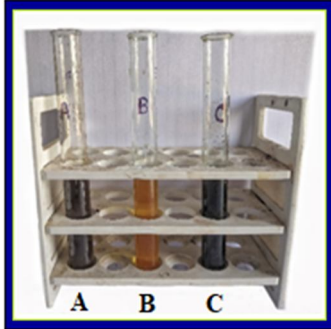

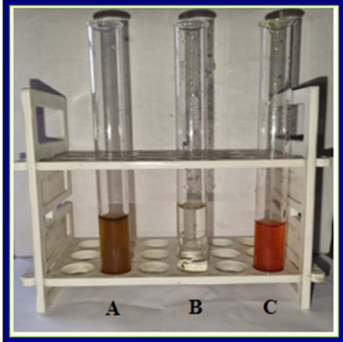
TABLE-3 : Water Soluble Extracts

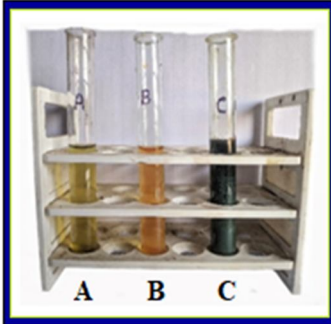
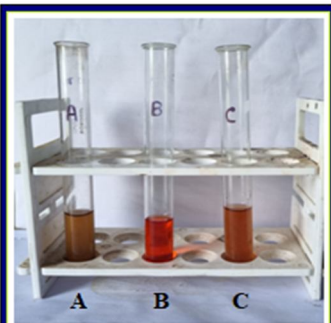
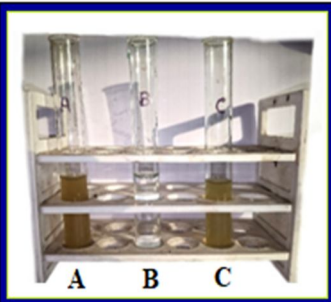
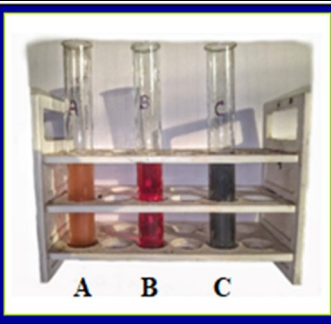
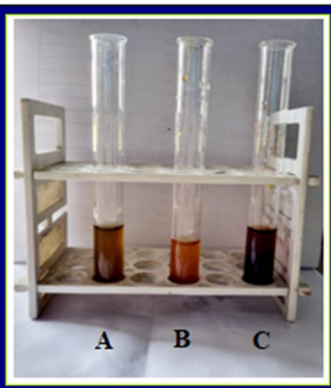
Ingredients	Amount taken in gm	Water quantity (ml)	Extract (gm)	Percentage (%)
Tulsi	1.02	50	0.144	14.4
Clove	1.01	50	0.2	20
Fennel	1.02	50	0.219	21.9
Turmeric	1.03	50	0.267	26.7
Ginger	1.01	50	0.224	22.4
Liquorice	1.02	50	0.28	28
Coriander	1.03	50	0.209	20.9
Cardamom	1.01	50	0.216	21.6
Betel leaves	1.03	50	0.186	18.6
Fenugreek	1.04	50	0.236	23.6
Mint	1.02	50	0.193	19.3


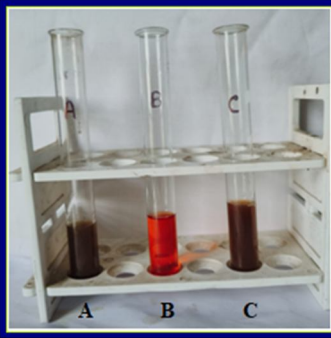
D. Phytochemical Screening

The chemical, identification tests of different herbal extract are given in the following

TABLE-4 Chemical Test Of Plant Extracts

Sr. No.	Name of herbs	Tests	Observation	Images
1	Tulsi	0.5 ml of crude extract was mixed with 2ml of glacial acetic acid containing 2-3 drops of 2% solution of FeCl ₃ . Then 2ml of concentrated H ₂ SO ₄ was poured into the mixture.	A brown ring at the interface indicated the presence of cardiac glycosides	
2	Clove	Aq. Extract + ferric chloride 5% solution	the cloves extract and eugenol standard turned light green	
3	Turmeric	1 or 2 ml of extract added with Wagner's reagent (dissolving 2gm of iodine and 6 gm of potassium iodine in 100ml of distilled water)	Reddish brown precipitate shows for the alkaloids	
4	Liquorice	Moistened with 80% H ₂ SO ₄	Orange yellow is observed for transformation of flavone glycoside Liquiritin to chalcone glycoside isoliquiritin	

5	Pudina	1ml of Aqueous extract and 2 drops of Ferric chloride($FeCl_3$) added with the extract	Green – blackish colour indicate presence of catechol tannins	
6	Cardamom	Aqueous extract of Elettaria cardamomum + volatile oil + Sudan III	Red colour due to volatile oil	
7	Fennel	2ml extract + 1ml Mayer's reagent (1.36gm mercuric chloride + 5gm potassium iodide in 100ml water)	Light green precipitate indicating presence of alkaloids	
8	Ginger	Aq. Extract of ginger + few drops of Wagner's reagent (dissolving 2gm of iodine and 6 gm of potassium iodine in 100ml of distilled water)	Reddish brown precipitation due to alkaloids	
9	Betel leaves	1 gm Powder form of Piper betel + 10 ml distilled water (5 min boil) + 2 drop 5% $FeCl_3$	Greenish black Precipitation due to tannin	

10	Fenugreek	4ml of aqueous extract + few drops of Wagner's reagent	Green precipitate confirms the present of alkaloids	
11	Coriander	Aq. Extract of <i>Coriandrum sativum</i> + Violet oil + Sudan III	Red colour due to volatile oil	

Here, A= Plant Extract, B= Reagent, C= Final Product

E. Formulation of herbal gargle

The herbal Mouth-gargle was prepared by the formula given in Table – 5

TABLE-5 Formulation Table

Ingredients	Function	Each 5 ml contains
Tulsi	Antibacterial	20%
Ginger	Stomachic	5%
Clove	Dental analgesic	10%
Liquorice	Sweetening agent	5%

Cardamom	Aromatic	3%
Turmeric	Expectorant	5%
Fenugreek	Antioxidant	5%
Fennel	Carminative	10%
Betel leaves	Pungent	3%
Pudina	Cooling agent	10%
Coriander	Carminative	3%
Sorbitol	Sweetening agent	10%
Glycerol	Co surfactant	5%
Sodium benzoate	Preservatives	1.25%
Water	Diluent	q.s

III. EVALUATION

- 1) *Colour*- Dark Brownish
- 2) *Odour*- Characteristics
- 3) *pH*- pH of prepared herbal gargle was measured by using Digital pH meter. The pH meter was previously calibrated using standard buffer solution. Collect about 1 ml of gargle and dissolved in 50 ml of distilled water and it's pH was found 5.84 at room temperature.
- 4) *Weight per ml*:

Table – 6: Weight per ml test

Sample quantity	Weight of empty volumetric flask	Weight of volumetric flask with formulation	Result (wt/ml)
25ml	42.21 gm	67.53 gm	1.01gm/ml

5) *Test for Microbiological growth*

The formulated mouth gargle was inoculated in the plates of agar media by streak plate method and a control was prepared. The plates were kept at 37°C for 24 hours in the incubator. After the incubation period plates were taken out and checked for microbial growth.

6) *Stability studies*

Stability test aims to ensure that the gargle formulations are usable and can maintain the same characteristics in the long term basis, before undergoing antibacterial assay

The formulation and preparation of a pharmaceutical product is incomplete without proper stability studies of the prepared product. This is done in order to determine the physical and chemical stability of the prepared product and thus determine the safety of the product at (30 ± 2°C, RH- 65±5°C)

TABLE-7 : Stability Studies Of Formulated Herbal Gargle

Sl. No.	Parameters	Observations			
		Initial	10 days	20 days	30 days
1	Colour	Dark brown	Dark brown	Dark brown	Dark brown
2	Odour	Characteristics	Characteristics	Characteristics	Characteristics
3	Consistency	Stable	Stable	Stable	Stable
4	Phase separation	Nil	Nil	Nil	Nil
5	pH	5.83	5.82	5.84	5.85
6	Homogeneity	Good	Good	Good	Good
7	Weight /ml	1.01 gm/ml	1.03 gm/ml	1.01 gm/ml	1.02 gm/ml

IV. RESULT & DISCUSSION

The look of gargle retained its colour and homogeneity after a one-month examination. The gargle's formulation showed no signs of phase separation. Gargle has maintained its pH and has a mildly acidic character. The specially prepared gargle proved active against a variety of human pathogens, including *S. aureus* (+ve) and *E. coli* (-ve).

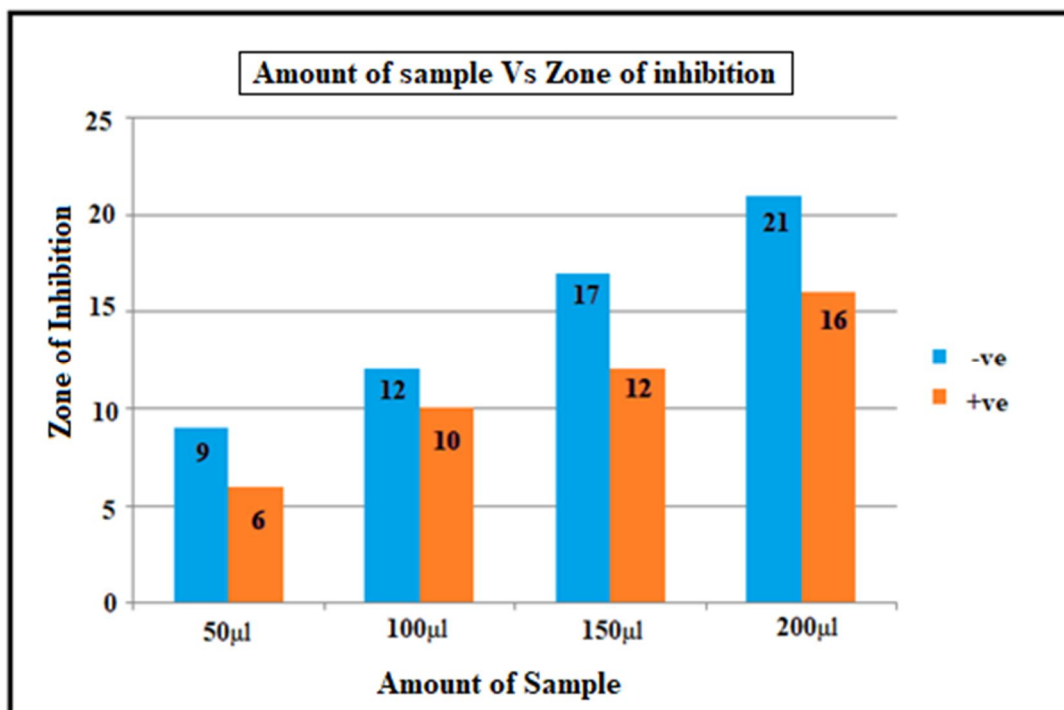
The formulation was useful against various microorganisms and shows good stability during various physicochemical test and mostly effective against gram negative bacteria instead of gram positive bacteria. This mouth-gargle entirely prepared from plants parts and safe for health.

TABLE-8 : Zone Of Inhibition

ORGANISM	ZONE OF INHIBITION (nm)			
	50 μ l	100 μ l	150 μ l	200 μ l
E. coli(-ve)	9 nm	12 nm	17 nm	21 nm
S. aureus(+ve).	6 nm	10 nm	12 nm	16 nm

For various gargle concentrations amount, the agar diffusion method was used to determine their antibacterial activity. For E. coli (-ve), the zone of inhibition was found to be 9 mm for 50 μ l, 12 mm for 100 μ l, 17 mm for 150 μ l, and 21 mm for 200 μ l, respectively. For S. aureus, the zone of inhibition was found to be 6 mm for 50 μ l, 10 mm for 100 μ l, 12 mm for 150 μ l, and 16 mm for 200 μ l, respectively. These findings demonstrated the significant antibacterial activity of the herbal mouthwash and the capability of the current product to prevent bacterial growth in the oral cavity.

TABLE- 9 ANTI-BACTERIAL ACTIVITY



V. CONCLUSION

The present liquid herbal gargle can work in long way to help people to cure the various throat disorder . Present herbal formulation is acceptable for a long period.

Furthermore the prepared herbal gargle were standardized by various physicochemical studies like pH, appearance of solution, consistency, phase separation and microbial study and all test results are in limit. So the prepared herbal formulation is very good and safe for any age group.

VI. ACKNOWLEDGEMENT

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VII. CONFLICT OF INTEREST

The authors declare that there is no conflict of interest to reveal.

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