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Optimization and Formulation of Mixed Niosome/Cyclodextrin Encapsulation Process for Béjaia Propolis Extract

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Abstract

This study focuses on the preparation and optimization of a mixed cyclodextrin and niosomal suspension containing propolis extract from the Melbou region. The experimental design was utilized to determine the key parameters influencing encapsulation efficiency and particle size. The extraction yield of propolis was found to be 12.16%. The flavonoid content, specifically quercetin, was quantified using UV-visible analysis.
The optimal niosomal suspension exhibited an encapsulation efficiency of 34.81% and an average particle size of 557.283 nm, compared to 426.80 nm for the placebo. Thermogravimetric analysis (TGA) revealed enhanced thermal stability of the encapsulated extract, with significant improvement compared to the unencapsulated extract. The niosomal formulation delayed the onset of mass loss at 220°C, indicating effective preservation of volatile and bioactive components.
These findings suggest that niosomal encapsulation can enhance the stability and bioavailability of propolis extract, offering promising applications in pharmaceutical and nutraceutical formulations.

Keywords: *Natural product, propolis extract, niosomes, mixte encapsulation, cyclodextrins.*

I. Introduction

For millennia, bee products, particularly propolis, have been used by humans for their numerous beneficial properties such as antimicrobial, anti-inflammatory, antioxidant, and wound healing activities [1]. Propolis is a resinous substance collected by bees from the buds and bark of certain trees and mixed with their salivary secretions [2]. It exhibits a characteristic balsamic odor and a range of colors. Despite its recognized biological properties, the application of propolis is often limited by its poor solubility in aqueous media, reduced bioavailability, and chemical degradation [3].
To overcome these limitations, encapsulation by cyclodextrins [4, 5]or niosomes [6] are a promising strategy. Cyclodextrins (CDs), natural oligosaccharides, can encapsulate hydrophobic molecules, thus enhancing their solubility, stability, and bioavailability. Concurrently, niosomes are nanocarriers capable of loading both hydrophilic and lipophilic substances, making them particularly suitable for drug delivery [7].
Mixed encapsulation, combining the benefits of CDs and niosomes, represents an innovative approach to optimize the delivery of active compounds. This method aims to improve the protection and efficacy of propolis formulations [8, 9].
In fact, Machado et al.(2018) explored the interest of niosomes prepared from Span 80 and Tween 80 enhanced with β-cyclodextrin (β-CD) or modified amphiphilic β-CD (Mod-β-CD) to encapsulate methyl orange (MO) and methyl yellow (MY). This modification improved dye encapsulation efficiency and release rates, especially for MO, without significant changes in vesicle size and morphology [8].
Our study focuses on the optimization and characterization of the mixed niosome/cyclodextrin encapsulation of propolis extract from Bejaia region. To the best of our knowledge, this is the first time this technique has been applied to Algerian propolis. The originality of this work lies in the dual use of CDs and niosomes for shared encapsulation of the propolis extract. This dual encapsulation aims to offer superior protection and enhance the bioavailability of propolis.

II. Material and methods

II.1. Material

II.1.1. Chemicals

Quercetin, aluminum chloride ($AlCl_3$), Span 60 and cholesterol were purchased from Sigma-Aldrich;
Methanol and ethanol (Biochem Chemopharma)
Sodium nitrite ($NaNO_2$) (Biochem Chemopharma)
Sodium hydroxide (NaOH) (Biochem Chemopharma)
PM-βCD were obtained from Orsan (France). All reagents were of analytical grade.

II.1.2. Raw propolis

The raw propolis is collected in the region of Melbou (Bejaïa) Algeria with geographical coordinate: 36°38′23″, 5°21′39″, in the month of March 2024.

II.2. Methods

II.2.1.Propolis extraction

Ethanolic extracts of propolis (EEP) are obtained using an agitation method. The chosen parameters include the amount of propolis (33.33 g), duration (41 minutes), with the volume and temperature of the solvent fixed at 100 ml and 50 °C, respectively [10].

II.2.2. Preparation of niosomal solutions with optimal conditions using experimental design

To determine the optimal conditions for the encapsulation process, a Box-Behnken experimental design were carried out.
The factors were: cyclodextrin concentration (C CD) [3.333-30 mg/ml], Span concentration (C Sp) [20-60 mg/ml], and cholesterol concentration (C Ch) [5-20 mg/ml]. The responses studied were encapsulation efficiency (EE) and the size of the formed niosomes. This method enabled us to optimize the formulation, achieving the best encapsulation conditions for the propolis extract. The experimental matrix is presented in the Table 1.
In this process, two distinct phases were prepared: an aqueous phase containing PM-βCD and the extract diluted in water, and an organic phase composed of cholesterol, Span 60, and ethanol.

II.2.3. Encapsulation efficiency: Quantification of flavonoids (Quercetin)

The quantification of flavonoids. such as quercetin aims to assess the concentration of these compounds in samples. Flavonoids are phytochemical compounds found in many plant-based foods such as fruits, vegetables, wine, and tea. Quercetin in particular is a well studied flavonoid known for its potential antioxidant, anti-inflammatory and other health benefits [11].
This method relies on the use of AlCl3 to form a yellow complex with flavonols and flavones. 400 µl of the extract are mixed with 120 µl of NaNO2 and left to rest for 5 minutes. Then 120 µl of AlCl3 solution are added to the mixture stirred and allowed to react for 6 minutes. After this period 800 µl of NaOH are introduced. The absorbance of the solution is measured at a wavelength of 510 nm. Simultaneously, a calibration curve for quercetin is established at different concentrations. This method allows for the precise quantification of quercetin in various samples providing valuable insights into the flavonoid content and potential health benefits of the analyzed extracts [10].

Table 1: The experimental matrix

Exp No	C CD (mg/ml)	C Sp (mg/ml)	C Ch (mg/ml)
1	3.33	20	12.5
2	30	20	12.5
3	3.33	60	12.5
4	30	60	12.5
5	3.33	40	5
6	30	40	5
7	3.33	40	20
8	30	40	20
9	16.67	20	5
10	16.67	60	5
11	16.67	20	20
12	16.67	60	20
13	16.67	40	12.5
14	16.67	40	12.5
15	16.67	40	12.5

II.2.4. Determination of niosome size

To determine the size of niosome suspensions using an optical microscope, a drop of the suspension is placed on a slide and covered with a coverslip. An ocular micrometer calibrated with an objective micrometer is used to measure the size of the niosomes. The divisions on the ocular micrometer, converted into length units through calibration, allow for the calculation of the niosome size by multiplying by the calibration factor.

II.2.5. Preparation of Optimum niosomal solution and its validation

We identified the optimal niosome manufacturing conditions for the encapsulation system. To validate the experimental design, the optimal formulation was prepared using 3.333 mg/ml of PM-βCD, 20 mg/ml of Span 60 and 20 mg/ml of cholesterol and the responses were evaluated (the blank sample or placebo prepared using the same conditions).

II.2.6. Characterization of optimal mixed niosomal solution

II.2.6.1. Thermogravimetric analysis (TGA)

Thermogravimetric analysis (TGA) was conducted using a Mettler Toledo thermogravimetric analyzer. Approximately

22 mg of extract. 76.5 mg of niosomal formulation. or 33.5 mg of placebo were analyzed. Measurements were taken from 40°C to 600°C at a heating rate of 20°C/min under inert atmosphere.

III. Results and discussion

III.1. Extraction yield of propolis

The extraction of bioactive compounds from propolis, such as flavonoids and phenolic compounds, was performed using a solvent evaporation method. This procedure produced a viscous brown extract, known as "ethanolic propolis extract". The extraction yield is expressed as a mass percentage, indicating the mass of dry extract obtained relative to the mass of raw propolis. The yield of the ethanolic propolis extract depends not only on the extraction techniques used but also on the chemical composition of the raw propolis itself. The yield of the ethanolic propolis extract from the Melbou region was determined at 12.16%.

III.2. Quantification of flavonoids in propolis extract (Quercetin)

III.2.1. Calibration curve

The results are presented in the form of a quercetin calibration curve (Figure 1).

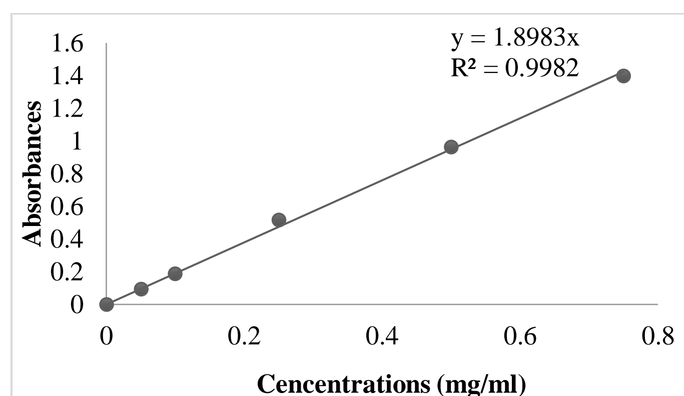

Figure 1: Quercetin calibration curve for flavonoid quantification.

The calibration line passes through the origin, providing a linear equation of the form y=a*x, where y is the absorbance, a is the slope, and x is the quercetin concentration. The equation is:y=1.8983*x and R^2=0.999.

The curve Abs=f(C) demonstrates the proportionality between the absorbance and quercetin concentration. This relationship is supported by the correlation coefficient R^2, which is close to 1 with a value of 0.999, confirming a very strong correlation between the two variables. Using this curve, the concentration of the propolis extract was determined to be 0.1275 mg/ml.

III.3. Determination of experimental design responses

Following the preparation of niosome suspensions (extract-PM-βCD-niosomes) by varying the quantities of Span 60 (C Sp) and cholesterol (C Ch) according to the experimental design, both the encapsulation efficiency (E.E) and particle sizes were determined (Table 2).

The results indicate that Span 60 has a significant impact on particle size, with a notable increase at higher quantities. PM-βCD also appears to influence particle size, although its effect is less pronounced than that of Span 60. Conversely, cholesterol has a relatively minor impact on particle size compared to the other components. The encapsulation efficiency increases with higher amounts of Span 60 and PM-βCD, while cholesterol does not significantly influence the encapsulation efficiency.

The results suggest that the use of Span 60 generally leads to the formation of larger and more stable niosomes due to its long alkyl chain. This enhances the encapsulation efficiency of hydrophobic active substances, such as propolis, due to the hydrophobic properties of Span 60 and the rigidity it imparts to the membrane. In contrast, PM-βCD improves the encapsulation capacity by increasing both stability and solubility. Cholesterol's effect is only apparent when its quantity is increased. Same results were found by Taouzinet et al [12] when studing the encapsulation of vitamin E by liposomes [12].

Mathematical Model

The modeling using the Box-Behnken experimental design allowed us to obtain the following model:

For particle size:

$$Size=1390.17+37.4849*CD-140.667*Span-11.0627*CHL-205.051*CD^2+151.424*Span^2-75.5692*CHL^2+7.31327*CD*Span+6.17966*CD*CHL+61.6237*Span*CHL.$$

For encapsulation efficiency:

$$EE=41.5452+3.66721*CD+2.01403*Span-0.0883176*CHL+2.36712*CD^2+0.619395*Span^2+6.17367*CHL^2-0.544675*CD*Span-2.33982*CD*CHL-2.00022*Span*CHL.$$

Statistical Analysis

The statistical parameters for particle size and encapsulation efficiency are summarized in Table 3. These parameters include R^2, Adjusted R^2, Lack of Fit, and Reproducibility. R^2 and Q^2, represent the model's ability to explain and predict the observed results, respectively. When these parameters are close to 1, it indicates that the model can explain the variation in the data and provide accurate predictions, confirming the model's validity.

These parameters demonstrate the reliability and validity of the model in explaining and predicting the particle size and encapsulation efficiency, supporting its use in further optimization studies.

Table 2: Determination of experimental design responses

Exp No	C CD (mg/ml)	C Sp (mg/ml)	C Ch (mg/ml)	Size (nm)	EE (%)
1	3.33	20	12.5	466.2	38.02
2	30	20	12.5	628.43	52.12
3	3.33	60	12.5	947.91	43.65
4	30	60	12.5	1050.56	54.01
5	3.33	40	5	1122.09	50.05
6	30	40	5	1229.34	64.53
7	3.33	40	20	1090.35	55.89
8	30	40	20	1201.75	54.79
9	16.67	20	5	1199.28	46
10	16.67	60	5	1311.45	60.1
11	16.67	20	20	973.33	53.69
12	16.67	60	20	1478.82	55.2
13	16.67	40	12.5	1427.4	43.18
14	16.67	40	12.5	1365.52	40.19
15	16.67	40	12.5	1427.4	40

Table 3: Statistical analysis values.

Parameters	R2	R2 Adj	Q2	Lack of Fit	Reproducibility
Size	0.985	0.959	0.775	0.216	0.984
EE	0.957	0.879	0.656	0.241	0.949

III.4. Propolis extract entrapped in CD/noisome caracterisation

III.4.1. validation of optimal noisomal solution

After preparing the niosomal suspensions according to the parameters defined in the experimental design. The particle size of the optimum reached 557.283 nm and 426.80 nm for placebo; on the other hand, the encapsulation efficiency was 34.81%.

According to our results an increase in particles size was observed in the former, confirming that the extract has been encapsulated into the niosomes vesicles.

III.4.2. Thermogravimetric Analysis (TGA)

The thermogravimetric analysis (TGA) (Figures 2) reveals distinct thermal degradation patterns for the propolis extract and the niosomal encapsulated propolis extract. The unencapsulated propolis extract shows a significant mass loss starting at approximately 50°C. This initial decrease is likely due to the evaporation of volatile compounds present in the propolis extract such as essential oils and alcohols. These compounds are known for their high volatility, which leads to their evaporation at lower temperatures [4]. The degradation process continues steadily up to 400°C, indicating the breakdown of various bioactive compounds within the extract.

In contrast, the TGA profile for the CD/niosomal encapsulated propolis extract demonstrates enhanced thermal stability. The mass loss does not begin until around 220°C, suggesting that the encapsulation effectively prevents the early evaporation of volatile components. This encapsulation effect is crucial as it indicates that the volatile and potentially bioactive compounds of the propolis extract are retained within the cyclodextrin and niosomes, thus enhancing their stability. Beyond 220°C, the degradation pattern of the encapsulated extract mirrors that of the placebo, indicative of the breakdown of the niosomal components such as cholesterol, Span 60, and cyclodextrin, eventually leading to total decomposition at around 400°C.

Comparing these results with existing literature, it is evident that encapsulation via niosomes significantly improves the thermal stability of bioactive compounds. For instance, Ghumman et al. (2023) observed that encapsulating curcumin in niosomes enhanced its thermal stability, preventing significant degradation up to 200°C, similar to our findings with propolis [13]. Similarly, Sun et al. (2018) demonstrated that the encapsulation of essential oils in cyclodextrins, liposomes or niosomes protected the volatile compounds from early evaporation, thereby preserving their bioactivity during thermal processing [14, 15].

These comparisons underscore the effectiveness of niosomal encapsulation in stabilizing thermally sensitive bioactive compounds. The delay in the onset of degradation for the encapsulated propolis extract highlights the potential of niosomes as a robust delivery system for enhancing the stability and efficacy of natural extracts in various applications, including pharmaceuticals and nutraceuticals.

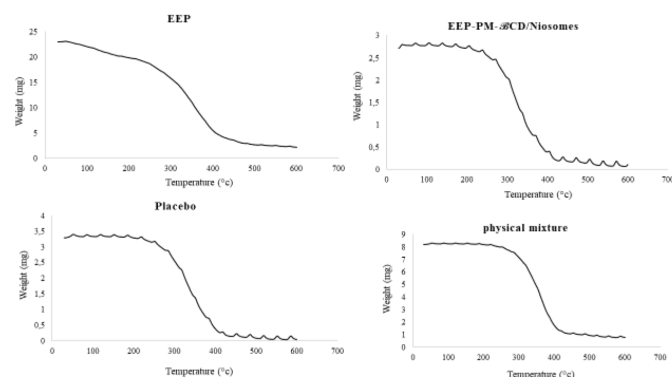

Figure 2: Thermogravimetric graphs of EEP, encapsulated EEP, placebo (blank) and physical mixture respectively.

IV. Conclusions

This study successfully prepared and optimized a CD/niosomal suspension containing propolis extract from the Melbou region (Béjaia).

The optimal CD/niosomal suspension demonstrated significant improvements in thermal stability compared to the unencapsulated extract, as confirmed by thermogravimetric analysis (TGA). The extraction yield of propolis from the Melbou region was determined to be 12.16%.

Using UV-visible analysistotal flavonoid content was determined, specifically quercetin, in the propolis extract. The encapsulation efficiency of the optimal CD/niosomal suspension was calculated to be 34.81%, with an average particle size of 557.283 nm, compared to 426.80 nm for the placebo.The enhanced thermal stability of the encapsulated extract, with a delayed onset of mass loss at 220°C, indicates effective preservation of volatile and bioactive components. These results highlight the potential of CD/niosomal encapsulation to enhance the stability and bioavailability of Algerian propolis extract, promising applications in pharmaceutical and nutraceutical formulations.

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Conflict of interest
The authors declare no conflict of interest. financial or otherwise.

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