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Research Article

Formulation Development and Evaluation of Polyherbal Mouthwash Containing Psidium Guajava L.

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Abstract:
The mouthwashes prepared from the herbal ingredients has shown great potential to overcome the chemical-based preparations. The combination of two or more herbal ingredients to form a polyherbal formulation has become an efficient approach to add value to the basic herbal formulation. In present study, the polyherbal mouthwash has been prepared using the leaves of Psidium guajava (guava), rhizomes of Curcuma longa (turmeric) and flower buds of Syzygium aromaticum (clove). All the three plant materials were collected and separately extracted. Phytochemical screening and quantitative estimation has been performed. The formulation has been prepared by optimization of different concentrations of used active ingredients. All the prepared formulations had undergone evaluation for organoleptic properties, pH and viscosity. The in-vitro antibacterial study has been performed by agar well diffusion method. The bacteria selected for the study were S. aureus, S. mutans and E. coli. The prepared mouthwash shows promising results in terms of zone of inhibition. When compared the prepared mouthwash with the marketed one, it shows greater zone of inhibition due to the synergistic effect produced by the multiple herbs used in the formulation. From the results obtained in this research work, it can be clearly stated that polyherbal mouthwash has become an effective way to improve the efficacy of herbal formulations. The combination of Psidium guajava (guava), Curcuma longa (turmeric) and Syzygium aromaticum (clove) in a mouthwash has shown promising results to improve the chances of usage of polyherbal mouthwash to commercially available chemical-based product.

Keywords: Psidium guajava, Curcuma longa, Syzygium aromaticum, Polyherbal formulation, Herbal Mouthwash

1. Introduction

1.1 Polyherbal formulations

The concept of Polyherbal formulation (PHF) deals with the use of multiple herbs in a medicinal preparation. The concept is found in Ayurvedic and other traditional medicinal systems where multiple herbs in a particular ratio may be used in the treatment of illness. This key traditional therapeutic herbal strategy exploits the combination of several medicinal herbs to achieve extra therapeutic effectiveness, usually known as polypharmacy or polyherbalism.[1]

The key advantages for Polyherbal formulations are:-

1. Polyherbal formulations are known to express high effectiveness in a vast number of diseases. The therapeutic effect of herbal medicines is exerted due to the presence of different phytoconstituents and the effects are further potentiated when compatible herbals are formulated together in PHFs.
2. They are usually found to have wide therapeutic range. Most of them are effective even at a low dose and safe at high dose, thus they have superior risk to benefit ratio.
3. Polyherbal formulations result in fewer side effects as compared to allopathic drugs.
4. They are a product of the nature, they are relatively cheaper, eco-friendly and readily available than allopathic drugs.
5. Their better affordability and greater accessibility account for increasing demand globally, especially in rural areas and some developing countries, where costly modern treatments are not available.
6. These formulations are preferred by physician due to the mechanism of synergism in this type of formulations.
7. They are more readily acceptable, culturally and socially.[2]

1.2 Mouthwash

Mouthwash is an aqueous solution which is most often used for control of plaque and is a medicated liquid which is held in the mouth and swished by the action of perioral musculature to eliminate the oral pathogens. It is a liquid which is held in the mouth passively or swilled around the mouth by contraction of the perioral muscles and/or movement of the head, and may be gargled, where the head is tilted back and the liquid bubbled at the back of the mouth. They are also known as oral rinse or mouth rinse or mouth bath.[3]

There are two main types of mouthwashes:

- **Therapeutic mouthwashes:** These have active ingredients that kill bacteria and can help reduce plaque, gingivitis, cavities and bad breath. Those that contain fluoride help prevent or reduce tooth decay.

- **Cosmetic mouthwashes:** These may temporarily control or reduce bad breath and leave your mouth with a pleasant taste, but don't reduce your risk of cavities or gum disease.

1.3 Herbal Mouthwash

Herbal mouthwashes are mouthwashes which are prepared from natural plant extracts. The use of herbal mouthwash has grown advantage over chemical mouthwashes due to their non-irritant and non-staining properties and it does not contain alcohol. The natural extracts present in these herbal mouthwashes are obtained from various plant leaves, fruits, seeds and various tree oils. They have very minimal or no side effects and they are less harmful. [4]

Herbal medicine has shown great potential as mouthwashes and they are preventive in their approach. The herbal extracts have anti-inflammatory effects and prevent bleeding, which is important in dental treatment. Antiseptics, antibacterial, antimicrobial, antifungal, antioxidant, antiviral, and analgesic agents derived from plants are of widespread interest in dentistry. Though herbal products have helped to control dental plaque and gingivitis, they have been used for a short time and only as an adjunct to other oral hygiene measures such as brushing and flossing.

Unlike most commercial cosmetic and therapeutic oral rinses, natural mouth rinses typically do not contain:

1. Alcohol
2. Stannous fluoride, a processed form of fluoride that stain teeth
3. Cetyl pyridinium chloride (CPC), which also can cause staining
4. Harsh chemical preservatives and dyes
5. Artificial colors and sweeteners.

1.4 Periodontal Disease

Periodontal, on etymology is divided as "Peri" means surrounding, "odonto" means tooth", collectively it means pertaining to the surrounding of teeths. Periodontal diseases are mainly the result of infections and inflammation of the gums and bone that surround and support the teeth.[5] In its early stage, the gums can become swollen and red, and they may bleed.

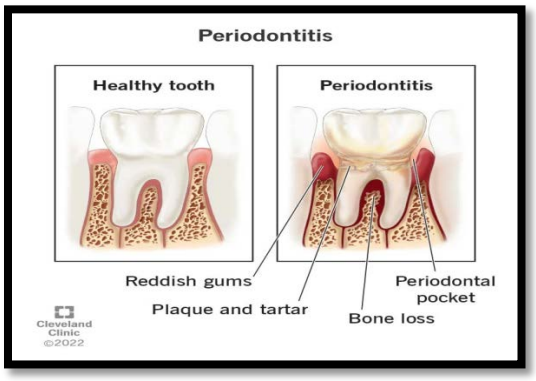

Figure 1.1: Comparison of Healthy Tooth and Periodontitis

2. Plant Profile

2.1 Psidium guajava L. (Guava)

Psidium guajava (common name-guava) is well known tropic tree which is abundantly grown for fruit. It has about 133 genera and more than 3,800 species. *Psidium guajava* and it's all parts have an old history of medicinal value. The plant is well known by a common name "Guava" in English. It has a huge content of antimicrobial and antibacterial compounds. [6]

Scientific Classification

Kingdom: Plantae
Order: Myrtales
Family: Myrtaceae
Genus: Psidium
Species: *P. guajava*
Binomial Name: *Psidium guajava* L. [7]

Pharmacological Actions

The pharmacological actions and the medicinal uses of aqueous extracts of guava leaves in folk medicine include the treatment of various types of gastrointestinal disturbances such as vomiting, diarrhea, inhibition of the peristaltic reflex, gastroenteritis, spasmolytic activity, dysentery, abdominal distention, flatulence and gastric pain. These extracts have also been indicated to cause disturbances of the central nervous system: insomnia, convulsions and epilepsy. Bronchitis, asthma attacks, cough, pulmonary diseases could be also treated with guava teas and could also be useful as anti-inflammatory and hemostatic agent. Moreover, aqueous extracts of guava leaves were described to be effective against a number of microbial strains and anti-rotavirus activity. [8]

Figure 2.1 Leaves of Psidium guajava

2.2 Curcuma longa L. (Turmeric)

Curcuma longa, or turmeric is a perennial herb and member of the Zingiberaceae (ginger) family and is cultivated extensively in Asia mostly in India and China. The rhizome, the portion of the plant used medicinally, yields a yellow powder.

Scientific Classification
Kingdom: Plantae
Order: Zingiberales
Family: Zingiberaceae
Genus: Curcuma
Species: *C. longa*
Binomial Name: Curcuma longa L. [9]

Pharmacological Actions
This review focuses on the medicinal and pharmacological properties of turmeric as anti-inflammatory, antioxidant, hepatoprotective, anticarcinogenic, antidiabetic, antimicrobial, antidepressant in addition to its use in cardiovascular disease, gastrointestinal and neurological disorders. [10]

Figure 2.2: Rhizomes of Curcuma longa

2.3 Syzygium aromaticum (Clove)

Syzygium aromaticum also known as cloves vary in length from about 1/2 to 3/4 inch and contain 14-20% essential oil. Cloves are strongly pungent due to their high content of eugenol, which can be extracted by distillation to yield the essential oil. Clove buds have been regarded as safe when taken orally for medicinal use. Cloves have been used by humans for medicinal applications for over two thousand years.

Scientific Classification:
Kingdom: Plantae
Order: Myrtales
Family: Myrtaceae
Genus: Syzygium
Species: *S. aromaticum*
Binomial Name: *Syzygium aromaticum* L. [11]
Pharmacological Actions

Clove is a medicinally important drug, reported to have a variety of different applications like antioxidant, antifungal, antiviral, antibacterial, anti-inflammatory, antithrombic, antipyretic, analgesic, anticonvulsant, antimycotic, insecticidal, antimutagenic, antiulcerogenic etc. The oil is used for treating a variety of health disorders including toothaches, indigestion, cough, asthma, headache, and stress and blood problems. Clove is used to treat various health conditions, including intestinal parasites, migraine headaches, colds, impotence, and gastrointestinal problems such as nausea, vomiting, diarrhea and gas.[12]

Figure 2.3: Flower buds of *Syzygium aromaticum*

3. Experimental Work

3.1 Collection and Procurement of Plant Material

Guava trees are available in abundance in India. The small branches of these trees were freshly cut. Leaves from the concerned branches procured were removed. Leaves of *Psidium guajava* were collected from Salakhedi, Ratlam (M.P.) in the month of November 2022.

The rhizomes of *Curcuma longa* and flower buds of *Syzygium aromaticum* were purchased from Patwa Brothers, a reputed vendor for herbal drugs in Ratlam (M.P.).

3.2 Identification and Authentication of Plant Materials

The collected plant materials were identified and authenticated by Head Botanist at Department of Botany.

3.3 Extraction of Plant Materials

Following procedure was adopted for the preparation of extract from the shade dried and powdered herbs [13]:

3.3.1 Defatting of Plant Materials

The collected leaves of *Psidium guajava*, rhizomes of *Curcuma longa* and flower buds of *Syzygium aromaticum* were cleaned thoroughly and separately washed in running water for about 10-15 min to remove all traces of dirt and extraneous contaminating material. Final wash was done with distilled water. The leaves of *Psidium guajava* were shade dried at room temperature. The shade dried plant material of *Psidium guajava*, rhizomes of *Curcuma longa* and flower buds of *Syzygium aromaticum* were coarsely powdered using a high-speed electric grinder for 15 min. The powder was transferred to separate sterile, airtight plastic containers with lids and each container was labeled with the name of the respective plant. The powdered plant material was subjected to extraction with petroleum ether using maceration method separately.[14] The extraction was continued till the defatting of the material had taken place. The drying, cleaning and defatting has been performed separately for all the three plant materials.

3.3.2 Extraction of Plant Material

After the defatting of plant materials, the extraction method has been performed. For all the three herbs, 20gm of powdered plant material has been taken in a 150ml Erlenmeyer flask and 100 ml of distilled water has been added in 3 separate flasks. The mixtures were wrapped in aluminum foil to avoid evaporation and exposure to light for 3 days at room temperature. The flasks were placed on a platform shaker at 70 rpm. After 3 days of soaking in solvent, the mixtures were transferred to 50 mL tubes and centrifuged for 10 min at 4,000 rpm at 25°C. The supernatant was collected and stored at 4°C until use. [15]

3.3.3 Determination of Percentage Yield

The percentage yield of yield of each extract was calculated by using formula:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of Powdered drug taken}} \times 100$$

The result of percentage yield has been shown in table 3.1

3.4 Qualitative Phytochemical Analysis

All the extracts were subjected to various qualitative tests to detect the presence of plant constituents [16]:-

a) Alkaloid
b) Flavonoid
c) Steroid
d) Terpenoid
e) Phenol
f) Proteins
g) Carbohydrates
h) Glycosides
i) Saponins

The qualitative results for *Psidium guajava, Curcuma longa and Syzygium aromaticum* extract has been shown below in table 3.2.

3.5 Quantitative Estimation of Phytoconstitutents

The main secondary metabolites which are responsible for Antibacterial and Antimicrobial activities are flavonoids and phenols, so the quantitative estimation has been performed only for them. The quantitative estimation result for all the three plants namely, *Psidium guajava*, *Curcuma longa* and *Syzygium aromaticum* has been shown in table 3.3.

3.5.1 Total phenolic content estimation

Principle: The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method [17].

Procedure: 2 ml of aqueous extract and each standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a UV/Vis spectrophotometer.

3.5.2 Total flavonoid content estimation

Principle: Determination of total flavonoids content was based on aluminium chloride method [18].

Procedure: 1 ml of 2% $AlCl_3$ solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm.

Table No. 3.1: Result of percentage yield of the prepared extracts

S. No.	Extract	Percentage Yield (%)
1.	*Psidium guajava*	16.2
2.	*Curcuma longa*	14.1
3.	*Syzygium aromaticum*	15.5

Table No. 3.2: Phytochemical test results for *Psidium guajava*

Phytochemical	Test	Observation	Result for Guava	Result for Turmeric	Result for Clove
Alkaloid	Mayer's Test	Dull White PPT	-	+	+
Flavonoid	Lead Acetate Test	Yellow colour PPT	+	+	+
Steroid	Liebermann-Burchard Test	Change of colour from blue to green	-	-	-
Terpenoid	Salkowski Test	Reddish-brown PPT	+	+	+
Phenol	Ferric Chloride	Blue-green or Black	+	+	+

		colour			
Protein	Xanthoproteic Test	Yellow colour	-	-	-
Carbohydrate	Molisch's Test	Reddish-violet ring at Junction	+	-	+
Glycoside	Legal's Test	Pink to blood Red colour	+	+	-
Saponin	Foam Test	Formation of stable foam	+	+	-

Table No. 3.3: Result of Quantitative Estimation of Phenol and Flavonoid

S. No.	***Psidium guajava* extract**	
1.	Total phenol (GAE) (mg/g)	147.42
2.	Total flavonoid (QE) (mg/g)	49.81
	***Curcuma longa* extract**	
1.	Total phenol (GAE) (mg/g)	128.68
2.	Total flavonoid (QE) (mg/g)	31.57

3.6 Formulation development of Polyherbal Mouthwash

Three different formulations of polyherbal mouthwash were developed as shown in Table 3.4. The mouthwash formula made use of three main herbal ingredients: *Psidium guajava* (Guava), *Curcuma longa* (Turmeric) and *Syzygium aromaticum* (Clove). Some minor non-herbal ingredients were also included, such as the sweetener, sodium benzoate, and salt. The minor components were used for preservation and for improving the taste. In order to test the antibacterial activity of the mouthwash herbs, different percentages of the herbal extracts were prepared. Meanwhile, the concentrations of the minor ingredients were fixed for all three mouthwashes. A similar amount of water was also added until the desired volume for the mouthwash was achieved.

Table 3.4: Different formulations of Prepared Polyherbal Mouthwash

Ingredient	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**
	Qty (in ml)					
Psidium guajava (10g/100ml)	30	20	20	25	25	35
Curcuma longa (10g/100ml)	10	20	15	20	10	10
Syzygium aromaticum (10g/100ml)	10	10	15	5	15	5
Water	40	40	40	40	40	40
Sucrose (10g/100ml)	3	3	3	3	3	3
Salt Solution (10g/100ml)	6.8	6.8	6.8	6.8	6.8	6.8
Sodium Benzoate (10g/100ml)	0.2	0.2	0.2	0.2	0.2	0.2
Total Volume	100	100	100	100	100	100

3.7 Evaluation of Prepared Polyherbal Mouthwash:

3.7.1 Colour and Odour: Physical parameters like odour and colour were examined by visual examination[20]. The results for organoleptic evaluation of all the prepared formulations has been described in the table 3.5.

Table No. 3.5: Result of Organoleptic characteristics of prepared formulations

Formulation	Colour	Odour
F1	Slight Yellow	Aromatic
F2	Yellow	Aromatic
F3	Slight Yellow	Pungent
F4	Slight Yellow	Aromatic
F5	Yellow	Pungent
F6	Slight Yellow	Aromatic

3.7.2 pH

pH of prepared herbal mouthwash was measured by using digital pH meter. The pH meter was calibrated using standard buffer solution and then the pH of prepared formulations was measured[21]. The results for the pH of all the prepared formulations has been described in the table 3.6.

Table No. 3.6: Result of pH of prepared formulations

Formulation	pH
F1	6.9
F2	6.7
F3	7.2
F4	7.5
F5	6.8
F6	7.1

3.7.3 Viscosity

Viscosity of the mouthwash was determined with the help of digital viscometer at 100 rpm with the spindle 6[22]. The results for the viscosity of all the prepared formulations has been described in the table 3.7.

Table No. 3.7: Result of Viscosity of prepared formulations

Formulation	Viscosity (in centi poise)
F1	1.49
F2	1.89
F3	2.01
F4	1.87
F5	2.62
F6	2.12

3.7.4 In-vitro Antibacterial Activity

In vitro antibacterial activity was performed on isolated colonies of *Streptococcus mutans*, *Staphylococcus aureus* and *Escherchia coli*. The Agar well diffusion technique was used for determining the zone of inhibition and minimum inhibitory concentrations (MIC). The strains of S. mutans, S. aureus and E. coli were inoculated in prefabricated agar plate. Plates were dried and 4 wells were made with the help of 6 mm agar well cutter. Hexidine (Chlorhexidine) mouthwash has been used as control. 25 µl, 50µl and 100 µl of prepared mouthwash was loaded in the three respective wells and control mouthwash was loaded in the final well. The agar plates were kept undisturbed to allow the passive diffusion of herbal mouth wash into the agar culture medium. Then the plates were incubated at

37°C for 24 hours. The zone of inhibition was calculated in mm[23].

The antibacterial activity was evaluated by agar diffusion method for different concentrations of mouthwash. The result of zone of inhibition for S. mutans, S. aureus and E. coli was shown in table 3.8. These results showed that the herbal mouthwash has significant antibacterial activity and the present preparation is able to inhibit bacterial growth in oral cavity.

Table No. 3.8: Comparative study of all the prepared formulations on all three bacteria

Formulation	*Staphylococcus aureus*			*Streptococcus mutans*			*Escherichia coli*		
	Zone of Inhibition in different dilutions (in mm)								
	25 µl	50 µl	100 µl	25 µl	50 µl	100 µl	25 µl	50 µl	100 µl
F1	5	8	11	7	9	12	11	15	18
F2	4	7	9	6	9	11	8	13	16
F3	6	9	11	5	8	12	9	12	15
F4	8	11	14	9	12	15	12	17	20
F5	6	8	9	6	9	12	7	13	17
F6	**8**	**12**	**15**	**9**	**13**	**17**	**13**	**18**	**22**

From this comparative evaluation of different formulations, it is evident that F6 is the best formulation. Further, this F6 formulation has been compared with the marketed Hexidine (Chlorhexidine) mouthwash to obtain the better results for the efficacy of prepared formulation. The results were shown in table 3.9 and graphically represented in fig. 3.1, 3.2 and 3.3.

Table No. 3.9: Comparative study of F6 formulation with Marketed formulation

Formulation	*Staphylococcus aureus*			*Streptococcus mutans*			*Escherichia coli*		
	Zone of Inhibition in different dilutions (in mm)								
	25 µl	50 µl	100 µl	25 µl	50 µl	100 µl	25 µl	50 µl	100 µl
F6 Formulation	8	12	15	9	13	17	13	18	22
Marketed Formulation	7	9	11	8	13	17	13	17	21

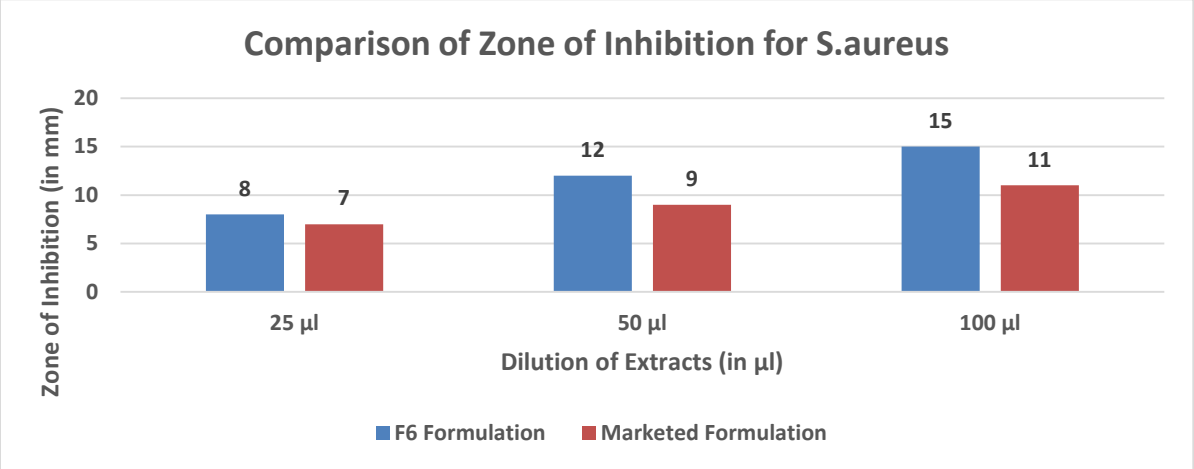

Figure 3.1: Comparison of Zone of Inhibition of S. aureus for F6 and Marketed formulation

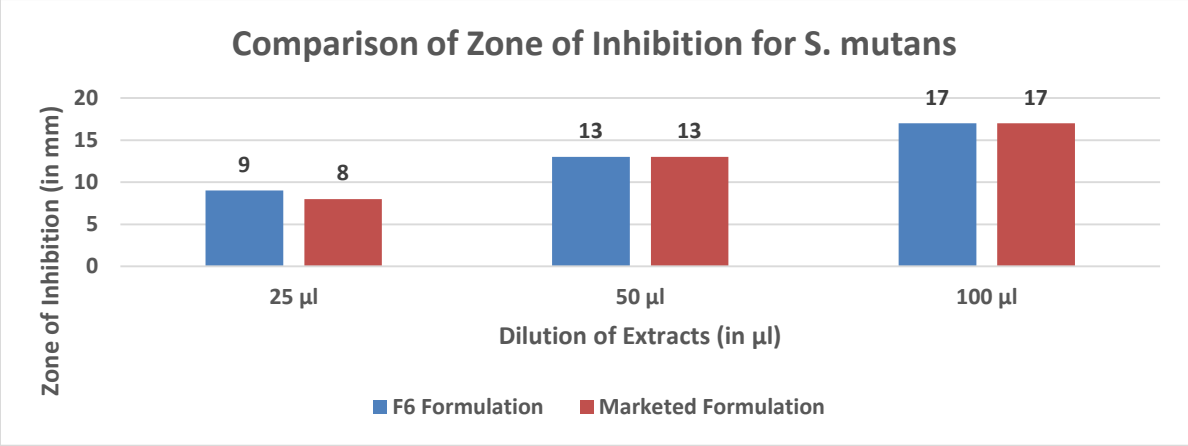

Figure 3.2: Comparison of Zone of Inhibition of S. mutans for F6 and Marketed formulation

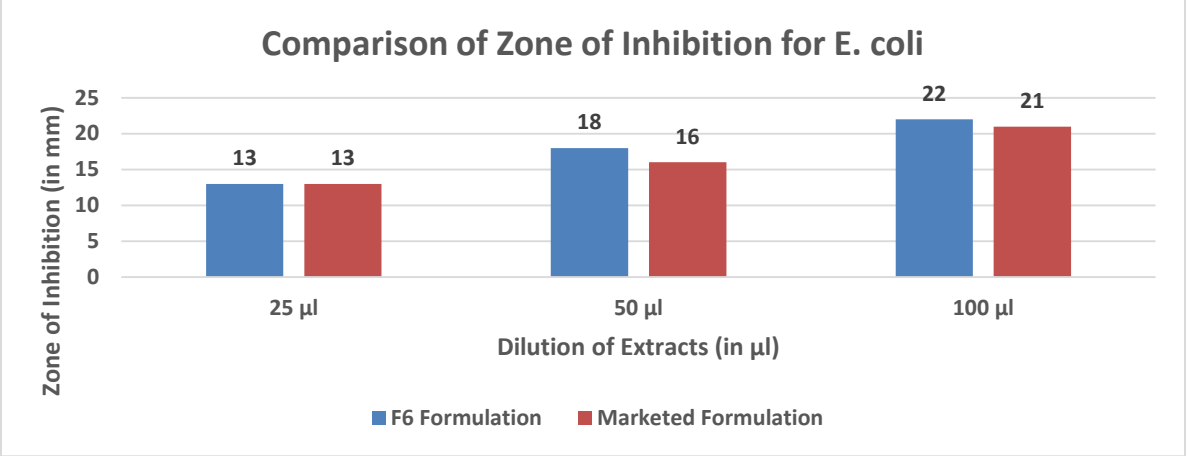

Figure 3.3: Comparison of Zone of Inhibition of E. coli for F6 and Marketed formulation

3.7.5 Stability Studies

The formulation and preparation of any 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. A general method for predicting the stability of any product is accelerated stability studies, where the product is subjected to elevated temperatures as per the ICH guidelines. A short term accelerated stability study was carried out for the period of 3 months for the prepared formulation. The samples were stored at under the following conditions of temperature as 3-5 °C, 25°C RH=60%, 40°C ±2% RH= 75%. Finally the samples kept under accelerated study were withdrawn on monthly intervals and were analyzed [24].

Table No. 3.10: Results of Stability studies

Temperature	Evaluation Parameter	Observation (months)			
		0	1	2	3
3-5 °C	Colour	Slight Yellow	Slight Yellow	Slight Yellow	Slight Yellow
	Odour	Aromatic	Aromatic	Aromatic	Aromatic

	pH	7.1	7.2	7.2	7.2
	Viscosity	2.12	2.12	2.13	2.12
25°C	Colour	Slight Yellow	Slight Yellow	Slight Yellow	Slight Yellow
	Odour	Aromatic	Aromatic	Aromatic	Aromatic
	pH	7.1	7.0	7.1	7.1
	Viscosity	2.12	2.11	2.11	2.10
40°C	Colour	Slight Yellow	Slight Yellow	Slight Yellow	Slight Yellow
	Odour	Aromatic	Aromatic	Aromatic	Aromatic
	pH	7.1	7.1	7.2	7.2
	Viscosity	2.12	2.11	2.11	2.12

The result for stability studies of stored F6 formulation is described in the table 3.10. When the formulation is kept at the temperature range between 3-5 °C shows there is no change in the colour, odour and viscosity of the prepared formulation while there is a slight change in the pH in the third month of the study.

When the formulation is kept at the temperature range between 25 °C RH=60% shows there is no change in the colour and odour of the prepared formulation while there is a slight change in the pH and viscosity in the second and third month of the study.

When the formulation is kept at the temperature range between 40 °C RH=75% shows there is no change in the colour and odour of the prepared formulation while there is a slight change in the viscosity in the second month of the study.

4. Summary and Conclusion

The mouthwashes prepared from the herbal ingredients has shown great potential to overcome the chemical-based preparations. The combination of two or more herbal ingredients to form a polyherbal formulation has become an efficient approach to add value to the basic herbal formulation.

In present study, the polyherbal mouthwash has been prepared using the leaves of *Psidium guajava* (guava), rhizomes of *Curcuma longa* (turmeric) and flower buds of *Syzygium aromaticum* (clove). The prepared mouthwash shows promising results in terms of zone of inhibition. When compared the prepared mouthwash with the marketed one, it shows greater zone of inhibition due to the synergistic effect produced by the multiple herbs used in the formulation.

In this research work, plant extracts has been prepared by maceration. The phytochemical screening has been performed for all the three plant materials. The extract has been screened for the presence of alkaloid, flavonoid, phenol, carbohydrate, steroids, glycosides, saponins and tannins. All the three plants has shown the presence of flavonoid and phenol which is an important phytoconstituent for the purpose of our study. The quantitative estimation for phenolic content and flavonoid content of all the three plant materials has been performed.

The formulation has been prepared by optimizing the different concentrations of active ingredients used in the study. The prepared mouthwash has been evaluated for the organoleptic parameters which shows the Slight yellow colour and aromatic smell of the prepared mouthwash. The pH has been measured as 7.1 for the selected formulation. The viscosity has been measured as 2.12 cP.

The in-vitro antibacterial study has been performed by agar well diffusion method. The bacteria selected for the study were S. aureus, S. mutans and E. coli. The zone of inhibition of F6 formulation has been found most effective. This selected formulation has been compared with the marketed formulation which shows

best results in terms of zone of inhibition for prepared formulation.

The accelerated stability studies has been performed for the selected formulation under the following conditions of temperature as 3-5°C, 25°C RH=60%, 40°C ±2% RH= 75%. The results shows no change in the organoleptic parameters, pH and viscosity of the formulation. The zone of inhibition also didn't shown any change and thus antibacterial activity also remains the same after three months.

From the above results obtained in this research work, it can be clearly stated that polyherbal mouthwash has become an effective way to improve the efficacy of herbal formulations. The combination of *Psidium guajava* (guava), *Curcuma longa* (turmeric) and *Syzygium aromaticum* (clove) in a mouthwash has shown promising results to improve the chances of usage of polyherbal mouthwash to commercially available chemical-based product.

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