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Original Research Article

FORMULATION AND EVALUATION OF ANTIMICROBIAL HERBOSOMAL GEL FROM CURCUMIN

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ABSTRACT

Curcumin, a bioactive compound derived from *Curcuma longa*, possesses potent antimicrobial and anti-inflammatory properties but suffers from poor solubility and bioavailability, limiting its therapeutic use in topical formulations. In this study, curcumin-loaded herbosomes were formulated and optimized to enhance its stability, skin permeation, and antimicrobial efficacy. Herbosomes were prepared using phospholipids and cholesterol via the solvent evaporation method, and various formulation parameters were optimized based on particle size and drug entrapment efficiency. The optimized formulation (F10) demonstrated a particle size of 220.47 nm and 71.12% entrapment efficiency. This formulation was then incorporated into a Carbopol-based gel matrix to prepare a topical herbosomal gel. Among the six gel formulations (G1–G6), G5 exhibited optimal physicochemical properties, including high drug content (99.45%), suitable pH (6.8), spreadability, and viscosity. The antimicrobial activity of the gel was evaluated against *Candida albicans* using the well diffusion method. The results showed that the herbosomal gel (G5) had significant antifungal activity, comparable to standard fluconazole. The study concludes that curcumin-loaded herbosomal gel is a promising approach for the development of stable, effective, and patient-friendly topical antimicrobial therapies.

Keywords: Curcumin, Herbosomes, Nanoparticles, Antimicrobial Gel, Topical Drug Delivery, Entrapment Efficiency, *Candida albicans*, Spreadability, Viscosity, Drug Content.

INTRODUCTION

In recent years, the development of plant-based therapeutic systems has gained significant attention due to their broad pharmacological activities and relatively lower side effect profiles compared to synthetic drugs. Curcumin, a hydrophobic polyphenolic compound derived from the rhizome of *Curcuma longa*, is a well-documented natural molecule with antioxidant, anti-inflammatory, anticancer, and antimicrobial activities (Aggarwal *et al.*, 2007; Anand *et al.*, 2007). Despite its therapeutic potential, curcumin's clinical utility is limited due to its low aqueous solubility, poor bioavailability, and instability at physiological pH (Bombardelli *et al.*, 1991; Gupta *et al.*, 2013).

To address these limitations, herbosomal technology a novel lipid-based drug delivery system has emerged as a promising approach. Herbosomes involve complexation of phytoconstituents with phospholipids to improve solubility, permeability, and biological stability, thereby enhancing bioavailability (Kalra *et al.*, 2010; Maiti *et al.*, 2007). These vesicular systems mimic biological membranes, making them suitable

for enhanced topical and systemic delivery of hydrophobic plant compounds like curcumin (Mun *et al.*, 2013).

Topical gels are widely used for localized drug delivery as they provide ease of application, targeted action, and reduce systemic side effects. When incorporated into a gel base, curcumin-loaded herbosomes can offer controlled release, better skin penetration, and improved antimicrobial activity (Priyadarsini, 2014). Curcumin has been shown to exhibit significant antimicrobial effects against a broad spectrum of pathogens including *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, and *Helicobacter pylori* (Tiwari *et al.*, 2012; Tyagi *et al.*, 2015).

In light of the increasing challenge of antimicrobial resistance and the need for safer, more effective topical agents, the current study aims to formulate and evaluate an antimicrobial herbosomal gel containing curcumin, with an emphasis on its physicochemical characteristics, stability, and *in vitro* antimicrobial efficacy.

MATERIALS AND METHODS

Materials

The preparation of the curcumin-loaded herbosomal gel involved the use of various analytical-grade chemicals sourced from reliable suppliers. Buffer components like hydrochloride, disodium hydrogen phosphate, and dipotassium hydrogen phosphate were obtained from S. D. Fine Chem. Ltd., Mumbai, while sodium hydroxide was procured from Annexe Chem Pvt. Ltd., Ahmedabad. Solvents such as methanol, ethanol, chloroform, dichloromethane, and polyethylene glycol were sourced from Qualigens Fine Chemicals, Mumbai. Key lipid components for herbosome formation included lecithin (HiMedia, Mumbai) and cholesterol (RFCL Ltd., New Delhi). For gel base preparation, Carbopol 940, methyl paraben (preservative), and triethanolamine (neutralizing agent) were also acquired from HiMedia and Qualigens, respectively. These materials were essential for the formulation, stabilization, and evaluation of the herbosomal gel.

Methods

Formulation development of Herbosomes

The Herbosomes was prepared with phospholipids: Cholesterol and Curcumin in the ratio of 1:.5:1, 1:1:1, 2:1.5:1, 2:2:1 respectively (Kidd, 2009). Weight amount of Curcumin and phospholipids and cholesterol were placed in a 100ml round-bottom flask and 25ml of dichloromethane was added as reaction medium. The mixture was refluxed and the reaction temperature of the complex was controlled to 50°C for 3 h. The resultant clear mixture was evaporated and 20 ml of n-hexane was added to it with stirring. The precipitated was filtered and dried under vacuum to remove the traces amount of solvents. The dried residues were gathered and placed in desiccators overnight and stored at room temperature in an amber colored glass bottle.

Characterization of Herbosomes

Entrapment efficiency

Herbosomes preparation was taken and subjected to centrifugation using cooling centrifuge (Remi) at 12000 rpm AT 4ºC (Hung *et al.*, 2007). The clear supernatant was siphoned off carefully to separate the non entrapped Curcumin and the absorbance of supernatant for non entrapped Curcumin was recorded at λ_{max} 428.0 nm using UV/visible

spectrophotometer (Labindia 3000+). Amount of Curcumin in supernatant and sediment gave a total amount of Curcumin in 1 ml dispersion. The percent entrapment was calculated by following formula:

$$\text{Percent Entrapment} = \frac{\text{Amount of drug in sediment}}{\text{Total amount of drug added}} \text{X } 100$$

Particle size and size distribution

The particle size, size distribution and zeta potential of optimized Herbosomes formulation were determined by dynamic light scattering (DLS) using a computerized inspection system (Malvern Zetamaster ZEM 5002, Malvern, UK) (Khan *et al.*, 2013). The electric potential of the Herbosomes, including its Stern layer (zeta potential) was determined by injecting the diluted system into a zeta potential measurement cell.

Formulation development of Herbosomal gel

In a beaker, measured amounts of methyl paraben, glycerin, polyethylene glycol, and Herbosomes were dissolved in roughly 35 ml of water and swirled at high speed using a mechanical stirrer (or sonicator). Then, while stirring, Carbopol 940 was gently added to the beaker containing the aforementioned liquid. The solution was neutralized by progressively adding triethanolamine solution while stirring constantly until the gel was formed (Nandgude *et al.*, 2008).

Evaluation of gel

Appearance and consistency

The physical appearance of gel formulations was visually examined for texture, and observations were made as shown in Table 4.

Washability

Formulations were applied to the skin, and then the ease and extent of washing with water were physically evaluated, with results similar to those shown in table 5.

Extrudability determination of formulations

Gel compositions were placed in collapsible metal tubes or collapsible aluminum tubes. The tubes were pushed to extrude the material, and the formulation's extrudability was tested (Bhowmik *et al.*, 2009).

Determination of Spreadability

Two standard-sized glass slides (6 ×2) one of the slides was covered with the gel formulation that was to be tested for spreadability. The second slide was positioned above the first in such a manner that the formulation was sandwiched between them for a total distance of 6 cm down the slide. The gel mixture between the two slides was traced uniformly to produce a thin layer by placing 100 grams of weight on the upper slide. The excess of the gel formulation clinging to the slides was scraped off and the weight was removed. The bottom slide was attached to the apparatus's board, and one end of the top slide was linked to a string to which a 20-gram force could be imparted using a simple pulley. The time it took for the upper slide to travel 6 cm and separate from the lower slide under the weight's direction was recorded. The experiment was performed six times, with the average of the results determined for each gel formulation:

$$\text{Spreadability} = \frac{m.l}{t}$$

Where, S=Spreadability (gcm/sec)

m = weight tied to the upper slide (20 grams)

l= length of glass slide (6cms).

t = time taken is seconds.

Determination of pH

A digital pH meter was used to determine the pH of the gels. One gram of gel was dissolved in 25 ml of distilled water, and the electrode was dipped in the gel mixture until a steady reading was obtained. It was also reported that she was always reading. Each formulation's pH readings were repeated two times (Queiroz *et al.,* 2009).

Drug content

Equivalent to 10mg of curcumin from herbosomal gel was taken in 10 ml volumetric flask and dilute upto 10ml with methanol, sonicate it for 10min, take 0.1ml from this and dilute upto 10ml and absorbance was measured at 428 nm. Calculate the percentage drug content using standard calibration curve (Panigrahi *et al.,* 2006).

Viscosity

The resulting gel studied for viscosity on Brookfield Synchrolectric Viscometer using Spindle No.7 at 50 RPM for comparative study. The angular viscosity was measured by gradually increase the RPM from 10 to 70 (Nawanopparatsakul *et al.,* 2005).

***In vitro* antimicrobial activity of gel**

Using the well diffusion method, the gel was tested against microbes on agar plates at concentrations of 10, 20, and 30µg/ml. After 48 hours of incubation at 37°C, the zones of inhibition were measured to assess antimicrobial activity (Bauer *et al.,* 1996).

RESULTS AND DISCUSSION

The present study aimed to develop and evaluate a curcumin-loaded herbosomal gel formulation for enhanced topical antimicrobial activity. The herbosomes were initially formulated by optimizing the ratios of phospholipids, cholesterol, curcumin, and dichloromethane as shown in Table 1. The preparation involved solvent evaporation followed by precipitation with *n*-hexane and vacuum drying. Twelve formulations (F1–F12) were prepared in three optimization phases: lipid-to-cholesterol ratio, curcumin concentration, and solvent volume.

Among these, Formulation F10 emerged as the most promising based on its particle size and entrapment efficiency, as reported in Table 3. F10 displayed the smallest particle size of 220.47 nm and a high entrapment efficiency of 71.12%, indicating better drug loading and a uniform nanoscale dispersion. Compared to other formulations like F1 (342.25 nm, 68.85%) and F6 (255.45 nm, 72.32%), F10 maintained an optimal balance between size and drug retention, making it suitable for topical application and skin permeation.

Based on the optimized formulation (F10), six herbosomal gel batches (G1–G6) were developed using varying concentrations of Carbopol 940 as shown in Table 2. The gels were evaluated for their physical characteristics including color, clogging, homogeneity, and texture, all of which remained consistent across batches each formulation appearing green, free from clogging, and smooth in texture (see Table 4).

The washability and extrudability of all gels were found to be acceptable (good washability and average extrudability), indicating that the gels could be easily applied and removed, maintaining patient compliance (as per Table 5). These features are critical for user-friendly topical drug delivery systems.

In terms of spreadability, pH, viscosity, and drug content, results are presented in Table 6. It was observed that formulation G5 showed the most favorable balance across parameters.

G5 demonstrated a spreadability of 18.8 gcm/sec, allowing even distribution on the skin surface. The pH of 6.8 was close to that of the skin, minimizing the risk of irritation. The viscosity of 2345 cps ensured the gel had adequate consistency for topical use neither too runny nor too stiff. Most importantly, G5 exhibited the highest drug content of 99.45%, indicating excellent drug incorporation and homogeneity in the gel matrix.

The antimicrobial efficacy of the optimized gel (G5) was tested against *Candida albicans* using the well diffusion method, and results are presented in Table 7. The zone of inhibition for G5 at 30 µg/ml was 14.5 mm, closely comparable to fluconazole, the standard antifungal agent, which showed a zone of 15.5 mm. This strong activity was retained even at lower concentrations (11.2 mm at 20 µg/ml and 9.7 mm at 10 µg/ml), suggesting that the curcumin-loaded herbosomal system effectively enhances the bioavailability and antifungal action of curcumin.

Table 1: Different formulations of Herbosomes

Formulation	Ratio of Phospholipids and Cholesterol	Curcumin Concentration (%)	Dichloromethane Concentration
Optimization of Phospholipids and Cholesterol			
F1	1:05	1	25
F2	**1:1**	**1**	**25**
F3	1:1.5	1	25
F4	1:2	1	25
Optimization of Drug Concentration			
F5	1:1	0.5	25
F6	**1:1**	**1.0**	**25**
F7	1:1	1.5	25
F8	1:1	2.0	25
Optimization of solvent concentration			
F9	1:1	1.0	10
F10	**1:1**	**1.0**	**25**
F11	1:1	1.0	50
F12	1:1	1.0	75

Table 2: Formulation of Herbosomal gel

Ingredients (%)	G1	G2	G3	G4	G5	G6
Herbosomes (eq. to)	1%	1%	1%	1%	1%	1%
Carbopol 940	0.5mg	0.75mg	1.0 gm	1.25 gm	1.5 gm	2.0 gm
Polyethylene Glycol	0.2ml	0.2ml	0.2ml	0.2ml	0.2ml	0.2ml
Methyl Paraben	0.08mg	0.08mg	0.08mg	0.08mg	0.08mg	0.08mg
Triethanolamine	1.0ml	1.0ml	1.0ml	1.0ml	1.0ml	1.0ml
Distilled Water (q.s)	100ml	100ml	100ml	100ml	100ml	100ml

***G= Herbosomal gel**

Table 3: Particle size and entrapment efficiency of drug loaded Herbosomes

Formulation Code	Particle size (nm)	Entrapment Efficiency (%)
F1	342.25	68.85
F2	**275.55**	**73.32**
F3	325.66	66.65
F4	320.12	69.98
F5	286.65	65.45
F6	**255.45**	**72.32**
F7	296.36	68.85
F8	274.65	63.22
F9	273.32	64.45
F10	**220.47**	**71.12**
F11	239.98	67.74
F12	240.12	65.23

Average of three determinations (n=3)

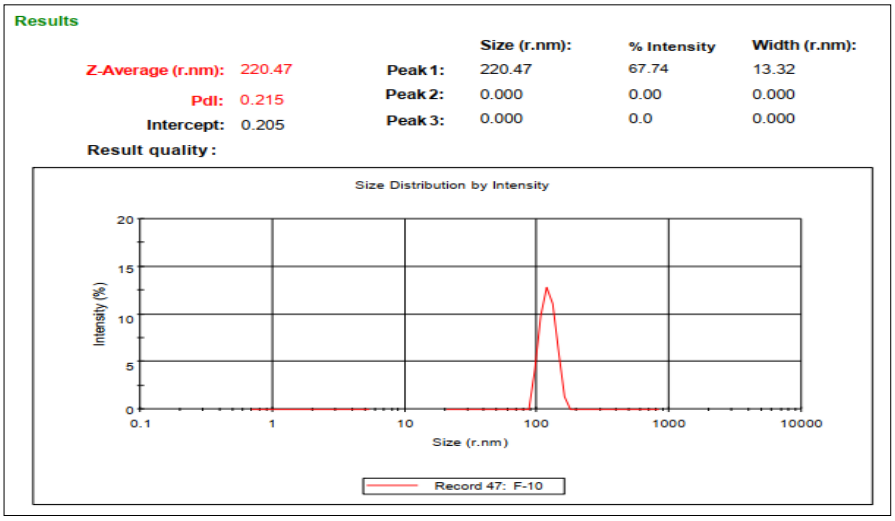

Figure 1: Particle size of optimized batch F10

Table 4: Results of physical appearance of Herbosomes gel

Formulation	Colour	Clogging	Homogeneity	Texture
G1	Green	Absent	Good	Smooth
G2	Green	Absent	Good	Smooth
G3	Green	Absent	Good	Smooth
G4	Green	Absent	Good	Smooth
G5	Green	Absent	Good	Smooth
G6	Green	Absent	Good	Smooth

Table 5: Results of washability and Extrudability of Herbosomes gel

Formulation	Washability	Extrudability
G1	Good	Average
G2	Good	Average
G3	Good	Average
G4	Good	Average
G5	Good	Average
G6	Good	Average

Table 6: Results of Spreadability, pH, Viscosity, % Drug content of Herbosomes gel

Formulation code	Spreadability (gcm/sec)	pH	Viscosity (cps)	% Drug content
G1	22.5±0.15	7.2±0.2	2365±25	92.25±0.32
G2	20.4±0.10	6.9±0.3	2145±32	96.65±0.25
G3	18.8±.26	7.5±0.1	2065±15	98.78±0.14
G4	20.2±0.24	7.3±0.4	2465±28	96.74±0.23
G5	18.8±0.32	6.8±0.3	2345±16	99.45±0.14
G6	16.4±0.14	5.5±0.2	2265±25	98.74±0.22

Average of three determinations (n=3)

Table 7: Antimicrobial activity of gel formulation (G5) against *Candida albicans*

S. No.	Standard /Formulation	Zone of inhibition (mm)		
		30µg/ml	20µg/ml	10µg/ml
1.	Fluconazole	15.5±0.94	12.6±0.5	10.8±0.84
2.	Gel optimized formulation (G5)	14.5±0.47	11.2±0.74	9.7±0.57

CONCLUSION

In conclusion, the study demonstrated that nano-encapsulation of curcumin in the form of herbosomes significantly improves its physicochemical properties and antifungal activity. Among all the formulations, G5, based on Formulation F10, proved to be the most efficient and pharmaceutically acceptable formulation, suitable for further clinical investigations.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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