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Formulation and Evaluation of Herbal Gel Containing Leaf Extract of *Mangifera indica*

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ABSTRACT

As a unique source for the treatment of infectious diseases in place of manufactured medicines, phytochemicals or the bioactive substances found in medicinal plants may be used. The luscious stone fruit known as the mango (*Mangifera indica* L.) is found all over the world, especially in tropical regions. It is a member of the Anacardiaceae family. The agar well diffusion method was used in this work to assess the antibacterial activity of the methanolic leaf extract of *Mangifera indica*. The extract exhibits the best and most active properties. Topical medications are frequently utilized nowadays to treat skin disorders. Here, an effort is undertaken to formulate and assess an herbal gel that contains methanolic *Mangifera indica* leaf extract. The continuous soxhlet hot technique of extraction is used. It evaluates the extract's MIC. With a 3^2 complete factorial design, gel is optimized. The pH, viscosity, extrudability, spreadability, drug content, and skin irritation studies of the gel were all evaluated. Stability tests were carried out in accordance with ICH recommendations. When the gel formulation was further compared to a commercial product, it was discovered that the results were stronger in every way. The optimized formulation was determined to comply with all of the parameters for a gel.

Keywords: Herbal gel, *Mangifera indica,* Antibacterial activity, Minimum Inhibitory Concentration, Optimization.

Introduction

Over 4000 years ago, the Ayurvedic and traditional medical systems began using the herb *Mangifera indica* (MI), commonly referred to as the mango. The Anacardiaceae family of flowering plants includes the genus Mangifera, which has roughly 30 species of tropical fruiting trees. Different portions of the mango tree are said to have a wide range of therapeutic benefits in ayurveda (Shah K. A et al., 2010). The growth of mango trees triggers a process known as carbon sequestration or carbon uptake, which is another crucial piece of mango tree knowledge. In mango tree climate zones around the world, the tree takes carbon dioxide from the air and uses it to build the trunk, branches, leaves, and fruit of the mango tree. During this process, the tree creates oxygen and releases it into the surroundings (Quintana S. E et al., 2021). In some cultures, young, tender green mango leaves are cooked and consumed. The leaves are also used to make tea and dietary supplements because they are considered to be extremely nutritive. Widely utilized in Ayurvedic and traditional Chinese medicine, the leaves of a specific type of mango called *Mangifera indica* are also used in other healing modalities (Mirza B et al., 2021). Diverse plant components and antioxidants are concentrated in mango leaves. Though preliminary, research suggests that the leaf of this tropical fruit may be helpful for weight loss, digestion, and skin health (Kumar M et al., 2021). Cooked mango leaves are frequently consumed in various regions.

Herbal gel is a solid, jelly-like substance that can range in qualities from soft and weak to strong and tough preparation. It is applied topically for many kinds of processes, such as protectors, antiseptics, and antimicrobials. Because they are more widely accepted culturally, are more compatible with the human body, and have fewer side effects, herbal medications have greatly improved for primary healthcare (Tollenaere M et al., 2022). A topical drug delivery system is a method of administering medication to the body by diffusing it through the skin layers. The efficiency of a topical application is largely dependent on the pace and volume of drug release from the base. Drugs used topically on the body are those used to cure a variety of conditions. Most frequently, a topical drug delivery method is applied to the skin, where the medication either treats the region of application or is taken up by the dermis and absorbed into the bloodstream (Talat M et al., 2021). Due to their advantages over cream and ointment, topical gels are a common dosage form for topical drug delivery and are used to treat skin conditions. They are made of a combination of the gelator, solvent, active ingredient, and additional excipients (Reddeman R. A et al., 2019).

Taxonomical Classification

Kingdom : Plantae

Subkingdom : Tracheobionta

Super division : Spermatophyta

Division : Magnoliophyta

Class : Magnoliopsida

Subclass : Rosidae

Order : Sapindales

Family : Anacardiaceae

Genus : Mangifera

Materials and Methods

Collection and Authentication of the plant material

The leaves of *Mangifera indica* were collected in the month of November - December 2022 from Alappuzh adistrict, Kerala, India and were authenticated by Dr. Sreeja Krishnan, Assistant Professor, Department of Post Graduate Studies and Research in Botany, Sree Narayana college, Cherthala.

Chemicals

Carbopol 940; Nice Chemicals Pvt. Ltd, Methanol; (Nice Chemicals Pvt. Ltd, Cochin), Cochin, Propylene Glycol; Chemdyescorporation, Gujarat, Poly Ethylene Glycol; Chemdyescorporation, Gujarat, Triethanolamine; Nice Chemicals Pvt. Ltd, Cochin, Methyl Paraben: and Chemdyescorporation, Gujarat, Propyl Paraben; Chemdyescorporation, Gujarat.

Preparation of methanolic extract of Mangifera *indica* leaf extract.

The obtained leaves were cleaned, airtight containers were used to keep them, they were dried in the shade, pulverized using a mechanical grinder, and they were cleaned again. It was extracted using the hot soxhlet method in a continuous process. The powdered leaves (70g) were defatted in a soxhlet apparatus at 60–80^{0}C using petroleum ether. Following defatting, 600 ml of methanol was used to continue the extraction. The extracted materials were collected, concentrated using a rotary evaporator, and kept in the refrigerator until needed. Extracts were weighed, and percentage yield and practical yield were determined in terms of air-dried raw material. The resulting extract was assessed using a preliminary phytochemical screening (Chirayath R. B et al., 2019).

Preliminary Phytochemical Screening

The standard qualitative techniques were used to identify the presence of several phytocomponents (Sarkar T et al., 2022).

MIC of extract (Tube Dilution Method)

A drug's minimum inhibitory concentration (MIC) is the lowest concentration at which it can stop a specific pathogen from growing. For efficient treatment, it is crucial to ascertain which pathogens an antibiotic is effective against (Abullais Saquib S et al., 2021). The MIC value is determined in this study using the tube dilution method, which involves making successive dilutions of extracts in liquid medium, which are then inoculated with standardized inoculums and incubated for a predetermined amount of time. The minimum inhibitory concentration is the amount of an antibiotic or test sample that must be present before an organism can develop.

***In-vitro* antibacterial activity of extract**

Using the agar-well diffusion method, the antibacterial activity of the *Mangifera indica* leaf extract against gram positive and gram negative microbiological strains was compared with the reference (Amoxicillin).

Pre-formulation study

a) Solubility study

The solubility of a compound is measured by how many grams it will dissolve in 100 grams of solvent at a certain temperature. The extract's solubility in various solvents, including water, methanol, ethanol, 0.1N HCl, 0.1N NaoH, distilled water, etc., was noted.

Determination of λmax

Using a UV double beam spectrophotometer, the extract's absorption maximum was measured. A solution of extract with a concentration of 10g/ml was made in phosphate buffer at a pH of 5.5, and it was scanned between 200 and 400 nm.

b) Preparation of calibration curve of extract

A stock solution containing 1000 g/ml has been produced by dissolving an accurately weighed 100 Mg extract in phosphate buffer pH 5.5 and then adjusting the amount to 100 ml. To obtain a concentration of 100 g/ml, 1 ml of the stock solution was transferred to a 100 ml volumetric flask and filled with phosphate buffer pH 5.5. To get solutions with concentrations of 2, 4, 6, 8, and 10 g/ml, respectively, aliquots of 0.2, 0.4, 0.6, and 0.8 ml from this

stock solution were placed into separate series of 10 ml volumetric flasks and built up to volume with phosphate buffer pH 5.5. At 241.5 nm, the absorbance was measured against a blank surface (Lerma-Torres J. M et al., 2019). The calibration curve for the extract was plotted using the absorbance readings.

Preparation of Gel

An exact amount of Carbopol 940 was weighed out and then continuously stirred into 50 ml of distilled water. The needed amount of methyl and propyl paraben was added to 5ml of distilled water and heated on a water bath to dissolve. Propylene glycol 400 and polyethylene glycol 200 were then poured into the solution once it had cooled. The additional amount of Mangifera indica methanolic extract needed was added to the aforementioned mixture, and the remaining distilled water was added to bring the volume to 100 ml. Finally, after thoroughly combining all of the ingredients in the Carbopol 940 gel with constant stirring and adjusting the pH of the skin to the desired range (6.8–7), triethanolamine was added drop by drop to the mixture to create the gel's desired consistency.

Table 1: Formulation chart

Sl.No.	Ingredients	Quantity for 50 gm
1	Carbopol 940	q.s
2	Methyl paraben	0.075 gm
3	Propyl paraben	0.015 gm
4	Propylene glycol	2.5 ml
5	Polyethylene glycol	7.5 ml
6	Extract	Q.S
7	Distilled water	Up to 50 ml

Optimization by 32 full factorial design

The concentration of the drug extract and the amount of carbopol 940 (X1 and X2, respectively) were chosen as the two independent variables in a 32 full factorial design. Prior research completed before putting the experimental design into practice is used to determine the amounts of the two components. Throughout the duration of the trial, all other formulation and processing variables remained unchanged. Design expert software version 10 optimized the preparation. The effects of the gelling agent and drug concentration were investigated, and all of the formulations were made and tested for a number of criteria. Data were entered into design-specific software, and a polynomial equation was produced. Viscosity of the formulation and antibacterial activity were the responses (dependent variables) examined.

Table 2: 3^2 full factorial design layout

Factors Independent variables	Coded levels			Responses Depended variables
	-1	0	1	
X1(Carbopol Con. %)	1	1.5	2	Y1- viscosity of gel
X2(drug Con. %)	2.5	5	10	Y2- antibacterial activity of gel

Evaluation of Optimized Gel

a) Physical appearance

The physical appearance's color, appear and application feel were all visually examined.

b) pH determination

Using a digital pH meter, the gel's pH was determined. After being dissolved in 100 ml of distilled water, one gram of gel was left to stand for two hours. The pH of the gel formulation was measured after fully submerging the electrodes. Calculated average results were used to determine the formulation's pH in triplicate.

c) Determination of extrudability

A metal tube that could collapse was filled with the gel compositions. The material was forced through the tubes to extrude it, and the formulation's extrudability was assessed by weighing how much material would be needed to pressurize a tube to extrude a 0.5-cm gel ribbon in 10 seconds (Kola-Mustapha A. T et al., 2020).

d) Determination of viscosity

By choosing the spindle number and rpm on the Brookfield viscometer, the viscosity of the created gel formulation was measured. A 50ml beaker containing 40g of the preparation was maintained there while the spindle groove was lowered, the rpm was set, and the dual reading was measured after three minutes. The obtained data was used to determine the viscosity using a factor. Three times the process was carried out, and the results were recorded as means.

e) Spreadability

It represents the size of the area to which a gel spreads easily when applied to skin or an affected area. The spreadability of a formulation affects its medicinal effectiveness as well. Spreadability is measured in terms of the number of seconds it takes for two slides to separate from the gel that is sandwiched in between them when a specific force is applied. Better spreadability is achieved with shorter gap times between two slides. The formula is used to calculate it.

S = M.L / T.

Where,

M = weight attached to upper slide.

The glass slide's length, L

T stands for the amount of time needed to divide the slides.

Results and Discussion

The extract contained carbohydrates, tannins, saponins, glycosides, resins, flavonoids, and alkaloids, as determined by preliminary phytochemical tests. It is very easily soluble in ethanol and dimethyl sulphoxide, according to a solubility study.

Using phosphate buffer as the solvent, a standard extract solution (10 g/ml) was scanned in a UV spectrometer between 200 and 400 nm. The highest absorption wavelength was discovered to be 271.5 nm. The phosphate buffer pH 5.5 at the max271.5 nm was used to produce the standard calibration curve for extract. The obtained calibration curve is linear.

Formulation and Optimization of Antibacterial *Mangifera indica* Gel

Incorporating carbopol, polyethylene glycol, propylene glycol, and extract, 9 formulations were developed. Nine formulations had different extract and carbopol concentrations.

Development of the optimum batch

The software generated 100 solutions based on statistical analyses, from which it chose the best batch out of 100. Table 7 presents the formula for the ideal batch.

Table 3: MIC value of extract

Organism	Samples	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.125 µg/ml	1.6 µg/ml
Bacillus subtilis	Extract	-	-	-	-	+	+	+
	Std	-	-	-	-	-	-	+
S.aureus	Extract	-	-	+	+	+	+	+
	Std	-	-	-	-	-	-	+
E.coli	Extract	-	-	-	+	+	+	+
	Std	-	-	-	-	-	-	+
Pseudomonas aeuroginosa	Extract	-	-	-	+	+	+	+
	Std	-	-	-	-	-	+	+

Table 4: Antibacterial activity of extract

Organism	Zone of inhibition (mm)			
	Standard		Ethanolic Extract	
	50 µg	100 µg	150 µg	200 µg
Bacillus subtilis	31	21	24	27
S.aureus	35	14	25	25
E.coli	20	10	15	17
Pseudomonas aeuroginosa	32	15	25	28

Table 5: Formulation chart

Formulation code	Carbopol (g)	Extract (%)	Methyl paaben(g)	Propyl paraben	Polyethylene glycol (ml)	Propylene glycol (ml)	Triethano lamine	Distilled water
F1	1	2.5	0.075	0.015	7.5	2.5	Q.S	Upto 50 ml
F2	1	5	0.075	0.015	7.5	2.5	Q.S	Upto 50 ml
F3	1	10	0.075	0.015	7.5	2.5	Q.S	Upto 50 ml
F4	1.5	2.5	0.075	0.015	7.5	2.5	Q.S	Upto50 ml
F5	1.5	5	0.075	0.015	7.5	2.5	Q.S	Upto50 ml
F6	1.5	10	0.075	0.015	7.5	2.5	Q.S	Upto50 ml
F7	2	2.5	0.075	0.015	7.5	2.5	Q.S	Upto50 ml
F8	2	5	0.075	0.015	7.5	2.5	Q.S	Upto50 ml
F9	2	10	0.075	0.015	7.5	2.5	Q.S	Upto50 ml

Table 6: Observed responses in 9 experimental run

Formulation code	Independent variables		Response variables	
	X1(g) Concentration of carbopol	X2(%) Concentration of extract	Y1(cp) viscosity	Y2 (mm) Antibacterial activity of gel
F1	1	2.5	18218	31
F2	1	5	18264	32
F3	1	10	18614	30
F4	1.5	2.5	21827	27
F5	1.5	5	21649	26
F6	1.5	10	21734	24
F7	2	2.5	24946	22
F8	2	5	24842	23
F9	2	10	24846	19

Table 7: Formula for Optimum batch

Number	Carbopol*	Extract*	Viscosity	Antibacterial activity	Desirability
1	*1.244*	*5.491*	19693.682	28.681	1.000

Figure 1: Optimized *Mangifera indica* gel

The evaluation studies reveal that it is a thick, opaque gel with a yellow tint. The pH value was 7. 1970 cps was the viscosity. It had been found that spreadability and extrudability were good. The study's findings on skin irritability yield a score of 0. The absence of skin discomfort in *Mangifera indica* gel is as definite.

Antibacterial activity of Gel

Gram positive and gram negative organisms were used as the test subjects for comparing the antibacterial activity of *Mangifera indica* gel to that of the commercial version of Amoxicillin (ALMOX). The final results were discovered to be better in every way.

Conclusion

For millennia, people have used herbal medicine to treat a variety of health problems. Since herbal medicine is a natural substance, our bodies can respond to it positively. On the other side, herbal medicine especially topical formulation may compel people to pay attention to what their bodies are trying to tell them and to target the source of pain or discomfort. The evaluation and research investigations have shown that Optimized *Mangifera indica* gel is good and capable of treating and relieving the problem area fast and efficiently. Unwanted side effects are minimized by; by-passing the digestive system.

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