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Effects of Enhancment Span-60 of *Vernonia amygdalina* Leaves Extract-loaded Niosomes

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ABSTRACT

***Introdiction**: Niosomes are a promising drug carrier because of their bilayer structure and the fact that they are produced by the self-association of nonionic surfactants and cholesterol in an aqueous phase. Niosomes are non-toxic, biodegradable, and compatible with human cells.* ***Aims**: This research aimed to create niosomes from V. amygdalina leaves and investigate how span 60 addition affects niosome properties.* ***Methods:** Extraction V. amygdalina using maceration, niosomes synthesized using a thin-film hydration process, and characterization performed by SEM, particle size, polydispersity index, zeta potential, and FTIR.* ***Conclusion:** V. amygdalina succesfully to loaded into niosomes. Span-60 with various concentration affected of characterization of niosomes. Increased span-60 increasing particle size, polydispersity index, and %EE.*

KEYWORDS: *Cholesterol, extract-loaded niosomes, niosomes characterization, nonionic surfactan, span 60*

INTRODUCTION

Niosomes are vesicular, novel drug delivery system, which can be used for the sustained, controlled as well as targeted delivery of drugs (Bhardwaj et al., 2020). Niosomes also contain a cholesterol core at their center. Niosomes are nonimmunogenic, biodegradable, and biocompatible. They have a long shelf life, a high level of stability, and the ability to deliver the medicine to the target site in a regulated and/or sustained way. These are all desirable qualities (Ag Seleci et al., 2016). Niosomes have been the subject of intense research over the past several years due to their possible use as a medication carrier (Ag Seleci et al., 2016; Li et al., 2014). It has been demonstrated that different nonionic surfactants can combine to generate niosomes, making it possible to encapsulate various medicines with varying soluble potential (Bayindir et al., 2015; Mehta & Jindal, 2015).

Niosomes have the ability to increase the bioavailability of medications by raising both their solubility in water and their permeability

across biological membranes. Niosomes are not water-soluble themselves (Cetin et al., 2022). Therefore, niosomes are very suitable to be used as carriers for the extract to enhance their efficacy, especially *Vernonia amygdalina* leaf extract.

It is widely acknowledged that *V. amygdalina* is among the most well-known plant species native to both Africa and Asia. Within the genus Vernonia, which has over a thousand different kinds of shrubs, this particular species is the one that is cultivated the most. Patients suffering from malaria drank a tea made from the bluish-green powder to reduce hiccups, fevers, kidney difficulties, and stomach disorders6. The bluish-green powder was developed by the Medical Traditional Healer Association in Rukararwe, which is located in Uganda. Vernoniosides extracted from the leaves of *V. amygdalina* have been shown to have anti-inflammatory effects in studies conducted on murine macrophage cell lines and on wild chimpanzees. These extracts have also been used to treat gastrointestinal ailments (Alaraa et al., 2017; Quasie et al., 2016).

The current study varies from previous ones in that it first creates an extract of V. amygdalina leaves, then loads it onto niosomes with active components, analyzes the loading of the extract's success, and finally characterizes them.. This study aimed to prepare niosome *V. amygdalina* leaves extract and investigation the effect of span addition on niosome characterization. Furthermore, they exhibit improved mechanical properties when compared to the span 60, giving them tremendous potential to improve the solubility and oral bioavailability of poorly soluble pharmaceuticals, as well as to improve drug permeability when applied topically.

METHODS

Material

We used afrika leaves harvested in the Samata district of Gowa, South Sulawesi, for this study. Gallic acid, cholesterol, and span 60 were supplied by Sigma-Aldrich. Folin-Ciocalteu, sodium carbonate, and chloroform by Merk. The rest of the chemicals were of analytical grade.

Extraction

The leaves of Afrika were washed, dried, sorted, and ground into powder. To obtain afrika leaves extract (ALE), the powder (100 g) was macerated with 1000 mL 96% ethanol and evaporated by rotary evaporator at 45°C, at 45-50 rpm.

Phytochemical screening

Phytochemical screening was carried out to identify the content of bioactive compounds in ALE including alkaloids, flavonoids, saponins, tannins, and steroids/triterpenoids referring to the Farnsworth procedure (Farnsworth, 1966).

Total phenolic content (TPC)

The total phenolic content was determined using a Folin-Ciocalteu reagent modified acc-

ording to Pourmorad's method (Pourmorad et al., 2006). Following the addition of each 0.5 mL extract to 5 mL of Folin-Ciocalteu reagent 10% and 4 mL of sodium carbonate 1 M, the mixture was allowed to incubate for 15 minutes. The absorbance was determined by measuring it at λ 765 nm. The results were expressed as mg gallic acid equivalents (GAE) per g extract (mg GAE/g) in accordance with the calibration curve comprised of gallic acid at concentrations ranging from 20-125 µg/mL as the standard.

Preparation of niosomes ALE (NS-ALE)

Niosomes were prepared following procedure by Abd-Elghany with slight modification(Abd-Elghany et al., 2022). In order to produce NS-ALE, a method known as thin-film hydration was applied. In a beaker with a circular bottom, Span 60 was dissolved in chloroform and methanol (1:1) at various concentrations (see Table I), and cholesterol was also dissolved in the mixture. The chloroform-methanol mixture was evaporated at a temperature of 60ºC under lowered pressure using a rotary evaporator spinning at a speed of 100 rpm in order to produce a dry, thin layer. After that, a final mass concentration of 10 mg/10 mL was accomplished by hydrating the thin film in PBS (pH 7.4) containing 10 mg of ALE. The multilamellar niosomes that were created were then sonicated for a period of twenty-four hours, which led to the formation of tiny vesicles. In the end, the niosomes were precipitated in PBS by employing a high-speed (4000 rpm 30 min), cooling centrifuge (Hettich EBA 21).

Characterization

Scanning electron microscopic (SEM)

SEM (JCM-600 plus, JEOL) had been used to evaluate the surface morphology of the proposed niosome formulation(Kamble et al., 2013).

Particle size, polydispersity index, and zeta-potential

DeltaTM nano C Particles Analyzer (Beckman Coulter) with a zeta-potential measurement facility were used to determine the niosomes' polydispersity index and particle size distribution. Particle size were read in 3000-30000 laser light intensity(Kamble et al., 2013).

Fourier transform infrared (FTIR) spectroscopy.

Using Fourier-transform infrared (FT-IR) spectroscopy (Nicollet iS 10 FTIR spectrometer, Thermo fisher), the possible interaction between Span 60, cholesterol, and ALE was studied for niosomes containing ALE throughout a wavenumber range of 4000 to 400 cm. This study was conducted for ALE-containing niosomes(Abou-Taleb et al., 2022).

Determination of entrapment efficiency

The entrapment efficiency (EE%) of the produced niosomes was determined by calculating the percent of the bioactive content that was entrapped within the centrifuged niosomes. This percentage matched to the total

amount of bioactive chemical (TPC) that was present in the first stage of the process. A spectrophotometric analysis was used to quantify the total phenolic content of NS-ALE, and the results were used into the calculation of EE%. The EE% was determined by applying the following equation:

EE% = [((Wi-Wf)/Wi] X 100

where; Wi was the amount of total phenolic content and Wf was the amount of free total phenolic content (Gunes et al., 2017).

Statistical analysis

GraphPad® software version 3 was used for statistical analysis, which included one-way ANOVA and post hoc Tukey's test. The statistical significance level was chosen at $P \leq 0.05$. To depict all data, mean ± standard deviation (SD) was used to represent all data.

RESULTS AND DISCUSSION

Extraction yield and phytochemical screening of ALE

The yields of the extracts, as well as the total phenolic content of ALE, are presented in Table 2. The yield of ALE 18.65%. The alkaloids, flavonoids, saponins, tannins, and steroid/triterpenoid were detected positively in ALE (Table 2). These results are consistent with those previously reported by Ali et al. (Ali et al., 2019).

Table 1. Composition of ALE noisomes.

Composition	Formula		
	F1	F2	F3
ALE (mg)	10	10	10
Span 60 (µmol)	1	2	3
Cholesterol (µmol)	1	1	1

TPC

The amounts of TPC were determined in the ALE as GAE and they were compared using the findings that are shown in Table 3. The TPC of ALE was showed highest than Harahap et al. (2021) reported (54.61 ± 0.94 mg GAE/g extract) but lower than Alara et al. (2017) reported (114.03 ± 1.25 mg GAE/g extract) (Alara et al., 2019; Harahap et al., 2021). We assumed that different extraction methods give different result. The soxhlet methods give optimum phenolic content than maceration (Alara et al., 2018).

Characterization NS-ALE

Niosomes made with cholesterol-Span 60 revealed, by SEM, that the vesicles they formed had a spherical shape. (figure 1). Previous research also reported that noisome using cholesterol:span showed spherical structure(Asaithambi et al., 2020; Kamble et al., 2013; Kumbhar et al., 2013). The size range of the prepared Span 60-based niosomes was on average between 400 ± 30 to 989 ± 10 nm, a polydispersity index of 0.276 ± 0.05 to 0.395 ± 0.04 and a zeta-potential of -30 ± 1.1

Table 2. Yield and phytochemic screening of ALE.

Sample	Yield (%)	Alkaloids	Flavonoids	Saponins	Tannins	Steroid/ triterpenoid
ALE	18.65%	+	+	+	+	+

Table 3. Total phenol content of ALE and NS-ALE.

Sample	TPC (mg GAE/g sample)
ALE	64.46 ± 0.40
F1	4.88 ± 0.98
F2	5.72 ± 0.64
F3	6.68 ± 0.540

n = 3

to -31.4 ± 2.3 mV, indicating to the formulation featuring high homogeneity as well as electrophoretic stability. Characterization of NS-ALE showed in Table 4.

The size of the vesicles varied drastically with surfactant concentration ranging from 1 to 3 µmol. However, 3 µmol of span 60 caused a significant increase in particle size (Table II). It's possible that the higher concentration is what's making the vesicles unstable, resulting in a very big particle size. The formula 1 had the smallest size (400.8 ± 1.3 nm). NS-ALE has a larger particle size than previous research by Zubairu et al. (2015) and Kemble et al. (2013).

In addition, the values of the zeta potential show how stable the niosomal formulations that were created are. A substantial negative zeta potential is a crucial signal of aggregation avoidance (Marianecci et al., 2012). A nanosuspension that is exclusively held together by electrostatic repulsion needs to have a zeta potential of at least ±30 mV in order to be considered physically stable. (Marianecci et al., 2012; Müller et al., 2001). However, a zeta potential of less than 20 mV is considered to be acceptable in the event of simultaneous electrostatic and steric stabilization(Jacobs & Müller, 2002; Müller et al., 2001). The Zeta-potential of all formulations in this analysis was negative. It's possible that this has something to do with knowing that cholesterol and surfactant molecules contain free carboxyl groups(Sjögren et al., 2005). The zeta-potential values of the various formulations did not differ from one another in a way that was statistically significant.

FTIR analysis

In Figure 2, we have a representation of the FTIR spectrum matching method that was applied in order to identify any potential interactions that may have occurred between ALE, excipients, and NS-ALE in the wavenumber range 4000-400 cm-1. Bands were found by the ALE at wavenumbers 3430.74, 2931.79, and 1627.42 cm-1. These wavenumbers correspond to the -OH group, the C-H group, and the C=C group, respectively. The spectra of Span 60 exhibited

Table 4. Comparative characters of NS-ALE.

Formulation	Particle size (nm)	Zeta potential (mV)	Polydispersity index	EE (%)
F1	400.8 ± 1.3^{a}	-30 ± 1.1^{a}	$0{,}276 \pm 0.02^{a}$	7.57 ± 0.52^{a}
F2	605.8 ± 2.1^{b}	-31.4 ± 2.3^{a}	$0{,}304 \pm 0.01^{b}$	8.88 ± 0.21^{b}
F3	989.8 ± 3.0^{c}	-31.1 ± 1.2^{a}	$0{,}395 \pm 0.01^{c}$	10.36 ± 0.31^{c}

* a – c, with the same letter means no significant difference ($p > 0.05$). n = 3

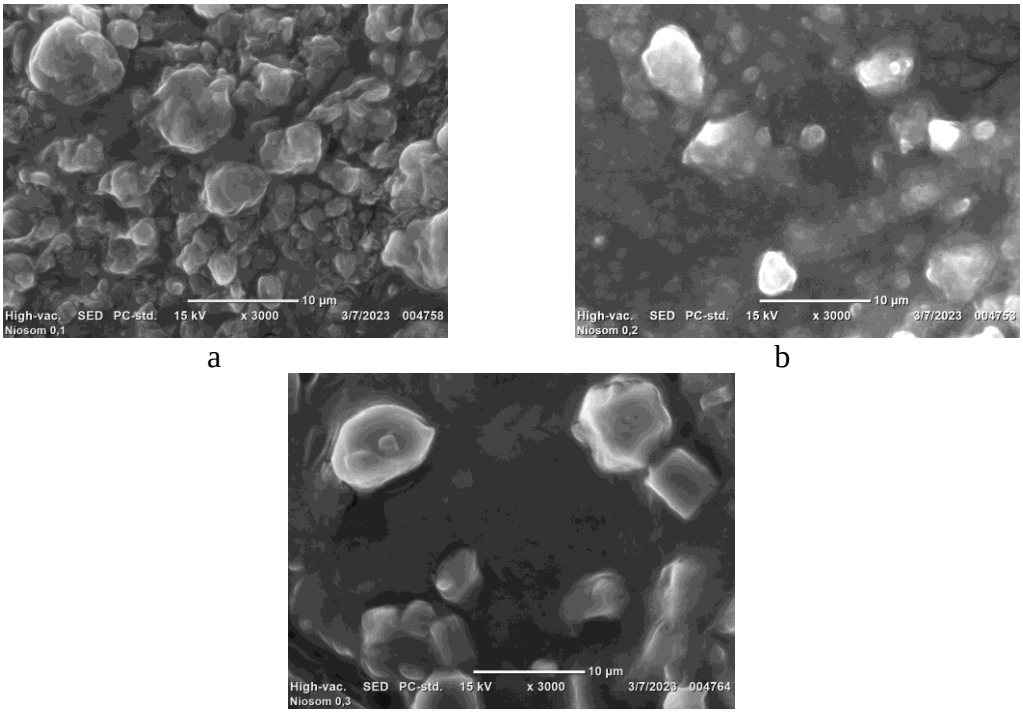

Figure 1. Scanning Electron Microscopy analysis of ALE loaded niosomes. a: F1; b: F2; c: F3

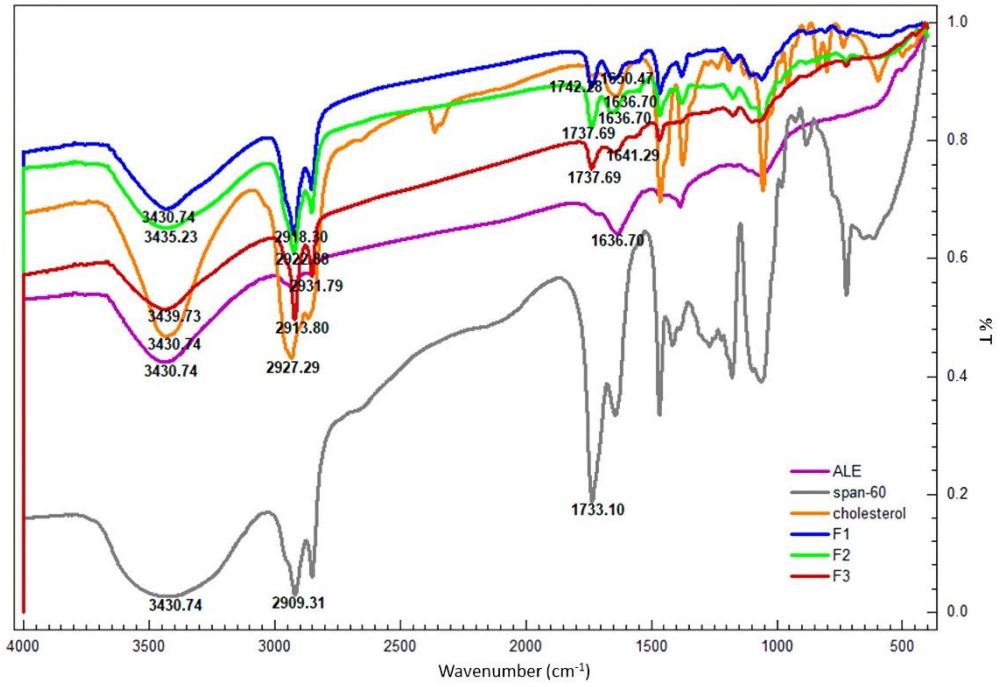

Figure 2. FTIR spectra of NS-ALE.

bands at wavenumbers 3430.74, 2909.31, and 1737.24 cm-1, each of which related to a different group: -OH, C-H, and -COO. The presence of bands at wavenumbers 3430.74 and 2927.29 cm-1 in cholesterol is indicative of -OH and C-H stretching, respectively. The physical mixing of the aforementioned components resulted in the presence of all of the bands at wavenumbers 3439.73-3430.74, 1742.31, and 2922.88-2913.80 cm-1, which correspond to -OH stretching, -COO stretching, and C-H stretching, respectively.

The findings revealed no interaction between ALE and the formulation's other excipients.

Entrapment efficiency

The %EE of NS-ALE was measured spectrophotometrically using the TPC technique. Table II displays the NS-ALE EE result. Formula 3 had the greatest %EE, with a value of 10.36 ± 0.31%. This research shown that when the concentration of span 60 increases, so does the%EE. Shehata et al. (2021) also reported the same thing, where the %EE increased as the span concentration increased (Shehata et al., 2021).

CONCLUSION

Through the utilization of the thin film hydration method, ALE-loaded niosomes were successfully synthesized. Morphological studies revealed that the NS-ALE were spherical vesicles that were closed. The characterisation of niosomes is affected by the addition of span-60 at varying doses. Particle size, polydispersity index, and % EE all rise when there is a higher concentration of span 60. In future, the developed niosomes will be evaluated for storage's effect on noisome characterization.

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