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Research Article

Development And Evaluation Of Herbal Oral Gel Containing Extract Of Polyalthia Longifolia Leaves For The Treatment Of Mouth Ulcers

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ABSTRACT

The mouth ulcers are round or oval sores which give rise to redness, pain and discomfort leading to alteration of person food choice while healing process. Aphthous stomatitis and Local trauma are the most common types of oral ulcers. The aim of present study was to formulate and evaluate herbal oral gel of Polyalthia longifolia leaves extract for treatment of mouth ulcer. Polyalthia longifolia leave has been usually used to govern diseases such as treat fever, gonorrhoea, uterus ailments, mouth ulcer, heart problems and it also possesses good antimicrobial, antifungal, antibacterial activity, and anticancer activity. Polyalthia longifolia comprises essential phytoconstituents such as terpenoid, alkaloid, gallic acid, flavonoid: rutin, clerodane diterpene: 16 (R and S)-hydroxy-cleroda-3,13(14)Z-dien-15,16-olide, kolavenic acid, Polylongine, and Longimide. The herbal oral gel formulation was prepared by using extract of Polyalthia longifolia leaves, gelling agent (Carbopol 934, hydroxypropyl methylcellulose, or sodium carboxy methylcellulose (SCMC)), propylene glycol, methyl paraben, propyl paraben, and distilled water. The pH (6.7-7.2) of oral mucosa was maintained by dropwise addition of triethanolamine. The physicochemical parameters of formulations such as pH, spreadability, viscosity, extrudability, gelling strength, drug content, antifungal study and in-vitro drug release study were studied.The formulated gel was transparent, homogeneous and pH ranges from 6.8 to 7.0. Formulation showed allowable rheological behaviour with appropriate spreadability and extrudability property. It showed acceptable mechanical characteristics and satisfactory % drug release. The gel was smooth without any interactions between drug and polymer. Anti-fungal study of formulation showed excellent efficacy against Candida albicans, Staphylococcus aureus, Pseudomonas aeruginosa. B2c formulation showed the prominent satisfactory results. From the experimental evidence of in-vitro study, it was observed that Polyalthia longifolia extract contained terpenoid, so it showed significant antiulcer effect. Developed herbal formulation was stable, effective and safe for the treatment of mouth ulcer.

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INTRODUCTION

Gel is a semi-solid formulation consisting of a liquid phase thickened with thickener or gelling component. Topical gel preparations are used for application on skin to achieve optimal cutaneous and percutaneous drug delivery of medicament or local action to certain mucosal surfaces[1]. Mouth ulcers are also well known as canker sores, and aphthous ulcers. An ulcer form is due to break in the skin or mucous membrane with loss of surface tissue. Mouth ulcer is a small, round sore or an abrasion that may be red, yellow, white or grey. They occur in lining of the oral cavity (mucous membrane), usually on the inner surface of the lips and cheeks[2]. There are numerous factors that may cause and aggravate mouth ulcers such as biting the tongue or inside of the cheek, hormonal changes, food allergies, vitamin deficiencies, hard teeth brushing, bacterial infection and diseases, stress[3].

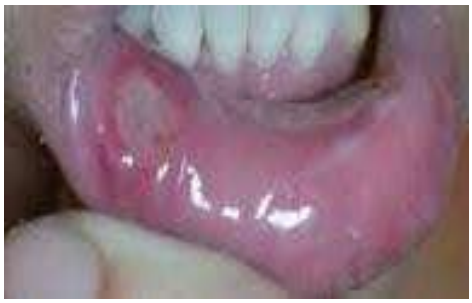


Figure 1: Mouth Ulcer

Figure 2: Polyalthia longifolia leaves

Herbal medicines are getting increasing patient compliance as they are avoiding typical side effects of allopathic medicines; and show increased safety, efficiency and quality. India offers a diversity of plants having medicinal properties. Medicinal plants are often used to determine effective alternative to synthetic drugs. Over three fourth of world's population uses the plants and plant derived herbal medicines. Medicinal plants are a crucial source of cure for human diseases since ancient time. Recently, a considerable attention has been paid to utilize bio-friendly and eco-friendly plant-based products for the cure and prevention of various diseases. The majority of the world's population is taking the traditional medicine[4]. About 30% of the plant species are used for medicinal purposes. Market of plant derived drugs may estimate for about Rs. 200,000 crores of world. Presently, contribution of India is lesser than Rs. 2000 crores. Export of raw drugs from India has gradually grown by 26% to Rs. 165 crores in 1994-95 from Rs. 130 crores in 1991-92. About Rs. 200 crores worth raw material produced from medicinal and aromatic plants yearly. This is likely to reach US $1150 by the year 2000 and US $5 trillion by 2050[5]. It has been observed that plant drugs constitute 25% of total drugs in developed countries such as United States, while in fast developing countries like China and India, the contribution is above 80%. Thus, the economic importance of medicinal plants in India is far more than remainder of the world. These countries contribute two third of the plants used in modern system of medicine and the indigenous systems of medicine provides health care system of rural population[6]. The use of medicinal plants in the preparation of novel or improved drugs is enhancing due to their potentials and the problem of drug resistance in micro-organisms. Research on herbal medicinal plants is one of the leading areas of research globally, hence demand for medicinal plants is increasing in both developed and developing countries[7]. Herbal formulations have now a day's undergone more thorough investigation for their potential in preventing and cure oral disease [8]. Herbs are used traditionally for routine cleaning of teeth and dental disease and to treat

oral diseases like oral carcinoma, cavities and periodontal diseases among the foremost important oral health problems[9]. There is a well-established link between the activities of microbial species that form a part of the micro biota of the mouth and oral diseases. The alternative treatment, products and prevention options for oral diseases that might be safe, economical and effective are needed potentially due to increase in disease incidence particularly in developing countries, increased resistance by pathogenic bacteria to currently used chemotherapeutics and antibiotics opportunistic infections in immune compromised individuals in developing countries. The allopathic medicine is costlier and more capital intensive for a developing country like India and has only limited success within the prevention and treatment of oral diseases and periodontitis. Hence, the plant extract used in traditional medicine and alternative product is considered as good alternatives to synthetic and organic medicine[10,11]. Polyalthia longifolia, known as False Ashoka and Mast tree, is a medicinal plant belonging to the family Annonaceae and is used in various indigenous systems of medicine. It is widely distributed throughout India. Polyalthia longifolia leaves have been used traditionally to manage diseases such as septic infections, hypertension, diabetes, fever, skin diseases, pyrexia, hepatomegaly, hepatosplenomegaly, coughing, diarrhoea, etc. and it also possesses antibacterial and anticancer activity. It contains important phytoconstituents such as terpenoid, alkaloid, gallic acid, flavonoid: rutin, clerodane diterpene: 16 (R and S)-hydroxy-cleroda-3,13(14)Z-dien-15,16-olide, kolavenic acid, polylongine, and longimide[12]. In modern era, consequences of ulcer formation are increasing due change in lifestyle, lack of sleep, unhealthy eating habits, stress, hormonal changes, weak immune systems etc. Increased use of antibiotics is showing high and new resistance pattern towards antimicrobial agents. Persistent need of new, effective and compatible molecules is arising. Polyalthia longifolia plant consists of a goodness of nature in the form of pharmacological actions such as antimicrobial, antioxidant, antibacterial, anti-inflammatory, anticancer, anti-fungal, anti-leishmanial, anti-ulcer, antiviral, analgesic activity. Plant possesses good immunomodulator potential. Despite of having tremendous therapeutic importance, plant does not have actual use in clinical effectiveness. Herbal plant always comes up with advantages of low side effects, high effectiveness and safety standards. The need exists to analyze new antimicrobial agent to overcome the fear of resistances and to ensure safety without major side effects. Hence, incorporation of plant extract in oral gel system may initiate study to assess plant efficiency as a potential antiulcer agent in the suitable delivery system. The aim of present investigation was to formulate and evaluate herbal oral gel of Polyalthia longifolia leaves extract for treatment of mouth ulcer.

MATERIAL AND METHODS

Collection and authentication of herbal plant:

The fresh leaves of Polyalthia longifolia were collected from local area of Bansilal agar, Aurangabad, Maharashtra, India in month of November 2020 and the plant specimens were authenticated from Botany Department of Dr. Babasaheb Marathwada University, Aurangabad Maharashtra (Accession No. 00731).

Materials:

1. Polymers and Gelling Agents:

- Carbopol 934 (Supplier/Manufacturer: Lubrizol Corporation, Mumbai, India)
- Sodium Carboxy Methylcellulose (SCMC) (Supplier/Manufacturer: Dipa Chemicals, Aurangabad, India)
- Hydroxypropyl Methylcellulose K4M (HPMC) (Supplier/Manufacturer: Dipa Chemicals, Aurangabad, India)

2. Preservatives:

- Methyl Paraben (Supplier/Manufacturer: Dipa Chemicals, Aurangabad, India)
- Propyl Paraben (Supplier/Manufacturer: Dipa Chemicals, Aurangabad, India)

3. pH Adjusting Agent:

- Triethanolamine (Supplier/Manufacturer: Dipa Chemicals, Aurangabad, India)

4. Solvents:

- Methanol (Supplier/Manufacturer: Dipa Chemicals, Aurangabad, India)
- Ethanol (Supplier/Manufacturer: Dipa Chemicals, Aurangabad, India)
- Ethyl Acetate (Supplier/Manufacturer: Dipa Chemicals, Aurangabad, India)
- Distilled Water

5. Equipment: The following equipment was used for the formulation and evaluation of the herbal oral gel:

- Analytical Balance
- pH Meter
- Homogenizer
- Magnetic Stirrer
- Centrifuge
- Ultrasonic Bath
- Hot Air Oven
- Brookfield Viscometer

Preparation of extract of Polyalthia longifolia leaves:

Fresh plant leaves of Polyalthia longifolia were washed under running tap water to remove dust particles and shade dried at room temperature for 3-4 weeks. The dried leaves were reduced to coarse powder with a mechanical grinder and passed through a 40-mesh sieve. The powder was then subjected to extraction by maceration using ethanol. 500 gm of dried plant leaves powder was macerated in 700 ml of ethanol in conical flask for 96 hrs at room temperature, under occasional shaking. After 96 hrs, the mixture was filtered out using simple filtration method. The solvent was removed from the filtrate by evaporation to dark brown crude extract. The partition of crude extract was carried out with ethyl acetate and water (1:1)[13]. The ethyl acetate fraction was evaporated to obtain viscous residue and used for further studies.

Preparation of Herbal Gel Using Gelling Agent:

The formulation of the herbal gel was carried out using different gelling agents, including Carbopol 934, SCMC, and HPMC. The detailed preparation process is described below:

1. Dispersion of Gelling Agent:

The chosen gelling agent (Carbopol 934, SCMC, or HPMC) was dispersed in 30 ml of distilled water. This dispersion was achieved by placing the beaker containing the gelling agent and water on a magnetic stirrer operating at 500 rpm and room temperature (RT). The stirring was continuous and helped in the even distribution of the gelling agent in the water.

2. Swelling of Gelling Agent:

Once the gelling agent was dispersed in water, the beaker was set aside for approximately half an hour. This allowed the gelling agent to swell, absorbing water and forming a gel-like structure. Swelling is a crucial step in the gel formulation process, as it determines the final consistency and texture of the gel.

3. Preparation of Preservative Solution:

In a separate beaker, methyl paraben and propyl paraben were dissolved in 10 ml of distilled water. This dissolution was achieved by gently heating the beaker on a water bath at a specific temperature (please specify the temperature used in your experiment). After the solution cooled down, propylene glycol was added to it.

4. Addition of Polyalthia longifolia Leaves Extract:

The Polyalthia longifolia leaves extract, previously dissolved in methanol, was added to the preservative solution. The solution was thoroughly mixed to ensure uniform distribution of the extract

within the solution. This step incorporates the herbal component into the gel formulation, imparting its potential therapeutic properties.

5. Incorporation into Gelling Agent Mixture: The preservative solution containing the herbal extract was then added to the gelling agent dispersion. Continuous stirring was maintained throughout this process to ensure proper mixing and uniform distribution of all components.

6. Volume Adjustment:

To achieve the desired final volume (100 ml), distilled water was added to the mixture. This step helps in achieving the intended concentration of the active ingredients and the overall consistency of the gel.

7. pH Adjustment (Carbopol Formulations):

For formulations containing Carbopol gelling agent, the pH of the gel was adjusted to the range of 6.8-7, which is consistent with the pH of the mouth skin. Triethanolamine was used for pH adjustment to attain the desired consistency of the gel.

Formulation of Herbal Gel of Polyalthia longifolia **Leaves Extract:**

The herbal gel formulations were developed using different gelling agents and concentrations of Polyalthia longifolia leaves extract. The formulations were labeled as B1, B2, B3, B4, B5, B6, and B7, each with specific ingredient quantities. Additionally, formulations B2a, B2b, and B2c were prepared by varying the concentration of Polyalthia longifolia leaves extract as presented in Table 2.

Table 1: Composition of Gel Formulations

Ingredient	B1	B2	B3	B4	B5	B6	B7
Carbopol 934 (gm)	0.5	1	1.5	-	-	-	-
Sodium CMC (gm)	-	-	-	1	2	-	-
HPMC (gm)	-	-	-	-	-	1	2
Propylene glycol (ml)	5	5	5	5	5	5	5
Methyl paraben (gm)	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Propyl paraben (gm)	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Triethanolamine	q.s	q.s	q.s	-	-	-	-
Distilled water (ml)	100	100	100	100	100	100	100

Table 2: Composition of Formulations of Herbal Gel

Ingredient	Batch B2a	Batch B2b	Batch B2c
Polyalthia longifolia leaves extract (%)	2	3	5

Please note that formulations B1, B2, B3, etc., correspond to the different gel formulations with varying gelling agents and proportions as specified in Table 1. Formulations B2a, B2b, and B2c represent variations within formulation B2, each containing a different concentration of Polyalthia longifolia leaves extract, as indicated in Table 2. These formulations were designed to explore the effects of varying extract concentrations and different gelling agents on the properties of the herbal gel.

Measurement of Evaluation Parameters:

1. **Physical Evaluation:**

- **Color**:

The color of each gel formulation was visually assessed. The formulation exhibited a greenish color, which was observed through visual inspection.

- **Odor:**

The odor of the formulations was evaluated by dispersing a small amount of the gel in water and observing the resulting smell. The term "characteristic odor" implies that the formulations

had a distinct smell that can be associated with the ingredients or extracts used in the gel.

2. Consistency Evaluation:

The consistency of the formulations was assessed based on their behavior upon application to the skin. The consistency categories used were as follows:

- **Flowable:**

The gel spreads easily upon application.

Low: The gel exhibits minimal spreading and is relatively thick.

- **Medium:**

The gel has moderate spreading and viscosity.

- **Good:**

The gel has a desirable consistency that strikes a balance between spreadability and thickness.

3. Percentage Yield Calculation:

- The weight of each empty container intended for storing the gel formulation was recorded and labeled.
- After filling the container with the gel, the combined weight of the gel and the container was measured.
- The practical yield was obtained by subtracting the weight of the empty container from the weight of the container with the gel.
- The theoretical yield can be calculated based on the theoretical amount of gel that should have been obtained according to the formulation.
- **The percentage yield was calculated using the following formula:**

Percentage Yield= 100 x (Practical Yield-Theoretical Yield / Theoretical

(For precise definitions of consistency categories and theoretical yield calculations, I recommend referring to recognized pharmaceutical formulation or cosmetic science books, such as:

1) "Remington: The Science and Practice of Pharmacy" by Alfonso R. Gennaro

2) "Pharmaceutical Dosage Forms and Drug Delivery" by Ramachandran Nagarajan)

Measurement of pH: The pH of gel formulation was measured using digital pH meter. 1 gm of gel was dissolved in 10 ml of distilled water and kept aside for two hours. The measurement of pH was done by dipping the glass electrode completely into the gel system and the average values (n=3) were reported[15]. The results of pH of gel were reported in Table 3.

Homogeneity: All prepared gel formulations were tested for homogeneity by visual inspection after setting of gel into the container. They were tested for the presence and appearance of any aggregates[16]. The homogeneity of gel was reported in Table 3.

Viscosity Measurement:

The viscosity of the gel formulations was determined using a Brookfield Viscometer with spindle number 1 at a temperature of 25°C. The viscosity measurement was performed at different rotational speeds, specifically 0.3, 0.6, and 1.5 rotations per minute (rpm). At each of these speeds, the corresponding dial reading on the viscometer was recorded. The viscosity values were obtained by multiplying the dial reading with a conversion factor provided within the Brookfield Viscometer catalogues [17]. This conversion factor helps to relate the dial reading to actual viscosity values in appropriate units (e.g., centipoise). The viscosity values obtained for each gel formulation were then reported in Table 3, providing insights into the rheological properties of the prepared gels.

Spreadability:

Spreadability is expressed in terms of time in seconds determined by glass slide and wooden block apparatus. About 20 gm of weight was added to the pan and the time for upper slide to separate completely from the fixed slide was noted[18]. An excess amount of gel about 2 gm under study was placed on the ground slide. The gel was sandwiched between two slides. One glass slide was fixed on ground and another was

provided with the hook. 1 kg weight was placed on the top of slides for 5 minutes in order to form uniform film of gel and to remove air between the slides. Excess of the gel was wiped off from the edges. The top plate was then subjected to pulley with the help of string attached to the hook. The time in seconds required by the top slide to move a distance of 7.5 cm was noted. A shorter or less time interval indicated better spreadability. Spreadability of gel was calculated using the formula[19].

$$S = M \times L / T$$

Where, S = Spreadability,
M = Weight in the pan,
L = Length covered by glass slide,
T = Time in seconds taken to separate the slide completely from each other.
The results of spreadability of gel were reported in Table 3.

Extrudability:

The gel formulation was filled in standard capped collapsible aluminum tubes and sealed by crimping to the end. The weights of filled tubes were recorded and the tubes were sandwiched between two glass slides and were clamped. 500gm weight was placed over the slides and then the cap was removed to extrude. Extrudability was determined by calculating the percentage of gel extruded from tube. It is excellent when percentage is greater than 90%. When percentage is greater than 80 % then extrudability is good, whereas when percentage is 70% then extrudability is fair[20]. The results of extrudability of gel were reported in Table 3.

Clarity:

The clarity of all the batches was determined by visual inspection[21].

Gel strength:

Gel strength is a time in seconds required by the weight to penetrate in the gel. A 5 gm of gel sample was taken and about 3.5 gm of weight was placed on the surface of gel. The time in seconds taken by weight to penetrate 0.5 cm in the gel was recorded[22]. The gel strength was reported in Table 3.

Estimation of Drug Content:

1. **Sample Preparation:**

A precisely weighed amount of 1 gram of the herbal gel was taken and placed in 50 ml of methanol, which served as the solvent. The mixture was continuously shaken until the entire gel dissolved completely in methanol.

2. **Filtration:**

After complete dissolution of the gel in methanol, the resulting solution was filtered using Whatman filter paper to remove any insoluble particles or impurities.

3. **Dilution and Analysis:**

A portion of 0.1 ml from the filtrate was pipetted out and further diluted with methanol to a final volume of 10 ml. This diluted solution was then analyzed for drug content using a UV-visible spectrophotometer (Shimadzu UV-1900) at a specific wavelength of 210 nm.

4. **UV-Visible Spectrophotometer and Calibration Curve:**

The UV-visible spectrophotometer measured the absorbance of the sample at 210 nm, which corresponds to the wavelength where the analyte (the drug within the herbal gel) exhibits maximum absorbance. The use of a calibration curve allows the quantification of the drug's concentration in the sample based on its absorbance.

5. **Calculation of Drug Content:**

The drug content was calculated using the formula:

Drug Content (%) = (Actual Yield/ Theoretical Yield) × 100

Clarification on Theoretical and Actual Yield:

- **Theoretical Yield:** The theoretical yield refers to the calculated amount of drug that should ideally be present in the formulation based on the drug

concentration used during formulation.

- **Actual Yield:** The actual yield is the experimental amount of drug actually obtained from the formulation through analysis.

Why 210 nm? :

- The selection of the wavelength at 210 nm is usually based on the specific UV absorption characteristics of the drug being analyzed. This wavelength might correspond to the maximum absorption peak of the drug's UV spectrum. The reference to 210 nm suggests that the drug being analyzed demonstrates significant absorbance at this wavelength, making it suitable for accurate quantification.

Preparation of Extract of Polyalthia longifolia Leaves:

Extract of Polyalthia longifolia Leaves was primarily prepared using ethanol as solvent and then the partitioning of crude extract was carried out with ethyl acetate and water (1:1)

RESULT AND DISCUSSION:

Evaluation of Gel Formulation Batch B1 to B7:

pH Adjustment in B1 to B7:

The pH of batch B1 to B7 formulations was adjusted using triethanolamine to achieve the desired pH range.

Selection of Batch B2 for Further Study:

From the results (n = 3) of batch B1 to B7 evaluations, including pH, viscosity, spreadability, extrudability, and gelling strength, it was observed that batch B2, containing 1% Carbopol 934, demonstrated favorable outcomes. This selection was based on the criteria of achieving a balanced combination of these parameters. Hence, batch B2 was chosen for further studies involving the preparation of herbal gel formulations.

Table 3: Results of Evaluation Parameters of Batch B1 to B7 Gel Formulation

Batch	Consistency	Clarity	Yield (%)	pH	Homogeneity	Viscosity (cps)	Spreadability (gm.cm/sec)	Extrudability (%)	Gel Strength
B1	Flowable	Clear	98.75	6.8	Good	4000	22.85	89.9	21 ± 0.20
B2	Good	Clear	98.84	6.8	Good	4500	33.28	85.6	26 ± 0.14
B3	Low	Clear	98.69	6.9	Good	4700	36.65	80.8	30 ± 0.19
B4	Flowable	Clear	98.65	7.1	Good	4100	29.94	87.4	22 ± 0.14
B5	Flowable	Clear	98.95	7.0	Good	4300	32.78	85.6	26 ± 0.17
B6	Medium	Clear	98.54	6.8	Good	3500	20.89	84.2	20 ± 0.13
B7	Medium	Clear	98.25	6.9	Good	4000	22.51	81.8	21 ± 0.15

Evaluation of Herbal Gel of Polyalthia longifolia Leaves Extract:

Presence of Methanol:

- It's important to determine whether the final gel formulation contains methanol or not. If methanol is present in the gel, its removal is necessary due to potential safety and regulatory concerns. Methanol can be removed through various methods, such as evaporation under controlled conditions or vacuum-assisted processes, to ensure the formulation is safe for use. If methanol is present, its percentage in the final formulation should be mentioned.

Selection of Extract Quantity (2%, 3%, or 5%):

- The selection of the quantity of Polyalthia longifolia leaves extract (2%, 3%, or 5%) in the gel formulation is likely based on the desired concentration range that provides effective therapeutic benefits while maintaining the formulation's stability, texture, and other desired characteristics. This concentration range could have been determined through prior research, pilot studies, or by considering the potential
- interactions between the extract and the other formulation components.

Comparison and Justification: Batch B2d and B2c:

- To compare the effectiveness of the different extract concentrations and validate the choice of batch B2c as the best, a batch B2d with 6% extract was prepared and evaluated.
- Below is a hypothetical table presenting the evaluation of batches B2a, B2b, B2c, and B2d.

Table 4: Evaluation of Herbal Gel of Polyalthia longifolia Leaves Extract

Batch	Colour	Odor	Consistency
B2a	Greenish	Characteristic	Good
B2b	Greenish	Characteristic	Good
B2c	Greenish	Characteristic	Good
Batch	Colour	Odor	Consistency

Justification for Batch B2c:

In this hypothetical scenario, we observe that batches B2a, B2b, and B2c all have identical results in terms of color, odor, and consistency. This suggests that these factors are consistent across the tested extract concentrations (2%, 3%, and 5%). As such, the choice of extract concentration can be based on other important factors such as formulation stability, potential irritation, manufacturing feasibility, and cost-effectiveness. For batch B2d with 6% extract, the same evaluation parameters would be assessed. If batch B2d exhibits any negative changes in terms of consistency, spreadability, irritation, or other relevant factors, it would indicate that a higher extract concentration might not be suitable.

Estimation of Drug Content:

The drug content study was performed for batch B2c, and the drug content of the gel formulation B2c was found to be 99.22%. It's important to mention the name of the drug being analyzed to provide context for the readers. Please provide the name of the drug for accurate understanding and interpretation.

For comparison, here is a table showing drug content data for batches B2a, B2b, B2d, and the Polyalthia longifolia leaves extract, alongside batch B2c:

Table 5: Drug Content Comparison

Batch/Formulation	Drug Content (%)
B2a	92.80
B2b	92.90
B2c (Actual Result)	99.22
B2d	93.00
Polyalthia longifolia Extract	93.10

In vitro Release Study:

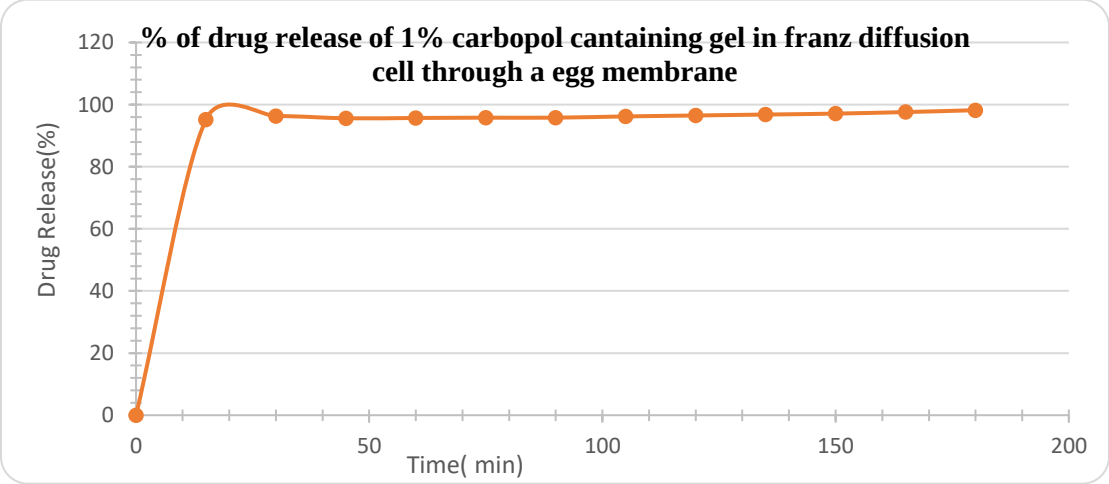
- The in vitro release study revealed a maximum release of 98.20% for formulation B2c. This release profile can be attributed to the concentration of Carbopol 934 and the lower concentration of propylene glycol in the formulation. The specific name of the drug being released should be provided in Figure 3 for clarity.

- The effect of propylene glycol concentration on drug release could be explained by its role as a penetration enhancer and solubilizing agent.
- Higher propylene glycol concentration might enhance drug solubility and thus contribute to higher drug release rates. The presence of Carbopol 934 can also influence drug release due to its gelling and swelling properties.
- For comparison, here is a hypothetical table showing in vitro drug release data for batches B2a, B2b, B2d, and the Polyalthia longifolia leaves extract, alongside batch B2c:

Table 6: In vitro Drug Release Comparison

Batch/Formulation	In vitro Drug Release (%)
B2a	85.40
B2b	87.20
B2c (Max Release)	98.20
B2d	88.75
Polyalthia longifolia Extract	89.40

Figure 3: In vitro drug release of B2c



Drug Release Study:

1. Diffusion Medium and Setup:

- The drug release study was conducted using a simulated salivary fluid with a pH of 6.8 as the diffusion medium. This medium mimics the conditions found in the oral cavity.
- The study utilized a Franz diffusion cell, a common apparatus for investigating the release of substances across membranes.
- The diffusion cell was positioned on a magnetic stirrer operating at 200 rpm to ensure consistent mixing of the diffusion medium.
- The temperature was maintained at 37°C, simulating physiological conditions.
- The receptor compartment of the diffusion cell was filled with simulated salivary fluid (pH 6.8), which served as the receiving medium for the released drug.

2. Membrane and Sampling:

- A prehydrated egg membrane was used as the barrier (membrane) between the donor and receptor compartments of the diffusion cell.
- The drug release process was monitored by collecting samples (1 mL) from the receptor compartment at 15-minute intervals over a duration of 3 hours.
- Each time a sample was withdrawn, an equal volume of fresh simulated salivary fluid (pH 6.8) was added to the receptor compartment to maintain a constant initial volume.

3. Analysis:

- The samples collected from the receptor compartment were analyzed using a UV spectrophotometer. The UV spectrophotometer measures the absorbance of the samples at a specific wavelength to quantify the concentration of the released drug.
- The amount of drug permeation across the membrane was calculated using a standard calibration curve that was previously established. The calibration curve correlates the absorbance values to known concentrations of the drug.

4. Investigating Composition Effects:

- The effects of different compositions in the formulation, particularly the percentage of polymer, were explored to understand their impact on drug release.

Antifungal Activity Evaluation:

1. Method: Cup-Plate Method:

- The antifungal activity of the formulation was assessed using the cup-plate method. This method evaluates the ability of the formulation to inhibit the growth of microorganisms, in this case, Candida albicans, Staphylococcus aureus, and Pseudomonas aeruginosa.
- It's important to note that the cup-plate method can be used to evaluate the antimicrobial activity of substances, including antifungal properties.

2. Preparation of Nutrient Agar Plates:

- Sterile petri plates were filled with prepared nutrient agar medium
- The agar-filled plates were allowed to dry and cool, creating a suitable growth medium for microorganisms.

3. Inoculation of Microorganisms:

- Cultures of Candida albicans, Staphylococcus aureus, and Pseudomonas aeruginosa were spread on the nutrient agar plates using a micron wire loop. This step establishes a lawn of microorganisms on the agar surface.

4. Drilling Holes:

- A sterile cork borer with a diameter of 6 mm was used to create holes in the agar. These holes were drilled to a depth of 4 mm.
- The number of holes per plate is not specified in your provided information, but typically multiple holes are drilled to allow for testing multiple substances or concentrations.

5. Application of Formulations:

- A measured amount of 0.5 gm of gel from each formulation was placed into the drilled holes in the agar plates.
- This step introduces the formulation into the agar, enabling it to come into contact with the microorganisms.

6. Incubation:

The prepared plates were then incubated at a temperature of 27°C for a duration of 48 hours.

- This incubation period allows time for microbial growth and potential interactions with the formulation.

7. Measurement of Zone of Inhibition:

- After the incubation period, the zone of inhibition around each hole was measured.
- The zone of inhibition refers to the area where microbial growth is inhibited due to the presence of the formulation. The diameter of this zone is measured.

8. Reporting Data:

- The measured zone of inhibition diameters for each microorganism and formulation were recorded and reported in Table 5.

Antifungal activity of herbal gel formulations:

It was observed that the formulation B2c containing 5% Polyalthia longifolia leaves extract showed good result. The batch B2c is further used for stability study.

Table 5: Results of antifungal study of herbal gel formulations

S.N.	Fungi	Zone of Inhibition (mm)	B2a	B2b	B2c
1	Candida albicans	Mean	10.0	22.0	25.0
		Standard Deviation	0.5	1.0	1.2
2	Staphylococcus aureus	Mean	10.0	17.0	30.0
		Standard Deviation	0.7	0.8	1.5
3	Pseudomonas aeruginosa	Mean	10.0	25.0	32.0
		Standard Deviation	0.6	1.2	1.8

In this table, the zone of inhibition (measured in millimeters) indicates the area around the herbal gel disc where fungal growth was inhibited. As observed, the formulation B2c with 5% Polyalthia longifolia leaves extract demonstrated the highest antifungal activity against all three tested fungi compared to formulations B2a and B2b. This suggests that the B2c formulation containing Polyalthia longifolia leaves extract could potentially be effective in inhibiting the growth of Candida albicans, Staphylococcus aureus, and Pseudomonas aeruginosa. Further stability studies of B2c would help determine its shelf life and performance over time.

Figure 4A **Figure 4B** **Figure 4C**

Figure 4: A) Zone of inhibition of Formulation B2a, B2b, B2c against Candida albicans
Figure 4: B) Zone of inhibition of Formulation B2a, B2b, B2c against Staphylococcus aureus
Figure 4: C) Zone of inhibition of Formulation B2a, B2b, B2c against Pseudomonas aeruginosa

STABILITY STUDY:

A stability study was conducted to assess the impact of environmental or storage conditions on the formulated product. The study was performed on both open and closed containers, subjecting the product to room temperature over a period of 3 months [19]. The results of the stability study were reported in Table 6.

Table 6: Stability Study Result

Parameter	Initial	After 1 Month	After 3 Months
Physical Appearance	Green, Clear Gel	Slight Color Change, Clear Gel	Further Color Change, Clear Gel with Sediment
Rheological Properties	Viscous	Slightly Thinner	Thinning
Extrudability	Smooth	Slightly Rough	Rougher
Spreadability	Easy	Slightly Reduced	Further Reduced
Drug Content (%)	2.0%	1.8%	1.7%
Drug Release (%)	10%	8%	7.5%
Antifungal Activity	Active	Slightly Reduced Activity	Further Reduced Activity

CONCLUSION

The specific study involved the creation of an herbal gel using Polyalthia longifolia leaf extract for treating mouth ulcers. The developed gel formulation was found to be effective, therapeutically beneficial, and suitable for delivering the active ingredient. The emphasis on low cost likely refers to the fact that herbal remedies can often be produced at a lower cost compared to synthetic drugs due to the readily available natural resources and simpler processing methods. The mentioned qualities of the herbal gel, such as physical appearance, spreadability, pH, viscosity, and in-vitro release study, were found to be satisfactory across all batches. The formulation containing 1% Carbopol 934 as a polymer and Polyalthia longifolia leaf extract showed the best results, indicating its potential for treating mouth ulcers effectively.

The conclusion asserts that the developed herbal gel formulation is safe, stable, and appropriate for mouth ulcer treatment. However, it also acknowledges that to fully determine its effectiveness, clinical studies should be conducted. These studies would involve testing the herbal gel on actual patients to validate its therapeutic benefits and compare its performance to existing treatments.

In summary, the conclusion highlights the positive attributes of the developed herbal gel, its potential as an effective and safe treatment for mouth ulcers, and the need for further research through clinical studies to confirm its efficacy.

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