RESEARCH ARTICLE

In-vitro Analysis of Antioxidant, Anti-inflammatory, Antidiabetic and Antimicrobial Studies of Kaempferol Ethosomes

Raghav S Shraddha[1], Bhavna Kumar[1*], Sethiya K Neeraj[1], Singhal Manmohan[1], Arya Swati[2]

[1]Faculty of Pharmacy DIT University, Dehradun, Uttarakhand, India.
[2]SGT College of Pharmacy, Shree Guru Gobind Singh Tricentenary University, Gurugram, Haryana, India.

ABSTRACT

Flavonoids have potent antioxidant, anti-inflammatory, antidiabetic and antimicrobial properties, and they generally report to have poor bioavailability and dissolution. This work emphasized on developing and characterizing kaempferol-loaded ethosomes and evaluating them for *in-vitro* assessments. The cold method was used for preparing kaempferol (KMP) ethosomes by means of soya lecithin, ethanol, and propylene glycol. The ethosomes were evaluated for vesicle size (253 ± 2.6), entrapment efficiency (91.05 ± 0.11%), zeta potential (-31.7 ± 1.12 mV), and drug release (89.91 ± 3.3%), compared with the release of pure drug (kaempferol), which was 48.96 ± 3.6% and showed an approximate double increment in the release rate. KMP ethosomes follow the Korsmeyer-Peppas model. The kaempferol ethosomes were further examined for their various *in-vitro* pharmacological activities as antimicrobial, anti-inflammatory, antidiabetic, and antioxidant in comparison with pure kaempferol. The results reported kaempferol loaded-ethosomes showed a strong anti-inflammatory, antimicrobial, antioxidant, and antidiabetic properties and thus, can be exploited as a viable drug delivery for various therapeutic applications.

Keywords: Antidiabetic, Anti-inflammatory, Antimicrobial, Antioxidant, Ethosomes, Kaempferol.

International Journal of Drug Delivery Technology (2024); DOI: 10.25258/ijddt.14.1.17

How to cite this article: Shraddha RS, Kumar B, Neeraj SK, Manmohan S, Swati A. *In-vitro* Analysis of Antioxidant, Anti-Inflammatory, Antidiabetic and Antimicrobial Studies of Kaempferol Ethosomes. International Journal of Drug Delivery Technology. 2024;14(1):118-125.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Diabetic foot ulcers (DFU) are a common issue affecting 15% of diabetic patients, leading to morbidity, death, and financial burdens. Infections cause skin damage, poor perfusion, and bacterial infections, ultimately resulting in sepsis. Advanced wound healing therapies use bioactive substances to impact healing processes.[1] Polyphenols have gained considerable attention over the past few decades as potential antidiabetic bioactives. They also possess various potentialities for treating cardiovascular, cancerous, neurodegenerative diseases and diabetes & its complications. Consequently, synthetic medicinal moieties have not shown significant potential in the treatment of diseases due to their many beneficial characteristics, including antioxidant and anti-inflammatory capabilities. Natural bioactives have become the most popular choice for treating diseases like diabetes, diabetic foot ulcers, and skin infections.[1-3] Advanced wound dressings containing bioactive substances can, directly and indirectly, impact healing processes.[4] Kaempferol, a phytoconstituent, has been suggested as an active moiety for diabetic foot ulcers due to its immune system suppression and various biological properties, including neuroprotection, cardioprotection, anti-inflammatory, antioxidant, antibacterial activity, and antidiabetic activity.[5-7] Kaempferol, despite its numerous health benefits, demonstrated low to moderate absorption and poor oral bioavailability (2%).[4-7] It is necessary to design innovative formulations to study the flavonoid biological applications and their efficiency in the therapeutic approach.[8,9] Novel approaches like ethosomes, liposomes, niosomes, micelles, conjugates, nanoparticles, and nano globules etc., are approaches for solubility and bioavailability enhancement of poorly soluble drug candidates.[10] Ethosomes are vesicles that range in size from nanometers to microns and are soft and malleable. They consist of water, ethanol (in high quantities), and phospholipids.[11,12] These are novel vesicular carriers that facilitate improved skin delivery and allow drugs to reach systemic circulation or deep skin layers.[13,14] Theoretically, ethanol fluidizes the stratum corneum's intercellular lipid bilayers, allowing flexible, soft vesicles to enter the disordered lipid bilayers.[11,15,16] As well as providing a controlled topical delivery system, lipid vesicles are used to solubilize poorly soluble drugs. According to studies, phospholipids and ethanol

work together in vesicular formulations to diffuse and permeate more into the lipid bilayers of the skin. Drugs that are both hydrophilic and lipophilic can be added to vesicles.[16,17] Despite their conceptual complexity, are known for their ease of setup, safety, and effectiveness, which can significantly expand their application.[16] In the current research, kaempferol loaded-ethosomes (KMP ethosomes) were developed to study the *in-vitro* antioxidant, antimicrobial, anti-inflammatory, and antidiabetic properties and the same were evaluated for vesicle size, drug entrapment, and drug release rates.

MATERIAL AND METHODS

Materials

KMP was purchased from Dhamtec Pharma and Consultants in Mumbai, India. Sigma-Aldrich (Germany) provided the chemicals as lipid (Soya lecithin), ethanol, propylene glycol, and cholesterol. All materials and compounds used in the research work were of analytical grades.

Method

Preparation of KMP ethosomes

Touitou *et al.* (2000) reported the cold method for developing ethosomes, which was used in the formulation of KMP ethosomes. According to Table 1, lecithin (at a specified amount) and KMP (200 mg) were dissolved in ethanol (10–37.5%), 0.5 mg/mL propylene glycol (PG), and a percentage of water, primarily with magnetic stirring for 1-hour at 700 rpm (Remi Equipment, Mumbai, India). A probe sonicator (Sonics-USA) was used to sonicate the solution three times for five minutes each (with a five-minute pause in between cycles). Additionally, the produced KMP ethosomes were refrigerated at 4ºC.[11,12]

Characterization of KMP ethosomes

- *Determination of vesicle size*

A zetasizer (Malvern Instruments, UK) was used to assess vesicle size (VS), polydispersity index (PDI), and zeta potential (ZP) using a computerized inspection system. The sample was mounted on a copper grid, covered with carbon, and stained with 1% aqueous solution of phosphotungstic acid and finally analyzed using transmission electron microscopy (TEM, FEI-Philips Tecnai) after the grid had been air dried.[18]

- *Fourier transforms infrared spectroscopy analysis*

Fourier transforms infrared spectroscopy (FTIR) (IR affinity-1, Shimadzu, Kyoto, Japan) of the ethosomal formulation and drug was used to identify the active functional groups in the drug. The dried samples were ground with potassium bromide before being mounted on a pellet under 3000 kg/cm^2 pressure to obtain the IR spectrum. The spectrum over 4000-400 cm-^{1} was developed using KBr disks. [11,12,17]

- *%Entrapment Efficiency and Drug loading*

For estimating %Entrapment Efficiency (%EE) and Drug loading (DL) (%), 200 mg of ethosomes were mixed into 100 mL of deionized water. KMP ethosomes were centrifuged at a rpm

of 12,000 for 45 minutes at 4°C (Remi-24 Cooling Centrifuge, India). After collecting the samples, they were washed with deionized water (n = 3) and analyzed for entrapment and loading efficiency using a UV spectrophotometer (Shimadzu UV-1800, Kyoto, 206-25400-38, Japan) at 365 nm.[19-21] The formula for %EE and DL is as follows:

$$\%EE = \frac{\text{Total amount of drug-Free drug}}{\text{Total amount of drug}} \times 100$$

$$DL\% = \frac{\text{Calculated drug content (mg)}}{\text{Theoretical drug content (mg)}} \times 100$$

- *Drug release studies of ethosomal formulation*

The KMP ethosomal formulation drug release profile was investigated using the dialysis method (MWCO-12000Da; SERVAPOR® Dialysis Membrane, Heidelberg, Germany).[18] The study was conducted using phosphate buffer saline (pH 7.4) with 0.5%, w/v sodium lauryl sulfate (SLS) as a release medium by USP dissolution apparatus I (Electrolab EDT08Lx). Samples were analyzed spectrophotometrically at 365 nm. DD-Solver software was used to plot a graph after the drug release profile of KMP ethosomes and fitted into various release kinetic models. The model that most closely matched the data was assessed using the linear regression coefficient (R^2).[18,19,22,23]

In-vitro Pharmacological Activity for KMP Ethosomes

Antioxidant activity

- *1.1-diphenyl-2-picryl hydrazyl radical (DPPH) assay*

The recommended procedure with a minor modification for free radical scavenging activity was employed to analyze the antioxidant capacity of pure KMP and KMP ethosomes. Each formulation was taken in different aliquots and diluted with methanol to make up to 1-mL. Test tubes were filled with a 0.1 mM 1.1-diphenyl-2-picryl hydrazyl radical (DPPH) solution, then incubated (30 minutes) and analyzed at 517 nm with a UV spectrophotometer. The %inhibition is determined by this formula.[19,24]

$$\text{Antioxidant activity} = \frac{\text{Abs Control - Abs Test}}{\text{Abs Control}} \times 100$$

Absorbance control = DPPH with methanol;
Absorbance test = DPPH with sample using KMP/KMP ethosomes.

- *Ferric reducing antioxidant power assay*

About 200 Mm mixture of phosphate buffer (pH 6.6) and potassium ferricyanide (1%, pH 6.6) in 1:1 ratio was taken with pure KMP and KMP ethosomes (10, 20, 40, 60, 80, and 100 µg/mL). Pure KMP, and KMP ethosomes samples were incubated (60ºC/20 minutes). The test samples were mixed with 10% trichloroacetic acid (TCA) and centrifuged at 3000 rpm for 8 minutes. The supernatant was added with ferric chloride (0.1%), and spectrophotometrically determined at 700 nm. The equation to get the percent of reducing power is as.[25]

$$\text{Antioxidant activity} = \frac{\text{Abs Control - Abs Test}}{\text{Abs Control}} \times 100$$

- *In-vitro antidiabetic activity of KMP ethosomes: α-amylase inhibition assay*

Utilizing 3,5-dinitrosalicylic acid (DNSA), amylase inhibition experiments for antidiabetic investigations were carried out. Before being dissolved in the buffer solution of sodium dihydrogen phosphate (0.02 M) and sodium chloride (0.006 M) at pH 6.9 (10–100 μg/mL), samples were dissolved in 10% dimethyl sulfoxide (DMSO). Furthermore, pure KMP and KMP ethosomes (20, 40, 60, 80, and 100 μg/mL) were combined with a solution of α-amylase (2 units/mL) and then incubated for 10 minutes at 30°C. Each tube received an addition of starch (1%) in water (w/v) for 3 minutes of incubation. A UV-visible spectrophotometer at 540 nm was used to check the absorbance. Below mentioned formula was used to determine the percentage inhibition of α-amylase activity.[24,25]

$$\% \text{ Inhibition} = \frac{\text{Abs Control - Abs Test}}{\text{Abs Control}} \times 100$$

The results were shown in terms of IC_{50}, which represents the concentration of the test substance (pure KMP and KMP ethosomes) necessary to cause 50% inhibition of the enzyme.

Anti-inflammatory activity

The albumin denaturation method with modification was used to determine the anti-inflammatory action of pure KMP and KMP ethosomes. The reaction mixture of 1% aqueous solution of bovine albumin fraction and test formulations (pure KMP + KMP ethosomes) in dose ranges of 10–100 or 100 μg/mL of diclofenac sodium can be used as a reference drug. The samples were measured at 660 nm by UV spectrophotometry, and the percentage of inhibition was calculated using the following formula.[24,26]

$$\% \text{ Inhibition} = \frac{\text{Abs Control - Abs Test}}{\text{Abs Control}} \times 100$$

Antimicrobial study

The antimicrobial efficacy of the two formulations containing pure KMP and KMP ethosomes was performed using strains of *Staphylococcus aureus* (MTCC96, MTCC737, MTCC740, MTCC1430, and NCTC6571). An experiment was carried out using the cup-plate method. Nutrient agar medium was added to a sterile petri plate that contained a microbe strain. Following that, an equivalent volume of KMP ethosomes (containing 8 μg/mL of KMP) and pure KMP solution (at the same concentration of KMP) was placed in the petri plate under aseptic conditions and incubated for 24 hours at 37°C in an incubator (Binder, Germany). After that, a graded scale was employed to define the zone of inhibition, with an aqueous solution of streptomycin sulfate serving as the standard.[27] For the antimicrobial study, this concentration was selected based on the reported minimum inhibitory concentration (MIC = 8 μg/mL) of KMP against quinolone-resistant MRSA strains. To obtain the necessary concentration of KMP solution, the required amount of pure KMP was dissolved in a small volume of DMSO, then the volume was made with sterile purified water.[28] Consequently, considering the reported MIC (1.56–6.25 μg/mL) of streptomycin against multiple strains of MRSA,[29] the aqueous solution of streptomycin was prepared in purified water to get a 6.25 μg/mL concentration.[30]

Statistical Analysis

All experiments were carried out in triplicates and expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

Formulation Development

Accordingly, to the outcome parameters such as small size, low PDI, relatively higher magnitude of ZP, and the highest %EE as mentioned in Table 1, the ethosome (F6) was chosen as the best one for further studies. Keeping the constant amount of KMP and propylene glycol (PG), it was found that at lower lecithin concentrations (253 mg, respectively) with the highest concentration of ethanol (37.5%), the smallest-sized vesicles (315.2 nm) with a low value of PDI (0.325), relatively high ZP (-31.3 mV), and encapsulation (89.05%) were obtained. Therefore, F6 was further prepared in triplicate and characterized. The prepared optimal formulation has shown promising results with VS of 253 ± 2.6 nm, a PDI of 0.345 ± 0.02, -31.7 ± 1.12 mV of ZP, and 91.05 ± 0.11% of encapsulation efficiency (%EE). Ethosomes are formed with stable and spherical shapes. Due to its hydrophilic and hydrophobic properties, PG enhances the solubility of KMP and ethosomal stability.

Size and Morphological Characterization of KMP Ethosomes

Earlier evidence reports that nanocarriers are the systems that can be used to determine pharmacological action of encapsulated drugs. For example, for antibacterial activity, the drug must cross the microbial cell wall *via* ion and/or protein channels. Thus, smaller particles may boost the cellular absorption of nanocarriers.[31,32]

The developed ethosomes formulation was evaluated for mean VS of 253 ± 2.6 nm with a uniform size distribution and a PDI value of 0.345 ± 0.02 (Figure 1 (a)). The PDI value suggested that the spacing between the vesicles was greater, which could provide sufficient repulsive forces and prevent unwanted aggregation and sedimentation.[33-35] The nanocarriers' ZP (surface charge) is also significant for demonstrating microbial growth inhibition. The ideal KMP ethosomes formulation had an average negative surface charge of -31.7 mV (Figure 1 (b)). The nano-carriers' colloidal stability is additionally enhanced by their high positive or negative surface charges. It is necessary to recognize how ethosomes behave in terms of morphology and surface characteristics to understand their drug-release kinetics. The TEM images of KMP ethosomes reflect that vesicles were homogenously

Table 1: Optimization of ethosomes formulations with different concentration of lipid and ethanol

Formulation code	Drug (mg)	Lipid (mg)	Ethanol (%)	PG (mg/mL)	VS (nm), PDI	ZP (mV)	EE (%)
FI	200	300	10	0.5	630.2, 0.723	- 26.8	66.5
F2	200	253	10	0.5	505.6, 0.515	-25.5	80.32
F3	200	300	20	0.5	415.3, 0.534	-23.8	65.25
F4	200	253	20	0.5	405.5, 0.346	-29.6	73.06
F5	200	300	37.5	0.5	358.3, 0.406	-28.4	82.05
F6	200	253	37.5	0.5	315.2, 0.325	-31.3	89.05
F7	200	300	30	0.5	345.3, 0.414	-29.4	84.02
F8	200	253	30	0.5	289.3, 0.323	-28.6	82.12
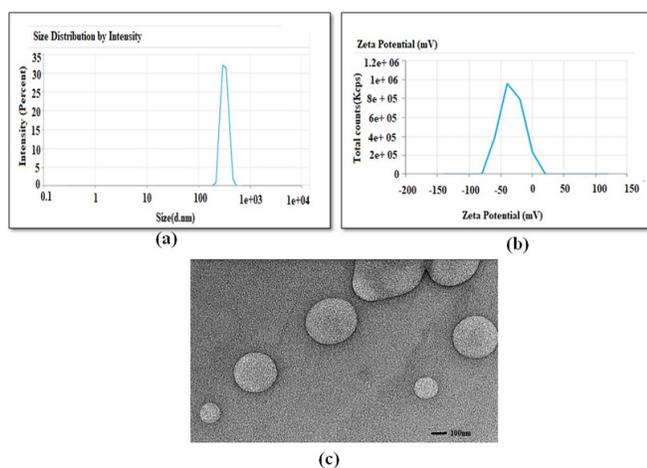
Figure 1: (a) Vesicular size; (b) Zeta potential (-31.7 mV ± 1.12); and (c) surface morphology (by TEM imaging) of the optimized KMP ethosomes at magnifications of 10 KX

Figure 2: Comparison of spectral data of FTIR for (a) pure KMP (b) Soya lecithin (c) KMP ethosomes

dispersed with uniform size, spherical in shape without any vesicular aggregation, as depicted in Figure 1(c).[18]

Fourier transforms infrared spectroscopy analysis

The interactions between components during the formation of ethosomes were analyzed by FTIR. Figure 2 depicts the FTIR spectra of pure KMP, soya lecithin, and KMP ethosomes. IR spectrum of pure KMP (a), peaks were found at 3598, 3296, and 3390 cm^{-1} (phenolic O-H stretching), 3184, 3080, and 3056 cm^{-1} (C-H stretching), and 1650 cm^{-1} (C=O stretching), as shown in Figure 2(a); (b) lipid (soya lecithin) showed peaks at 3409, 3299, and 1648 cm^{-1}, which corresponded to the O-H stretching frequency and C=O stretching frequency. Stretching vibrational peaks at 1226 and 1050 cm^{-1} are due to the presence of C-O and C-C bonds present in a molecule, as shown in Figure 2(b); (c) KMP ethosomes showed peaks at 1045 cm^{-1} (C-C bonds), 1650 cm^{-1} (C=O stretching), 2980 cm^{-1} (-C-H stretching), 3310 and 3694 cm^{-1} (OH stretching), as presented in Figure 2(c). The drug peak's intensity decreases and eventually disappears in the spectra of KMP ethosomes when compared to the drug and lipid spectra, as illustrated in Figure 2(c), demonstrating the encapsulation property of drug in the core of ethosomes.[19] As a result of the weak intermolecular interactions between the molecules of KMP and lipid, these observations support the formation of a KMP ethosomes formulation.

%EE & DL determination

The (%EE) of the developed formulations (F1-F8) was ranged from 66.5 to 89.05%, as represented above in Table 1. The amount of lecithin played an important role in encapsulating the lipophilic KMP into the core of the vesicles. The average amounts of lecithin (253 mg) with ethanol (37.5%) resulted in smaller-sized vesicles with the highest encapsulation of KMP. The optimized KMP ethosomes had an encapsulation efficiency of 91.05 ± 0.11%. The core structure of the ethosomes may be related to the higher encapsulation efficiency of KMP ethosomes. The lecithin and KMP are both the core components in this nanostructure. Both had affinity for each other and are lipophilic. Thus, a higher amount of KMP was entrapped within the core of the lecithin matrix. The maximum drug loading efficiency of optimized KMP ethosomes is 48.23 ± 2.1%.

Drug release studies of ethosomal formulation

The release studies provide significant insight into the behavior of KMP ethosomes formulations, which can be correlated to *in-vivo* studies. As illustrated in Figure 3(a), an *in-vitro* drug release analysis was conducted through a dialysis bag at physiological pH (Phosphate buffer saline, pH 7.4 with 0.5%, w/v SLS) to evaluate release patterns of KMP from ethosomes with the pure KMP. The KMP ethosomes exhibited high drug release (89.91%) at 1440 minutes as compared to the pure
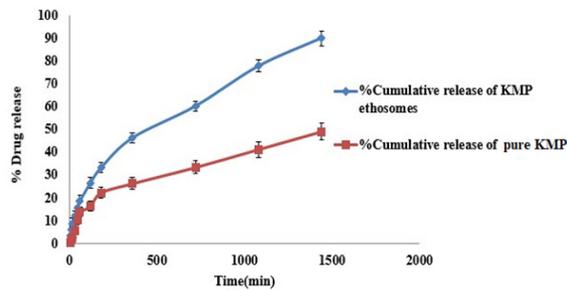
Figure 3(a): *In-vitro* release profile of pure KMP and KMP ethosomes. The results are the mean of at least three readings with standard deviations (Mean ± SD, $n = 3$)
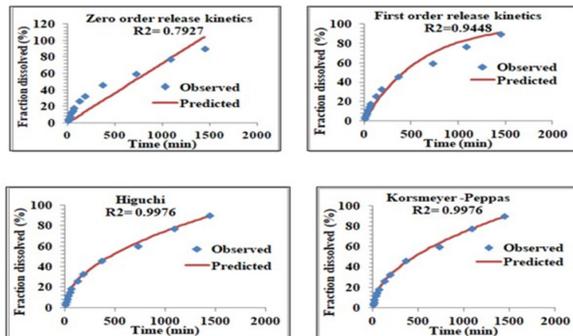
Figure 3(b): The following mathematical models were used to evaluate the release profile of KMP ethosomes F6: (a) Zero order; (b) First order; (c) Higuchi; (c) Korsmeyer- Peppas

KMP gel, which showed only 48.96% release of KMP at the same time duration. Thus, ethosomes showed more sustained release of KMP, which was significantly higher ($p < 0.05$) than the counter-control formulation. According to the kinetic modeling, the R^2 value of the Korsmeyer-Peppas model is higher than that of the other models, as shown in Figure 3(b). [36]

In vitro Pharmacology of KMP Ethosomes: Antioxidant Activity

(a) DPPH assay

The antioxidant potential of pure KMP and KMP ethosomes, reveals that the antioxidant potential is directly proportional to KMP concentration. Antioxidant potential increased as the concentration of KMP increased. At all concentrations, KMP ethosomes had significantly higher activity than pure KMP. KMP ethosomes demonstrated maximum activity at a concentration of 60 µg/mL ($92.06 \pm 2.3\%$), whereas pure KMP demonstrated $75.19 \pm 2.2\%$ at the same concentration as shown in Figure 4(a). The significant high activity in KMP ethosomes is due to its high solubility. KMP is a potent antioxidant and a powerful scavenger of free radicals. The increased solubility of KMP in ethosomes enhanced the antioxidant activity when soya lecithin and ethanol were present.[19,24]

(b) FRAP assay

The antioxidant capacity of pure KMP and KMP ethosomes was assessed using the FRAP technique. Between 10 to 100 µg/mL, a concentration-dependent effect was seen. As
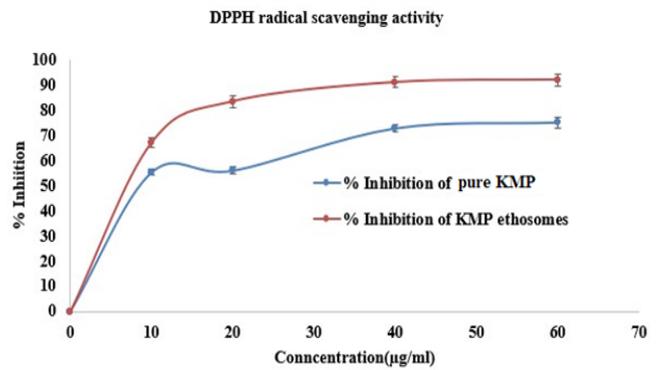
Figure 4(a): DPPH radical scavenging activity of pure KMP and KMP ethosomes

demonstrated in Figure 4(b), there was a substantial difference in activity between the pure KMP and KMP ethosomes, with the strongest effect occurring at 100 µg/mL. While KMP is more soluble after being incorporated into ethosomes, KMP ethosomes have stronger antioxidant activity.[25]

Antidiabetic Activity: α-Amylase Inhibitory Assay

Pure KMP and KMP ethosomes antidiabetic activity was investigated by inhibiting α-amylase enzyme as shown in Table 2. A substance's potency to inhibit a specific biological or biochemical function is a measure of its half-maximal inhibitory concentration (IC_{50}). According to the results shown in Figure 5, pure KMP and KMP ethosomes significantly inhibited amylase activity. Pure KMP and KMP ethosomes concentrations were found to vary between 20, 40, 60, 80, and 100 µg/mL with ranges of 51.23 to 73.55% and 69.42 to 85.12%. The IC_{50} value was found to be 2.40 ± 1.2 µg/mL for KMP ethosomes and 3.25 ± 3.2 µg/mL for pure KMP, respectively. At doses of 0.1, 0.5, and 1.0 µg/mL, the reference standard acarbose inhibited α-amylase activity by 32.54 ± 0.11, 74.29 ± 0.2, and $90.22 \pm 0.09\%$, respectively.[24, 25]

Anti-Inflammatory Study

The albumin denaturation method determines the anti-inflammatory potential for pure KMP and KMP ethosomes as depicted in (Figure 6). Pure KMP and KMP ethosomes were found to inhibit albumin denaturation in a concentration-dependent manner in the 20 to 100 µg/mL range. About
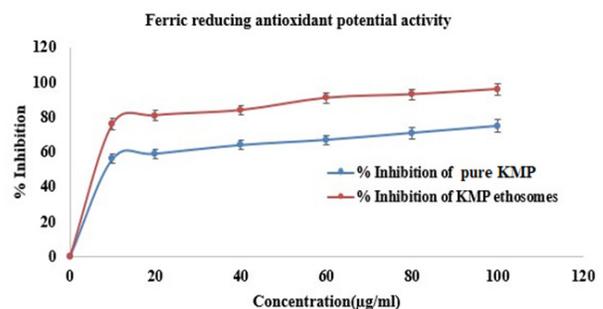
Figure 4(b): FRAP antioxidant activity of pure KMP and KMP ethosomes

Table 2: α-Amylase inhibitory assay of pure KMP and KMP ethosomes

Sample	Concentration (µg/mL)	%Inhibition	IC_{50} (µg/mL)
Pure kaempferol	20	51.23 ± 2.1	3.25 ± 3.2
	40	64.46 ± 2.2	2.58 ± 1.13
	60	68.59 ± 2.5	2.42 ± 1.22
	80	71.90 ± 2.1	2.31 ± 2.12
	100	73.55 ± 3.7	2.26 ± 2.5
KMP ethosomes	20	69.42 ± 2.5	2.40 ± 1.2
	40	71.90 ± 2.3	2.31 ± 1.2
	60	75.20 ± 2.7	2.21 ± 2.1
	80	78.51 ± 2.5	2.12 ± 1.2
	100	85.12 ± 2.9	1.95 ± 1.5
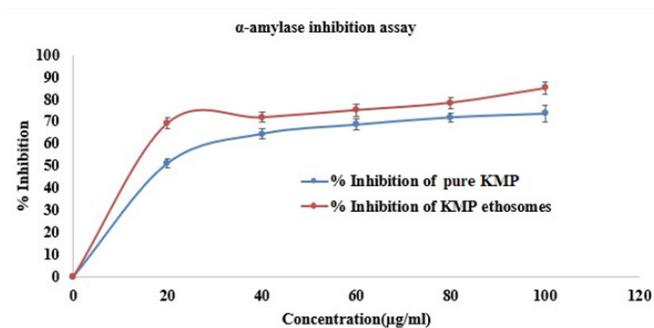
Figure 5: α-Amylase inhibitory assay of pure KMP and KMP ethosomes

100 µg/mL of diclofenac sodium (NSAID) was used as a standard, demonstrating a maximum inhibitory effect of 90.35 ± 0.5%. Pure KMP and KMP ethosomes (at a concentration of 100 µg/mL) exhibit maximum inhibitions of 65.78 ± 3.2 and 88.42 ± 3.5%, respectively. Compared to pure KMP, KMP ethosomes shows a maximum anti-inflammatory inhibitory effect in concentration dependent on albumin denaturation.[24,26] Protein denaturation has an irregular procedure that may be linked to an alteration in electrostatic hydrogen bonding, disulfide bonding, or hydrophobic bonding.[37] Protein denaturation generates autoantigens in certain diseases, such as cancer, rheumatic arthritis, and diabetic conditions
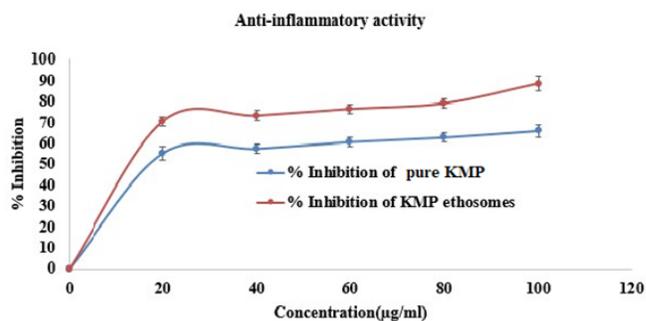
Figure 6: Anti-inflammatory activity of pure KMP and KMP ethosomes

that promote inflammation. Thus, by inhibiting protein denaturation, inflammatory conditions can be alleviated. KMP reduces pro-inflammatory cytokines such interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), IL-1β, and NF-Kappa B.[5,38] As a result, utilizing KMP ethosomes to regulate diabetes and other metabolic diseases can effectively reduce inflammation.[39]

Antimicrobial Study

The cup-plate method was used to compare the antimicrobial properties of KMP ethosomes and pure KMP to a standard streptomycin sulfate aqueous solution as a control against various strains of *S. aureus* (MTCC 96, MTCC 737, MTCC 740, MTCC 1430, and NCTC 6571). The findings are summarized in Table 3. Pure KMP had zone of inhibitions (ZOIs) of 11.62 ± 0.86, 12.49 ± 0.65, 12.24 ± 0.82, 12.56 ± 0.88, and 10.52 ± 0.85 mm against MTCC 96, MTCC 737, MTCC 740, MTCC 1430, and NCTC 6571, respectively. KMP ethosomes had ZOIs of 15.76 ± 0.83, 16.66 ± 0.50, 16.23 ± 0.40, 16.94 ± 0.79, and 14.49 ± 0.90 mm against the same strains of *S. aureus*. Streptomycin was utilized as a reference, with zones of inhibition of 18.05 ± 0.37, 18.96 ± 0.58, 15.83 ± 0.82, 18.87 ± 0.42, and 17.31 ± 0.42 mm against *S. aureus* (MTCC 96, MTCC737, MTCC740, MTCC1430, and NCTC6571). This investigation found that KMP ethosomes have stronger antimicrobial effects than pure KMP.[19] The increased solubility and permeability of KMP from KMP ethosomes as compared to pure KMP was responsible for the drug's increased antimicrobial activity, as KMP ethosomes become more absorbable as their surface area increases in the nanoformulation state.[27,24] The KMP, a flavonoid (a plant-produced secondary metabolite), exhibited

Table 3: Zone of inhibition produced by pure KMP and KMP ethosomes, compared to standard streptomycin against different strains of *Staphylococcus aureus*. The results are the mean of at least three readings with standard deviation (Mean ± SD, *n* = 3)

S. aureus (Strains)	Antimicrobial activity of KMP-containing formulations as compared to streptomycin		
	Diameter of zone of inhibition (mm), Mean ± SD, n = 3 by		
	Pure KMP	KMP ethosomes	Streptomycin
MTCC 96	11.62 ± 0.86	15.76 ± 0.83	18.05 ± 0.37
MTCC737	12.49 ± 0.65	16.66 ± 1.17	18.96 ± 0.58
MTCC740	12.24 ± 0.82	16.23 ± 0.40	15.83 ± 0.82
MTCC1430	12.56 ± 0.88	16.94 ± 0.79	18.87 ± 0.42
NCTC6571	10.52 ± 0.85	14.49 ± 0.90	17.31 ± 0.42

strong antimicrobial activities against the tested methicillin-resistant *staphylococcus aureus* (MRSA) strains by disrupting bacterial energy metabolism, decreasing pathogenicity, causing distraction of cellular reliability and integrity, outflow of cell contents, and ultimately cell death.[28] The current study's findings were supported by recent research in which KMP was discovered to have a more persistent regulating impact on bacterial infections than thiodiazole-copper.[40]

CONCLUSION

KMP ethosomes were successfully prepared using soya lecithin and ethanol using cold. The reduced vesicle size of KMP ethosomes enhances the solubility of poorly soluble KMP as evaluated from *in–vitro* release studies. The formulations have shown significant differences with pure KMP when analyzed for antioxidant, antidiabetic, anti-inflammatory, and antimicrobial activities. It was concluded that developing KMP ethosomes using the cold method is an easy, quick, stable, and sustainable process. α-amylase inhibition demonstrated the KMP ethosomes; potential antidiabetic activity, and DPPH and FRAP assays confirmed that they have higher antioxidant activity than pure KMP. Finally, compared to pure KMP, the formulation demonstrated significantly improved antimicrobial activity as well as anti-inflammatory activity. Ethosomes are a novel drug delivery system that can be the targeted drug delivery system for various diseases such as skin infections, foot ulcers, etc.

ACKNOWLEDGEMENT

REFERENCES

1. Zhang P, Lu J, Jing Y, Tang S, Zhu D, Bi Y. Global epidemiology of diabetic foot ulceration: a systematic review and meta-analysis. Annals of medicine. 2017; 49(2):106-16. DOI:10.1080/07853890.2016.1231932

2. Barjesteh T, Mansur S, Bao Y. Inorganic nanoparticle-loaded exosomes for biomedical applications. Molecules. 2021; 26(4):1135. DOI:10.3390/molecules26041135.

3. Grip J, Steene E, Engstad RE, Hart J, Bell A, Skjæveland I, Basnet P, Škalko-Basnet N, Holsæter AM. Development of a novel beta-glucan supplemented hydrogel spray formulation and wound healing efficacy in a db/db diabetic mouse model. European Journal of Pharmaceutics and Biopharmaceutics. 2021;169:280-91. DOI:10.1016/j.ejpb.2021.10.013

4. Özay Y, Güzel S, Yumrutaş Ö, Pehlivanoğlu B, Erdoğdu İH, Yildirim Z, Türk BA, Darcan S. Wound healing effect of kaempferol in diabetic and nondiabetic rats. Journal of Surgical Research. 2019; 233:284-96. DOI:10.1016/j.jss.2018.08.009

5. Chen AY, Chen YC. A review of the dietary flavonoid, kaempferol on human health and cancer chemoprevention. Food chemistry. 2013;138(4):2099-107. DOI:10.1016/j.foodchem.2012.11.139

6. Ashrafizadeh M, Tavakol S, Ahmadi Z, Roomiani S, Mohammadinejad R, Samarghandian S. Therapeutic effects of kaempferol affecting autophagy and endoplasmic reticulum

7. Raghav SS, Kumar B, Sethiya NK, Kaul A. A Mechanistic Insight on Phytoconstituents Delivering Hypoglycemic Activity: A Comprehensive Overview. Future Pharmacology. 2022;2(4):511-46. DOI:10.3390/futurepharmacol2040032

8. M Calderon-Montano J, Burgos-Morón E, Pérez-Guerrero C, López-Lázaro M. A review on the dietary flavonoid kaempferol. Mini reviews in medicinal chemistry. 2011;11(4):298-344. DOI:10.2174/138955711795305335.

9. Dabeek WM, Marra MV. Dietary quercetin and kaempferol: Bioavailability and potential cardiovascular-related bioactivity in humans. Nutrients. 2019; 11(10):2288. DOI: 10.3390/nu11102288.

10. Mohammed BS, Al Gawhari FJ. Transethosomes a novel transdermal drug delivery system for antifungal drugs. International Journal of Drug Delivery Technology. 2021; 11(1):238-243.

11. Touitou E, Dayan N, Bergelson L, Godin B, Eliaz M. Ethosomes—novel vesicular carriers for enhanced delivery: characterization and skin penetration properties. Journal of controlled release. 2000; 65(3):403-18. DOI: 10.1016/s0168-3659(99)00222-9.

12. Touitou E, Godin B, Dayan N, Weiss C, Piliponsky A, Levi-Schaffer F. Intracellular delivery mediated by an ethosomal carrier. Biomaterials.2001; 22(22):3053-9.DOI:10.1016/s0142-9612 (01)00052-7.

13. Bhalaria, M, Naik S, Misra AN. Ethosome – a novel delivery system of antifungal drugs in treatment of topical fungal diseases. Indian Journal of Experimental Biology 2009; 47(5):368-75

14. Muslim RK, Maraie NK. Perspective Impact of Gelling Agents on the Mechanistic Behavior for the Topical Delivery of Flufenamic Acid Nano-Ethosomal Dispersion. International Journal of Drug Delivery Technology. 2022; 12(2):789-797.

15. Singh A, Rathore P, Shukla M, Nayak S. Comparative studies on skin permeation of miconazole using different novel carriers. International Journal of Pharmaceutical Sciences and Research. 2010;1(9):61-6. DOI: 10.13040/IJPSR.0975-8232.1(9-S).61-66.

16. El-Shenawy AA, Abdelhafez WA, Ismail A, Kassem AA. Formulation and characterization of nanosized ethosomal formulations of antigout model drug (febuxostat) prepared by cold method: *In vitro*/ex vivo and *in vivo* assessment. Aaps Pharmscitech. 2020; 21:1-3. DOI: 10.1208/s12249-019-1556-z.

17. Chandran SC, Shirwaikar A, Kuriakose MR, Sabna NS. Development and evaluation of ethosomes for transdermal deliveryof Fluconazole. Journal of Chemical, Biological and Physical Sciences. (JCBPS) 2011; 2(1):254.

18. Dara T, Vatanara A, Meybodi MN, Vakilinezhad MA, Malvajerd SS, Vakhshiteh F, Shamsian A, Sharifzadeh M, Kaghazian H, Mosaddegh MH. Erythropoietin-loaded solid lipid nanoparticles: Preparation, optimization, and in vivo evaluation. Colloids and Surfaces B: Biointerfaces. 2019; 178:307-16. DOI:10.1016/j.colsurfb.2019.01.027.

19. Zafar A, Alruwaili NK, Imam SS, Alsaidan OA, Ahmed MM, Yasir M, Warsi MH, Alquraini A, Ghoneim MM, Alshehri S. Development and optimization of hybrid polymeric nanoparticles of apigenin: Physicochemical characterization, antioxidant activity and cytotoxicity evaluation. Sensors. 2022; 22(4):1364. DOI: 10.3390/s22041364

20. Dave V, Sharma S, Yadav RB, Agarwal U. Herbal liposome for the topical delivery of ketoconazole for the effective treatment of seborrheic dermatitis. Applied Nanoscience. 2017:973-87. DOI: 10.1007/s13204-017-0634-3

stress. Phytotherapy research. 2020; 34(5):911-23. DOI: 10.1002/ptr.6577.

21. Mamatha P, Bhikshapathi DVRN. Preparation and In-vitro Evaluation of Pemigatinib Nanosponges Tablets by Box-Behnken Design. International Journal of Pharmaceutical Quality Assurance. 2023;14(3):791-800.

22. El-Menshawe SF, Kharshom R, El Sisi A. Preparation and optimization of buccal propranolol hydrochloride nanoethosomal gel: a novel approach for enhancement of bioavailability. Journal of Nanomedicine & Nanotechnology.2017;8(2):1000435. DOI:10.4172/2157-7439.1000435

23. Zhang Y, Huo M, Zhou J, Zou A, Li W, Yao C, Xie S. DDSolver: an add-in program for modeling and comparison of drug dissolution profiles. The AAPS journal. 2010; 12:263-71. DOI: 10.1208/s12248-010-9185-1

24. Mahendran G, Manoj M, Prasad KR, Bai VN. Antioxidants, anti-proliferative, anti-inflammatory, antidiabetic and antimicrobial effects of isolated compounds from Swertia corymbosa (Grieb.) Wight ex CB Clark–An in vitro approach. Food Science and Human Wellness. 2015 ;4(4):169-79. DOI:10.1016/j. fshw.2015.08.003

25. Pulipaka S, Suttee A, Kumar MR, Kasarla R. Exploration of In-vitro Antidiabetic Activity of ZnO NPs and Ag NPs Synthesized using Methanolic Extracts of Alpinia mutica and Tradescantia spathaeca Leaves. International Journal of Pharmaceutical Quality Assurance. 2023;14(3):464-469.

26. Swati A, Negi PS and Meena HS. A Comparative evaluation of in vitro anti-inflammatory and antifungal activity of Ganoderma lucidum strains DARL-4 and MS-1. International Journal of Green Pharmacy. 2018; 12:S126-S130.

27. Gulluce M, Sahin F, Sokmen MÜ, Ozer H, Daferera D, Sokmen AT, Polissiou M, Adiguzel AY, Ozkan Hİ. Antimicrobial and antioxidant properties of the essential oils and methanol extract from Mentha longifolia L. ssp. longifolia. Food chemistry. 2007; 103(4):1449-56. DOI:10.1016/j.foodchem.2006.10.061

28. Morimoto Y, Aiba Y, Miyanaga K, Hishinuma T, Cui L, Baba T, Hiramatsu K. CID12261165, a flavonoid compound as antibacterial agents against quinolone-resistant Staphylococcus aureus. Scientific Reports. 2023;13(1):1725. DOI: 10.1038/s41598-023-28859-8

29. Kondo S, Ikeda Y, Hattori S, Hamada M, Takeuchi T. Susceptibility of methicillin-resistant Staphylococcus aureus to various antibiotics. Classification by aminoglycoside-modifying enzymes and antibiotics active against MRSA. The Japanese Journal of Antibiotics. 1991; 44(11):1211-5. PMID: 1664469

30. Kornder JD. Streptomycin revisited: molecular action in the microbial cell. Medical hypotheses. 2002; 58(1):34-46. DOI: 10.1054/mehy.2001.1450.

31. Sharma D, Rajput J, Kaith BS, Kaur M, Sharma S. Synthesis of ZnO nanoparticles and study of their antibacterial and antifungal properties. Thin solid films. 2010; 519(3):1224-9. DOI:10.1016/j. tsf.2010.08.073

32. Ilk S, Saglam N, Özgen M. Kaempferol loaded lecithin/chitosan nanoparticles: Preparation, characterization, and their potential applications as a sustainable antifungal agent. Artificial cells, nanomedicine, and biotechnology. 2017; 45(5):907-16. DOI:10. 1080/21691401.2016.1192040

33. Pool H, Quintanar D, de Dios Figueroa J, Mano CM, Bechara JE, Godínez LA, Mendoza S. Antioxidant effects of quercetin and catechin encapsulated into PLGA nanoparticles. Journal of nanomaterials. 2012;2012:86. DOI:10.1155/2012/145380

34. Barbieri S, Sonvico F, Como C, Colombo G, Zani F, Buttini F, Bettini R, Rossi A, Colombo P. Lecithin/chitosan controlled release nanopreparations of tamoxifen citrate: loading, enzyme-trigger release and cell uptake. Journal of Controlled Release. 2013; 167(3):276-83. DOI:10.1016/j.jconrel.2013.02.009

35. Souza MP, Vaz AF, Correia MT, Cerqueira MA, Vicente AA, Carneiro-da-Cunha MG. Quercetin-loaded lecithin/chitosan nanoparticles for functional food applications. Food and bioprocess technology. 2014:1149-59. DOI: 10.1007/s11947-013-1160-2

36. Elmas A, Akyüz G, Bergal A, Andaç M, Andaç Ö. Mathematical modelling of drug release. Research on Engineering Structures and Materials. 2020;6(4). DOI:10.17515/resm2020.178na0122

37. Dharmadeva S, Galgamuwa LS, Prasadinie C, Kumarasinghe N. In vitro anti-inflammatory activity of Ficus racemosa L. bark using albumin denaturation method. Ayu. 2018; 39(4):239. DOI: 10.4103/ayu.AYU_27_18

38. Wall C, Lim R, Poljak M, Lappas M. Dietary flavonoids as therapeutics for preterm birth: luteolin and kaempferol suppress inflammation in human gestational tissues in vitro. Oxidative medicine and cellular longevity. 2013. DOI:10.1155/2013/485201

39. Yang Y, Chen Z, Zhao X, Xie H, Du L, Gao H, Xie C. Mechanisms of Kaempferol in the treatment of diabetes: A comprehensive and latest review. Frontiers in Endocrinology. 2022; 13:990299. DOI:10.3389/fendo.2022.990299

40. Li AP, He YH, Zhang SY, Shi YP. Antibacterial activity and action mechanism of flavonoids against phytopathogenic bacteria. Pesticide Biochemistry and Physiology. 2022; 188:105221. DOI: 10.1016/j.pestbp.2022.105221