

## Formulation of curcumin- and quercetin-loaded nanoethosomes and evaluation of their antioxidant, antibacterial, and anti-inflammatory potential with dermal irritation assessment in chickens

Fatima Ashraf <sup>1</sup>, Arslan Muhammad Ali Khan <sup>2\*</sup>, Hifsa Batool <sup>3</sup>, Kiran Batool <sup>4</sup>, Syed Mubashir Azeem <sup>5</sup>, Junaid Iqbal <sup>6</sup>, Anas Jahangir <sup>7\*</sup>, Ramla Shabbir <sup>8</sup>, Hamna Nasar <sup>9</sup>

<sup>1</sup> Department of Biochemistry, Women University, Multan, Pakistan

<sup>2</sup> Department of Parasitology, University of Agriculture Faisalabad, 38040, Pakistan

<sup>3</sup> Department of Human Nutrition and Dietetics, University of Lahore, Pakistan

<sup>4</sup> Department of Biochemistry, Bahauddin Zakariya University, Multan, Pakistan

<sup>5</sup> Rashid Latif Khan University, Pakistan

<sup>6</sup> Faculty of Veterinary and Animal Sciences, Gomal University, Dera Ismail Khan, Pakistan

<sup>7</sup> Foundation University Islamabad, Pakistan

<sup>8</sup> Faculty of Pharmacy, The University of Lahore, Lahore, Pakistan

<sup>9</sup> Department of Pharmacy, Quaid-i-Azam University, Islamabad, Pakistan

### Article info

Received: 21 August 2026

Received in revised form: 23 September 2026

Accepted: 27 September 2026

Published online: 07 October 2026

### Keywords

Curcumin  
Nanoethosomes  
Quercetin  
Topical drug delivery  
Flavonoids  
Chicken

### \* Corresponding authors:

Arslan Muhammad Ali Khan

Email: [arslanrajpootkhan374@gmail.com](mailto:arslanrajpootkhan374@gmail.com)

Anas Jahangir

Email: [anas.jahangir@fui.edu.pk](mailto:anas.jahangir@fui.edu.pk)

### Reviewed by:

Awais Ibrahim

Institute of Zoology, University of the Punjab Lahore, Pakistan

Consent to publish the name of second reviewer could not be obtained

### Abstract

Quercetin and curcumin are strong natural antibacterial and antioxidant compounds. However, their topical use is restricted due to their low stability, poor solubility, and insufficient skin penetration. The purpose of this study was to develop nanoethosomes loaded with curcumin and quercetin and assess their antibacterial efficacy, antioxidant activity, and physicochemical characteristics for topical administration. The ethanolic injection method was used to create the nanoethosomes, which were then characterized for entrapment efficiency, zeta potential, particle size, and polydispersity index (PDI). The DPPH assay was used to measure antioxidant activity, while zone of inhibition, MIC, and MBC studies were used to measure antibacterial efficacy against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*. The findings indicated that the nanoethosomes loaded with quercetin had particle size of  $128.7 \pm 16.4$  nm, PDI of  $0.545 \pm 0.05$ , and zeta potential of  $-34.8 \pm 0.6$  mV, whereas those loaded with curcumin measured  $155.3 \pm 1.2$  nm, PDI  $0.186 \pm 0.07$ , and zeta potential  $-42.2 \pm 0.3$  mV. Curcumin's entrapment efficiency was  $92.1 \pm 2.1\%$ , whereas quercetin's was  $84.6 \pm 1.8\%$ . The curcumin-loaded nanoethosomes exhibited antibacterial activity against *S. aureus*, *P. aeruginosa*, and *E. coli*, with inhibition zones of  $21.5 \pm 1.1$ ,  $20.1 \pm 0.9$ , and  $22.3 \pm 0.8$  mm, respectively. Similarly, at  $50 \mu\text{g/mL}$ , both formulations showed 65 and 54% inhibition of BSA protein denaturation, respectively. These results demonstrated strong antioxidant activity, high stability, excellent drug loading, and broad-spectrum antibacterial activity, underscoring their promise as secure and efficient topical delivery methods for natural medicines.

This is an open access article under the CC Attribution license (<http://creativecommons.org/licenses/by/4.0/>)

## 1. Introduction

As a result of increasing prevalence of pathogenic bacteria, the exploration of medicinal plants as potential antioxidant and antibacterial agents has gained considerable attention in recent years (Ike et al. 2025; Merdana et al. 2025; Trung and Nhan 2025). To fight drug-resistant pathogenic bacteria, natural bioactive substances have been used due to their safety profiles and have gained more attention in pharmaceutical research (El-Saadony et al. 2025; Karabacak et al. 2025; Phupaboon et al. 2025; Ullah et al. 2025; Masood et al. 2025). Flavonoids such as quercetin and curcumin have gained significant attention in recent years due to their diverse pharmacological properties, including antioxidant, anti-inflammatory, antimicrobial, antiparasitic, and anticancer activities (Azeem et al. 2023; Haryati et al. 2025; Mustafa et al. 2025; Naeem et al. 2025; Satybaev et al. 2025; Singh et al. 2025a).

Despite their therapeutic potential, the clinical application of these compounds is limited by their poor aqueous solubility, instability, rapid metabolism, and low bioavailability (Teng et al. 2023; Fatima 2025; Kunu et al. 2025). Thus, there is a need to develop an effective drug delivery approach that can enhance the solubility of active compounds and also improve skin penetration.

A cutting-edge vesicular drug delivery technology based on nanoethosome has emerged to overcome the drawbacks of topical carriers and traditional liposomes (Singh et al. 2025b). Nanoethosomes, which are made of phospholipids, ethanol, and water, are highly flexible and deformable, which facilitates their passage through the skin barrier more successfully (Sguizzato et al. 2021; Ricci et al. 2024). In addition to increasing vesicle flexibility, ethanol also alters the stratum corneum's lipid structure, which improves transdermal medication

delivery (Akl et al. 2024). Furthermore, nanoethosomes provide several benefits, such as enhanced physicochemical stability, high drug loading capacity, and nanoscale particle size (Nasr et al. 2022). Their small size enables close contact with the skin surface, and by inhibiting vesicle aggregation their negative surface charge helps maintain colloidal stability (Elsayed et al. 2021).

Quercetin and curcumin can neutralize reactive oxygen species and lessen oxidative damage. Further, both flavonoids have broad-spectrum antibacterial properties that work against both Gram-positive and Gram-negative bacteria (Shome et al. 2022; Bashir et al. 2025; Sarfraz et al. 2025). They function by disrupting bacterial cell membranes, inhibition protein synthesis, and interfering with the function of nucleic acids. Thus, the two bioactive compounds (quercetin and curcumin) in a single carrier system may offer synergistic pharmacological effects, particularly in antioxidant and antimicrobial applications (Hasan et al. 2022). Therefore, this study focuses on the preparation and characterization of quercetin- and curcumin-loaded nanoethosomes using the ethanolic injection method. The formulated systems were evaluated for particle size, zeta potential, polydispersity index, entrapment efficiency, antioxidant activity, antibacterial efficacy, drug-release behavior, and ex vivo skin permeation to assess their suitability as topical delivery vehicles.

## 2. Materials and Methods

### 2.1 Chemicals and reagents

The chemicals and reagents used in the study (quercetin, curcumin, ethanol, soya lecithin, triethanolamine, and DPPH or 2, 2-diphenyl-1-picrylhydrazyl) were purchased from Sigma-Aldrich, USA.

### 2.2 Formation of nanoethosomes

Nanoethosomes were prepared using the cold method. Briefly, soya lecithin (3% w/v) was added to an ethanol (40% v/v) solution under constant stirring (900 rpm) at room temperature. Curcumin and quercetin were added to the prepared ethanol- lipid mixture and stirred until dissolved. Then, distilled water was added dropwise to the solution under constant stirring, and the resultant dispersion was sonicated at 100W and 20 kHz for 10 min to decrease vesicle size (Nasr et al. 2022). Thereafter, the prepared nanoethosomes were equilibrated at 25 °C for 50 min and then stored at 4 °C for further analysis.

### 2.3 Characterization of nanoethosomes

#### 2.3.1 Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared spectroscopy (FTIR) was conducted to analyze the functional groups of the synthesized nanoparticles. A small quantity of the dried sample was directly applied onto the sample holder of the Bruker Alpha FTIR spectrometer equipped with an attenuated total reflectance (ATR) accessory. The sample was scanned over a spectral range of 4000 to 400 cm<sup>-1</sup> (Salman et al. 2020).

#### 2.3.2 Zeta potential and particle-size analysis

Zeta potential and particle size analyses were conducted using a Zetasizer Nano ZS (Malvern Instruments, UK) to evaluate the surface charge and size distribution. Zeta potential was measured through electrophoretic light scattering, which quantifies the electrophoretic mobility of particles in an electric field to infer surface charge and colloidal stability. Particle size and polydispersity index (PDI) were evaluated by dynamic light scattering (DLS), based on the detection of fluctuations in scattered light due to the Brownian motion of particles.

All measurements were carried out at a controlled temperature of 25 °C (Lu et al. 2018).

#### 2.3.3 Entrapment efficiency (%EE)

The nanoethosomal dispersion was subjected to high-speed centrifugation to separate unencapsulated (free) drug from the nanoethosomes. The absorbance of the supernatant containing the free drug was measured using a UV-Visible spectrophotometer at wavelengths of 372 and 425 (Ryu et al. 2021). Entrapment efficiency was then computed using the formula:

$$\%EE = [(Total\ drug - Free\ drug) / Total\ drug] \times 100$$

#### 2.3.4 Antioxidant activity

Antioxidant potential was measured by the DPPH (2, 2-diphenyl picrylhydrazyl) scavenging method. 1 mL of prepared nanoethosomes (concentration range of 10-50 µg/mL) was mixed with 2 mL of DPPH solution, and then the mixture was incubated at room temperature for 20 min. After that, the wavelength was measured at 517 nm (Salman et al. 2020).

#### 2.3.5 Antibacterial activity assay

Antibacterial potential was determined by the agar well diffusion method. The agar medium was prepared, and pathogenic bacterial strains (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*) were spread over the plates. Six millimeter diameter wells were formed in the agar medium. Thereafter, 100 µL of nanoethosomes were loaded into the wells, and then the plates were incubated at 37 °C for 24 h. The antibacterial potential was determined in millimeters of inhibitory zones around the wells (Erhonyota et al. 2023).

#### 2.3.6 Anti-inflammatory activity by bovine serum albumin assay

The anti-inflammatory activity of the prepared nanoethosomes at different concentrations (10-50 µg/mL) was assessed by the bovine serum albumin assay. 1 mL of the nanomaterial was added to 1 mL of 5% (w/v) bovine serum albumin solution, and the mixture was heated at 40 °C for 30 min. After that, the mixture was cooled to room temperature, and the absorbance of the solution was measured at 660 nm (Bailey-Shaw et al. 2017).

#### 2.3.7 Primary dermal irritation assessment and ex vivo skin permeation study

The prepared nanogel was applied for 24–48 h to evaluate its dermal safety and irritation potential. Further, using a Franz diffusion cell setup with excised chicken skin, the permeation behavior of the nanogel was monitored, and drug diffusion across the membrane was quantified. A total of 12 male chickens, 15 days of age and weighing 400-600 g, were divided into four groups, with three birds in each. The fourth group, specifically prepared for primary dermal irritation assessment, was provided with a combined nanoethosomal formulation containing both quercetin and curcumin. The nanoethosomal formulations were applied topically at a concentration of 50 µg/mL, using a volume of 0.5 mL per animal. All birds were housed in well-cleaned and ventilated cages (23-27 °C) with relative humidity of 50-70%. The scoring system was based on visible erythema, scored on a scale of 0-4. The procedure was adopted as described by Ali et al. (2022), who used mice for this purpose.

### 2.4 Statistical analysis

Data were expressed as the mean ± standard deviation (SD). Statistical

comparisons among groups were performed using one-way ANOVA. A *p*-value of less than 0.05 was considered statistically significant.

### 3. Results

#### 3.1 FTIR analysis

FTIR analysis of quercetin-loaded nanoethosomes exhibited different absorption bands linked to functional groups of both phospholipids and quercetin, showing the incorporation into the vesicular system. A peak around 3341  $\text{cm}^{-1}$  suggested the properties of O-H stretching and the presence of hydroxyl groups in the ethosome structure. Moreover, a peak near 2920  $\text{cm}^{-1}$  is linked to the C-H stretching vibration of the phospholipid alkyl chain. Furthermore, the peak at 1650  $\text{cm}^{-1}$  was attributed to the C=O stretching vibration of the flavonoid carbonyl functional group, whereas the band in the area of 1600-1500  $\text{cm}^{-1}$  was ascribed to aromatic stretching, a major property of quercetin structure. The bands in the 1250-1000  $\text{cm}^{-1}$  range indicated C-O and C-O-C stretching, reflecting the presence of phenolic and ether groups (Fig. 1).

Furthermore, the FTIR analysis of curcumin-loaded nanoethosomes represented the peaks corresponding to functional groups of both phospholipid and curcumin, confirming the incorporation of curcumin into the vesicular system. An absorption band at 3323  $\text{cm}^{-1}$  showed the presence of hydroxyl groups in the ethosomal matrix. A peak around 2921  $\text{cm}^{-1}$  was ascribed to C-H stretching vibrations of alkyl chains, confirming the presence of phospholipids. Moreover, the peak at 1635  $\text{cm}^{-1}$  showed the C=O stretching of the diketone structure of curcumin. Furthermore, a peak at 1053  $\text{cm}^{-1}$  is associated with C-O-C stretching, showing the presence of ether groups (Fig. 2). Overall, the observed absorption bands were consistent with the characteristic functional groups of the formulation components. These observations suggests that FTIR analysis was used for functional group characterization; however, it was not considered sufficient to establish chemical stability, the absence of degradation, or specific molecular interactions.

#### 3.2 Zeta analysis

The size of particle, zeta potential, and polydispersity index (PDI) of blank, curcumin-loaded, and quercetin-loaded nanoethosomes were measured to estimate the influence of drug incorporation on the

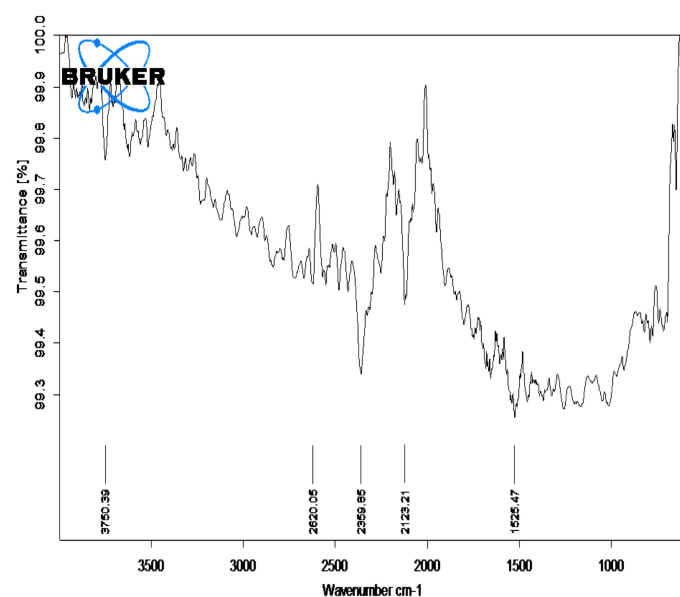


Fig. 1. FTIR of quercetin-containing nanoethosomes

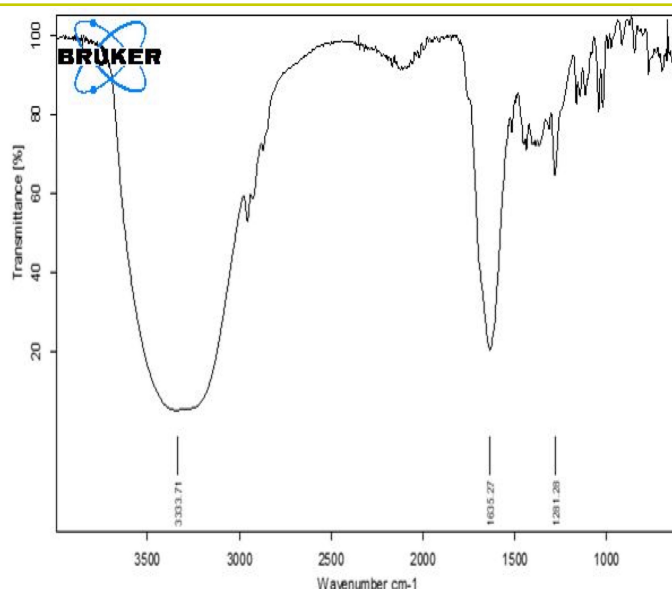


Fig. 2. FTIR of curcumin-containing nanoethosomes

vesicular system (Fig. 3). The blank formulation showed a particle size of 110 nm, a zeta potential of -31.7 mV, and a narrow size distribution (PDI= 0.210). However, quercetin-loaded nanoethosomes showed an increased vesicle size (128.7 nm) and PDI (0.545). Further, the zeta potential was -34.8 mV. Moreover, the curcumin-loaded nanoethosomes showed the largest size (155.3 nm) and low PDI (0.186); however, the zeta potential decreased (-42.2 mV). Both nanoethosomal formulations exhibited strong encapsulation; however, the curcumin-loaded nanoethosomes showed higher entrapment (92.1%) as compared to quercetin-loaded nanoethosomes (84.6%) (Table 1).

#### 3.3 Antioxidant potential

All the samples showed concentration-dependent DPPH scavenging activity (Fig. 4). The antioxidant activity of curcumin-loaded nanoethosomes was enhanced from 10 to 50  $\mu\text{g/mL}$ . At 50  $\mu\text{g/mL}$ , the curcumin-loaded nanoethosomes showed 80% DPPH radical scavenging activity, whereas quercetin-loaded nanoethosomes showed 74% activity. These findings indicate concentration-dependent radical-scavenging activity under DPPH assay conditions.

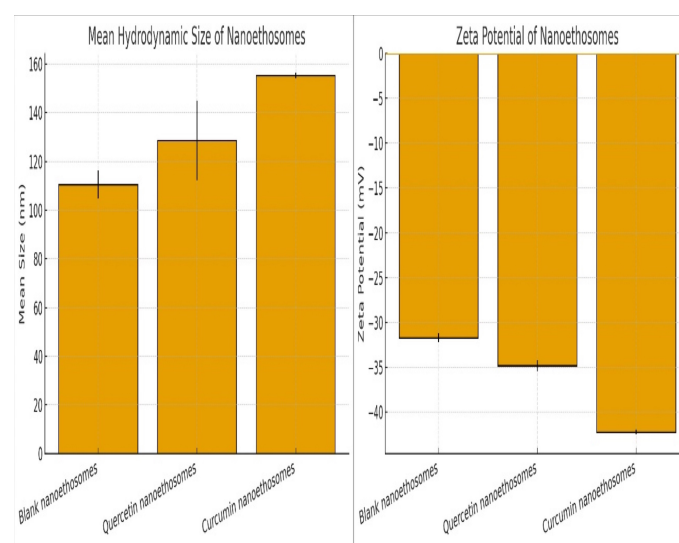


Fig. 3. Zeta size and potential of nanoethosomes

Table 1. Entrapment efficacy of nanoethosomes

| Formulation                    | Entrapment efficiency (%) Mean $\pm$ SD |
|--------------------------------|-----------------------------------------|
| Quercetin-loaded nanoethosomes | 84.6 $\pm$ 1.8                          |
| Curcumin-loaded nanoethosomes  | 92.1 $\pm$ 2.1                          |

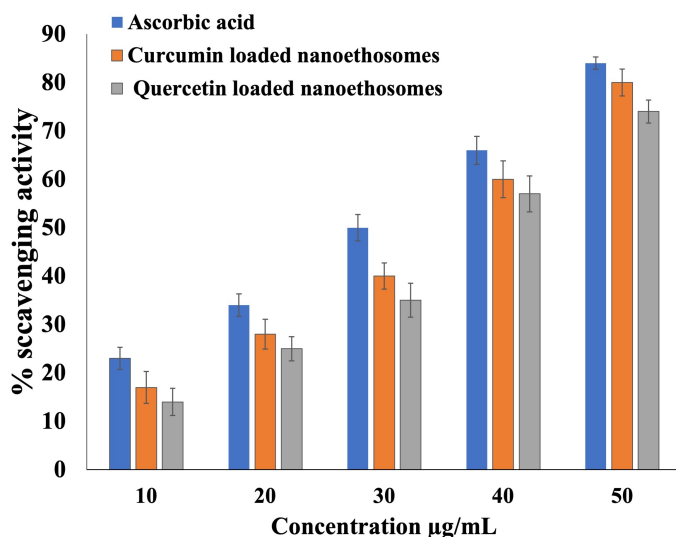


Fig. 4. Antioxidant activity of curcumin- and quercetin-loaded

### 3.4 Antibacterial potential

The antibacterial activity of quercetin- and curcumin-loaded nanoethosomes was evaluated against *S. aureus*, *P. aeruginosa*, and *E. coli*. For this purpose, the agar well diffusion method was adopted. Briefly, the bacterial cultures were prepared and inoculated onto the appropriate agar medium to obtain a uniform bacterial pattern. Wells of 6mm diameter were prepared in the inoculated agar plates, and 100 µL of each nanoethosome was added to each well. After that, the plates were incubated at 37 °C for 24 hours. The diameter of the inhibition zones was measured in millimeters.

The curcumin-loaded nanoethosomes showed higher antibacterial potential as compared to quercetin nanoethosomes. The zones of inhibition by curcumin-loaded nanoethosomes against pathogenic bacterial strains (*S. aureus* = 21.5  $\pm$  1.1 mm, *P. aeruginosa* = 20.1  $\pm$  0.9 mm, and *E. coli* = 22.3  $\pm$  0.8 mm) are shown in Fig. 5. Furthermore, the antibacterial activity was evaluated by determining the minimum

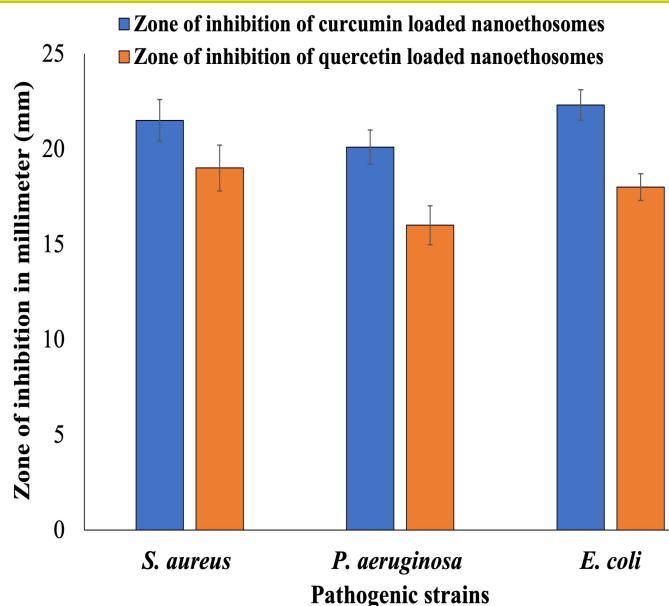


Fig. 5. Inhibitory zones of quercetin- and curcumin-loaded

inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The values are given in Table 2. The antibacterial responses were recorded for each bacterial strain, and all experiments were performed using three independent replicates (n=3). However, because the nanoethosome formulations contained 40% ethanol and neither ethanol-only vehicle control nor the blank nanoethosome controls were not included, the contribution of ethanol and the nanoethosomal carrier to the observed antibacterial activity cannot be excluded.

### 3.5 Anti-inflammatory potential

Both curcumin- and quercetin-loaded nanoethosomal formulations showed concentration-dependent inhibition of BSA protein denaturation (Fig. 6). At highest concentration of 50 µg/mL, the curcumin-loaded and quercetin-loaded nanoethosomes exhibited 65% and 54% inhibition, respectively.

### 3.6 Primary dermal irritation

The erythema scale (0-4), (0 = none, 1 = light pink, 2 = dark pink, 3 = light red, and 4 = dark red) showed that the control exhibited irritation at different times (8, 16, and 24 h). However, group 2 (quercetin nanoformulation), group 3 (curcumin nanoethosomes), and group 4 (combination of both) showed normal skin appearance (Fig. 7). This

Table 2. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of quercetin and curcumin-loaded nanoethosomes

| Bacterial strain              | Formulation                    | MIC (µg/mL) | MBC (µg/mL) |
|-------------------------------|--------------------------------|-------------|-------------|
| <i>Staphylococcus aureus</i>  | Quercetin-loaded nanoethosomes | 25          | 50          |
|                               | Curcumin-loaded nanoethosomes  | 20          | 40          |
| <i>Pseudomonas aeruginosa</i> | Quercetin-loaded nanoethosomes | 30          | 60          |
|                               | Curcumin-loaded nanoethosomes  | 25          | 50          |
| <i>Escherichia coli</i>       | Quercetin-loaded nanoethosomes | 25          | 50          |
|                               | Curcumin-loaded nanoethosomes  | 20          | 40          |

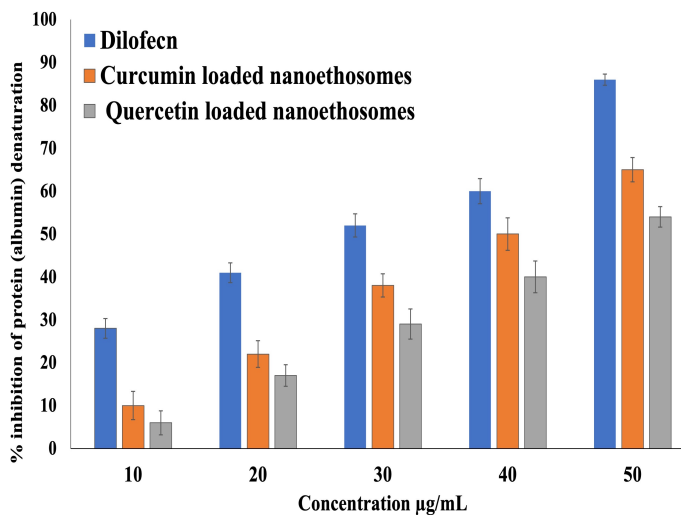


Fig. 6. Anti-inflammatory potential by bovine serum albumin assay of prepared nanoethosomes

pattern confirms that the model was sensitive (strong response in the control) while all nanoethosomal treatments were non-irritant under the test conditions. The lack of late-onset redness at 24 hours also argues against cumulative irritation or sensitization. Overall, these findings support the dermal safety of the quercetin, curcumin, and combined nanoethosomal formulations for topical use.

4. Discussion

Medicinal plants and their phytochemicals are considered as potential alternatives against bacterial infections (Mba et al. 2025; Khang et al. 2025; Shehzad et al. 2025), but their efficiency can be increased further by blending them with some other suitable agents (Shahzad et al. 2024; Okoye et al. 2025; Zhao et al. 2025). The current research shows the effective preparation of the quercetin- and curcumin-loaded nanoethosomes as innovative topical delivery systems with good physicochemical properties, high drug loading, and improved

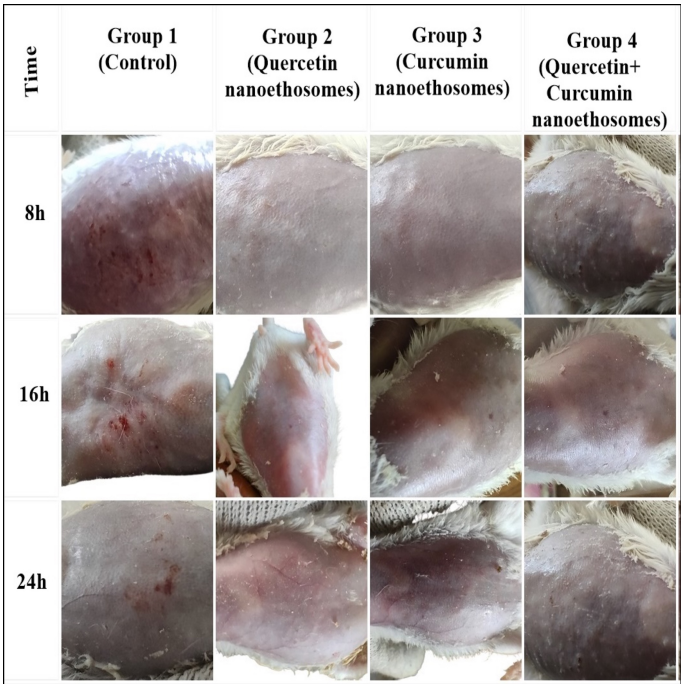


Fig. 7. Primary dermal irritation

biological activity. Their small size, highly deformable vesicular structure, and the ability of ethanol to deform facilitates penetration through the skin barrier, making nanoethosomes especially valuable in delivering poorly soluble natural compounds across the skin barrier (Emanet and Ciofani 2023). The size of nanoscale vesicles, negative surface charge, and satisfactory polydispersity values indicated favorable physicochemical characteristics of formulated nanoethosomes (Midekessa et al. 2020). These qualities are necessary to maintain high levels of topical activity and interaction with stratum corneum (Menon et al. 2012).

FTIR analysis suggested that quercetin and curcumin were readily incorporated into the nanoethosomes bilayer without any indication of chemical degradation or incompatibility. The retention of characteristic functional group peaks, along with slight inclination and broadening of the bands, indicate physical encapsulation that involve hydrogen bonding and electrostatic interactions as opposed to covalent modification (Pasieczna-Patkowska et al. 2025). These molecular interactions may be beneficial, since they help stabilize the vesicular structure and retain the inherent bioactivity of the loaded compounds (Pop et al. 2015). The lack of new peaks also indicates that both flavonoids have a high level of chemical stability during the process of formulation, which is very important in the case of natural products that are likely to degrade (Espíndola 2023). The higher entrapment efficiency of curcumin than quercetin may be linked its lipophilicity and subsequent attachment to the phospholipid bilayer that reduces the loss of drugs into the external aqueous solution (Kuche et al. 2019). A high entrapment efficiency has been shown to achieve high drug delivery with low dosage, enhance therapeutic consistency, and minimize wastage on formulations (Shen et al. 2017; Matalqah et al. 2025). However, the entrapment efficiency of quercetin was also relatively higher and hence, confirmed the applicability of nanoethosomes for entrapment of both flavonoids.

The DPPH assay showed that nanoethosome encapsulation enhanced the free-radical scavenging activity of both compounds. These high, dose-dependent antioxidant activities indicate that the nanoethosome carrier has no effect on the availability of active sites and even higher dispersion and stability of the antioxidants (Mazzotta et al. 2021). The scavenging activity was higher in nanoethosomes loaded with curcumin as compared to those loaded with quercetin due to phenolic and diketone-based radical neutralization mechanisms exhibited by curcumin (Mazzotta et al. 2021). The antioxidant activity comparable with that of ascorbic acid at elevated levels demonstrates the potential of these formulations in the treatment of oxidative stress-associated skin diseases (Oresajo et al. 2012). For instance, the flavonoid-based nanoethosomes exhibit greater free-radical scavenging potential (Ricci et al. 2024). Antibacterial assays showed broad spectrum action against Gram-positive and Gram-negative bacteria, with curcumin-loaded nanoethosomes showing greater efficacy against all the strains tested. This increased antibacterial activity is explained by the fact that nanoethosomal penetration, disruption of the cell membrane induced by ethanol, and intracellular delivery of the bioactive compounds work in conjunction (Emanet and Ciofani 2023). The lower values of MIC and MBC of the curcumin formulations imply strong interactions with bacterial membranes and have better bactericidal properties (Shome et al. 2022). Further, it was previously reported that liposomes (lipid-based nanomaterials) showed potential antibacterial activity and considerably inhibit pathogenic bacterial

growth (Ghosh and De 2023).

Furthermore, the prepared nanoethosomal formulations showed anti-inflammatory potential. The results suggested that these formulations may suppress inflammatory mediators, such as tumor necrosis factors (El-Mahdy et al. 2020). Thus, the combination of these therapeutic activities suggests that these formulations may help control the inflammatory and infectious diseases.

Dermal irritation tests established the safety of the quercetin- and curcumin-loaded nanoethosomes because none of the subjects experienced any erythema or other negative effects of the skin during the study period. This observation is especially important considering the fact that ethanol level is relatively high in ethosomes, which may even be hazardous at high concentrations (Abdulbaqi et al. 2016). Lack of irritation reveals that the optimized formulation provides a balance between the enhancement of penetration and compatibility with the skin, and it is suitable for recurrent topical use (Jain et al. 2022; Narsa et al. 2024). Future studies should encompass the long-term stability analyses and in vivo efficacy evaluation in disease-specific models.

## 5. Conclusions

The curcumin- and quercetin-loaded nanoethosomes were effectively formulated, exhibiting the favourable physicochemical characteristics, greater entrapment efficacy, potential antioxidant and anti-inflammatory activities, and good antibacterial potential. The nanoethosomal system enhanced the stability and bioactivity of both compounds, demonstrating promising potential as an effective topical delivery platform for natural therapeutics.

## Declarations

**Funding:** Not applicable

**Conflict of interest:** The authors declare no conflicts of interests

**Acknowledgements:** None

**Data Availability:** All the associated data are available from the corresponding author upon reasonable request

## References

- Abdulbaqi IM, Darwis Y, Khan NAK, Assi RA, Khan AA. (2016). Ethosomal nanocarriers: the impact of constituents and formulation techniques on ethosomal properties, in vivo studies, and clinical trials. *International Journal of Nanomedicine* 2016(11): 2279-2304. <https://doi.org/10.2147/IJN.S105016>
- Akl MA, Eldeen MA, Kassem AM. (2024). Beyond skin deep: phospholipid-based nanovesicles as game-changers in transdermal drug delivery. *AAPS PharmSciTech* 25(6):184. <https://doi.org/10.1208/s12249-024-02896-6>
- Ali A, Ali A, Rahman MA, Warsi MH, Yusuf M, Alam P. (2022). Development of nanogel loaded with lidocaine for wound-healing: Illustration of improved drug deposition and skin safety analysis. *Gels* 8(8): 466. <https://doi.org/10.3390/gels8080466>
- Azeem M, Hanif M, Mahmood K, Ameer N, Chughtai FRS, Abid U. (2023). An insight into anticancer, antioxidant, antimicrobial, antidiabetic and anti-inflammatory effects of quercetin: A review. *Polymer Bulletin* 80(1): 241-262. <https://doi.org/10.1007/s00289-022-04091-8>
- Bailey-Shaw YA, Williams LAD, Green CE, Rodney S, Smith AM. (2017). In-vitro evaluation of the anti-inflammatory potential of selected Jamaican plant extracts using the bovine serum albumin protein denaturation assay. *International Journal of Pharmaceutical Sciences Review and Research* 47(1): 145-153.
- Bashir A, Munir A, Hira H, Akram R, Ghafoor A, Mustafa A, Bisma, Jameel M. (2025). Green guardians: plant-derived antioxidants and their role in oxidative stress control. *Agrobiological Records* 22: 114-128. <https://doi.org/10.47278/journal.abr/2025.054>
- El-Mahdy MM, Hassan AS, El-Badry M, El-Gindy GEDA. (2020). Performance of curcumin in nanosized carriers niosomes and ethosomes as potential anti-inflammatory delivery system for topical application. *Bulletin of Pharmaceutical Sciences Assiut University* 43(1): 105-122. <https://doi.org/10.21608/bfsa.2020.93599>
- El-Saadony MT, Saad AM, Mohammed DM, Korma SA, Alshahrani MY, Ahmed AE, Ibrahim SA. (2025). Medicinal plants: bioactive compounds, biological activities, combating multidrug-resistant microorganisms, and human health benefits-a comprehensive review. *Frontiers in Immunology* 16: 1491777. <https://doi.org/10.3389/fimmu.2025.1491777>
- Elsayed MM, Okda TM, Atwa GM, Omran GA, Abd Elbaky AE, Ramadan AEH. (2021). Design and optimization of orally administered luteolin nanoethosomes to enhance its anti-tumor activity against hepatocellular carcinoma. *Pharmaceutics* 13(5): 648. <https://doi.org/10.3390/pharmaceutics13050648>
- Emanet M, Ciofani G. (2023). Ethosomes as promising transdermal delivery systems of natural-derived active compounds. *Advanced NanoBiomed Research* 3(10): 2300020. <https://doi.org/10.1002/anbr.202300020>
- Erhonyota C, Edo GI, Onoharigbo FO. (2023). Comparison of poison plate and agar well diffusion method determining the antifungal activity of protein fractions. *Acta Ecologica Sinica* 43(4): 684-689. <https://doi.org/10.1016/j.chnaes.2022.08.006>
- Espíndola C. (2023). Some nanocarrier's properties and chemical interaction mechanisms with flavones. *Molecules* 28(6): 2864. <https://doi.org/10.3390/molecules28062864>
- Fatima E. (2025). Extraction of bio-active compounds from plant sources using novel extraction techniques-MAE (Microwave Assisted Extraction), UAE (Ultrasonic Assisted Extraction), and SFE (Supercritical fluid Extraction): An insightful review. *Trends in Animal and Plant Sciences* 6: 54-65. <https://doi.org/10.62324/TAPS/2025.080>
- Ghosh R, De M. (2023). Liposome-based antibacterial delivery: an emergent approach to combat bacterial infections. *ACS Omega* 8(39): 35442-35451. <https://doi.org/10.1021/acsomega.3c04893>
- Haryati T, Herliatika A, Sinurat AP, Wina E, Purba M, Puastuti W. (2025). Efficacy of clove leaves, mangosteen peel extract and liquid smoke as feed additives for native chickens. *International Journal of Veterinary Science* 14(1): 107-112. <https://doi.org/10.47278/journal.ijvs/2024.214>
- Hasan AA, Tatarskiy V, Kalinina E. (2022). Synthetic pathways and the therapeutic potential of quercetin and curcumin. *International Journal of Molecular Sciences* 23(22): 14413. <https://doi.org/10.3390/ijms232214413>
- Ike OO, Ekugba CU, Ezenyilimba BN, Onwumelu IJ, Okonkwo AP, Ejivade OM, Ezejesi HC, Okonkwo JC, Nwankwo CA. (2025). Strain effect on hematological indices of broiler chicks fed graded levels of *Phyllanthus amarus* leaf extract. *Agrobiological Records* 19: 50-55. <https://doi.org/10.47278/journal.abr/2025.006>
- Jain AK, Jain S, Abourehab MA, Mehta P, Kesharwani P. (2022). An insight on topically applied formulations for management of various skin disorders. *Journal of Biomaterials Science, Polymer Edition* 33(18): 2406-2432. <https://doi.org/10.1080/09205063.2022.2103625>
- Karabacak M, Mammadov E, Maharramov M, Özkök D, Seyidov M, Memmedov B, Ganbarov H, Kanbur M. (2025). Some medicinal plants used in animal health in Nakhchivan Autonomous Republic. *International Journal of Veterinary Science* 14(5): 945-956. <https://doi.org/10.47278/journal.ijvs/2025.055>
- Khang TN, Binh HT, Dao VTT, Thao LTT, Duy TT. (2025). Antibacterial and antifungal abilities of *Tacca* (*Tacca leontopetaloides* L. Kuntze) leaf extraction

- and its application in fresh mango preservation. *International Journal of Agriculture and Biosciences* 14(4): 556-564. <https://doi.org/10.47278/journal.ijab/2025.034>
- Kuche K, Bhargavi N, Dora CP, Jain S. (2019). Drug-phospholipid complex—a go through strategy for enhanced oral bioavailability. *AAPS PharmSciTech* 20(2): 43. <https://doi.org/10.1208/s12249-018-1252-4>
- Kunu W, Meekrasae R, Peethong O, Khanma S, Insamran Y, Patathananone S. (2025). Phytochemical analysis and effectiveness of ripened *Spondias pinnata* fruit extracts against antibiotic-resistant mastitis-causing bacteria. *International Journal of Veterinary Science* 14(1): 196-203. <https://doi.org/10.47278/journal.ijvs/2024.225>
- Lu PJ, Fu WE, Huang SC, Lin CY, Ho ML, Chen YP, Cheng HF. (2018). Methodology for sample preparation and size measurement of commercial ZnO nanoparticles. *Journal of Food and Drug Analysis* 26(2): 628-636. <https://doi.org/10.1016/j.jfda.2017.07.004>
- Masood A, Nadeem T, Manzoor MU, Owais M, Sohrani MIK, Saeed Z, Rizwan M, Balouch RA, Awais QM. (2025). In vitro evaluation of *Apocynum Cannabinum* against different bacteria. *Continental Veterinary Journal* 5(2): 213-218. <http://dx.doi.org/10.71081/cvj/2025.051>
- Matalqah S, Lafi Z, Mhaidat Q, Asha N, Yousef Asha S. (2025). Applications of machine learning in liposomal formulation and development. *Pharmaceutical Development and Technology* 30(1): 126-136. <https://doi.org/10.1080/10837450.2024.2448777>
- Mazzotta E, Orlando C, Muzzalupo R. (2021). New nanomaterials with intrinsic antioxidant activity by surface functionalization of niosomes with natural phenolic acids. *Pharmaceutics* 13(6): 766. <https://doi.org/10.3390/pharmaceutics13060766>
- Mba Anthonia Nkiru, Onwumelu IJ, Benjamin N, Emelugo, Chiedo CC, Ezejesi, HC, Ejivade OM, Okonkwo JC. (2025). Effect of different inclusion levels of *Phyllanthus amarus* leaf extract on growth performance of three strains of broiler. *Trends in Animal and Plant Sciences* 5: 54-58. <https://doi.org/10.62324/TAPS/2025.064>
- Menon GK, Cleary GW, Lane ME. (2012). The structure and function of the stratum corneum. *International Journal of Pharmaceutics* 435(1): 3-9. <https://doi.org/10.1016/j.ijpharm.2012.06.005>
- Merdana IM, Lazuardi M, Susilowati S, Mustofa I, Hamid IS, Yuniarti WM, Soeharsono, Samirana PO. (2025). Mode of action of medicinal plants to enhance fecundity and treat infertility in female animal models: rats, mice and rabbits. *International Journal of Agriculture and Biosciences* 14(6): 1184-1195. <https://doi.org/10.47278/journal.ijab/2025.102>
- Midekessa G, Godakumara K, Ord J, Viil J, Lattekivi E, Dissanayake K, Fazeli A. (2020). Zeta potential of extracellular vesicles: toward understanding the attributes that determine colloidal stability. *ACS Omega* 5(27): 16701-16710. <https://dx.doi.org/10.1021/acsomega.0c01582>
- Mustafa S, Saleem MI, Shahid S, Nazar M, Zaka F, Kaleem QM, Butt AA, Mahfooz A, Tahir S, Khan AMA. (2025). Antibacterial activity of plant essential oils against *Staphylococcus aureus* isolated from bovine mastitis. *Agrobiological Records* 22: 97-105. <https://doi.org/10.47278/journal.abr/2025.052>
- Naeem F, Ahmed R, Malik M, Hayat D, Saeed Z, Zahid A, Adnan M, Rauf A, Nazir M, Haneef A. (2025). In vitro evaluation of medicinal plant extracts against *Corynebacterium pseudotuberculosis*: A potential remedy for caseous lymphadenitis in small ruminants. *Continental Veterinary Journal* 5(2): 127-133. <http://dx.doi.org/10.71081/cvj/2025.057>
- Narsa AC, Suhandi C, Afidika J, Ghaliya S, Elamin KM, Wathoni N. (2024). A comprehensive review of the strategies to reduce retinoid-induced skin irritation in topical formulation. *Dermatology Research and Practice* 2024(1): 5551774. <https://doi.org/10.1155/2024/5551774>
- Nasr AM, Moftah F, Abourehab MA, Gad S. (2022). Design, formulation, and characterization of valsartan nanoethosomes for improving their bioavailability. *Pharmaceutics* 14(11): 2268. <https://doi.org/10.3390/pharmaceutics14112268>
- Okoye CS, Attama AA, Osonwa UE, Uronnachi EM. (2025). Antioxidant and hepatoprotective effects of New *Bouldia laevis* extract and its homeopathic formulations in streptozotocin-induced diabetic rats. *Trends in Animal and Plant Sciences* 5: 76-84. <https://doi.org/10.62324/TAPS/2025.067>
- Oresajo C, Pillai S, Manco M, Yatskayer M, McDaniel D. (2012). Antioxidants and the skin: understanding formulation and efficacy. *Dermatologic Therapy* 25(3): 252-259. <https://doi.org/10.1111/j.1529-8019.2012.01505.x>
- Pasieczna-Patkowska S, Cichy M, Flieger J. (2025). Application of Fourier transform infrared (FTIR) spectroscopy in characterization of green synthesized nanoparticles. *Molecules* 30(3): 684. <https://doi.org/10.3390/molecules30030684>
- Phupaboon S, Matra M, Prommachart R, Totakul P, Wanapat M. (2025). In vitro fermentation studies through synergistic effects of antioxidant phytonutrients derived from encapsulated medicinal plants. *International Journal of Agriculture and Biosciences* 14(6): 1113-1121. <https://doi.org/10.47278/journal.ijab/2025.089>
- Pop OL, Vodnar DC, Socaciu C. (2015). Encapsulation field polymers: Fourier Transform Infrared Spectroscopy (FTIR). In: Mishra M, editor, *Encyclopedia of biomedical polymers and polymeric biomaterials*, 11 Volume Set. CRC Press, Boca Raton. Pp. 3451-3467. <https://doi.org/10.1201/9781351237970>
- Ricci A, Stefanuto L, Gasperi T, Bruni F, Tofani D. (2024). Lipid nanovesicles for antioxidant delivery in skin: Liposomes, ufasomes, ethosomes, and niosomes. *Antioxidants* 13(12): 1516. <https://doi.org/10.3390/antiox13121516>
- Ryu S, Park S, Lee HY, Lee H, Cho CW, Baek JS. (2021). Biodegradable nanoparticles-loaded PLGA microcapsule for the enhanced encapsulation efficiency and controlled release of hydrophilic drug. *International Journal of Molecular Sciences* 22(6): 2792. <https://doi.org/10.3390/ijms22062792>
- Salman M, Tariq A, Ijaz A, Naheed S, Hashem A, Abd-Allah EF, Javed MR. (2020). In vitro antimicrobial and antioxidant activities of *Lactobacillus coryniformis* BCH-4 bioactive compounds and determination of their bioprotective effects on nutritional components of maize (*Zea mays* L.). *Molecules* 25(20): 4685. <https://doi.org/10.3390/molecules25204685>
- Sarfraz S, Rehman S, Shah A, Iqbal A, Younis MU. (2025). Antibacterial efficacy of Tulsi (*Ocimum sanctum*) leaf extract against multidrug-resistant poultry pathogens. *Trends in Animal and Plant Sciences* 6: 141-146. <https://doi.org/10.62324/TAPS/2025.087>
- Satybaev B, Valitova N, Kushaliyev K, Nurzhanova F, Kairgalieva G, Bayantassova S, Kozhayeva A, Mendybayeva E. (2025). Therapeutic efficacy of phytopreparation for the prevention and treatment of varroosis and noseosis of honey bees in Western Kazakhstan. *International Journal of Agriculture and Biosciences* 14(6): 1168-1177. <https://doi.org/10.47278/journal.ijab/2025.087>
- Sguizzato M, Esposito E, Cortesi R. (2021). Lipid-based nanosystems as a tool to overcome skin barrier. *International Journal of Molecular Sciences* 22(15): 8319. <https://doi.org/10.3390/ijms22158319>
- Shahzad A, Tahir A, Kashif M, Arshad M, Nasir A, Abbas M. (2024). Ethnopharmacological use of *Moringa oleifera*: an updated review. *Continental Veterinary Journal* 4(1): 250-260.
- Shehzad T, Abeer K, Dua RU, Aslam RS, Umar M, Fatima R, Sarfraz F, Hadier MZ, Zaheer M, Khan AMA, Usmani MW. (2025). The antibacterial and antiparasitic activity of the moringa-derived phytochemicals and nanoparticles. *Continental Veterinary Journal* 5(1): 82-92. <http://dx.doi.org/10.71081/cvj/2025.040>
- Shen S, Wu Y, Liu Y and Wu D. (2017). High drug-loading nanomedicines: progress, current status, and prospects. *International Journal of Nanomedicine* 2017(12): 4085-4109. <https://doi.org/10.2147/IJN.S132780>
- Shome S, Talukdar AD, Upadhyaya H. (2022). Antibacterial activity of curcumin and its essential nanoformulations against some clinically important

- bacterial pathogens: A comprehensive review. *Biotechnology and Applied Biochemistry* 69(6): 2357-2386. <https://doi.org/10.1002/bab.2289>
- Singh M, Verma M, Pandey S, Kumar R, Khan F, Pandey P. (2025a). Anticancer potential of quercetin, epigallocatechin gallate, kaempferol, apigenin, and curcumin against several human carcinomas. *Endocrine, Metabolic & Immune Disorders-Drug Targets* 25(14): 1148-1159. <https://doi.org/10.2174/0118715303320523240910072723>
- Singh JP, Saini G, Singh B, Tiwari G. (2025b). Nano-formulation approaches to enhance transdermal drug delivery-An updated review of nanovesicular carrier "Transethosomes". *Pharmaceutical Nanotechnology* 13(4): 739-757. <https://doi.org/10.2174/0122117385306281240427073651>
- Teng H, Zheng Y, Cao H, Huang Q, Xiao J, Chen L. (2023). Enhancement of bioavailability and bioactivity of diet-derived flavonoids by application of nanotechnology: A review. *Critical Reviews in Food Science and Nutrition* 63(3): 378-393. <https://doi.org/10.1080/10408398.2021.1947772>
- Trung TT, Nhan P. (2025). Using garlic powder supplements in the diet of growing crossbred rabbits; evaluation of weight gain, physiological and biochemical blood parameters, and the excretion of *Escherichia coli* in feces. *International Journal of Veterinary Science* 14(6): 1093-1099. <https://doi.org/10.47278/journal.ijvs/2025.086>
- Ullah U, Mumtaz MZ, Jaffar TH, Khaskheli MA, Imran A, Zaib AA, Shehzad S, Pervaiz H, Bibi A, Malik B, Haleem M, Fakhir M, Sarwar W, Alvi AH, Zahra FT, Qadir F, Razzaq A. (2025). Phytochemical constituents with antimicrobial activity from ethanolic extract of *Lawsonia inermis* leaves and stem extracts. *Agrobiological Records* 20: 11-17. <https://doi.org/10.47278/journal.abr/2025.015>
- Zhao Y, Zhang M, Bhandari B, Li C. (2025). Development of special nutritional balanced food 3D printing products based on the mixing of animals/plants materials: research progress, applications, and trends. *Critical Reviews in Food Science and Nutrition* 65(30): 6985-7009. <https://doi.org/10.1080/10408398.2025.2457420>

#### Citation

Ashraf F, Khan AMA, Batool H, Batool K, Azeem SM, Iqbal J, Jahangir A, Shabbir R, Nasar H. (2026). Formulation of curcumin- and quercetin-loaded nanoethosomes and evaluation of their antioxidant, antibacterial, and anti-inflammatory potential with dermal irritation assessment in chickens. *Letters in Animal Biology* 06(2): 146 – 153. <https://doi.org/10.62310/liab.v6i2.432>