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Advancement in Herbal Drug Delivery: Preparation and Characterization of Transfersomes Containing *Azadirachta indica*, *Aloe vera*, and *Curcuma longa* Extracts for Skin Applications**Preeti Pandey¹, Vivekanand A.Kashid²**¹Research Scholar, Bhagwant University, Ajmer-305004, India²Research Guide, Bhagwant University, Ajmer-305004, India.**Article Information**

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The increasing demand for natural and effective remedies in skin care and therapeutic applications has led to a surge in the use of plant-based extracts. *Azadirachta indica* (Neem), *Aloe vera*, and *Curcuma longa* (Turmeric) are among the most widely studied plants, known for their potent anti-inflammatory, antimicrobial, and wound-healing properties. However, the bioavailability and effectiveness of these herbal extracts are often limited by poor absorption when administered conventionally. To address this issue, transfersomes—nanosized lipid vesicles designed for enhanced skin penetration—have emerged as a promising drug delivery system. In this study, we developed transfersomal formulations incorporating *Azadirachta indica*, *Aloe vera*, and *Curcuma longa* extracts to enhance their transdermal delivery. The transfersomes were prepared using the thin-film hydration method, employing phospholipids, cholesterol, and an edge activator (Tween 80) to form flexible and nanosized vesicles. The formulations were characterized in terms of physicochemical properties, including size, surface charge, encapsulation efficiency, and in vitro release profile. Preliminary phytochemical screening of the extracts confirmed the presence of bioactive compounds such as flavonoids, alkaloids, saponins, and steroids, which contribute to the therapeutic potential of these plants. The transfersomal formulations exhibited enhanced stability, improved drug release, and increased skin penetration, suggesting their potential for effective topical treatments of skin disorders. This research demonstrates the promising role of transfersomal technology in optimizing the therapeutic efficacy of herbal formulations for dermatological applications.

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INTRODUCTION:

Herbal medicines have long been a cornerstone of healthcare systems worldwide, offering diverse bioactive compounds with therapeutic potential. Among the most studied medicinal plants are *Azadirachta indica* (Neem), *Aloe vera*, and

Curcuma longa (Turmeric), all of which are recognized for their wide array of pharmacological properties. *Azadirachta indica*, commonly known as Neem, is famed for its antifungal, antibacterial, and anti-inflammatory effects and has been utilized for centuries to treat skin ailments, infections, and wounds (Hossain et al., 2020). *Aloe vera*, with its soothing, anti-inflammatory, and hydrating properties, is widely used in the management of burns, skin irritations, and other dermatological conditions (Surjushe et al., 2008). *Curcuma longa*, known for its active compound curcumin, exhibits antioxidant, anti-inflammatory, and antimicrobial properties, making it beneficial in the treatment of skin diseases and other inflammatory conditions (Chainani-Wu, 2003).

Despite their proven efficacy, the bioavailability of these herbal extracts is limited when used in conventional formulations. This limitation arises primarily due to poor absorption across biological barriers such as the skin, which reduces their therapeutic impact. To address this challenge, nanocarriers like transfersomes have gained attention. Transfersomes are advanced drug delivery systems consisting of phospholipids, cholesterol, and edge activators, which form highly flexible, nanosized vesicles capable of improving the penetration of active compounds across the skin (Touitou et al., 2000). These vesicles, with their unique properties, can enhance the transdermal delivery of bioactive molecules, thereby improving the bioavailability and therapeutic effectiveness of herbal extracts.

The therapeutic use of plant-derived extracts has been extensively studied for centuries due to their proven medicinal properties. Among these, *Azadirachta indica* (Neem), *Aloe vera*, and *Curcuma longa* (Turmeric) have garnered significant attention owing to their bioactive compounds and their ability to treat a variety of ailments, particularly skin-related disorders.

Azadirachta indica is a versatile plant renowned for its broad spectrum of pharmacological properties, including antimicrobial, anti-inflammatory, antiviral, and antidiabetic effects. Research has highlighted the efficacy of Neem in treating various skin diseases, such as fungal infections, acne, and psoriasis. Studies by Gnanasekaran et al. (2012) and Vasudevan et al. (2015) indicate that Neem extracts possess potent antifungal properties, which have been utilized in developing topical treatments for skin conditions like athlete's foot and eczema. Additionally, *Azadirachta indica* has shown promise as an anti-inflammatory agent due to the presence of compounds like azadirachtin and nimbin, which inhibit inflammatory pathways (Fadaka et al., 2018).

Similarly, *Aloe vera* is widely acknowledged for its beneficial effects on skin health. It has been studied for its ability to accelerate wound healing, reduce skin irritation, and possess moisturizing properties. According to Hegazy et al. (2015), Aloe vera gel is effective in promoting skin regeneration and soothing burns, and its polysaccharide-rich composition aids in tissue repair. Moreover, Aloe vera's antifungal and anti-inflammatory properties have been confirmed by studies, including those of Ali et al. (2017), who demonstrated its utility in treating inflammatory skin conditions like acne vulgaris.

Curcuma longa (Turmeric) has also been extensively studied for its active compound curcumin, which has shown anti-inflammatory, antioxidant, and antimicrobial properties. Curcumin has been proven to modulate immune responses, reduce oxidative stress, and combat microbial infections (Anand et al., 2007). Moreover, its wound-healing abilities have been explored by Mishra et al. (2014), who found that curcumin accelerates healing processes by reducing inflammation and promoting collagen formation. However, its clinical use is limited by poor bioavailability and low absorption. Therefore, enhancing its delivery through modern drug delivery systems has become a focus of research (Khanna et al., 2014).

While these herbal extracts offer considerable therapeutic benefits, their bioavailability and effectiveness can be limited when administered conventionally. To overcome these challenges, innovative drug delivery systems such as transfersomes have been developed. Transfersomes are phospholipid vesicles that, when combined with an edge activator like Tween 80, significantly enhance the penetration of active compounds through the skin (Touitou et al., 2000). Their ability to deliver active substances efficiently into deeper skin layers has been demonstrated in various studies, such as those by Mahajan et al. (2015), which investigated the transdermal delivery of herbal compounds using transfersomes. The study suggested that these vesicles improve the pharmacokinetic profile of active herbal components, thereby increasing their therapeutic efficacy.

Additionally, recent advancements have shown that transfersomes can improve the solubility and stability of herbal extracts, making them more effective for treating chronic skin conditions like eczema, acne, and psoriasis (Agarwal et al., 2016). This makes the combination of *Azadirachta indica*, *Aloe vera*, and *Curcuma longa* with transfersomal technology a promising approach to enhance the therapeutic potential of these herbal formulations.

This study aims to develop and characterize transfersomes encapsulating *Azadirachta indica*, *Aloe vera*, and *Curcuma longa* extracts. The thin-film hydration method, a well-established technique for preparing flexible nanosized vesicles, was employed in this research. The main objective is to assess the physicochemical properties, phytochemical composition, and transdermal delivery potential of these herbal-loaded transfersomes. Through this investigation, we aim to contribute to the development of more effective herbal formulations with enhanced therapeutic

outcomes for skin-related conditions.

MATERIAL & METHODS:

The study utilized a combination of herbal and laboratory-grade chemicals. *Azadirachta indica*, *Aloe vera*, and *Curcuma longa* were collected fresh from a nearby botanical garden. Laboratory reagents included trichloroacetic acid, methanol, ethanol, petroleum ether, chloroform, and phosphate buffer. Excipients and formulation agents such as wool fat, hard paraffin wax, white paraffin wax, cetostearyl alcohol, starch, sodium lauryl sulphate, and triethanolamine were also used. Methyl paraben served as a preservative, while ascorbic acid and thiobarbituric acid were used for antioxidant and biochemical assays. Distilled water was used throughout all procedures.

Collection and Authentication of plant Materials:

Fresh leaves of *Azadirachta indica*, *Aloe vera*, and roots of *Curcuma longa* were collected, washed thoroughly under running water, and shade-dried at room temperature for 7–10 days. Once dried, they were ground into a fine powder, and approximately 50 grams of each was weighed for extraction.

Soxhlet Extraction:

The Soxhlet apparatus, consisting of a round-bottom flask, extractor, and condenser, was assembled. Approximately 200–300 mL of ethanol was added to the flask as the extraction solvent. The powdered plant material was packed into a cellulose thimble and placed inside the extractor. Upon heating, ethanol vaporized, condensed, and continuously percolated through the plant powder, extracting phytochemicals over 6–8 hours until the siphon tube solvent turned colorless, indicating exhaustion. The extract was then concentrated using a rotary evaporator under reduced pressure or gentle heating, and the semisolid residue was stored in an airtight container at 4°C for further use.

Physicochemical Estimations:

Physicochemical parameters relevant to the powdered material were determined to assess its quality and composition.

Determination of Ash values:

Ash values are determined to evaluate the purity and quality of herbal materials by quantifying the inorganic content, including minerals and impurities like sand or silica.

Determination of Total ash Value:

The total ash value determines the amount of inorganic residue present in a sample after incineration. About 2–3 g of powdered material is accurately weighed and placed in a tared crucible.

It is then incinerated in a muffle furnace at 450–600°C until a white or gray ash is obtained, indicating the removal of carbon. The crucible is cooled in a desiccator and weighed. Heating, cooling, and weighing are repeated until a constant weight is achieved to ensure accuracy. Calculate the total ash content using the formula:

$$\text{Total Ash (\%)} = [(\text{Weight of Ash} / \text{Weight of Sample}) \times 100]$$

Determination of acid Insoluble ash Value:

The acid-insoluble ash value quantifies the portion of ash that remains undissolved in dilute hydrochloric acid. The total ash is transferred to a beaker, mixed with 25 mL of 5% HCl, and gently boiled for 5 minutes. The mixture is then filtered using ashless filter paper, and the residue is washed with hot water to remove acid traces. The filter paper with residue is transferred to a crucible and incinerated at 450–600°C until carbon-free. After cooling in a desiccator, the final weight is recorded. Calculate the acid-insoluble ash content using the formula:

$$\text{Acid-Insoluble Ash (\%)} = [(\text{Weight of Residue} / \text{Weight of Sample}) \times 100]$$

Determination of water Soluble ash Value:

The water-soluble ash value indicates the amount of inorganic salts soluble in water. The total ash is transferred to a beaker, mixed with 25 mL of distilled water, and gently boiled for 5 minutes. The mixture is filtered using ashless filter paper, and the residue is thoroughly washed with hot water. The filter paper with the residue is placed in a crucible and ignited at 450–600°C until carbon-free. After cooling in a desiccator, the residue is weighed. The water-soluble ash is calculated by subtracting the residue weight from the total ash weight. Calculate the water-soluble ash content using the formula:

$$\text{Water-Soluble Ash (\%)} = [(\text{Weight of Residue} / \text{Weight of Sample}) \times 100]$$

Loss on Drying:

Loss on Drying (LOD) determines the moisture and volatile content in a sample, which affects its stability and quality. About 2–5 g of the sample is weighed into a pre-weighed, clean, and dry container. It is then dried in an oven at 105°C (or a specified temperature) until a constant weight is achieved. For heat-sensitive substances, drying can be done in a vacuum oven or desiccator. After each drying cycle, the sample is cooled in a desiccator and reweighed until no further weight loss occurs. Calculate the loss on drying using the formula:

$$\text{Loss on Drying (\%)} = [(\text{Initial Weight of Sample} - \text{Final Weight of Sample}) / \text{Initial Weight of Sample}] \times 100$$

Determination of alcohol soluble extractive value:

The alcohol-soluble extractive value measures the content of alcohol-soluble compounds such as alkaloids, glycosides, and flavonoids. About 5 g of the powdered sample is placed in a glass-stoppered flask with 100 mL of 90% ethanol. The mixture is shaken occasionally for 6 hours and then allowed to stand for 18 hours. After filtration, 25 mL of the filtrate is evaporated to dryness in a pre-weighed dish using a water bath. The residue is dried in an oven at 105°C for 1 hour, cooled in a desiccator, and weighed.

Calculate the alcohol-soluble extractive value as:
Alcohol-Soluble Extractive Value (%) =

$$\frac{\{\text{Weight of residue}\}}{\{\text{Weight of air-dried sample}\}} \times 100$$

Preliminary phytochemical studies:

Preliminary phytochemical screening was conducted to detect the presence of major bioactive compounds in the plant extracts. These tests help identify constituents like alkaloids, flavonoids, tannins, saponins, steroids, and triterpenoids, which contribute to the therapeutic potential of plant materials.

1. Test for Alkaloids:

- Mayer's Test: A yellowish precipitate with Mayer's reagent indicates alkaloids.
- Dragendorff's Test: An orange or reddish-brown precipitate signifies alkaloid presence.
- Wagner's Test: A reddish-brown precipitate confirms alkaloids.

2. Test for Saponins

- Froth Test: Persistent froth after shaking with water suggests saponins.
- Persistent Froth Test: Froth stability for 15–30 minutes confirms saponins.
- Emulsion Test: Stable emulsion with oil or butanol indicates saponins.

3. Test for Tannins and Phenolics:

- Ferric Chloride Test: A greenish-blue or black color suggests tannins/phenolics.
- Lead Acetate Test: A yellowish or white precipitate confirms tannins.
- Gelatin Test: A white precipitate with gelatin solution indicates tannins.

4. Test for Flavonoids

- Shinoda Test: A pink or red color with magnesium and HCl indicates flavonoids.
- Alkaline Reagent Test: A yellow coloration with NaOH confirms flavonoids.
- Ferric Chloride Test: A color change to yellow/orange indicates flavonoids.

5. Test for Steroids:

- Liebermann-Burchard Test: A green or blue-green color after adding acetic anhydride and sulfuric acid indicates steroids.
- Salkowski Test: A reddish-brown ring at the interface of chloroform and sulfuric acid confirms steroids.

6. Test for Triterpenoids:

- Salkowski Test: A reddish-brown or pink color at the interface after adding Salkowski reagent to chloroform extract indicates triterpenoids.

Preparation of Transfersomes:

Transfersomes loaded with *Azadirachta indica*, *Aloe vera*, and *Curcuma longa* extracts were prepared using the thin-film hydration method, a technique widely used for developing flexible and nanosized vesicular systems. In this process, phospholipids (lecithin), cholesterol, and an edge activator (Tween 80) were dissolved in ethanol to form a homogenous lipid solution, into which the herbal extracts were incorporated. This mixture was then subjected to rotary evaporation at 40–45°C under reduced pressure to remove the solvent, resulting in a thin, dry lipid film on the inner wall of a round-bottom flask. The film was hydrated with phosphate buffer (pH 7.4) under constant agitation, allowing the lipid molecules to self-assemble into bilayer vesicles encapsulating the active ingredients. The hydrated suspension was then probe-sonicated for 5–10 minutes to reduce vesicle size, improving uniformity and enhancing transdermal penetration potential. Finally, the transfersomal suspension was stored in sterile, airtight containers at 4°C to maintain stability until further formulation.

Ingredients for Transfersome Preparation

Ingredient	Function	% w/w
<i>Azadirachta indica</i> extract	Antifungal agent	5%
<i>Aloe vera</i> extract	Wound healing, moisturizer	5%
<i>Curcuma longa</i> extract	Antifungal	5%
Phospholipids (Lecithin)	Vesicle formation	4%
Edge activator (Tween 80)	Enhances vesicle flexibility	1%
Cholesterol	Stabilizes vesicles	1%
Ethanol	Solvent for lipid phase	4%
Phosphate Buffer (pH 7.4)	Hydration medium	Q.S. to 100%

RESULT & DISCUSSION:

Macroscopic studies:

Azadirachta indica (Neem) leaves are lanceolate with serrated edges, typically 20–40 cm long, exhibiting a strong bitter odor and taste. Fresh leaves are dark green, while dried ones turn brownish-green, with possible contaminants such

as plant debris and dust. Aloe vera leaves are thick, fleshy, and elongated, ranging from 30–50 cm, emitting a mild fresh odor and a slightly bitter, mucilaginous taste. The fresh leaves are green, and the inner gel is transparent, with potential contaminants like soil particles and plant fibers.

Curcuma longa (Turmeric) rhizomes are cylindrical, 3–7 cm long, and have an earthy, pungent odor and a bitter taste. Fresh rhizomes are yellow-orange, turning bright yellow when dried, with contaminants including dust, fibers, and traces of other rhizomes.

Table 1: Organoleptic characters of plants Azadirachta indica leaves and Aloe vera, and Curcuma longa extract:

S.No	Parameters	Observations of Azadirachta indica (Neem)	Observations of Aloe vera	Observations of Curcuma longa (Turmeric)
1.	Shape	Leaves: Lanceolate, serrated edges	Thick, fleshy, long leaves	Rhizome: Cylindrical, branched
2.	Size	Leaves: 20-40 cm long	Leaves: 30-50 cm long	Rhizome: 3-7 cm long
3.	Odour	Strong, bitter	Mild, fresh	Earthy, slightly pungent
4.	Taste	Bitter	Slightly bitter, mucilaginous	Pungent, slightly bitter
5.	Colour	Fresh leaves: Dark green, Dry leaves: Brownish-green	Fresh leaves: Green, Gel: Transparent	Fresh rhizome: Yellow-orange, Dry powder: Bright yellow
6.	Foreign organic matter	Presence of other plant debris, dust possible	May contain soil particles, fibers	Possible contamination with dust, fibers, or other rhizomes

Physicochemical Standardization of Proposed Plant Drug:

The standardization of Azadirachta indica leaves, Aloe vera, and Curcuma longa extracts was evaluated based on various physicochemical parameters.

Table 2 Standardization parameters of Azadirachta indica leaves, Aloe vera, and Curcuma longa extracts

S.No	Parameters % w/w	Azadirachta indica leaves (% w/w)	Aloe vera (% w/w)	Curcuma longa (% w/w)
1	Ash value	7.25	12.51	8.58
2	Foreign organic matter	0.88	1.15	0.90
3	Water soluble ash	4.52	3.75	3.88
4	Acid insoluble ash	0.45	0.85	0.55
5.	Moisture content	5.05	7.10	9.10

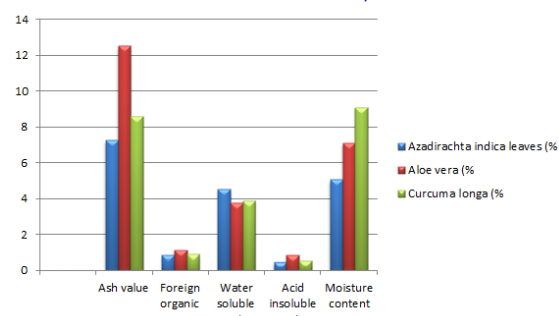


Fig: Graph of Standardization parameters of Azadirachta indica leaves, Aloe vera and Curcuma longa extracts

Preliminary Phytochemical Analysis of Extracts

The phytochemical analysis of the ethanol extracts of Azadirachta indica leaves, Aloe vera leaves, and Curcuma longa roots revealed the presence of various bioactive compounds.

Table 3: Phytochemical Profile of Azadirachta indica leaves, Aloe vera leaves and Curcuma longa extracts

S.No	Chemical Tests	Azadirachta indica Leaves Extract (Ethanol)	Aloe vera Leaves Extract (Ethanol)	Curcuma longa Roots Extract (Ethanol)
1.	Tests for Steroids and Triterpenoids:			
	• Liebermann-Burchard Test	+ (Positive)	- (Negative)	+ (Positive)
	• Salkowski Test	+ (Positive)	- (Negative)	+ (Positive)
2.	Test for Saponins:			
	• Foam Test	+ (Positive)	+ (Positive)	- (Negative)
3.	Tests for Alkaloids:			
	• Hager's Test	+ (Positive)	- (Negative)	+ (Positive)
	• Mayer's Test	+ (Positive)	- (Negative)	+ (Positive)
4.	Tests for Glycosides:			
	• Borntrager's Test	+ (Positive)	- (Negative)	+ (Positive)
	• Keller Killiani Test	- (Negative)	+ (Positive)	- (Negative)
5.	Tests for Tannins and Phenolic Compounds:			
	• Gelatin Test	+ (Positive)	- (Negative)	+ (Positive)
	• Ferric Chloride Test	+ (Positive)	+ (Positive)	+ (Positive)
6.	Tests for Flavonoids:			
	• Ferric Chloride Test	+ (Positive)	+ (Positive)	+ (Positive)
	• Alkaline Reagent Test	+ (Positive)	+ (Positive)	+ (Positive)
7.	Tests for Proteins:			
	• Biuret Test	- (Negative)	+ (Positive)	- (Negative)

	• Xanthoproteic Test	- (Negative)	+ (Positive)	- (Negative)
8.	Test for Carbohydrates:			
	• Fehling Test	- (Negative)	+ (Positive)	- (Negative)

Evaluatory parameters of Colour, Odour and Consistency in Transfersomes

The colour, odour, and consistency of different formulations were evaluated to assess their physical characteristics.

Table: 3 Parameters of Colour, Odour and Consistency in formulations:

S.No	Parameters	Transfersomes
1.	Colour	Greenish-brown
2.	Odour	Herbal, mild
3.	Consistency	Smooth, thick

CONCLUSION:

In conclusion, the study successfully developed and characterized herbal-loaded transfersomes utilizing *Azadirachta indica*, *Aloe vera*, and *Curcuma longa* extracts through the thin-film hydration method. The macroscopic and physicochemical evaluations of the raw plant materials revealed their distinct physical properties, including shape, size, odor, and taste, as well as the presence of foreign organic matter. The standardization of these extracts confirmed the quality and purity of the herbal materials, with values for ash content, foreign organic matter, and moisture content being within acceptable limits. Preliminary phytochemical analysis highlighted the presence of various bioactive compounds such as alkaloids, saponins, flavonoids, and steroids, which contribute to the therapeutic efficacy of these extracts. The formulation of transfersomes exhibited promising characteristics, including a greenish-brown color, mild herbal odor, and smooth, thick consistency, making them suitable for transdermal drug delivery. These findings underscore the potential of using herbal extracts in advanced drug delivery systems like transfersomes, providing enhanced therapeutic benefits through improved bioavailability and sustained release of active ingredients.

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CONFLICT OF INTEREST:

The author declares that there is no conflict of interest regarding the publication of this research.

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