



# Development of a Novel Freeze-dried Mulberry Leaf Extract-based Transfersome Gel

## Dondurularak Kurutulmuş Dut Yaprığı Ekstresi Bazlı Yeni Bir Transferzom Jelinin Geliştirilmesi

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### ABSTRACT

**Objectives:** Nowadays, antioxidants are important for health-related concerns related to acne vulgaris. Acne vulgaris is interrelated with the development of free radicals that interact with cells. Mulberry leaves contain phenolic compounds, including antioxidants such as quercetin. An antioxidant is a scavenger of free radicals. The current study addresses the development of a mulberry leaf extract-based transfersome gel containing quercetin by a thin-layer hydration method for topical antioxidant delivery. The process was optimized by encapsulating the drug in a variety of transfersome formulations.

**Materials and Methods:** Batch optimization was carried out by particle size and zeta analysis, entrapment efficiency (%), polydispersity index, *in vitro* drug release, and drug content analysis.

**Results:** The optimized batch MF5 provided 86.23% entrapment efficiency of quercetin in the vesicles and 95.79% drug release. It furnished a spherical shaped vesicle with an average diameter of 118.7 nm and zeta potential of -45.11 mV. The MG1 formulation provided superior antioxidant activity, drug content, and entrapment efficiency, *ex vivo* drug release, spreadability, homogeneity, and stability to MG2. The presence of quercetin in the extract and gel formulation was confirmed by using high performance thin layer chromatography.

**Conclusion:** It is evident from this study that a mulberry leaf extract-based transfersome gel is a promising prolonged delivery system for quercetin and has reasonably good stability characteristics. This research recommends that mulberry leaf extract-based transfersome gel can potentially be used in the treatment of acne vulgaris through a transdermal drug delivery system.

**Key words:** Transfersomes, mulberry leaves, quercetin, antioxidant activity, transfersome gel

### ÖZ

**Amaç:** Günümüzde antioksidanlar, akne vulgaris bağlantılı sağlık sorunları için önemlidir. Akne vulgaris, hücrelerle etkileşime giren serbest radikallerin gelişimi ile ilişkilidir. Dut yaprakları, kuersetin gibi antioksidanlar da dahil olmak üzere fenolik bileşikler içerir. Bir antioksidan, serbest radikal temizleyicisidir. Bu çalışma, topikal antioksidan taşınımı için ince tabaka hidrasyon yöntemi ile kuersetin içeren dut yaprağı ekstresi bazlı transferzom jeli geliştirilmesini ele almaktadır. İşlem, ilacı çeşitli transferzom formülasyonları ile enkapsüle ederek optimize edildi.

**Gereç ve Yöntemler:** Seri optimizasyonu için partikül boyutu, zeta analizi, yükleme etkinliği (%), polidispersite indeksi, *in vitro* ilaç salımı ve ilaç içeriği analizleri gerçekleştirildi.

**Bulgular:** Optimize edilmiş MF5 serisinin, enkapsülasyon etkinliği %86,23 ve kuersetin salımı %95,79 olarak bulundu. Ortalama çapı 118,7 nm ve zeta potansiyeli -45,11 mV olan küresel şekilli morfolojiye sahip oldukları tespit edildi. MG2 formülasyonuna göre MG1 formülasyonu üstün antioksidan aktivite, ilaç içeriği, enkapsülasyon etkinliği, *ex vivo* ilaç salımı, dağılılabilirlik, homojenlik ve stabilite sağladı. Ekstre ve jel formülasyonunda kuersetin varlığı, yüksek performanslı ince tabaka kromatografisi kullanılarak doğrulandı.

**Sonuç:** Bu çalışmada, dut yaprağı ekstresi bazlı transferzom jelin, kuersetin için ümit verici uzun süreli ilaç salım sistemi olduğu ve oldukça iyi stabilite özelliklerine sahip olduğu belirlendi. Bu araştırma, dut yaprağı ekstresi bazlı transferzom jelin potansiyel olarak bir transdermal ilaç taşıyıcı sistem aracılığıyla akne vulgaris tedavisinde kullanılabileceğini önermektedir.

**Anahtar kelimeler:** Transferzomlar, dut yaprakları, kuersetin, antioksidan aktivite, transferzom jel

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## INTRODUCTION

In the modern era, acne is the most common skin disease worldwide.<sup>1</sup> The research scenario indicates that approximately 85% of the world's population between 11 and 30 years is affected at some point in their lifetime. Approximately 4.8 million peoples per year are affected by acne.<sup>2,3</sup> Principally, it is a multifactorial disease of the pilosebaceous unit, which manifests as comedones or severe inflammatory lesions<sup>4,5</sup> in the skin, mainly on the face. This is related to the elevated rate of sebum excretion, abnormal proliferation of keratinocytes, overload production of male hormone androgens, oil-producing glands on the face, and inflammatory response initiated by bacterial antigens and cytokines.<sup>6</sup> In addition, oxidative stress is a major factor responsible for skin diseases such as acne. Principally, oxidative stress is initiated by free radicals/reactive oxygen species (ROS).<sup>7,8</sup> In acne vulgaris, the sebum contains hydroxyl radicals, nitrous oxide, and ROSs such as superoxide, which creates irritation during acne, inflammation, etc. Interestingly, active ingredients in plants contain exceptional antioxidant capacity, and scientific reports have revealed that herbal formulations showed the ability to repair damage caused by ROS. Additionally, various scientific reports have revealed that natural antioxidants show fewer adverse effects than synthetic antioxidant compounds.<sup>9</sup> The encapsulation of antiacne drugs in vesicular and particulate delivery systems is in the pioneering stage, and the substitution approach is being taken to minimize their side effects while preserving their efficacy. Presently, the development of a novel drug delivery system has the goal of high therapeutic activity along with patient compliance while conquering the penetration difficulties associated with transdermal drug delivery systems.<sup>9</sup> Traditionally, *Morus alba* L. (mulberry), generally known as a medicinal plant, has been used. The main content of mulberry leaf extract, which is normally referred to as phenolics such as quercetin [quercetin 3-(6-malonylglucoside)], isoquercetin, rutin, and some other flavonoids, has been identified in mulberry leaves.<sup>10</sup> It has been used to treat inflammation, cough, hypertension, cancer, and fever due to its medicinal value. The polyphenolic compounds in mulberry leaves provide its antioxidant properties by scavenging free radicals and guard many organs against oxidative stress.<sup>11</sup> The utilization of mulberry leaves in formulation development can offer several advantages such as easy availability, low cost, non-toxic formulation, and enhancement of sericulture farming. Plenty of literature has revealed that quercetin reduces the production of interleukin-6 and the expression of metalloproteinase-1 and, consequently, reduces inflammation and fibroblast proliferation.<sup>12</sup> Also, as an antioxidant, quercetin scavenges ROS and ultimately repairs damaged cells. Over the past few decades, sustained and efficient drug delivery systems have gained the noteworthy attention of researchers; transdermal drug delivery is one such system that offers productive significance and advantages.<sup>13</sup> The novel era of drug delivery introduced the use of transfersomes, developed by Gregor Cevc in the year 1991. Transferomes consist of a

hydrated core surrounded by an ultra-deformable lipid layer complex.<sup>14</sup> Generally, transfersomes are made by intercellular sealing of lipids, which increases their flexibility, reduces the risk of absolute vesicle rupture in the skin, and permits transfersomes to penetrate the natural water gradient across the epidermis following application to the skin. Transferomes can thus be utilized for the delivery of synthetic and herbal drugs.<sup>15,16</sup> Recently, considerable attention has been focused on developing a new lipidic nanovesicle-based transdermal drug delivery system. Transfersomes are deformable and flexible and have a high affinity for penetration through the skin to the systemic circulation. Transdermal administration of transfersome vesicles offers a great advantage over other vesicles; therefore, we attempted to develop a transfersomal gel formulation of mulberry leaf extract. Subsequently, prepared transfersome gels were evaluated for entrapment efficiency, particle morphology, particle size, zeta potential, polydispersity index (PDI), swelling index, viscosity and pH behavior, fourier transform infrared spectroscopy (FTIR), antioxidant activity, deformability index, and penetration using Franz diffusion cells. High performance thin layer chromatography (HPTLC) was used to analyze the level of active substances contained in the extract. In conclusion, novel transfersomal prepared gel could be used to explore the application of natural mulberry leaf extract containing an antioxidant (quercetin) for the treatment of acne-like skin diseases. Also, it could overcome the drug resistivity and adverse effects of currently challenging acne therapy.

## MATERIALS AND METHODS

### *Material*

Mulberry leaves were procured from a local market in Indapur, Maharashtra. Ethanol AR (70%) and TWEEN80 were from Loba Chemie (Pvt. Mumbai). PHOSPHOLIPON 90G, Carbapol 940, PROPYLENE GLYCOL, and Formic acid were from Merk Life Sciences. Diethyl ether, Ethyl acetate, Toulene, Petroleum ether, 2,2-diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid, and quercetin were from Yucca Enterprises (Mumbai).

### *Processing and extraction of mulberry leaves*

Mulberry leaves were collected from the local market in Indapur, (MS) India, in July. Briefly, the collected leaves were washed systematically under running water to remove soil and other debris adhered to them. Clean leaves were dried, ground, and passed through a sieve ASTM #30. Mulberry leaf powder (50 g) was subjected to Soxhlet extraction using 70% Ethanol AR as the solvent.<sup>17</sup> Finally, the extract was subjected to physical and phytochemical characterization.

### *Freeze drying (FD)*

The mulberry leaf extract solution was frozen at -20°C for 24 h and then dried using a lyophilizer (Labconco, United Kingdom) at 0.013 mbar pressure and -49°C. The obtained extract powder was milled by mortar and pestle to achieve a fine powder.



### *In vitro drug release of transfersomes*

An *in vitro* drug release study was performed by using modified Franz diffusion cells. A dialysis membrane (Hi-Media, Molecular weight 5.000D) was arranged between the receptor and donor compartments. Subsequently, the transfersomes of mulberry leaf extract were kept in the donor compartment, and the receptor compartment was filled sufficiently with phosphate buffer, pH 7.4 (25 mL). The diffusion cells were maintained at  $37\pm 0.5^\circ\text{C}$  with constant stirring at 40 rpm throughout the experiment. At 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, and 360 min intervals, 5 mL aliquots were withdrawn from the receiver compartment through the side tube and again filled with 5 mL and analyzed for drug content by UV-Vis spectroscopy.<sup>25</sup>

### *Preparation of transfersome gel*

Herein, we used the optimized batch of transfersomes (MF5) for preparation of the gel. A 10% (w/v) transfersome suspension was weighed for gel formation. Carbapol 940 was added to purified water with stirring and allowed to hydrate for 24 h. The transfersome suspension was dispersed in the hydrated carbapol 940 slurry and stirred continuously for 30 min. Then, propylene glycol was added slowly to the slurry. The pH of the formulation was adjusted with triethanolamine, and the same procedure was carried out for the control extract-based gel (MG2) (Table 2).<sup>26</sup>

### *Evaluation of MG and MG2 gel formulation*

MG1 and MG2 gel formulations were subjected to organoleptic evaluation, FTIR, homogeneity rate, pH and viscosity measurements, flow property measurements, drug content analysis,<sup>27</sup> and %EE.<sup>28</sup>

### *Homogeneity rate*

The homogeneity of the formulated gel was determined by pressing a small amount of both gels (MG1 and MG2) between the thumb and the index finger. The uniformity was a resolute as a harmonized or not.<sup>29</sup>

### *Spreadability*

A spreadability test of MG1 and MG2 gels was carried out by pressing 0.5 g of the final formulation. Briefly, a sample of the gel from each batch was pressed between 2 translucent spherical glass slides, and the highest degree of spreading was permitted by leaving them for 5 min. The diameter of the formed circle was calculated to articulate the spreadability of the formulated gel.<sup>30</sup>

### *HPTLC analysis*

HPTLC analysis was performed by application of the std. and isolated quercetin, and MG1 formulation. The samples were prepared by using methanol. Subsequently, the analysis of quercetin was performed on the HPTLC plate of silica gel 60F254 (5 cm x10 cm) using a mixture of toluene:ethyl

acetate:formic acid as the mobile phase in proportions of 5:4:0.2. Finally, the quercetin dark brown colored bands were identified and confirmed via  $R_f$ .<sup>31</sup>

### *Limit of detection (LOD) and limit of quantification (LOQ)*

LOD and LOQ are 2 important terms used to describe the smallest concentration of an analyte that can be reliably measured by an analytical procedure. The LOD and LOQ of compounds were determined based on  $R_f$  values as well as UV/Vis spectral overlaying of respective standard compounds. The LOD was determined based on the lowest concentration detected by the instrument from each of 2 standards, while the LOQ was determined based on the lowest concentration quantified in the samples.

The determination of LOD and LOQ was calculated using the formula,

$$\text{LOD} = \frac{1}{4} 3.3(\text{SD}/\text{S}) \dots \dots \dots (3)$$

$$\text{LOQ} = \frac{1}{4} 10(\text{SD}/\text{S}) \dots \dots \dots (4)$$

Where,

SD: Standard deviation of the response

S: Slope

### *Drug content (%)*

Accurate quantities of MG1 and MG2 gel were measured into in separate beakers, lysed with 50 mL methanol for 15 min using ultrasonication, and centrifuged at 10,000 rpm for 30 min ( $25^\circ\text{C}$ ). The clear supernatant was collected, added to 10 mL methanol, and diluted with pH 7.4. The quercetin content was calculated from the absorbance determined using a UV spectrophotometer at 246 nm.

### *Ex vivo release studies*

Fresh hairless abdominal goat skin was collected from a slaughterhouse and used for release studies after peeling the skin from the underlying cartilage placed inside the receptor compartment.<sup>32,33</sup> In brief, using a Franz diffusion cell apparatus, the drug release studies were performed. Goat skin was placed between the donor and receptor compartments. The receptor compartment was filled with the phosphate buffer and ethanol mixture in a ratio of 8:2 (15 mL) at  $37\pm 0.5^\circ\text{C}$ , and gel samples were exposed to the donor compartment. Receptor compartment containing dissolution media was continuously stirred using magnetic stirrer at 25 rpm, which help to avoid the saturation of dissolution media during the penetration of the active content.<sup>34</sup> For the calculation of percent *ex vivo* drug release, sampling was performed at different time intervals.

### *Statistical analysis*

All outcomes in this work are expressed as a mean  $\pm$  SD. A paired Student's t-test was used for the comparison of percent

**Table 2. Transfersome gel of mulberry leaf extract (MG1) and control extract gel (MG2)**

Composition	Transfersomes	Extract	Carbapol 940	Triethanolamine	Propylene glycol	Water
Concentration (% w/w)						
MG1	Equal to 10% extract	-	1	0.1	12.5	Add 100
MG2	-	10	1	0.1	12.5	Add 100

*ex vivo* drug release of 2 related gel samples (MG1 and MG2). Differences at  $p < 0.05$  was considered significant.

#### *Ex vivo penetration test*

An *ex vivo* penetration test of the transfersome gel was carried out on fresh hairless abdominal goat skin through a Franz diffusion cell apparatus assembly. The goat skin was assembled between the donor and receptor compartment with an effective diffusion area of 2.26 cm<sup>2</sup> and a cell volume of 25 mL. Briefly, the receptor compartment contained phosphate buffer and ethanol (8:2) media (15 mL) at body temperature  $37 \pm 0.5^\circ\text{C}$  and the media in the receptor compartment was stirred. The gel was placed in the donor compartment, and samplings were carried out periodically for 24 h from the receptor compartment. Simultaneously, the sink condition was maintained, and the collected samples were subjected to UV spectrophotometry analysis. Based on the experimental findings, the permeation coefficient was calculated by using the cumulative amounts of drug permeated per unit area ( $\mu\text{g}/\text{cm}^2$ ) vs time graph. The transdermal flux was calculated from the slope of the linear portion of the graph.

#### *Stability study*

MG1 and MG2 formulations were stability tested for 3 months at  $4^\circ\text{C} \pm 2^\circ\text{C}$  and  $40^\circ\text{C} \pm 2^\circ\text{C}$  75% $\pm$ 5% RH stations for determination of the physical and chemical stability of the formulations (as per International Council for Harmonisation guidelines).

## RESULTS

#### *Characterization of mulberry leaf extract*

The extraction was performed by the successive hot continuous Soxhlet extraction method. The ethanolic extract of mulberry leaves was greenish in color. The phytoconstituent analysis revealed the presence of sterols, tannins, phenols, and alkaloids. The pH of the extract was 6.5.

#### *TLC analysis*

TLC fingerprinting of mulberry leaf extracts was performed along with std. quercetin under UV 254. It showed that spots representing std. quercetin and isolated quercetin had  $R_f$  values of 0.38 and 0.31, respectively (Figure 1). In conclusion, quercetin analysis using TLC confirmed the presence of quercetin in the extract.

Subsequently, the UV spectra of std. quercetin was observed at 254 nm, which confirmed the purity and presence of quercetin in the prepared sample (Figure 2a). Also, the UV spectra of isolated quercetin from mulberry leaf extract were obtained at 246 nm and confirmed the existence of quercetin in the extract (Figure 2b).

#### *Calibration curve of standard quercetin and isolated quercetin*

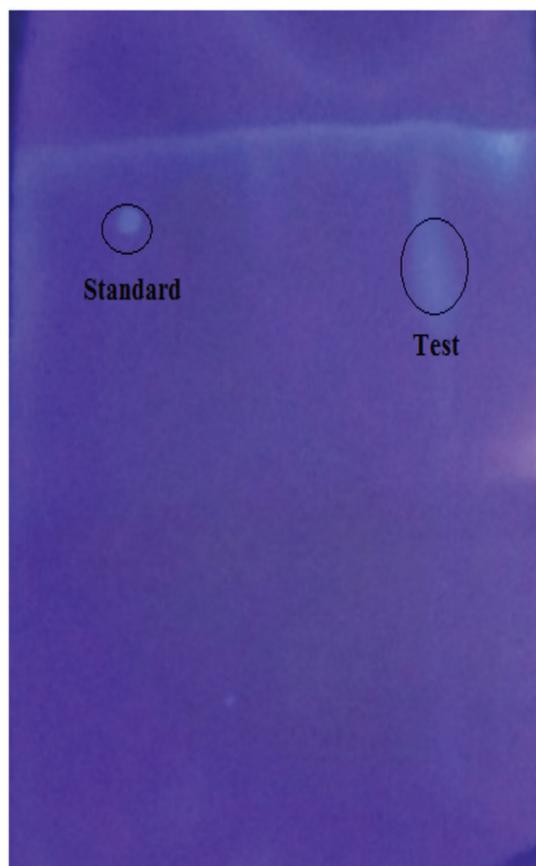
The calibration curve of the std. (Figure 3a) and isolated quercetin (Figure 3b) showed linearity along with 0.999 and 0.996  $R^2$ , respectively, providing confirmation of the purity of quercetin.

#### *FTIR spectra of std and quercetin isolated from mulberry extract*

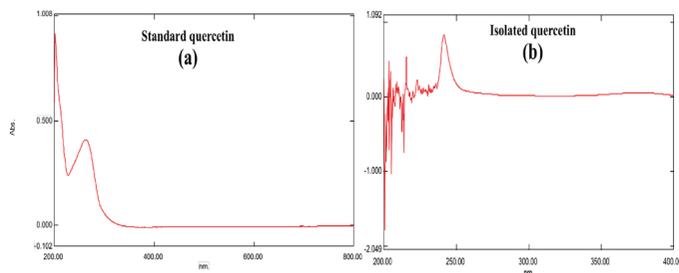
FTIR investigations revealed the presence of quercetin. In brief, std. quercetin showed the peak of O-H stretching vibration at 3387.47 cm<sup>-1</sup>, whereas O-H bending of phenol function was detectable at 1316 cm<sup>-1</sup>. The C=C aromatic ring stretch band was at 1605.47 cm<sup>-1</sup>. The in-plane bending band of in aromatic hydrocarbon was detectable C-H at 1446.70 cm<sup>-1</sup>. The C=O aryl ketonic stretch absorption was evident at 1659.83 cm<sup>-1</sup>. Bands at 1165.63 cm<sup>-1</sup> was attributable to the C-O stretching in the aryl ether ring (Figure 4a). The observed frequencies for isolated quercetin from the extract of mulberry leaves are shown in Figure 4b. It showed the O-H (strong) stretching vibration at 3288.26 cm<sup>-1</sup>, and C-H stretching vibration at 2916.94 cm<sup>-1</sup>. The C=O aryl ketonic stretch absorption was evident at 1731.51 cm<sup>-1</sup>. The in plane bending band of in aromatic hydrocarbon was detectable C-H bending at 1415.10 cm<sup>-1</sup>. Bands at 1026.95 cm<sup>-1</sup> was attributable to the C-O stretching in the aryl ether ring at 1026.95 cm<sup>-1</sup>. Overall, it provides confirmation of quercetin present in the extract (Figure 4b). These pragmatic frequencies confirmed that the isolated fraction was quercetin in reference to std. quercetin.

#### *Antioxidant activity*

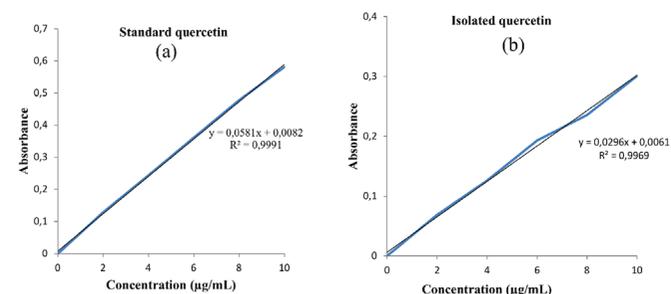
The antioxidant activity of the FD extract showed an excellent result in contrast to standard ascorbic acid. Quercetin isolated from mulberry leaves, ascorbic acid (std.), and std. quercetin furnished antioxidant activity of 67.2%, 83.20%, and 69.54%



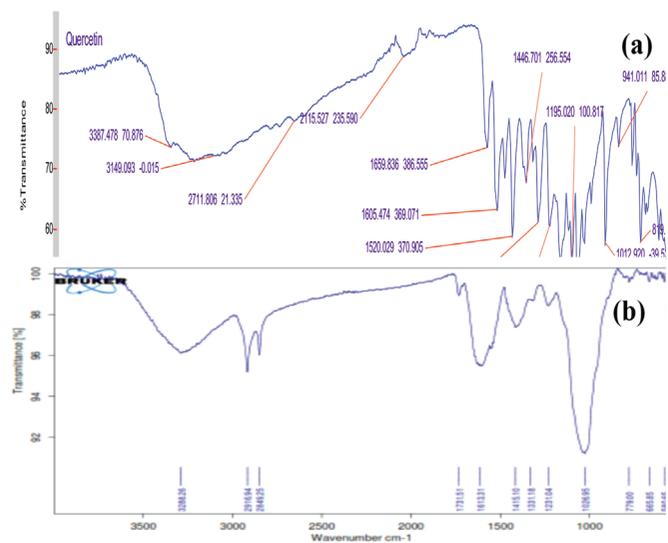
**Figure 1.** TLC plate developed under UV light at 254 nm  
TLC: Thin layer chromatography, UV: Ultraviolet



**Figure 2.** UV spectra of standard quercetin (a) isolated quercetin (b)  
UV: Ultraviolet



**Figure 3.** Calibration curve of standard quercetin (a) and isolated quercetin (b)



**Figure 4.** FTIR spectra of standard quercetin (a) and isolated quercetin (b)  
FTIR: Fourier transform infrared spectroscopy

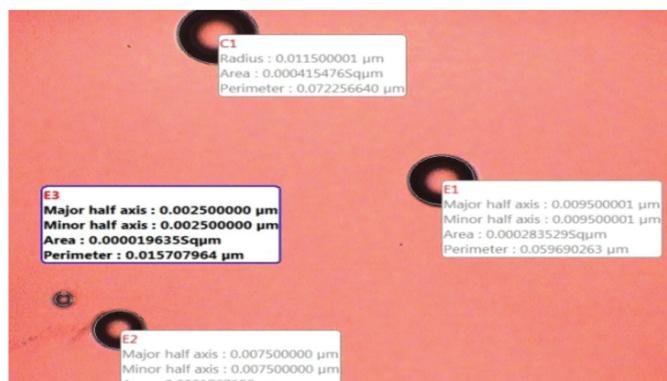
consistently. In conclusion, free- radical scavenging capacity of isolated quercetin and standard quercetin was found similar.

*Characterization and optimization of transfersomes*

*Shape of the vesicle*

The shape of the vesicle by Motic microscopy revealed no aggregation or irregularities in transfersomes, and the spherical structures of vesicles were observed in the range of 110 to 460 nm in diameter. Hence, the successful construction of transfersome vesicles was confirmed (Figure 5).

*Optimization of transfersomes*



**Figure 5.** Unsonicated transfersomes vesicles by Motic microscopy

*Particles size analysis and PDI*

The size distribution of the transfersome suspension was determined by a particle size analyzer (nanoplus) which works by photon correlation spectroscopy (Table 3). Owing to the surfactant, the vesicle size observed was within the range of 114.5 to 416.6 nm. The PDI values of the formulation were observed in the range of 0.270 to 0.628. Based on the particle size and PDI value, it was concluded that the particle size distribution was consistent within the formulation.

*Zeta potential*

The Zeta potential provides knowledge of particle aggregation or flocculation in suspension. Herein, the transfersome batch (MF1 to MF6) showed a zeta potential in the range of -21.19 to -45.11 mV. These results specify the stability of the transfersomes in a suspension.

*Percent entrapment efficiency*

Table 3. Characterization and optimization of prepared transfersomes						
Batch no	Particle size	Zeta potential	PDI	%EE	Deformability index	
MF1	381.9 nm	21.19 mV	0.511	68.23±1.2	3.52±0.9	
MF2	214.5 nm	-44.56 mV	0.421	65.23±1.1	3.63±0.5	
MF3	268.3 nm	-21.91 mV	0.448	76.23±0.9	2.25±1.3	
MF4	401.9 nm	-22.39 mV	0.270	77.65±1.6	3.5±2.1	
MF5	118.7 nm	-45.11 mV	0.389	86.23±2.1	1.03±0.8	
MF6	416.6 nm	-27.73 mV	0.628	80.23±1.8	2.05±1.5	

n=3, ± standard deviation, PDI: Poly dispersity index, EE: Entropment efficiency

%EE was calculated by using the ultracentrifugation method. In brief, the un-entrapped drug was separated and the amount of it calculated. Subsequently, the EE was calculated for transfersomes. From the estimation of the %EE, the MF1 to MF6 showed 57.65 to 86.23 %EE.

#### *In vitro* release of transfersomes

The determination of percent drug release in phosphate (pH 7.4) was carried out by using the slope of quercetin in phosphate buffer (pH 7.4) calibration curve (Figure 6). The comparison *in vitro* cumulative release from batches MF1 to MF6 in the diffusion study is shown in Figure 7; it was 90.34%, 92.07%, 91.69%, 92.03%, 95.79%, and 91.42%, respectively. Among all the batches, MF5 showed the highest extended-release of 95.79% after 6 h (Figure 7). Herein, based on the %EE, particle size analysis, zeta potential, and PDI, we selected the MF5 batch as the optimized batch and used it for further processes of gel formulation.

#### FTIR spectra of transfersomes

The FTIR spectra of transfersome gels showed peaks for O-H stretching, C-H stretching, C-O stretching, C=O stretching, C-H bending, and C-O stretching around 3334 cm<sup>-1</sup>, 2923 cm<sup>-1</sup>, 2853 cm<sup>-1</sup>, 1620 cm<sup>-1</sup>, 1453 cm<sup>-1</sup>, and 1035 cm<sup>-1</sup>, respectively, which is

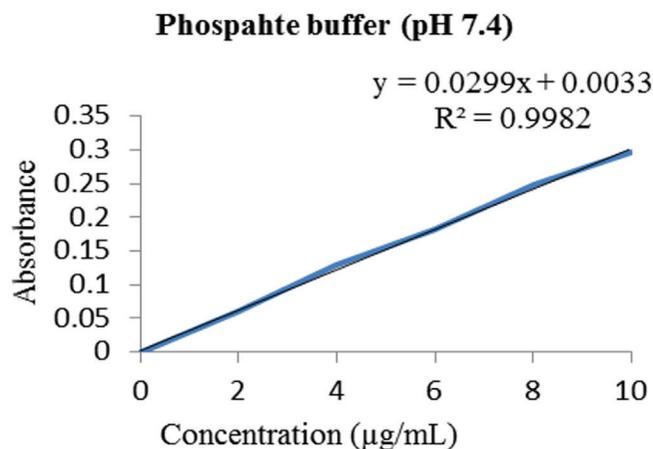


Figure 6. Calibration curve of extract (pH 7.4)

#### *In vitro* release profile of Transfersomes

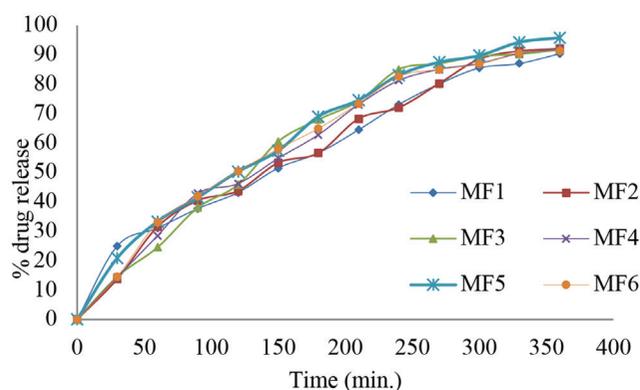


Figure 7. *In vitro* release profile of transfersomes

about same as for std. quercetin FTIR. The appearance of the above peaks in Figure 8 confirmed the presence of quercetin and other compounds. No interaction was found between quercetin and the excipients used in formulation development.

#### Evaluation of MG1 and MG2

The MG2 gel showed a greenish color and a glossy appearance due to direct contact between the extract and the gel-forming agent. The MG1 gel furnished a slightly greenish color and a transparent as well as glossy appearance, because the extract was entrapped in the lipid vesicles. Both gels gave off a somewhat sweet odor and demonstrated exceptional homogeneity.

#### Measurement of the viscosity of MG1 and MG2

The viscosity of MG1 and MG2 gel formulations were determined by using a Brookfield Viscometer at different time intervals (Figure 9). MG1 and MG2 showed a remarkable result for viscosity. This was because of carbopol, and it could be advantageous to resist drug leakage. Concurrently, the pH of the gel was determined with a digital pH meter (Figure 10), and it showed that MF1 gave a constant pH after 6 h and in the case of MF2, variation in the pH was observed.

#### Swelling index

The MG1 gel showed good water-holding capacity. Herein, hydrogen bonding facilitates the formation of a structure that

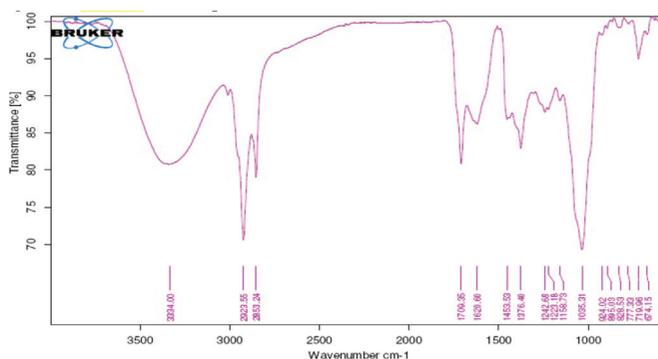


Figure 8. FTIR spectra of transfersomes  
FTIR: Fourier transform infrared spectroscopy

#### Viscosity of gel

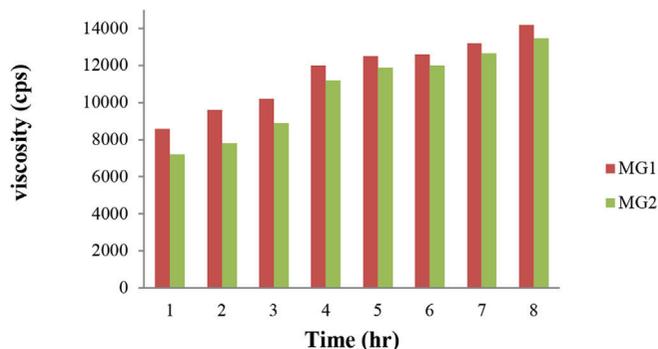


Figure 9. Viscosity of MG1 and MG2 gel

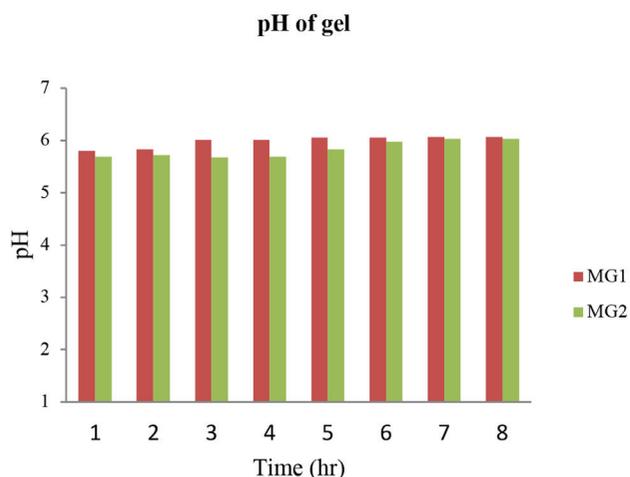


Figure 10. pH of MG1 and MG2 gel

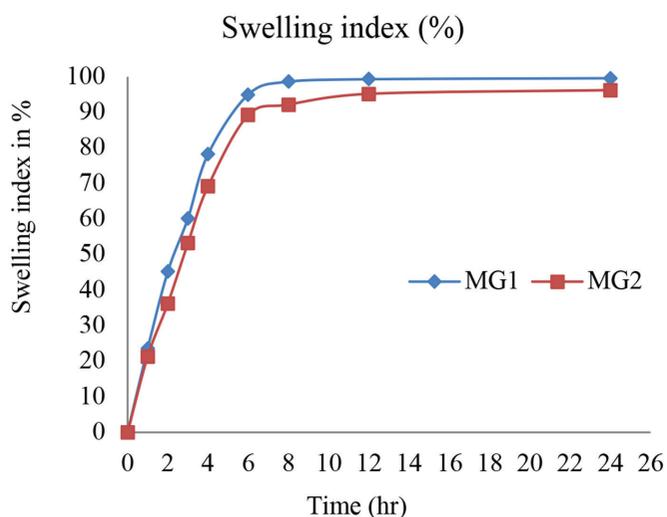


Figure 11. Swelling index of transfersomal gel

allows swelling of the excipients. A comparison of MG1 and MG2 showed a swelling index of up to 99.61% and 96.27%, respectively (n=3, Figure 11).

#### Flow properties

The flow properties of MG1 and MG2 gels were computed at different day intervals. It was observed that the MG1 gel showed better flow properties than the MG2 as shown in Table 4. Outcomes of the flow properties concluded that the MG1 provides exceptional tensile strength, elongation rate, and spreadability. MG1 also exhibited a good homogeneity rate as

compared with the MG2 batch.

#### Entrapment efficiency, drug content, and antioxidant activity

The % drug content of the MG1 and MG2 formulations was found to be 98.23% and 89.52%, respectively (Figure 12a). Moreover, the MG1 gel formulation showed superior antioxidant activity (66.72%) as compared with the MG2 gel formulation (59.23%) as shown in Figure 12a. The MG2 gel antioxidant activity and drug content were found to be quite a bit less; this may have been because of the extract becoming degraded during the manufacturing process of the gel. The MG1 and MG2 gel formulation showed 85.6% and 81.20% EE. The formation of multi-laminar vesicle complexes in transfersomes enhances the %EE (Figure 12b).

#### HPTLC analysis

Optimized parameters for std quercetin, isolated quercetin, and the gel formulation (MG1) containing quercetin by HPTLC at 246 nm are reported in Table 5. The spectrum scan of std. quercetin is comparable with that of an isolated compound and the formulation containing quercetin (Figure 13). For the extract, the retention time was found to be 8.4 min, which coincided with standard quercetin. The results of tests carried on standard and isolated Quercetin and the formulation are summarized in Figure 14. It confirmed the presence of quercetin in the gel formulation, along with the absence of an interaction between excipients and quercetin.

#### LOD and LOQ

The LOD were determined to be 0.25 and 0.23 ng/spot, and the LOQ was found to be 0.6 and 0.5 ng/spot for std quercetin and isolated quercetin, respectively. The values remained quite similar for both compounds, which revealed the sensitivity of the method.

#### Ex vivo percent drug release

The *ex vivo* percent drug release of MG1 and MG2 was carried out in phosphate buffer pH 7.4 through goat skin using Franz diffusion cells (Figure 15). The dissolution profile of MG1 (96.86%) showed an excellent drug release as compared with the MG2 (88.23%) up to 24 h. A statistically significant test for comparison of the *ex vivo* release of MG1 and MG2 was performed by a paired t-test. By conventional criteria, the considered difference between MG 1 (transfersome gel) and MG 2 (control gel) was statistically significant at the level of  $p < 0.05$ .

Table 4. Flow properties of MG1 and MG2

Properties	MG1		MG2	
	0 days	10 days	0 days	10 days
Homogeneity rate (1/10 mm)	60.54±1.6	99.58±2.9	59.23±1.2	99.03±3.6
Tensile strength (kg/cm)	30.25±0.9	35.62±1.7	30.01±1.9	33.87±2.8
Elongation rate (%)	200.21±1.4	282.3±2.5	199.56±1.6	276.2±3.1
Spreadability (cm)	9.80±0.9	9.98±0.9	8.01±0.9	8.26±0.9

n=3, ± standard deviation

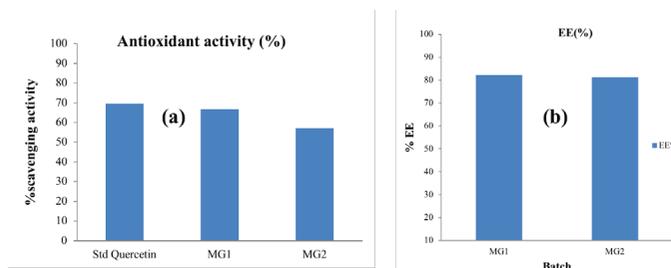


Figure 12. (a, b) Antioxidant properties and %EE of MG1 and MG2 gel  
EE: Entropment efficiency

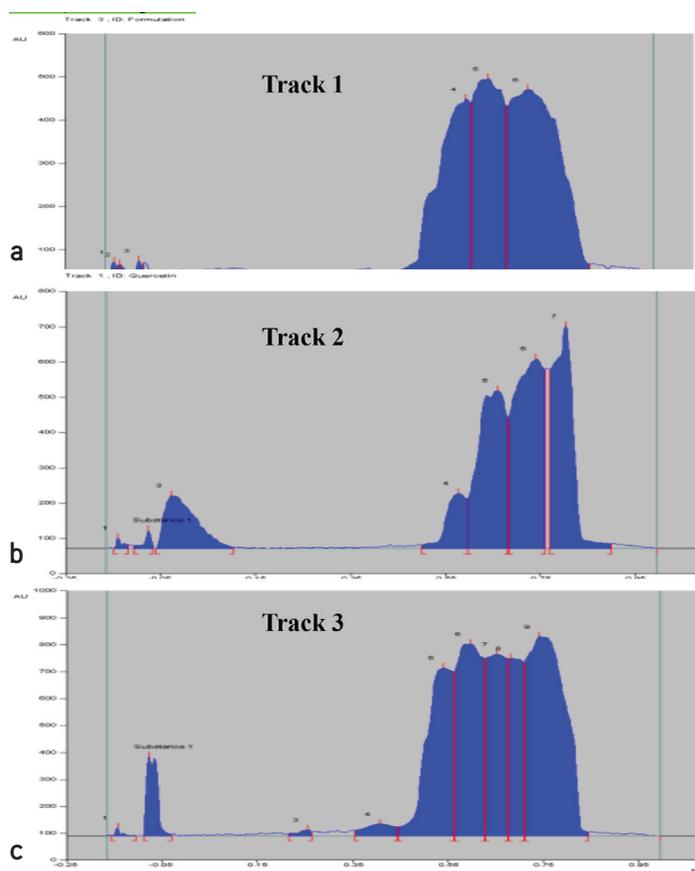


Figure 13. a) standard quercetin (track 1), b) isolated quercetin (track 2) and c) gel formulation (track 3)

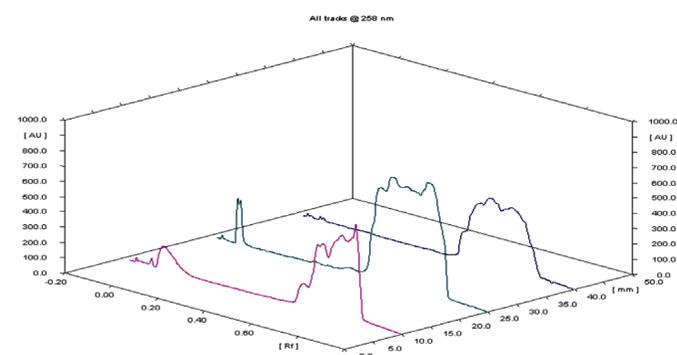


Figure 14. Overlay at 246 nm of standard quercetin, isolated quercetin, and formulation (MG1) in HPTLC  
HPTLC: High performance thin layer chromatography

### Ex vivo permeation test

The MG1 gel showed better transdermal flux as compared with the MG2 ( $35.52 \pm 3.02$  and  $26.01 \pm 2.02$ , respectively). Moreover, a superior permeation coefficient was shown for the MG1 ( $0.016 \pm 0.0009$ ) than the MG2 ( $0.012 \pm 0.0003$ ) gel formulation.

### Stability studies

After 3 months of stability testing at  $4 \pm 2^\circ\text{C}$  and  $40 \pm 2^\circ\text{C}$  in sealed glass ampules, negligible drug leakage was confirmed. Based on observation, it was concluded that the MG1 gel formulation was more stable at  $4 \pm 2^\circ\text{C}$  as compared with the  $40 \pm 2^\circ\text{C}$ . Furthermore, leakage of the drug from the MG1

### Ex vivo percent drug release

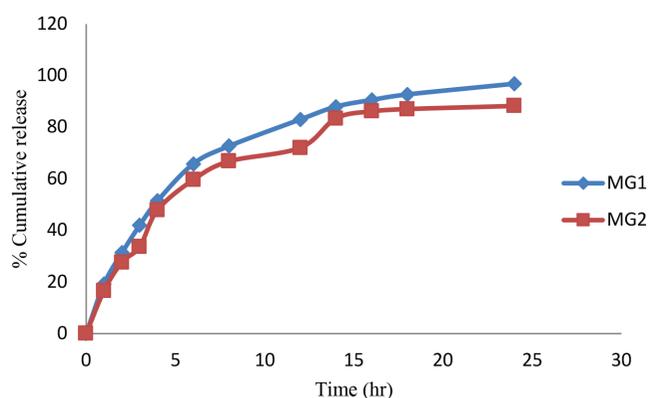


Figure 15. Dissolution profile of MG1 and MG2 gel

Table 5. Optimized Parameters of HPTLC for quercetin isolation

Parameters	Description	Parameters	Description
Stationary phase	Merck Silica gel 60 F254 HPTLC pre-coated plates	Plate size	4.0 cm x10.0 cm
Mode of separation	Normal phase	Development chamber	Camag twin trough chamber
Mobile phase	Ethyl acetate: toluene: formic acid (4:3.5:0.5v/v/v)	Bandwidth	7.0 mm
Chamber saturation	30 min	Space between the bands	7.0 mm
Sample applicator	Camag linomat V	Syringe	Hamilton, 100.0 $\mu\text{L}$
Distance from the edges of the plat	13.0 mm	Rate of a sample application	150 nL/sec
Lamp and wavelength	Deuterium, 246 nm	Development distance	85.0 mm
Densitometric scanner	Camag scanner IV equipped with win-CATS planar chromatography manager software version 1.4.7		

HPTLC: High performance thin layer chromatography

batch was found to be minimum as compared with the MG2 gel formulation at both stability stations. The viscosity of the carbopol-containing gel prevented the movement and fusion of transfersomes, which resulted in the low drug leakage in gel formulation (MG1). As compared with the drug release of the MG1 gel formulation at zero days after stability, it demonstrated excellent product stability. As a result, there is no major variation seen in MG1, before and after stability (Table 6). Hence, the MG1 transfersome-based gel formulation is more stable without causing any incompatibility, and it shows promising potential for topical application.

**Table 6. Stability evaluation of transfersomal gel after 3 months**

Properties MG1	Gel formulation		
	MG2		
(4°C±2°C)	a- Color	Slightly yellowish	Greenish
	b- pH	5.62±0.23	5.89±0.95
	c- Viscosity (CPS)	14005±230	13202±456
	d- EE (%)	84.96±2.35	78.35±3.69
	e- Drug content (%)	98.01±2.61	89.40±3.10
(40°C±2°C)	a- Color	Slightly yellowish	Greenish
	b- pH	5.95±1.02	6.23±1.63
	c- Viscosity (CPS)	13750±412	12889±362
	d- EE (%)	83.69±4.02	73.23±5.06
	e- Drug content (%)	97.96±00.97	84.25±2.96

n=3, ± standard deviation, EE: Entrapment efficiency

## DISCUSSION

Gels are a semi-solid dosage form system of drug delivery and constitute a method of good repute among novel pharmaceutical dosage forms. Nowadays, herbal nano lipid vesicle-based gel formulations are gaining attention due to their safe and effective use. In this study, the transfersomes of mulberry leaf extract were prepared by a thin-layer hydration method. Numerous investigations have revealed that quercetin has exceptional antioxidant potential and decreases the production rate of interleukin-6 and the expression of metalloproteinase-1. Consequently, it reduces inflammation and fibroblast proliferation during the healing process. Moreover, plenty of literature claims that ROS is a major factor in skin diseases (example; acne vulgaris), and antioxidant have scavenging potential against ROS. Synthetic antioxidant agents have some adverse/side effects. Thus, the nontoxic nature of herbal antioxidants such as quercetin can be effective in the treatment of acne vulgaris<sup>12</sup>. Herein, the mulberry leaves containing the active antioxidant quercetin were isolated by using TLC and HPTLC, confirmed by using UV spectroscopy and FTIR, and compared by using std quercetin. The fruitful outcomes of TLC and UV spectroscopy provide evidence of successful isolation and confirmation of the purity of quercetin. The FTIR spectra of

std. quercetin, isolated quercetin, and the gel formulation (MG1) showed the confirmation of quercetin content and excipient compatibility. In the development of a transfersome formulation, various factors are important. The sizes and shape of the vesicle mainly depend on the concentration of phospholipon 90G and the concentration of surfactants. An increase in the concentration of surfactant increases the EE of vesicles and ultimately prevents drug leakage.<sup>35,36</sup> Outcomes of the experiment are tabulated in Table 3, which reveals that the transfersomes prepared by using a specific concentration of tween 80 and phospholipid showed a superior result as compared with other formulations. Also, the proper proportion of excipients in batch MF5 showed the highest value of %EE (86%). Principally, the particle size of vesicles is an important parameter in the formulation of transfersomes. Generally, particle size distribution is based on volume. Moreover, the dynamic light scattering DLS technique determines the PDI value, which generally ranges from 0 to 0.6. If the value of PDI >0.6, it indicates that the sample has a very broad size distribution. The results showed that the PDI value of each batch changed with particle size (Table 3). The highest PDI value of MF6 was caused by the large particles, which were prone to aggregation.<sup>26,37,38</sup> Zeta potential is an important parameter to describe the stability of the dispersion system. It is used to measure the magnitude of the electrostatic potential or repulsive force among the same electrical charge of particles in suspension. Also, it gives an idea that particles in suspension undergo aggregation or flocculation. Various scientific reports revealed that the zeta potential is stable when more positive than of +30 mV or more negative than -30 mV.<sup>24,26,39</sup> Herein, the results revealed that the MF5 transfersomes were more stable than other formulations because their zeta potential was comparatively high. A negative value of zeta potential might be derived from the lipid composition in the formula. Phosphatidylcholine is a zwitterionic compound with an isoelectric point of 6-7. Also, phosphate buffered saline at pH 7.4 was used as the hydrating medium in the process of vesicle formulation. In that, the pH was slightly higher than the isoelectric point of phosphatidylcholine and due to this, the phosphatidylcholine carried a negative charge.<sup>24</sup> The deformability index is an important parameter in transfersome preparation and generally is used to examine the flexibility of transfersomes. The deformability index value is influenced by the concentrations of phospholipid and surfactant used. The use of excessive surfactant can lower the deformability index value as it can lead to the formation of micelles. At a larger index value of deformability, the transfersomes will be more flexible, which will allow them to penetrate through skin pores smaller than themselves.<sup>40</sup> The results show that MF5 transfersomes had the lowest deformability index. Based on the transfersome characterization results, batch optimization was carried out and the optimal batch further formulated into a gel. The formula selected was a batch with the highest percentage of drug entrapped, a uniform nano-size distribution, a value of the PDI more than ±0.30, and a value of zeta potential more negative than -30 mV. Based on the summary results, MF5 transfersomes was chosen because they had a spherical shape, the highest

percentage of drug entrapped ( $86.23 \pm 2.1\%$ ), a PDI of 0.389, a zeta potential value of  $-45$ , a Dv90 particle size of 118.7 nm, and a deformability index of  $1.03 \pm 0.8$ . Simultaneously, mulberry leaf extract-based gel (MG2) was prepared for evaluation and comparison with MG1. FTIR of the MG1 gel confirmed that there was no interaction between quercetin and excipients. Besides, the MG1 gel formulation showed satisfactory flow properties, viscosity, and stable pH. Also, MG1 gel showed a superior swelling index and the homogeneity rate. The antioxidant assay of MG1 gel showed that the good antioxidant activity as compared with the MG2. In the MG2 formulation, there may be a chance of degradation or interaction between quercetin and excipients and, due to that, the antioxidant activity is slightly diminished. HPTLC analysis was used for further phytochemical and pharmacological investigation of the gel formulation. In the present study, the phytochemical constituent (quercetin) was identified and confirmed based on the color zone obtained during the HPTLC analysis. The color of the zone with isolated quercetin and transfersomes gel formulation the color of the reference compound (std. quercetin) under daylight and UV light after derivatization in the chromatogram confirmed the presence of stable and pure quercetin. This detailed chemical profile may be useful in the identification and quality evaluation of drugs concerning plants. *Ex vivo* percent drug release results conducted on transfersome and control gels showed a significant difference ( $p < 0.05$ ). Furthermore, the penetration rate of MG1 was higher vs MG2. These fruitful findings could be related to the negative charge of transfersomes.<sup>41</sup> These negative charges generated the weak electrostatic repulsion between transfersomes and intercellular components of the skin. Subsequently, this repulsion accelerates the penetration of negatively charged transfersomes through follicles of different skin layers. Additionally, the rapid penetration of transfersomes into the intact part of the basal area of follicles occurred.<sup>42</sup> Herein, the hydration and rehydration temperature, as well as rpm used for the development of transfersomes are key parameters in the penetration mechanism of transfersomes. Also, the phase transition temperature of the surfactant, hydrophile-lipophile balance value of the surfactant, saturation, and unsaturation of alkyl chain length play major roles in the penetration mechanism. The prepared M1 gel showed less drug release as compared with the control formulation. A possible reason involved less leakage from vesicles of transfersomes and greater hydration temperature than the gel to liquid phase transition temperature, and along with that, it provides a superior %EE.<sup>43,44</sup> The stability study revealed that the MG1 gel formulation had admirable stability at  $4^\circ\text{C} \pm 2^\circ\text{C}$  and  $40^\circ\text{C} \pm 2^\circ\text{C}$ . The outcomes of the present investigation confirmed that transfersomes of mulberry leaf extract in a gel system offer a new substitute for transdermal drug delivery. Thus, based on the prolific findings, the MG1 gel formulation could be utilized in the treatment of acne.

## CONCLUSION

The present study revealed the development of a novel transfersome gel of mulberry leaf extract, with enhanced

antioxidant activity as compared with MG2 gel. The optimized batch of transfersomes confirms the excellent zeta potential, particle size, EE (%), PI, deformability index, and *in vitro* percent drug release. Moreover, the MG1 gel *ex vivo* drug release and penetration studies indicated that the developed transfersome gel formulation may serve as a promising carrier for better penetration through the skin as compared with the MG2. Although MG1 gel demonstrated good homogeneity, spreadability, excipient compatibility, and drug content as compared with the MG2, the percent EE and antioxidant properties of MG1 were admirable. Consequently, MG1 batch transfersome gel of mulberry leaf extract offers tremendous antioxidant potential, which creates new opportunities for the topical application for the treatment of acne vulgaris.

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