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FORMULATION AND EVALUATION OF NOVEL HERBAL MOUTH GEL OF ALOE BARBADENSIS, AZADIRACHTA INDICA AND CURCUMA LONGA FOR HEALING OF MOUTH ULCER

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ABSTRACT

Aphthous Stomatis or mouth ulcer is the most common condition that we encounter clinically the lesions are single or multiple superficial and deep sealed and are associated with microbial invasions. Herbal gel formulated were stable, safe, and effective over to synthetic formulations for the treatment of mouth ulcer. Mouth ulcer often causes pain irritation of the sores salty, spicy, and sour food items and may cause discomfort while healing occurs due to use of chemical formulation. This project focused on the preparation of a herbal mouth ulcer healing gel because better cultural acceptability, better compatibility, with human body and less side effects. The gel was prepared by using alcoholic extract of Aloe barbadensis and Azadirachta indica leaves and extract powder of turmeric. Developed formulation were transparent, homogeneous and pH ranges from 6-8. Formulation showed acceptable rheological behaviour with applicable spreadability and Extrudability properties, however further clinical studies are required to established clinical efficacy of prepared herbal gels.

KEYWORDS: *Mouth Ulcer, Aloe Barbadensis And, Azadirachta Indica, Gel, Wound-Healing*

INTRODUCTION

Gels are mainly semi-solid formulations having a liquid phase that has been thickened with some other components. Topical gel preparations are used for the skin application or percutaneous penetration of medicament or local action to certain mucosal surfaces. Mouth ulcers are small sores or an abrasion that develops in mouth or at the base of gum. Mouth ulcers are also known as canker sores or aphthous ulcer. A break or breach in the mucous membrane, that lines within the mouth is also recognised as a mouth ulcer. It generally arises as a yellow or white colour depression in mouth. Mouth ulcers are usually generated by a number of causes, such as biting the inner layer of cheek, food allergies, hard teeth brushing, hormonal changes, vitamin deficiencies, bacterial infection and diseases .[1,41]



Fig 1: Mouth ulcer.

Causes Of Mouth ulcer [46]

There is no definite etiology and pathology known for mouth ulcer; although some factors are considered important which include nutritional deficiencies such as iron, vitamins especially B12 and C, poor oral hygiene, infections, stress, indigestion, mechanical injury, skin disease etc. Some other factor include such as:

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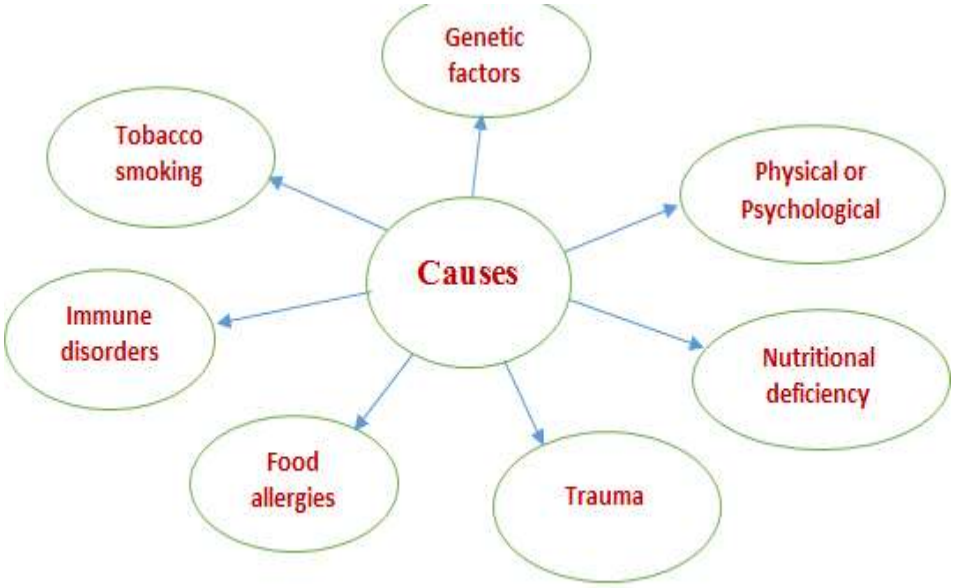


Fig 2: Causes of mouth ulcer

SATMANT OF PROBLEM

- The development of a polyherbal gel formulation offers several advantages:
- Targeted Delivery: Gels can be applied directly to the affected area, ensuring localized treatment.
- Enhanced Stability: Proper formulation can enhance the stability and shelf-life of the active ingredients.([Academia][5])
- Improved Patient Compliance: Gels are easy to apply and generally well-accepted by patients.

Pharmacognistic Investigation Herbal drug Profile

1.Aloe barbedensis (Aloe Vera)



Fig 2: Aloe barbedensis Leaves

Pharmacognostic study

Synonym: Aloe

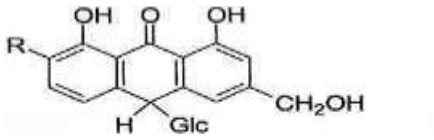
Aloe Vera: The biological source of Aloe Vera is Aloe barbadensis. It belongs to the family Xanthorrhoeaceae.

Morphology: Aloe Vera is a stemless or very short stemmed plant growing to 60–100 cm (24–39 in) tall, spreading by offsets. The leaves are thick and fleshy, green to grey-green, with some varieties showing white flecks on their upper and lower stem surfaces. The margin of the leaf is serrated and has small white teeth. The flowers are produced in summer on a spike up to 90 cm (35 in) tall, each flower being pendulous, with a yellow tubular corolla 2–3 cm (0.8–1.2 in) long. Like other Aloe species, Aloe Vera forms Arbuscular mycorrhiza, a symbiosis that allows the plant better access to mineral nutrients in soil. Plant part used: Leaves, flowers, stems, roots, fruits, seed.

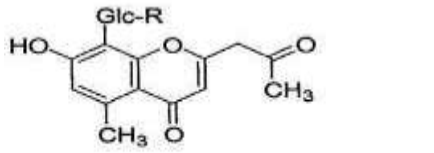
Chemical constituents: The chemical constituents in Aloe Vera are Anthraquinones, Saccharides, Prostaglandins and fatty acids. Others: Enzymes, amino acids, vitamins, minerals. Other compounds: Cholesterol, triglycerides, steroids, uric acid, lignins, beta-sitosterol, gibberellin, salicylic acid.

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Uses: It is analgesic, antibacterial, antiviral, antifungal, antioxidant immune modulating, antiseptic, anti-inflammatory. Aloe vera is used in the sites of periodontal surgery, toothpick injuries, chemical burns, aphthous ulcers, gum abscesses, dry socket, lichen planus, benign pemphigus and gingival problems associated with AIDS, leukaemia, migratory glossitis, geographic tongue and burning mouth syndrome, denture sore mouth, candidiasis, desquamative gingivitis, vesiculobullous diseases, acute monocytic leukemia, xerostomia.[33,48]



R=H, Barbaloin; R=OH, homonataloin

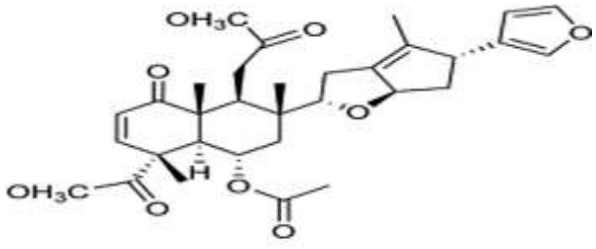


R=H, Aloesin; R=*p*-coumaroyl, Aloeresin A

2.Azadirachta indica (Neem)



Pharmacognostic study:
Common Name – Neem[50]
Botanical Name –Azadirachta Indica
Azadirachta indica Leaves of Azadirachta indica, commonly called as neem, belonging to family Meliaceae
Morphology. Neem is a medium-sized tree, reaching 15 to 30 m in height, with a large rounded crown up to 10-20 m in diameter. It is mainly evergreen but sometimes shed its leaves during the dry season. Neem has a deep taproot and is a mycorrhizal-dependent species. Neem leaves are medium to large in size and elongated to oblong in shape, averaging
20-40 centimetre in length. The vibrant green leaves are smooth and glossy with sharp, serrated edges.
Chemical Constituents are rich in several phytoconstituents such as nimbin, nimbidin, nimbolide, and limonoids, quercetin and sitosterol. Leaves contain mixture of compounds including nimbin, nimbanene, 6 desacetylnimbinene, nimbandiol, nimbolide, ascorbic acid, n-hexacosanol and different amino acids, and nimbiol and several other types of ingredients. In addition to this, the bark also contains nimbin, nimbinin, and nimbidin.
Uses They have very strong antibacterial, antifungal and anti-inflammatory, Wound healing activity and are quite commonly.[50]



Nimbin

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3.Curcuma longa (Turmeric)

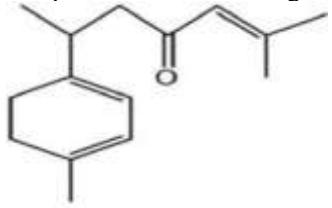


Fig 4: Curcuma longa Rhizome

Pharmacognostic study
Common name: Curcuma[49]
Synonyms: Saffron, Haldi
Turmeric: The biological source of Turmeric is Curcuma longa which belongs to the family Zingiberaceae. Evaluation of turmeric has been done for gastric and duodenal antiulcer activity in rats. Volatile oil of Curcuma longa possess antiinflammatory and anti-arthritic activities. Water and fat soluble extracts of curcumin exhibited strong antioxidant activity comparable to vitamins C and E.

Morphology: Turmeric is a perennial herbaceous plant that reaches up to 1 m (3 ft 3 in) tall. Highly branched, yellow to orange, cylindrical, aromatic rhizomes are found. The leaves are alternate and arranged in two rows. They are divided into leaf sheath, petiole, and leaf blade. From the leaf sheaths, a false stem is formed. The petiole is 50 to 115 cm (20–45 in) long. The simple leaf blades are usually 76 to 115 cm (30–45 in) long and rarely up to 230 cm (91 in). They have a width of 38 to 45 cm (15 to 18 in) and are oblong to elliptical, narrowing at the tip Plant part used: Rhizomes and stem.

Chemical constistuents: Phytochemical components of turmeric include diaryl heptanoids, a class including numerous curcuminoids, such as curcumin, demethoxycurcumin, and bisdemethoxycurcumin. Curcumin constitutes up to 3.14% of assayed commercial samples of turmeric powder (the average was 1.51%); curry powder contains much less (an average of 0.29%).Some 34 essential oils are present in turmeric, among which turmerone, germacrone, atlantone, and zingiberene are major constituents.49
Uses: Most turmeric is used in the form of rhizome powder to impart a golden yellow color. It is used in many products such as canned beverages, baked products, dairy products, ice cream, yogurt, yellow cakes, orange juice, biscuits, popcorn color, cereals, sauces, and gelatin. It is a principal ingredient in curry powders. Although typically used in its dried, powdered form, turmeric also is used fresh, like ginger. It has numerous uses in East Asian recipes, such as pickle that contains large chunks of soft turmeric, made from fresh turmeric. 34



α-turmerone

HYPOTHESIS

- **Aloe vera**: Aloe vera has demonstrated anti-inflammatory and wound-healing properties. Clinical studies have shown that Aloe vera can accelerate the healing of oral ulcers and reduce pain and inflammation.
- **Neem**: Neem possesses antibacterial, antifungal, and anti-inflammatory effects. Its compounds have been found to modulate immune responses and reduce inflammation, which may contribute to the healing of oral ulcers.
- **Turmeric**: Curcumin, the active compound in turmeric, has significant anti-inflammatory and antimicrobial activities. Studies indicate that curcumin accelerates wound healing and reduces pain and inflammation in oral ulcer models.

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Aim and Objective

To formulate and evaluate a novel herbal mouth gel containing Aloe vera (Aloe barbadensis), Neem (Azadirachta indica), and Turmeric (Curcuma longa) for the effective healing of mouth ulcers.

Objectives

1. To extract and standardize active compounds from Aloe vera, Neem, and Turmeric.
2. To develop a stable and bioavailable herbal mouth gel formulation incorporating these extracts.([Enago][1])
3. To assess the antimicrobial activity of the formulated gel against common oral pathogens.
4. To evaluate the gel's anti-inflammatory and wound-healing properties in vitro.
5. To conduct a clinical trial to determine the gel's efficacy in reducing ulcer size and pain in patients with mouth ulcers.
6. To analyze patient compliance and acceptability of the herbal mouth gel.

LITERATURE REVIEW

- Aloe Vera (Aloe barbadensis)**

Efficacy: A meta-analysis of randomized controlled trials involving 847 participants indicates that Aloe vera significantly accelerates the healing of oral ulcers, reducing both ulcer size and pain intensity compared to placebo treatments .([PubMed][1])
Clinical Trials: A randomized clinical trial demonstrated that Aloe vera gel outperforms amlexanox 5% oral paste in reducing ulcer size and pain on the fifth day of treatment .([PubMed][2])
Safety: Aloe vera is generally considered safe for topical application, with minimal adverse effects reported in clinical studies .

- Neem (Azadirachta indica)

Properties: Neem exhibits antimicrobial and anti-inflammatory effects, which may contribute to the healing of oral ulcers.
Clinical Evidence: While specific studies on neem's effect on oral ulcers are limited, its established properties suggest potential benefits in oral mucosal healing.

- Turmeric (Curcuma longa)

Active Compound: Curcumin, the active compound in turmeric, has demonstrated significant anti-inflammatory and antimicrobial activities.
Animal Studies: Studies indicate that curcumin accelerates wound healing and reduces pain and inflammation in oral ulcer models .

Advantages of Herbal Medicine

- Herbal medicines have a long history of use and better patient tolerance and public acceptance.
- The cultivation and processing of medicinal herbs are eco-friendly.
- Prolong and apparently uneventful use of herbal medicines is safe and efficacious.35

Properties of Gel

- Ideally, the gelling agent must be inert, safe and cannot react with other formulation constituents.
- The gelling agent should produce a sensible solid-like nature at the time of storage, which is easily broken when exposed to shear forces produced by squeezing the tube, trembling the bottle, or at the time of topical application.
- It should have suitable anti-microbial agent.
- The topical gel must not be sticky
- The apparent viscosity or gel strength increases with an increase in the effective crosslink density of the gel. However, a rise in temperature may increase or decrease the apparent viscosity, depending on the molecular interactions between the polymer and solvent.
- They exhibit the mechanical characteristics of the solid state.
- Each component is continuous throughout the system.

Method of prepration of Gel

1.Gel can be prepared by three method.

Three methods can be used:

2.Cold method

After cooling water to 4 to 100 degrees, it was poured into a mixing vessel. The geling agent was added slowly and agitated until the complete solution was reached. Temperatures below 100 °C were maintained during the melting process. A solution of the drugs was slowly added while mixing gently. The liquid should be transferred to container and allowed to warm to room temperature, where it will become a clear gel.

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"In this project we are going to use cold method for the preparation of herbal gel of Azadirachta indica, Aloe Barbedensis and curcuma longa."

3.Dispersion method

Stirring the gelling agent in water at 1200 rpm for 30 minutes dispersed the gelling agent. The nonaqueous solvent was used to dissolve the drug. The preservative was also added. Continuous stirring was performed while adding this solution to the gel above.

4.Fusion method

This method involves the use of various waxy materials as gallant in a non-polar medium. In this method, waxy materials are melted and drugs are added. A uniform gel was formed by stirring slowly until it was dissolved.

Polymer and expients used in gel

Gelling agent – Carbopol 934, gelatin, tragacanth ,hydroxypropyl cellulose

Commonly Used Gelling Agents

- Acacia, Pectin, Starch,Tragacanth
- Xanthan gum Alginic acid (seaweed)
- Animal/vegatable fats: cocoa butter,Gelatin
- Bentonite, veegum (magnesium aluminum silicate)
- Carboxymethylcellulose (CMC) and other cellulose derivatives
- Carbomer resins (Carbopols)
- Colloidal silicon dioxide

Gelling agents are gel-forming agents when dissolved in a liquid phase as a colloidal mixture forms a weakly cohesive internal structure. They are organic hydrocolloids or hydrophilic inorganic substances.32

Preservatives Preservative are those chemical used alone or in combination to prevent the growth of microrganism in solution e.g Methyl paraben , Propyl paraben.31

Buffering agent Buffers are compound or mixture of compounds that by their presence in the solution resist changes in the pH upon the addition of small quantities of acid or alkali Trietholamine.

Moisturizers – glycerin 30

Moisturizer preparation used to prevent dryness

Antioxidants

Antioxidant are compound that inhibit oxidation a chemical reaction that can prouce free radicals and chain reaction that may damage the cells of organism Bases e.g Vitamin c,lutein

Binders

Binder excipients hold the ingredient of a formulation together e.g Tragacanth , Gelatin

MATERIAL AND METHODS

Chemicals

Ethanol, Carbopol 934, distilled water, methyl paraben and propyl paraben, Propylene glycol, Triethanolamine,

Chemicals used in formulation of gel

Carbopol 934,Methyl paraben, and Trietholanamine collected from the Research lab fine chem industries mumbai Glycerin are collected from Vikash pharma.

Equipment's and Instrumentations:

Digital balance, pH meter, Magnetic stirrer, Digital water bath, Autoclave, Hot air oven, Incubator, Spreadability Apparatus

Collection of materials:

The leaves of Azadirachta indica, Aloe barbadensis were collected from the medicinal garden and rhizomes of Curcuma longa were collected from the local area.

Formulation of gel:

A sufficient amount of Carbopol 934 was soaked in distilled water overnight, and then mixed with distilled water with continuous stirring using a mechanical stirrer. Another solution containing varying concentrations of Ethanolic extract of Azadirachta indica EEZ (ml) Ethanolic extract of aloe (EEA) (ml) Turmeric Rhizome extract and the required quantity of methyl paraben and propyl paraben were added with continuous stirring. Propylene glycol was also added to the solution. This prepared solution was further mixed with Carbopol 934 solution thoroughly with continuous stirring, volume was made upto 30ml with water and the pH was adjusted by addition of triethanolamine to obtain gel of required consistency. Seven formulations (F1 to F7) of the herbal gel were prepared.

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Table 1: Formulation of Herbal Mouth Gel Azadirachta indica, Aloe Vera& Curcuma Longa

Ingredients	F1	F2	F3
Ethanolic extract of aloe (EEA) (ml)	4	4	4
Ethanolic extract of Azadirachta indica EEZ(ml)	3	3	3
Turmeric Rhizome extract	3	3	3
Carbopol 934	10	11	12
Methyl Paraben (g)	0.2	0.2	0.2
Propyl Paraben (g)	0.1	0.1	0.1
Triethanolamine	0.3	1.4	1.2
Propylene glycol(ml)	1	1	1
Glycerine	0.50	0.30	0.46
Water (ml)	Upto 30 gm	Upto 30 gm	Upto 30 gm

METHOD OF PREPARATION OF HERBAL GEL

After cooling water to 4 to 100 degrees, it was poured into a mixing vessel. The Gelling agent carbapol 934 is added slowly and agitated until the completesolution istemperatures below 100 °Cwere maintained during the melting process. A solution of the drugs extarct Ethanolic extract of Azadirachta indica EEZ(ml)Ethanolic extract of aloe (EEA) (ml)Turmeric Rhizome extract is slowly added while mixing gently. The liquid should be transferred to container and allowed to warm to room temperature, where it will become a clear gel.



Fig 5. Novel Herbal Mouth gel of Aloe barbadensis, Azadirachta indica and Curcuma longa

Plan of Work: Herbal Mouth Gel for Mouth Ulcer Treatment

1. Pre-Formulation (Week 1)
Collection & Authentication: Obtain fresh Aloe vera, Neem, and Turmeric. Authenticate species through botanical identification.
Extraction: Prepare ethanolic extracts of Aloe vera and Neem; aqueous extract of Turmeric.
Phytochemical Screening: Identify active constituents responsible for therapeutic effects.

2. Formulation Development (Week 2)
Gel Base Preparation: Use Carbopol 934 as a gelling agent.([IJPS Journal][1])

3. Evaluation (Week 3)
Physical Evaluation: Assess color, odor, consistency, and homogeneity.
pH Measurement: Determine pH to ensure compatibility with oral mucosa.
Viscosity & Spreadability: Evaluate rheological properties to ensure ease of application.
Antimicrobial Activity: Test against common oral pathogens using agar diffusion method

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4. Clinical Evaluation (Weeks 4–5)
Ethical Approval: Obtain necessary ethical clearance for clinical trials.
Patient Selection: Recruit individuals with diagnosed mouth ulcers.
Treatment Protocol: Apply herbal gel as per standardized dosage regimen.
Monitoring: Assess ulcer size, pain reduction, and healing progression.
Data Analysis: Statistical evaluation of clinical outcomes.

5. Documentation & Reporting (Week 6)
Compilation of Data: Organize experimental and clinical data.
Analysis & Interpretation: Interpret findings in the context of existing literature.
Report Preparation: Draft comprehensive report detailing methodology, results, and conclusions.

EVALUATION OF GEL

Physical evaluation:

Physical parameters such as color, odour and consistency were checked visually.

Color: The color of the formulations was checked by visual inspection.

Consistency: The consistency of formulations was checked by applying on skin.

Odour: The odour of the formulations was checked by mixing the gel in water and observing the smell. Physical evaluations of gel formulations were reported. 3

Measurement of pH:

The pH of herbal gel formulations were determined by using digital pH meter. 1 gm. of gel was taken and dispersed in 10 ml of distilled water and keep aside for two hours. The measurement of pH of formulation was carried out in three times and the average values are reported. pH of gel formulation was reported.

Homogeneity: All developed gel formulations were tested for homogeneity by visual inspection after the gels have been set in to the container. They were tested for their presence and appearance of any aggregates.

Spreadability:

Spreadability is expressed in terms of time in seconds taken by two slides to slip off from gel that is placed in between the slides under the direction of certain load. If the time taken for separation of two slides is less then better the spreadability. Spreadability is calculated by using the formula:

Formula

S = M. L / T

Where

M = weight tied to upper slide

L = length of glass slides

T = time taken to separate the slides

S= Spreadability

Percentage Yield

Weight the empty container in which the gel formulation was stored again weight the container with the gel formulation to obtain the practical yield subtract the weight of empty container with the container with gel formulation. then the percentage yield was calculated by the formula given below

$$\text{Percentage yield} = (\text{Practical Yield} / \text{Theoretical yield}) \times 100$$

Antimicrobial Activity

The antimicrobial activity was studied using the well diffusion method. Out of formulation F7 gel containing the F6 shown the highest zone of inhibition and it comparable with the marketed oracool gel formulation both against E. coli. The both product no inhibited growth. The result shown in fig.

All the prepared gel formulations were evaluated for parameters such as physical appearance, pH, homogeneity, spread ability and viscosity. The observation reveals that the gels were having smooth texture and were elegant in appearance. The pH of all prepared gels was found to be in range of 6.5-7.0. All the gels showed good spreadability. Also from the above data it was observed that increase the concentration of plant extract increases the spreadability. All the prepared gels showed good homogeneity with absence of lumps. The developed preparations were much clear and transparent. The viscosity of all the developed gels was found to be excellent and within the range.

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Physical Evaluation

Physical parameters such as colour, odour and consistency were checked visually. Check the colour of gel yellowish colour and texture good F1 to F3.

Formulation	F1	F2	F3
Colour	Yellowish	Yellowish	Yellowish
Texture	Good	Good	Good
Odour	Characteristic	Characteristic	Characteristic

Table 2: Physical Evaluation of gel formulation

pH

1 gm. of gel mix in 10 ml water mix and check the pH all F1 to F3 gels.

Homogeneity

All developed gel formulations were tested for homogeneity by visual inspection after the gels have been set in to the container.

Spreadability

Spreadability is expressed in terms of time in seconds taken by two slides to slip off from gel that is placed in between the slides under the direction of certain load.

Formulation	Spreadability (g.cm/sec)
F1	6.3
F2	6.9
F3	75

Percentage Yield (%)

Weight the empty container in which the gel formulation was stored again weight the container with the gel formulation to obtain the practical yield subtract the weight of empty container with the container with gel formulation.

Formulation	F1	F2	F3
Percentage Yield (%)	96.9	98.2	95.4

Antimicrobial Activity



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Fig 6: Antimicrobial Activity of herbal gel Formulation

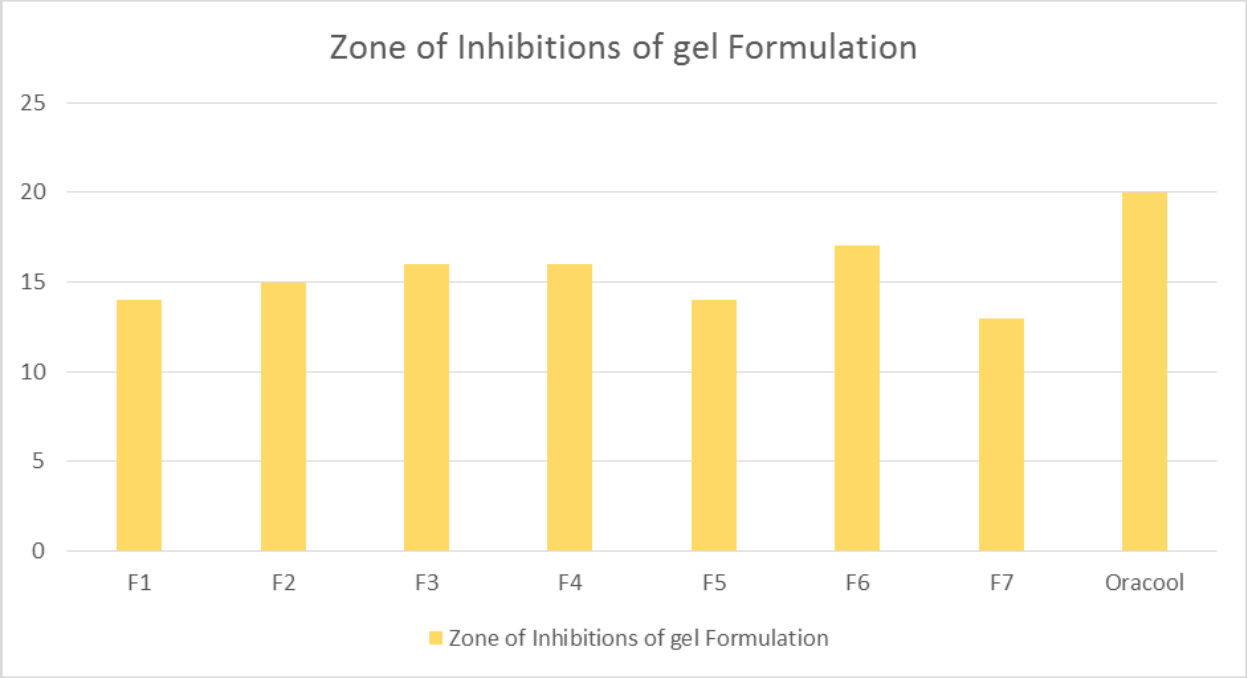


Fig 7: Zone of Inhibition of gel formulation

CONCLUSIONS

Nowadays there is a lot of demand for herbal formulations in the market due to their cost effectively and absence of any side effects. From the above experimental data it is clear that a gel formulation with herbal ingredients such as aloe, neem and, haldi has good characteristics, viscosity and also possesses a good antimicrobial activity which is necessary in the management of mouth ulcers**.** Natural remedies are more acceptable in the belief that they are safer with lesser side effect than the synthetic medicines. Nowadays herbal formulation have increasing demand in the world market. New herbal gel formulation having good antimicrobial activity as well as anti-inflammatory activity so it is safe, stable and good for mouth ulcer treatment.

Expected Outcome

1. Enhanced Healing of Mouth Ulcers
2. Antimicrobial and Anti-inflammatory Effects
3. Improved Oral Health Parameters

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4. Safety and Patient Compliance
5. Scientific Validation and Market Potential

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