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Formulation and Evaluation of Herbal Gel Containing Leaf Extract of Azadirachta Indica

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Abstract:

In Ayurvedic System Medicine, the entire *Azadirachta indica* (Neem) plant is frequently used to treat diabetes and a variety of skin conditions. Various chemical compounds that are utilized as pesticides and insecticides have been provided by nature for this plant. Neem is a significant herb in Ayurveda with anti-fungal, anti-bacterial, anti-diabetic, anti-viral, and anti-helminthic activities. Neem (*Azadirachta indica*), a plant of the Meliaceae family, is thought to have a health-promoting effects since it is a high source of antioxidants. Nowadays, topical medicines are routinely used to treat skin conditions. Here, an effort is made to formulate and assess an herbal gel that incorporates leaf extract from *Azadirachta indica* that has been methanolic. The continuous soxhlet hot extraction method has been used. It assesses the MIC of the extract. Gel is optimized using a 3^2 full factorial design. Evaluations were done on the gel's pH, viscosity, extrudability, spreadability, medication content, and skin irritation investigations. According to ICH recommendations, stability tests were conducted. It was found that the outcomes were stronger in every way when the gel formulation was further compared to a commercial product. Every requirement for a gel was found to be met by the optimized formulation. The research is a doctrinal and analytical study that is made on leaf extract of *Azadirachta Indica* and the very objective of the study is to impart a better understanding of the medicinal use as well as side effect of the extract to the reader as well as the members of pharmaceutical community.

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

Introduction:

Azadirachta indica, also referred to as margosa, neem, nimtree, or Indian lilac, is a member of the Meliaceae family of mahogany trees. It belongs to one of the two species of the genus Azadirachta (Ouerfelli et al., 2022). Although it originated in the Indian subcontinent and some regions of Southeast Asia, it has since become naturalized and is now grown in tropical and subtropical regions all over the world. Neem oil is made from the plant's fruits and seeds (Naz H et al., 2022). A Hindustani noun called nm is derived from the Sanskrit word nimba. The margosa tree is renowned for its ability to withstand drought. It typically thrives in regions with sub-arid to sub-humid weather and 400-1,200 mm (16-47 in) of yearly precipitation. It can thrive in areas with annual rainfall of less than 400 mm, although in those situations, it is highly dependent on ground water levels (Venmathi Maran B. A et al., 2021). Margosa can grow in a variety of soil types, although it prefers deep, sandy soils that have good drainage. It is a typical tropical to subtropical tree that thrives in temperatures between 70 and 90 degrees Fahrenheit (21 to 32 °C). It can withstand temperatures up to very high levels but cannot endure temperatures below 5 °C (41 °F). Margosa is one of the very few shade-giving trees that can grow in dry, arid regions, such as the southern, coastal regions of India and Pakistan (Sengupta P et al., 2017). The trees don't care at all about the water's quality and can survive on the smallest trickle of water, no matter how bad it is (Ali E et al., 2021). Margosa trees are frequently utilized for shade in India and other tropical nations where the Indian diaspora has spread. They can be found along streets, surrounding temples, schools, and other public structures, or in the backyards of the majority of people (Gupta S. C et al., 2017). Large expanses of land are used to plant trees in extremely dry regions.

A solid, jelly-like substance known as gel can be either soft and weak or strong and rigid in production (Dutta D et al., 2022). For a variety of processes, including protectors, antiseptics, and antimicrobials, it is applied topically. Herbal medicines have substantially improved for primary healthcare because they are more widely accepted culturally, are more compatible with the human body, and have less adverse effects (Bhinge S. D et al., 2017). A topical drug delivery system is a way to give the body medicine by letting it permeate the

layers of skin. The rate and amount of medication released from the base determine how effective a topical treatment will be in major part. Drugs that are applied topically to the body are used to treat a wide range of illnesses (Aziz N et al., 2020). Most typically, a topical drug delivery method is used to apply medication to the skin, where it either treats the area where it is administered or is absorbed by the dermis and enters the bloodstream (Shahtalebi M. A et al., 2018). Topical gels are a typical dosage form for topical drug delivery and are used to treat skin disorders because they have advantages over cream and ointment. The gelator, solvent, active component, and other excipients are all combined to produce them.

Taxonomical Classification:

Kingdom : Plantae
Subkingdom : Melioideae
Scientific name : Azadirachta
Division : Magnoliophyta
Class : Magnoliopsida
Order : Sapindales
Family : Meliaceae
Genus : Azadirachta excelsa

Materials and Methods:

Collection and Authentication of the plant material: The leaves of *Azadirachta indica* were collected in the month of October – November 2022 from Alappuzha a district, 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 Azadirachta *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 (80g) were defatted in a soxhlet apparatus at 60–80^{0}C using petroleum ether. Following defatting, 700 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.

Preliminary Phytochemical Screening: The standard qualitative techniques were used to identify the presence of several phytocomponents (Raju, D., & Jose, J. 2019).

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. 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 (Blum F. C et al., 2019). 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 *Azadirachta 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.

b) 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.

c) 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. 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 Azadirachta 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 Independentvariables	Coded levels			Responses Depended variables
	-1	0	1	
X_1(Carbopol Con. %)	1	1.5	2	Y1- viscosity of gel
X_2(drug Con. %)	1.25	2.5	5	Y2- antibacterialactivity 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.

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, phenolic compounds, 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 272.5 nm. The phosphate buffer pH 5.5 at the max 272.5 nm was used to produce the standard calibration curve for extract. The obtained calibration curve is linear.

Formulation and Optimization of Antibacterial *Azadirachta 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 3 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	30	20	22	25
S.aureus	33	12	23	24
E.coli	19	11	13	15
Pseudomonas aeuroginosa	31	14	24	27

Table 5: Formulation chart

Formulation code	Carbopol (g)	Extract (%)	Methyl paaben(g)	Propyl paraben	Polyethylene glycol (ml)	Propylene glycol (ml)	Triethanolamine	Distilled water
F1	1	1.25	0.075	0.015	7.5	2.5	Q.S	Upto 50 ml
F2	1	2.5	0.075	0.015	7.5	2.5	Q.S	Upto 50 ml
F3	1	5	0.075	0.015	7.5	2.5	Q.S	Upto 50 ml
F4	1.5	5	0.075	0.015	7.5	2.5	Q.S	Upto50 ml
F5	1.5	2.5	0.075	0.015	7.5	2.5	Q.S	Upto50 ml
F6	1.5	5	0.075	0.015	7.5	2.5	Q.S	Upto50 ml
F7	2	1.25	0.075	0.015	7.5	2.5	Q.S	Upto50 ml
F8	2	2.5	0.075	0.015	7.5	2.5	Q.S	Upto50 ml
F9	2	5	0.075	0.015	7.5	2.5	Q.S	Upto50 ml

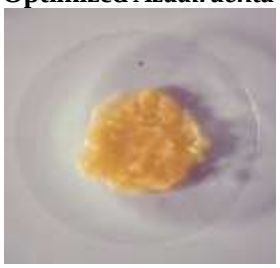
Table 6: Observed responses in 9 experimental run

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	1.25	16346	30
F2	1	2.5	16352	31
F3	1	5	16572	29
F4	1.5	5	19613	26
F5	1.5	2.5	19782	25
F6	1.5	5	19816	23
F7	2	1.25	22329	21
F8	2	2.5	22568	22
F9	2	5	22984	18

Table 7: Formula for Optimum batch

Number	Carbopol*	Extract*	Viscosity	Antibacterial activity	Desirability
1	*1.186*	*2.562*	19782.843	25.468	1.000

Figure 1: Optimized *Azadirachta indica* gel



According to the evaluation studies, it is a thick, opaque gel with a slight yellow color. 7 was the pH level. Viscosity measured at 1978 cps. Extrudability and spreadability were discovered to be favorable. Skin irritation is measured in the study and is given a score of 0. The *Azadirachta indica* gel's absence of skin irritation is as assured.

Antibacterial activity of Gel: The antibacterial activity of *Azadirachta indica* gel and the commercial version of Amoxicillin were compared using both gram positive and gram negative organisms as test subjects. The final outcomes were found to be preferable in every manner.

Conclusion:

Numerous biologically active substances found in medicinal plants are beneficial for extending lifespans and treating disease. Substances including terpenoids, flavonoids, sterols, simple phenolic compounds, proteins, enzymes, lipids, oils, carbohydrates, etc. Natural resources still serve as the foundation of the primary healthcare system and are the source of synthetic and conventional herbal medication. It has been determined through evaluation and research studies that Optimized *Azadirachta indica* gel is effective and may quickly and effectively treat and relieve the problem region. *Azadirachta indica* should be able to target the problem area as a topical gel for quick treatment and relief. It also prevents undesirable side effects by bypassing the GI tract.

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