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Formulation and Evaluation of Anti-Microbial Polyherbal Gel Loaded With Tripleurospermum Disciforme, Tagetes Minuta, and Retama Raetam Plant Extracts

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ABSTRACT

The is expected to yield herbal formulations with significant antimicrobial and antifungal properties, utilizing Tripleurospermum disciforme, Targets minute, Retama raetam plants And additional compounds. The evaluation of the prepared herbal formulation will provide insights into their chemical Characteristics and physicochemical parameters, which may contribute to the development of effective antimicrobial and antifungal agent.Animal studies using healthy rats and rabbits are anticipated to demonstrate the safety and suitability of the polyherbal gel formulations for further development and potential therapeutic use.The polyherbal gel formulations will be evaluated for their pH, appearance, homogeneity, viscosity, and spreadability, providing essential information for their formulation and application. Antimicrobial assay results will indicate the effectiveness of the polyherbal gel formulation against Escherichia coli, offering potential alternatives to conventional antimicrobial medications. In vitro drug release kinetics analysis will help identify the release patterns of the herbal formulations which is crucial for controlling drug release and optimizing therapeutic outcomes. The study aims to provide valuable insights into the antimicrobial actions of the polyherbal gel, making it a potential candidate for traditional medicine and further research in this field.

KEYWORDS: Polyherbal gel formulation, Tripleurospermum disciforme, Targets minute, Retama raetam, antimicrobial, against Escherichia coli.

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INTRODUCTION

Various ailments have been treated and cured in India using medications with natural origins [1]. In addition, Indian folk medicine has a variety of prescriptions for treating many medical conditions, including ulcers, ulcerative colitis, leprosy, diarrhea, scabies, venereal illness, skin infections, and snake bites [2]. For a variety of disorders, more than 80% of the global population still uses conventional treatments [3]. Many Indian medicinal plants are thought to have a variety of pharmacological effects because they contain different groups of photo chemicals. Gels have been used for a very long time as topical treatments in both cosmetics and medicine. Gels have become more popular in the principal class of semisolid arrangements, increasing the number of aesthetic and scientific preparations [4]. Natural formulations that include two or more plants are known as polyherbal formulations (PHF). Skin fungus infection is one of the most prevalent dermatological conditions nowadays. There are several treatment alternatives available, including liquid dose, semisolid dose, and stable dosage formulations. The amazing effectiveness of polyherbal formulations in treating a variety of diseases is what draws people to them. In comparison to creams and ointments, topical administration in peculiar locations of gels gives substantial benefits of a quicker discharge of medication straight to the action site, irrespective of the drug's water solubility [5,6]. Alkaloids, anthraquinone glycosides, tannins, triterpenes, flavonoids, and steroids are among the phytochemically abundant compounds found in Tripleurospermum disciform, Targets minuta, and Retama return [7,8]. All three plants have a history of beingused medicinally, particularly for their antibacterial, antimicrobial, antioxidant, and wound-healing abilities [9,10].Tripleurospermum disciforme (C.A.Mey.) is the scientific name for the "Plain Chamomile" of the Asteraceae family [11]. Iran is home to this plant, which is utilized in Iranian herbal medicine as a sedative, anti-inflammatory, and relaxing agent., for weariness, and muscular discomfort [12]. It is also utilized in stress management. Although there are a lot of traditional and customary applications for it in Iran, there aren't many records of its antibacterial properties [13,14]. Recent research has shown that this species' essential oil possesses anti-inflammatory [15,16], antispasmodic as well as antiseptic [17,18], antifungal [19,20], antibacterial [21,22], and antioxidant properties [23,24]. Although Tagetes minuta, a tall, erect marigold plant endemic to South America's southern half with tiny blooms, belongs to the genus Tagetes.

NEED OF THE STUDY

India has an extensive heritage of medical knowledge based on plants. The usage of plant-based medicines is steadily gaining popularity across the globe. The benefit of employing medicinal plants as a form of therapy for ailments is supported by the traditional Indian medical systems of Ayurveda and Siddha [1]. 80% of the world's population depends mostly on traditional medicine, according to 1993 research by the Director of WHO Traditional Medicine., which is primarily plant-based, particularly

for their basic medical requirements. In India, 70% of people are said to use traditional medicine as their main form of healthcare. In comparison to contemporary medications, which have estimated annual sales of US$2.5 billion, significant corporations now produce herbal medicines with an estimated annual turnover of US$300 million. The use of herbal medicine has grown in significance on a worldwide scale, both medically and economically [47].

OBJECTIVES

In this study we will evaluate and produce a polyherbal gel composed of plant extracts from Tripleurospermum disciform, Targets minute, and Retama raetam.

- To prepare and synthesize the polyherbal gel of Tripleurospermum disciform, Targets minute, and Retama raetam.
- To identify the phytoconstituents of Tripleurospermum discifome, Targets minuta, and Retama raetam plant extracts.
- To study physicochemical parameters of herbal formulations.
- To evaluate prepared herbal formulations for antimicrobial activity.
- To compare the antimicrobial activity of the polyherbal gel with conventional antimicrobial medications to assess its potential as a traditional medicine for combating infections.

MATERIAL AND METHODS

Selection and collection of plant

The plant Retama raetam was selected on the basis of Ethano – botanical survey. The plant was selected for its anti- arthritic activity. The flowerss of Retama raetam were collected from Department of Botany Amravati University.

Authentication of Plant

Taxonomical Position

Table 3.1: Showing the Taxonomy Profile of the Plant

Kingdom	Plantae
Phyllum	Tracheophytes
Class	Angiosperms
Order	Fabales
Family	Fabaceae
Genus	Retama
Species	R.raetam

Retama raetam is a species of flowering plant in the family Fabaceae, native to northern Africa from the Western Sahara to Sudan, Sicily, Israel, Sinai Peninsula, the Palestine region and Saudi Arabia, and widely naturalized elsewhere.

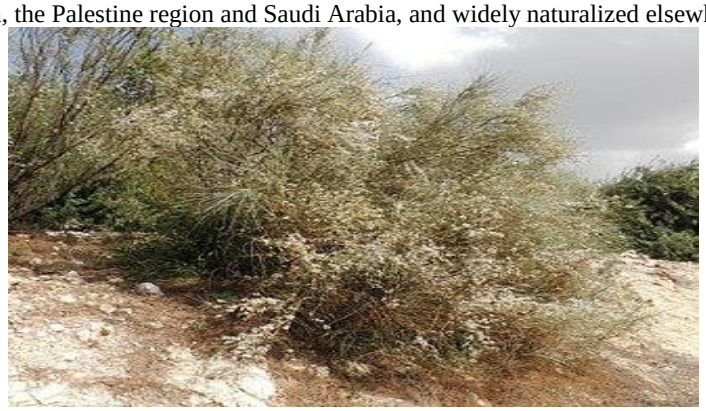

Fig 3.1: Natural Habitat of Retama raetam

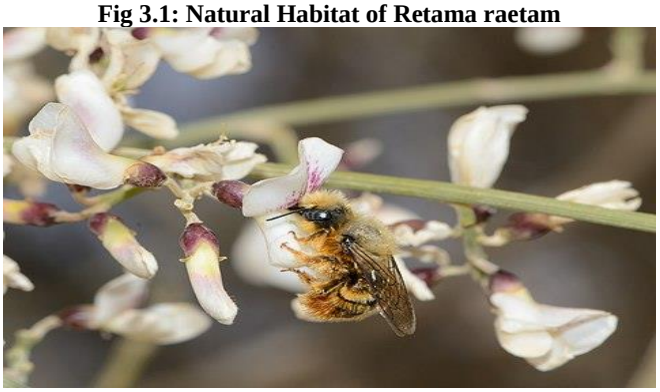

Fig 3.2: Flowers with pollinating bee, Osmia gracilicornis

Morphological Description

An evergreen stem-assimilating desert plant, the white weeping broom is a shrub that grows to about 3 m. and may be 6 m. across. The plants are grey-green with slender, drooping branches; the young plants are wispy, with a single stem and strong taproot. The leaves, which are very small (about 6-7 mm. long), simple, subsessile and narrow (only 1 mm. wide), drop quickly and the plant remains leafless for most of the year. The flowers are 8-10 mm. long, white and pea-like, appearing close to the stem in clusters of 3-15. The hairless grape-shaped seed pod (10-15 mm. diameter) contains one or two kidney-shaped seeds, which are about 6.5 mm. long and may be yellow, green, brown or black. The fruit is an indehiscent pod with one seed of a dark colour, 12-15 mm. long and 7-10 mm. wide. Flowering takes place in the spring between March and May

Geographical Distribution
Local: Northern Algerian Sahara. Regional: North Africa.
Global: The plant is native on maritime sands in the Mediterranean region and on sandy sites in the Sahara.

Ecology
Retama raetam, grows on sandy soils (dune slope/dune base) and in dry conditions (rainfall around 100 mm. per year).

Status
According to the IUCN criteria this Saharo-Mediterranean species falls into the "C" category. The plant is not threatened and appears on the floristic list of several protected sites listed by the UNEP World Conservation Monitoring Centre.

Part used
The stems, leaves and flowers, collected in the spring and prepared as an infusion, a decoction and mixed with other plants. It can be taken by mouth, or used externally as a poultice.

Constituents
Flavonoids, quinolizidine alkaloid.

Pharmacological action and toxicity
Diuretic activity and hypoglycaemic actitvity. The fruits of Retama raetam are considered toxic and thought to provoke hallucinations. Ingesting the plant to produce an abortion has sometimes led to poisoning and even death.

Pharmacopeias
Not relevant for this species.

Pharmaceutical products
Not relevant for this species.
Traditional medicine and local knowledge

- ✓ It is used as an abortifacient, anthelmintic, antiseptic, purgative, sedative, and vulnerary.
- ✓ The flowers are an important source of fodder for dromedaries; when taken in excess this can lead to dangerous urinary problems.
- ✓ When eaten during drought this can lead to abortion, and gives a bitter taste to the milk.
- ✓ The plant is a valuable legume shrub producing good fuel wood. It is also used to stabilise sand dunes.
- ✓ In Morocco, the stems and leaves are crushed and mixed with honey and given orally as an emetic.
- ✓ A decoction of the leaves is given as a purgative and anthelmintic.
- ✓ In Tissint (Morocco), the powdered leaves and flowers are used to heal circumcision wounds and as an antiseptic for wounds, skin rash and pruritus.
- ✓ In Marrakech the plant is crushed in either milk or butter and used for the same purposes.
- ✓ The decoction is used as a massage for pruritus and scabies (human and animal).
- ✓ The roots are used in fumigation as an abortifacient.
- ✓ Likewise, an infusion of the leaves and flowers can help produce an abortion; it is used with great caution since it can lead to poisoning.

Extract preparation of the plant Retama raetam
Flowers were collected during the vegetative stage, rinsed with distilled water then air-dried for two weeks and ground to a fine powder in a Mettler AE 200 (Dangoumau type) grinder. Extraction was performed using solvents of increasing polarity (petroleum ether, ethyl acetate, acetone/water 60/40, V/V and water) using a simple a beaker for the solid–liquid extraction and a separatory funnel for the liquid–liquid extraction. At each extraction step, 50 g of plant material was mixed with 250 ml of solvents. First, the samples were extracted by petroleum ether to remove lipophilic compounds and then the resulting residues were extracted with acetone 60%. The acetonic extract was further portioned in separating funnel using ethyl acetate and water, yielding four fractions (petroleum ether, acetone 60%, ethyl acetate and water)

Table 3.2: Percentage yield of different flower extracts of Retama raetam

Name of the plant	Solvent system used	Wt. of dry powder	Volume of solvent	Wt. of extract	% yield
Retama raetam	Pet. ether	600 gm	1100 ml	20.2 gm	3.0%
	Ethyl acetate	600 gm	1100 ml	26.3 gm	3.9%
	Methanol	600 gm	1100 ml	43.1gm	6.0%

Organoleptic evaluation of Retama raetam

The plant Retama raetam flowers were investigated for their colour, odour, and taste

Table 3.3: Showing Organoleptic evaluation of Retama raetam

Parameters	Retama raetam
Colour	Brown
Taste	Astringent
Odour	Pleasant

Table 3.4: Organoleptic evaluation of extracts of Retama raetama flowers

Extract	Colour	Appearance	Taste	Smell
Pet ether	Light brown	Semi solid	Bitter	Sting
Ethyl acetate	Greenish brown	Semi solid	Bitter	Sting
Methanol	Dark brown	Semi solid	Bitter	Sting

Loss on drying

The weight of the dried powder and fresh sample were measured, and the percentage of water lost and drying-related loss was computed. Water loss as a percentage was computed.

Table 3.5: Percentage loss in weight of plant materials on drying

Plant Species	Wt. of plant material	Wt. of plant material after drying	Loss in wt. on drying	%Loss in weight
Retama raetam	4000 grams	2800 grams	1200 grams	40%

Solubility

Different solvents, such as chloroform, acetone, ethanol, and water, were used to test the solubility of Tabernaemontanadivaricata Pet. ether, ethyl acetate, and methanol flowers extracts.

Table 3.6: Solubility determination of Retama raetam extract in different solvents

Extract	Chloroform	Acetone	Methanol	Ethanol	Water
Pet ether	Soluble	Soluble	Soluble	Not Soluble	Not Soluble
Ethyl acetate	Soluble	Soluble	Soluble	Soluble	Soluble
Methanol	Soluble	Soluble	Soluble	Soluble	Soluble

Determination of total ash values

An accurately weighted sample of the plant Retama raetam weighing about 10g was added to a silica crucible that had already been fired in order to calculate the total ash value. Evenly spread out the material, light it, and then gradually raise the temperature to 500–600 °C until the substance turns white, signifying the reduction of carbon. With weight, let cool in a desiccator. With reference to the air-dried sample, determine the percentage of ash (Chaturvedi et al., 2012).

Table 3.7: Showing ash content of the plant Retama raetam

Name of the Plant	Weight of powdered material	After burning in the crucible(ash)	%age of content
Retama Raetam	15gm	0.95gm	0.95×100/10=9.5%

Plant extracts qualitative phytochemical analysis

A preliminary phytochemical investigation was conducted on several extracts of flowers. To identify the presence or absence of different active substances such as glycosides, carbohydrates, phenolic compounds, alkaloids, flavonoids, saponins, lipids or fixed oils, protein, tannins, and amino acids, the extracts were divided into several sections (Khandelwal., 2005; Kokate., 1994).

Tests for Sugar and Fat

Molish Examination
A test tube was filled with two milliliters of aqueous extract and two drops of an alcoholic α-naphthol solution. A milliliter of concentrated sulphuric acid was then carefully blended along the test tube's walls. The development of a violet ring at the junction indicates the presence of carbohydrates.

Fehling's Examination
One milliliter each of Fehling's A and B solutions were combined with one milliliter of aqueous extract in a test tube, which was then heated in a water bath for ten minutes. Red precipitate development indicates the presence of lowering sugar.

Benedict's examination
Benedict's reagent and extract were combined in an equal volume test tube and heated in a water bath for five to ten minutes. The test solution becomes green, yellow, or red to indicate the presence of reducing sugar, depending on how much of it is there.

Examinations for Alkaloids
The extract was mixed with diluted hydrochloric acid, given a good shake, and then filtered. The filtrate was used for the subsequent experiments.

Mayer Test
2-3 ml of filtrate was added, along the test tube's sides, along with a few drops of Mayer's reagent. The presence of alkaloids is suggested by the formation of a white or creamy precipitate.

Hager Exam
In a test tube, 1-2 ml of filtrate was mixed with a few drops of Hager's reagent. Precipitate that takes on a yellow hue indicates the presence of alkaloids.

Wagner Exam
A test tube was filled with 1-2 milliliters of filtrate and a few drops of Wagner's reagent. Alkaloids can be detected by the formation of a reddish-brown precipitate.

Test for steroids and triterpenoids.
In Salkowski's test, a Chloroform was added to the extract, which was then filtered. The filtrate was subsequently given a few drops of strong sulfuric acid, shaken, and let to stand. If the bottom layers become red, there is sterol present. The layer at the bottom that is golden yellow indicates the presence of triterpenes.

Libermann-Burchard Examination
Chloroform was combined with the extract. This solution was heated to a boil, then cooled with a few drops of acetic anhydride added. It was introduced to the test tube through the sidewalls with strong sulfuric acid. Brown rings appear where two layers meet; if the upper layer turns green, this indicates the presence of steroids; if the hue turns deep red, this indicates the presence of triterpenoids.

Examinations for Flavonoids
Lead Acetate Test
To the extract, a few drops of lead acetate solution were added. The formation of a yellow precipitate could be a sign that flavonoids are present.

Examine Alkaline Reagen A few drops of sodium hydroxide were added to the extract in a different test tube. Flavonoids are present when a bright yellow color develops and then turns colorless when a few drops of diluted acid are added.
Examinations for Phenolic and Tannin Compounds

The Ferric Chloride Test
In distilled water, a small amount of extract was dissolved. This solution was mixed with 2 milliliters of a 5% ferric chloride solution. The existence of blue, green, or violet color production is indicated by phenolic chemicals.

PREPARATION OF POLYHERBAL GEL

The polyherbal gel was prepared using the cold mechanical stirring method as per the composition given in table First, Carbopol 934 was weighed accurately according to each batch formulation and dispersed slowly into approximately 50% of the required amount of purified water under continuous stirring to avoid lump formation. The dispersion was allowed to hydrate and swell for about 4 to 6 hours at room temperature.

In a separate beaker, the herbal extracts Ripleurospermum disciform (1 g), Tagetes minuta (1 g), and Retama raetam (1 g) were accurately weighed and dissolved in a small volume of purified water to form a uniform extract solution. To this solution, propylene glycol (10–15 g depending on the batch) was added as a humectant and permeation enhancer. Additionally, disodium EDTA (0.05 g) was added to stabilize the formulation against metal ion contamination. Preservatives, namely methylparaben (0.2 g) and propylparaben (0.02 g), were dissolved separately in a small amount of hot water (~70°C) and then added to the herbal mixture to ensure microbial stability.

The herbal-extract-propylene glycol mixture was then slowly added to the hydrated Carbopol dispersion under continuous stirring using a mechanical stirrer until a homogeneous gel base was obtained. After thorough mixing, triethanolamine was added dropwise to the formulation to adjust the pH of the gel to the desired range of 6.5 to 7.0, which is ideal for topical application. The pH was monitored using a calibrated pH meter. Finally, the total volume was made up to 100 g with purified water, and the gel was mixed uniformly until a clear or semi-transparent gel of desired consistency was obtained. The prepared gels were transferred into clean, labeled containers and stored at room temperature for further evaluation.

Table 6.1: Composition of Polymerbal Gel

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
Tripleuro spermum Extract	1 g	1 g	1 g	1 g	1 g	**1 g**	1 g	1 g
Tagetes Minuta Extract	1 g	1 g	1 g	1 g	1 g	**1 g**	1 g	1 g
Retama Raetam Extract	1 g	1 g	1 g	1 g	1 g	**1 g**	1 g	1 g
Carbopol 934	0.5 g	0.75 g	1 g	1.25 g	0.5 g	**0.75 g**	1 g	1.25 g
Propylene Glycol	10 g	10 g	10 g	10 g	15 g	**15 g**	15 g	15 g
Disodium EDTA	0.05 g	0.05 g	0.05 g	0.05 g	0.05 g	**0.05 g**	0.05 g	0.05 g
Methylparaben	0.2 g	0.2 g	0.2 g	0.2 g	0.2 g	**0.2 g**	0.2 g	0.2 g
Propylparaben	0.02 g	0.02 g	0.02 g	0.02 g	0.02 g	**0.02 g**	0.02 g	0.02 g
Triethanolamine	q.s.	q.s.	q.s.	q.s.	q.s.	**q.s.**	q.s.	q.s.
Purified Water	q.s. to 100 g	q.s. to 100 g	q.s. to 100 g	q.s. to 100 g	q.s. to 100 g.	q.s. to 100 g	q.s. to 100 g	q.s. to 100 g

EVALUATION OF POLYHERBAL GEL

Appearance

The physical appearance of each gel formulation was visually inspected under natural light. Parameters such as color, clarity, and presence of particulate matter were observed. The gel was examined for any signs of phase separation, grittiness, or air entrapment. A small amount of gel was placed on a glass slide and spread gently to further assess its transparency and smoothness.
pH Measurement

The pH of the polyherbal gels was measured using a digital pH meter (previously calibrated with standard buffer solutions of pH 4.0 and 7.0). About 1 g of gel was dispersed in 10 mL of distilled water, stirred gently to form a uniform dispersion, and the pH electrode was immersed into the solution. The pH value was recorded once it stabilized. The test was carried out in triplicate to ensure consistency, and the acceptable pH range for topical use was 6.5 to 7.0.

Viscosity

The viscosity of the gel formulations was determined using a Brookfield Viscometer equipped with an appropriate spindle (spindle no. 64) at a speed of 10 rpm. Approximately 50 g of gel was transferred into a clean beaker, avoiding air bubbles. The spindle was immersed in the sample and allowed to rotate for a fixed time. The viscosity, expressed in centipoise (cPs), was recorded from the viscometer dial once the reading stabilized. Higher viscosity indicates thicker gel consistency.

Homogeneity

The homogeneity of the gel was assessed by visual inspection and tactile examination. A small quantity of gel was squeezed from the container and applied on a glass slide. The formulation was checked for uniform distribution of extracts, absence of grittiness, and smooth texture using both visual observation and gentle rubbing between fingers. A homogeneous gel ensures even distribution of active ingredients throughout the formulation.

Spreadability

Spreadability was evaluated to determine the ease with which the gel spreads over the skin. This test was performed using the slip and drag method. A fixed amount (1 g) of gel was placed between two glass slides. A standard weight (500 g) was placed on the upper slide for 5 minutes to compress the sample uniformly. The top slide was then pulled with a string attached to a pan, and the time taken to slip off the slide over a 10 cm distance was recorded. Spreadability (S) was calculated using the formula:
S=M×LTS = \frac{M \times L}{T}S=TM×L
Where:

- M = Weight tied to upper slide (g)
- L = Distance moved (cm)
- T = Time taken (s)

Higher spreadability indicates better application on skin.

Extrudability

Extrudability is the ability of the gel to be expelled from the container under applied force. This was tested by filling 20 g of gel in a collapsible aluminum tube. The tube was pressed from the bottom, and the amount of gel extruded in 10 seconds was measured. The ease and consistency of gel coming out was also observed. Extrudability was graded as excellent, good, or poor based on smooth and continuous flow.

In Vivo Skin Irritation Study

The in vivo skin irritation study was conducted using healthy albino rats (or rabbits) weighing between 150–200 g and aged 6–8 weeks. Prior to the test, the dorsal surface of each animal was shaved carefully over an area of approximately 4 cm^2, 24 hours before application of the test formulation, to ensure no hair or visible injuries interfered with the evaluation. A total of three animals were used per formulation, and ethical clearance was obtained from the Institutional Animal Ethics Committee (IAEC) before initiating the study.

For the test, approximately 0.5 g of the polyherbal gel was applied evenly on the shaved area of the skin. The application site was then covered with sterile gauze and secured with a non-irritating adhesive tape or bandage to maintain contact for 4 hours. After this period, the covering was gently removed, and any residual formulation was wiped off without washing the area. The site was then observed for signs of erythema (redness) and edema (swelling) at 1, 24, 48, and 72 hours after removal of the patch. The severity of skin reaction was assessed and scored using the Draize scoring system. Erythema and edema were each graded on a scale from 0 to 4, where 0 indicated no reaction and 4 indicated severe reaction (such as eschar formation or intense swelling). A control was maintained on a separate site of the animal, where a placebo base gel (without herbal extracts) was applied to compare and rule out the effect of excipients. Based on the observations at each time point, a Primary Irritation Index (PII) was calculated by summing the individual scores of erythema and edema for all animals and dividing by the total number of observations. The results were interpreted as follows: a PII of 0–0.4 indicated no irritation, 0.5–1.9 indicated slight irritation, 2.0–4.9 moderate irritation, and 5.0–8.0 indicated severe irritation. All procedures were carried out in accordance with the CPCSEA guidelines, ensuring ethical handling and minimal discomfort to the animals.

RESULT & DISCUSSION

The plant material was collected from .....(Table 3.1). It was shade dried and its Organoleptic properties were evaluated. Organoleptic properties of powdered Retama raetam showed brown colour and possess astringent taste along with pleasant odour as shown in (Table 3.3 and 3.4). Percentage loss of the plant material after drying was calculated and was found 40% loss in weight (Table 3.5) and the ash content was also calculated (Table 3.7) whereas the percentage yield with different solvents from non-polar to polar solvents was done, using pet. ether, ethyl acetate and methanol (Table 3.2).

The results of qualitative phytochemical analysis of the crude powder of rhizomes of Retama raetam are shown in Table 4.1. Methanolic extracts showed the presence of alkaloids, terpenoids, flavonoids, phenols, tannins, carbohydrate, glycosides and saponins. On the other side, ethyl acetate extracts showed the presence of carbohydrates, alkaloids, saponins, flavonoids, triterpenoids, steroids, and tannins. While in petroleum ether extracts only carbohydrates are present and other phyto-components like glycosides, tannin, saponins, protein and amino acids were absent.

Solubility of different extracts of Retama raetam was evaluated using different solvents viz. Chloroform, Acetone, Methanol, Ethanol and Water and results were recorded (Table 3.6).

Table 4.1 Phytochemical evaluations of different extracts of Retama raetam flowers

S. No.	Experiment	Result		
		Pet Ether Extract	Ethyl acetate extract	Methanolic extract
Test **for Carbohydrates**				
1.	Molisch's Test	-ve	+ve	+ve
2.	Fehling's Test	-ve	+ve	+ve
3.	Benedict's Test	-ve	+ve	+ve
Test for Protein & Amino acids				
	Biuret's Test	-ve	-ve	-ve
	Ninhydrin test	-ve	-ve	-ve
Test for Glycosides				
	Borntrager Test	-ve	+ve	+ve
	Killer killaniTest	-ve	+ve	+ve
Test for Alkaloids				
1	Mayer's Test	-ve	+ve	+ve
2	Hager's Test	-ve	+ve	+ve
3	Wagner's Test	-ve	+ve	+ve
Test for Saponins				
	Froth Test	-ve	+ve	+ve
Test for Flavonoids				
	Lead acetate	-ve	+ve	+ve
	Alkaline reagent test	-ve	+ve	+ve
Test for Triterpenoids and Steroids				
	Libermann-Burchard Test	+ve	+ve	+ve
	Salkowski Test	-ve	+ve	+ve

Test for Tannin and Phenolic Compounds				
	Ferric Chloride Test	-ve	+ve	+ve
	Gelatin Test	-ve	+ve	+ve
	Lead acetate Test	-ve	+ve	+ve

EVALUATION OF POLYHERBAL GEL

The prepared polyherbal gel formulations (F1–F8) were evaluated for key physicochemical parameters including appearance, homogeneity, pH, viscosity, spreadability and extrudability. The results are summarized below.

Appearance

The physical appearance of each gel formulation was visually inspected under natural light. The visual inspection of the gels revealed that F1, F2, and F5 were clear and light green, indicating good clarity and minimal particle dispersion, likely due to lower concentrations of Carbopol. F3, F6 were slightly opaque, while F4, F7, and F8 appeared opaque, which can be attributed to the higher levels of Carbopol (1–1.25 g). Opaqueness increases with increasing polymer content, which thickens the gel matrix and reduces light transmission. The results are shown in table

Homogeneity

The homogeneity of the gel was assessed by visual inspection and tactile examination. Formulations F1, F2, F5, and F6 exhibited smooth and uniform texture, without grittiness or phase separation. In contrast, F3, F4, F7, and F8 showed uniform but slightly denser consistency, which is acceptable and indicates good mixing of the extracts with the gel base. No lumps or phase separation were observed in any batch, confirming good compatibility of herbal extracts with the base. The results are shown in table

pH Measurement

The pH of the polyherbal gels was measured using a digital pH meter. The pH values of all batches ranged from 6.5 to 6.7, which is within the acceptable range for topical application (ideal skin pH is ~5.5–7.0). This ensures non-irritancy and skin compatibility. Minor variations reflect the natural acidic or basic tendencies of the herbal extracts used, and pH was adjusted with triethanolamine as needed. The results are shown in table

Table: Appearance, Homogenisity and pH of Polyherbal Formulation (F1 to F8)

Parameter	F1	F2	F3	F4	F5	F6	F7	F8
Appearance	Clear, light green	Clear, light green	Slightly opaque	Opaque	Clear, greenish	Slightly opaque	Opaque	Opaque
Homogeneity	Smooth	Smooth	Smooth	Uniform	Smooth	Smooth	Uniform	Uniform
pH	6.5	6.6	6.7	6.6	6.5	6.6	6.7	6.6

Viscosity

Viscosity plays a vital role in determining the consistency, application properties, and retention of topical gel formulations on the skin. The viscosity of the prepared gels was measured using a Brookfield viscometer and results are reported in centipoise (cPs).

Table: Viscosity of Polyherbal Gel Formulation (F1 to F8)

Batch	Viscosity (cPs)
F1	4215 ± 132.35
F2	5234 ± 145.19
F3	6154 ± 140.84
F4	7218 ± 152.37
F5	4341 ± 128.29
F6	5462 ± 135.63
F7	6255 ± 144.14
F8	7338 ± 151.68

The study revealed that, viscosity increased with increasing concentrations of Carbopol 934, as expected. Batches F1 and F5 with 0.5 g of Carbopol showed the lowest viscosity 4215 ± 25 and 4341 ± 28 cPs, respectively, indicating a lighter and more fluid texture. Batches F4 and F8, which contained the highest amount of Carbopol (1.25 g), exhibited the highest viscosity values of 7218 ± 46 and 7338 ± 51cPs, producing a thicker and more structured gel matrix. Formulations with moderate Carbopol concentrations (0.75–1 g) in F2, F3, F6, F7 showed intermediate viscosities, balancing spreadability and stability. The addition of PEG 400 in batches F5 to F8 did not significantly affect the viscosity but may contribute to improved texture and moisture retention. High viscosity is generally preferred for sustained skin adherence, but overly viscous gels may feel sticky or be difficult to apply uniformly. Therefore, batches with moderate viscosity (F6) are considered as ideal. The results are shown in table and figure

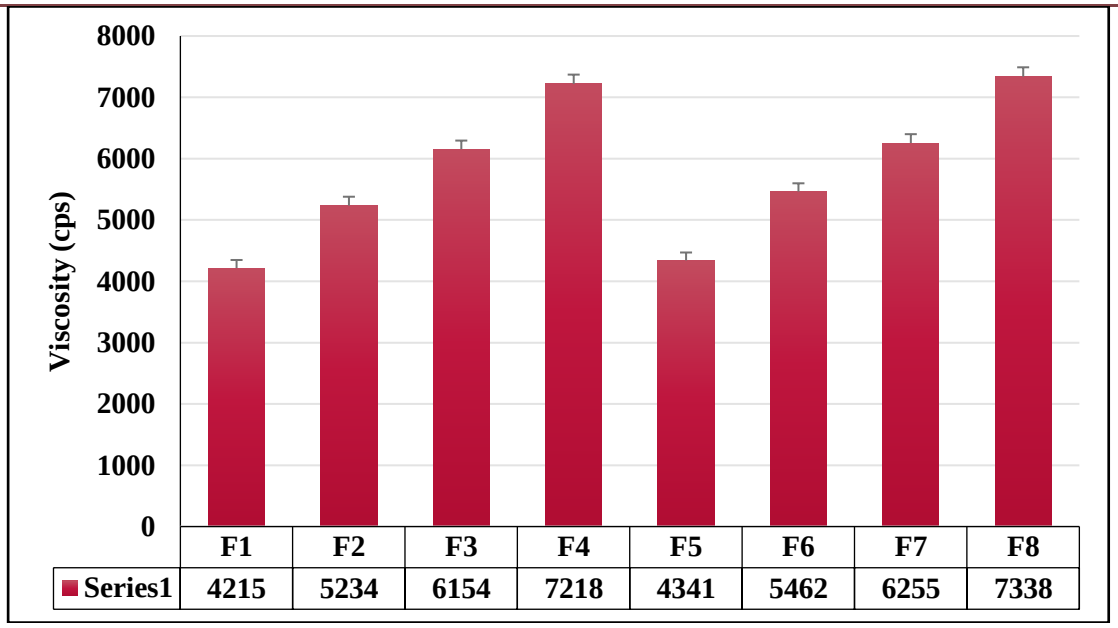

Figure : Viscosity (cps) of Polyherbal Gel Formulation (F1 to F8)

Spreadability

Spreadability measures the gel's ability to evenly distribute over the skin under minimal pressure. It reflects user-friendliness and determines patient compliance. The results of spreadability, measured in g·cm/s, are as shown in table

Table: Spreadability Test of Polyherbal Gel Formulation (F1 to F8)

Batch	Spreadability (g·cm/s)
F1	7.2±0.05
F2	6.9 ±0.04
F3	6.3 ±0.06
F4	5.5 ±0.03
F5	7.0 ±0.05
F6	6.5 ±0.04
F7	5.9 ±0.07
F8	5.2 ±0.04

From the spreadability study it was noticed that, spreadability decreases with an increase in viscosity. This inverse relationship is evident across all batches. Batch F1 and F5 showed the highest spreadability values (7.2 and 7.0 g·cm/s), indicating their excellent ability to cover skin surfaces easily. These gels had the lowest viscosity, making them easy to apply but potentially prone to running or less retention.

Batch F4 and F8, the most viscous gels, showed the lowest spreadability (5.5 and 5.2 g·cm/s), suggesting difficulty in spreading and potentially reduced patient acceptance.

Batch F2 and F6 provided ideal spreadability (6.9 and 6.5 g·cm/s) with moderate viscosity, offering a balanced texture neither too runny nor too stiff.

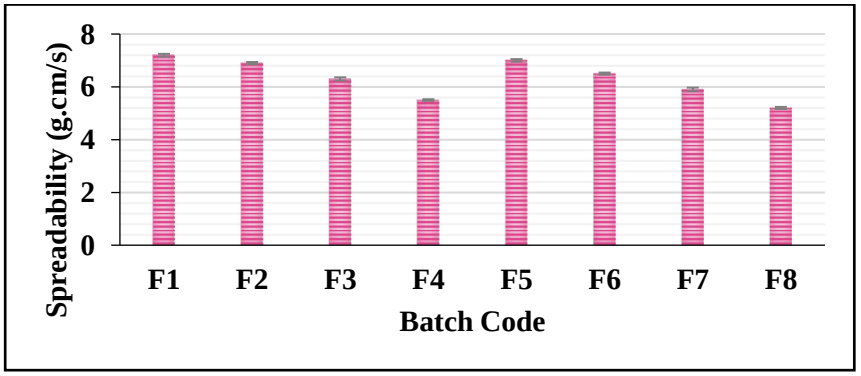

Figure : Spreadability (g.cm/s) Study of Polyherbal Gel Formulation (F1 to F8)

Extrudability

The extrudability of the prepared polyherbal gel formulations (F1–F8) was evaluated to assess the ease with which the gel can be expelled from the container under standard conditions. This is a crucial parameter that influences user convenience, product dispensing, and dosing accuracy in semi-solid formulations. The test was conducted using a collapsible tube containing 20 g of gel, and the amount extruded in 10 seconds under consistent pressure was measured. The results are presented as mean ± standard deviation from three determinations.

Table: Extrudability Test of Polyherbal Gel Formulation (F1 to F8)

Batch	Extrudability (g/10 ses)
F1	10.5 ± 0.3
F2	9.8 ± 0.2
F3	8.9 ± 0.3
F4	7.5 ± 0.2
F5	10.2 ± 0.2
F6	9.5 ± 0.2
F7	8.7 ± 0.3
F8	7.2 ± 0.3

The extrudability values ranged from 7.2 ± 0.3 g (F8) to 10.5 ± 0.3 g (F1), reflecting a clear trend correlating viscosity and polymer concentration with the ease of extrusion. Formulations F1 and F5, which contained the lowest concentration of Carbopol 934 (0.5 g), exhibited the highest extrudability (10.5 ± 0.3 g and 10.2 ± 0.2 g, respectively). These formulations were less viscous, allowing the gel to flow smoothly and effortlessly from the container with minimal pressure. Such characteristics are desirable for patient-friendly topical applications, especially for individuals with reduced hand strength or for elderly users. In contrast, formulations F4 and F8, which contained the highest concentration of Carbopol (1.25 g), showed significantly lower extrudability values (7.5 ± 0.2 g and 7.2 ± 0.3 g, respectively). The high viscosity in these formulations made the gel more difficult to expel, which may be inconvenient for users and could lead to product wastage or inconsistent dosing. These observations confirm that as viscosity increases, extrudability decreases, due to the increased resistance of the gel matrix to flow under applied pressure. Formulations F2, F3, F6, and F7, which had moderate Carbopol concentrations (0.75–1.0 g), demonstrated balanced extrudability values in the range of 8.7 to 9.8 g/10 sec, indicating a favorable compromise between consistency and ease of use. Among them, F2 and F6 offered the most optimal values (9.8 ± 0.2 g and 9.5 ± 0.2 g), aligning well with their moderate viscosity and good spreadability, which makes them suitable candidates for optimized topical delivery.

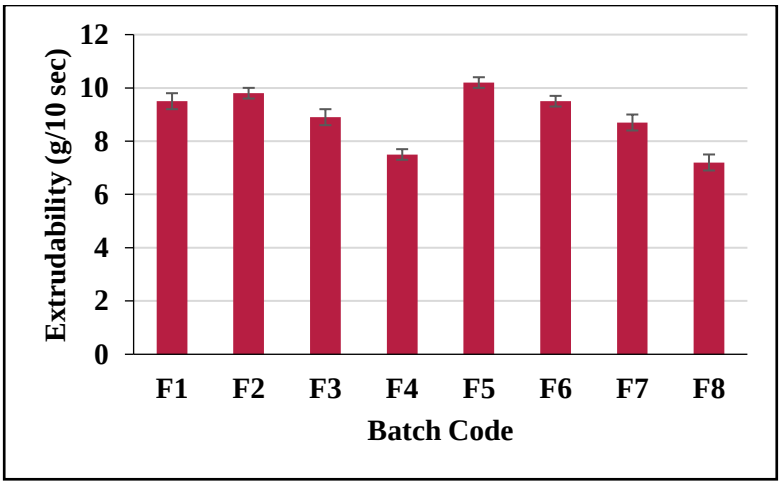

Figure : Extrudability Study of Polyherbal Gel Formulation (F1 to F8)

The extrudability test confirmed that gel viscosity, primarily governed by Carbopol concentration, directly affects the ease of dispensing. F2 and F6 stood out as the most balanced formulations, providing an ideal combination of application consistency and user convenience, making them promising for further development.

In Vivo Skin Irritation Study

The in vivo skin irritation study was performed on albino rats to evaluate the dermal safety of polyherbal gel formulations F1 to F8 using the Draize test. Each formulation was applied to a shaved dorsal skin area, and observations were recorded at 1 hour, 24 hours, 48 hours, and 72 hours post-application. The severity of skin reactions was assessed based on erythema (redness) and edema (swelling) scores, and the Primary Irritation Index (PII) was calculated for each batch to quantify the level of irritation.

Table: In Vivo Skin Irritation Study

Batch	Observation Time Points	Erythema Score	Edema Score	Skin Reaction	Primary Irritation Index (PII)	Interpretation
F1	1h, 24h, 48h, 72h	0	0	None	0.00	Non-irritant
F2	1h: slight erythema (1), resolved by 24h	1 (1 animal)	0	Slight redness	0.10	Non-irritant
F3	1h: erythema (1), resolved by 24h	1 (2 animals)	0	Slight redness	0.17	Non-irritant
F4	1h–24h: erythema (2), edema (1), resolved by 48h	2	1	Mild redness & swelling	0.58	Slight irritation
F5	1h, 24h, 48h, 72h	0	0	None	0.00	Non-irritant
F6	1h: very slight erythema (1), resolved by 24h	**1 (1 animal)**	**0**	Very slight, self-resolving	**0.08**	Non-irritant (Optimized)
F7	1h: erythema (1), edema (1), resolved by 48h	1 (2 animals)	1 (2 animals)	Mild redness & swelling	0.33	Non-irritant to slight
F8	1h–24h: erythema (2), edema (1), resolved by 72h	2	1	Mild persistent redness	0.75	Slight irritation

Among all tested formulations, F6 demonstrated the most favorable dermal safety profile, with only very slight erythema observed in a single animal at 1 hour, which completely resolved by 24 hours. There was no edema reported at any time point. The corresponding PII value for F6 was 0.08, categorizing it as non-irritant according to standard irritation scales. The minimal and self-limiting nature of the erythema in F6 highlights its excellent compatibility with skin and supports its selection as the optimized formulation.

Other formulations such as F1, F2, F3, and F5 also showed low or negligible irritation, with PII values ranging between 0.00 to 0.17, confirming their non-irritant nature. However, F2 and F3 did show slight erythema in one or two animals, respectively, though all symptoms resolved within 24 hours without progressing to edema. F5, like F1, showed no irritation at all time points, further indicating good dermal tolerance. In contrast, F4, F7, and F8 showed signs of mild irritation. F4 and F8, which had higher concentrations of Carbopol 934 (1.25 g), exhibited both erythema and edema during early observation periods. F8 displayed persistent erythema up to 72 hours, with a relatively high PII of 0.75, classifying it as slightly irritating. F7 showed mild redness and swelling in two animals, with a PII of 0.33, placing it in the non-irritant to slight irritant category.

CONCLUSION

These findings suggest that higher Carbopol concentrations may contribute to increased skin sensitivity, potentially due to the more viscous and occlusive nature of these formulations. In summary, the in vivo irritation study confirms that all formulations fall within acceptable safety limits, with F6 being the most balanced in terms of skin tolerance, formulation consistency, and therapeutic potential. Its low PII, minimal transient erythema, absence of edema, and fast skin recovery rate collectively designate F6 as the optimized batch for further development as a safe and effective polyherbal gel for topical application.

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