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Formulation, optimization and evaluation of niosomal suspension loaded with *Cinnammomum zeylanicum* and *Zingiber officinale* for allievating symptoms of PCOS

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Abstract

Often characterized by insulin resistance, hyperandrogenism, and chronic anovulation, Polycystic Ovary Syndrome (PCOS) is a common endocrine condition affecting women of reproductive age. Side effects and insufficient symptom alleviation are common problems with current treatments. The goal of this study is to reduce the symptoms of PCOS by formulating, optimizing, and testing a niosome suspension containing bioactive components from Zingiber officinale (ginger) and Cinnamomum zeylanicum (cinnamon). The ether injection approach was used to create niosomes, which improve the stability and bioavailability of medications enclosed. Several formulation parameters were methodically optimized, such as the kind of surfactant, the amount of cholesterol. The in vitro release kinetics, zeta potential, encapsulation efficiency, and particle size of the resultant niosome suspension were all measured. Niosome suspensions are significant because they have the ability to get around the drawbacks of traditional medication delivery methods. Therapeutic results can be improved by increasing the solubility, preventing degradation, and achieving a regulated release of bioactive chemicals by encasing them in niosomes. The potential of this method in controlling PCOS is further supported by the use of natural extracts, such as cinnamon and ginger, which are known to have anti-inflammatory, antioxidant, and insulin-sensitizing characteristics.

Key words : PCOS, Niosome, Suspension, *Cinnammomum zeylanicum, Zingiber officinale.*

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PCOS is a complex endocrine condition that affects a considerable proportion of women who are fertile. Estimates of its prevalence vary from 6% to 20% worldwide. PCOS is a condition marked by hyperandrogenism, polycystic ovaries, and recurrent anovulation. It is linked to a wide range of symptoms, such as irregular menstrual periods, infertility, hirsutism, acne, and obesity. Furthermore, insulin resistance and PCOS are frequently associated, which raises the risk of type 2 diabetes and cardiovascular illnesses. Even with PCOS's high frequency and serious health consequences, available treatments are still inadequate and frequently only partially relieve symptoms while having unfavorable side effects[1,2].

A rising interest in natural chemicals with possible therapeutic benefits has resulted from the hunt for safe, well-tolerated, and effective medicinal options. Known for their anti-inflammatory, antioxidant, and insulin-sensitizing qualities are two such botanicals: Zingibern officinale (ginger) and Cinnamomum zeylanicum (cinnamon). Both ginger and cinnamon have strong anti-inflammatory and antioxidant properties and have been demonstrated to increase insulin sensitivity and lower fasting blood glucose levels. As such, they are both viable options for the treatment of PCOS symptoms.[3]

Nevertheless, the limited bioavailability and stability of these bioactive molecules restricts their clinical utility. Niosomes are vesicular systems made of cholesterol and non-ionic surfactants that present a promising way around these restrictions. Both hydrophilic and lipophilic compounds can be encapsulated by niosomes, which increases their bioavailability, prevents degradation, and permits regulated release. For the encapsulation and administration of extracts containing cinnamon and ginger, niosomes present an appealing delivery mechanism.[4]

The purpose of this study is to formulate, optimize, and evaluate a niosome suspension that contains extracts from Zingibe rofficinale and Cinnamomum zeylanicum to help with PCOS symptoms. The ether injection method, which is well-known for yielding vesicles with a restricted size distribution and good encapsulation effectiveness, will be employed to generate the niosomes. To produce the ideal niosome suspension, a methodical strategy will be used to optimize the formulation variables, such as surfactant and cholesterol concentration.Then, the zeta potential, encapsulation efficiency, and particle size of the optimized niosome suspension will be assessed. The goal of the study is to show that the niosome-based delivery system is a novel and efficient treatment option for PCOS, which is a common condition, by considerably reducing its symptoms.[5]

Materials :

Cinnammomum Zeylanicum and *Zingiber Officinale* extracts were purchased from AMSAR PRIVATE LTD, Indore, Madhya Pradesh, India.Non ionic surfactant and Cholesterol were supplied by ANALAB FINE CHEMICALS, Pimpri-Chinchwad,Pune, Maharastra, India and were used without any modification.

Preformulation study of drugs :

The Goal of Preformulation research is to determine the physicochemical characteristics

of drugs. Preformulation criteria such as organoleptic properties (table-1), phytochemical testing (table-3) and solubility studies (table-2) were assessed for both drugs. Additionally investigations were conducted utilizing a variety of analytical tools like including FTIR, DSC, UV spectroscopy and HPTLC.

UV Visible spectroscopy :

Standard curve of cinnamon and ginger :

By plotting concentration v/s absorbance, a calibration curve was created using ethanol in the concentration range of 2,4,6,8, and 10 µg/ml. The estimated regression coefficient values for the two medications were determined to be 0.993 and 0.999, respectively. A graphical illustration of the calibration curve for cinnamon and ginger is provided in Figures 1 and 2.

FTIR studies :

The IR absorption spectra of both drugs was measured in the 4000-200 cm range. Purity was rated based on the key peaks. Infrared absorption spectra reveal functional groups unique to a specific molecule. Figures 3 and 4 show graphical representations of cinnamon and ginger IR spectra.

Preparation of Cinnammomum zeylanicum and Zingiber officinale loaded niosomes:

Niosomes were prepared using ether injection method. Accuretly weighed amounts of Cholesterol and Surfactant were dissolved in 8ml of diethyl ether. Accuretly weighed amount of herbal extract were dissolved in 2 ml of methanol. The methanolic Solution were added to the solution of Surfactant and Cholesterol. The resultant Solution was added drop by drop to Phosphate buffer pH 7.4 heated at 60-65^{o} C using a syringe with continuous stirring. The resultant suspension was stirred for another one hour to get perfectly spherical niosomes in the suspension. Batches were formulated and the optimized batch was selected based on the best results.

Formulation and optimization of Cinnammomum zeylanicum and Zingiber officinale loaded niosomes:

Using Design Expert Software, the *Cinnammomum zeylanicum* and *Zingiber officinale* loaded formulation were formulated and optimized. We considered many other design strategies before deciding on the randomised central composite design since it is suited for thoroughly examining every potential impact of every variable on response. Two independent variable were chosen, namely the surfactant concentration (X1) and the cholesterol concentration (X2).The dependent variable were the percentage of entrapment and the particle size. To choose an optimised batch, 13 formulation batches were made and assessed. Table-5 displays the stages of noisome optimization as well as the independent and dependent variable.[6]

Evaluation of Niosomes :

Vesicle size analysis :

Using a digital microscope and pixel pro software, the size shape and lameller character of the vesicles in the sonicated formulation were examined. A stage micrometer

was used to calibrate the microscope. A drop of the surface after appropriate dilution, and the slide was examined under a 10x magnification using a labomed microscope.

Entrapment efficiency :

To separate niosomes from non-entrapped medication, niosomal formulation was centrifuged at 10000 rpm at 4°C for 30 minutes using a cooling centrifuge. The amount of free drug in the supernatant was measured using a UV spectrophotometer (Schimadzu, UV 1700, Japan) at 372 and 288 nm after centrifugation. The formula used to compute the percentage of drug entrapment in niosomes was then applied.

Percent drug entrapment = (Total drug - drug in supernatant / Total drug) x 100

In vitro Drug release :

A predetermined volume of the niosome suspension was poured into the dialysis tube, and both ends were tightly sealed. The filled tube was then submerged in a buffer solution in a beaker that had been brought to pH 7.4, which was meant to mimic physiological conditions. Throughout the experiment, the beaker was kept at 37°C and gently shaken with a low-speed shaker to closely replicate physiological circumstances.

Samples of the release medium were taken out at prearranged intervals, usually lasting one to twenty-four hours. To keep sink conditions stable, each removed sample was replaced with an equivalent volume of brand-new, pre-warmed release medium. The amount of medicine released was then measured by UV spectrophotometry.[7,8]

Organoleptic properties :

Table-1. Organoleptic properties of the extracts

Organoleptic properties	Cinnamon	Ginger
Colour	Reddish brown colour	Light brown
Odour	Woody, dominant	Fresh, woody, citrus
Texture	Soft and crumbly	Crumbly and fibrous
Taste	Spicy, warm	Warm, spicy, slightly sweet

Solubility profile :

Table-2. Solubility profile of the extracts

Solvent	Cinnamon	Ginger
Distilled water	Sparingly soluble	Sparingly soluble
Ethanol	Soluble	Soluble
Methanol	Soluble	Soluble
Phosphate buffer	Completely soluble	Complete soluble

Phytochemical analysis :

Table-3. Phytochemical analysis of the extracts

Phytoconstituent	Cinnamon	Ginger
Flavanoids	Present	Present
Saponins	Present	Present
Tannins	Present	Absent
Alkaloids	Present	Present
Terpenoids	Present	Present
Glycoside	Absent	Absent

Optimization of cinnamon and ginger niosomes :

Trial batches (fig. 9) were evaluated to find the optimized batch (fig. 10) which gave the perfect evaluation results.

Effect of variables on vesicle size :

The vesicle size of the niosomes depends on the type of the surfactant being used. Span 60 gives large vesicles with a larger

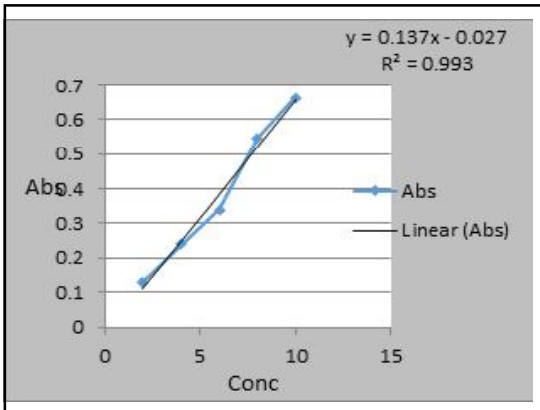

Fig. 1. Calibration curve of cinnamon

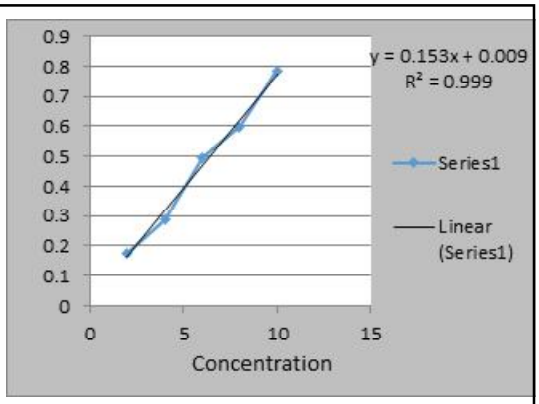

Fig. 2. Calibration curve of ginger

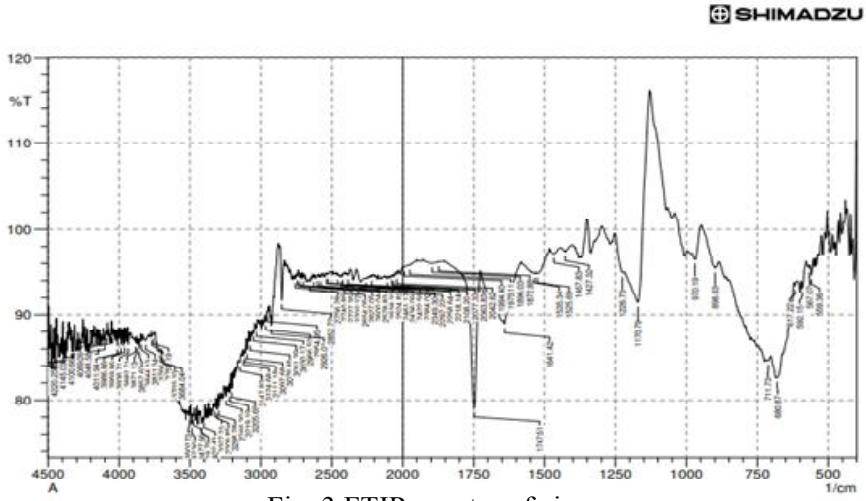

Fig. 3 FTIR spectra of cinnamon

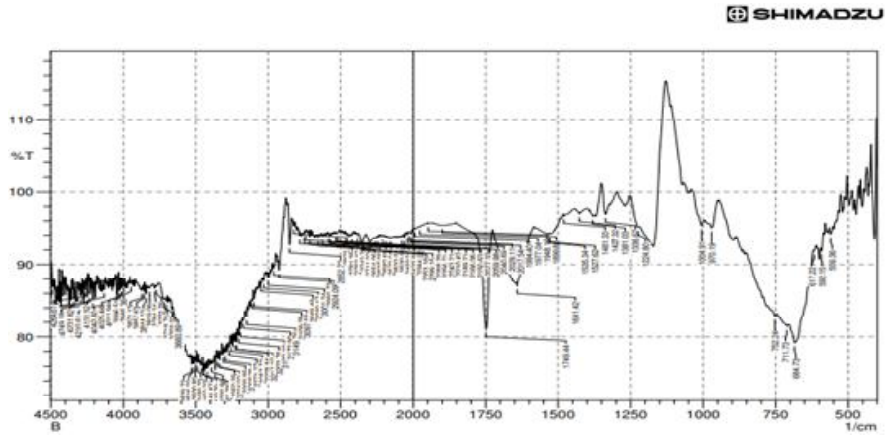

Fig. 4. FTIR spectra of ginger

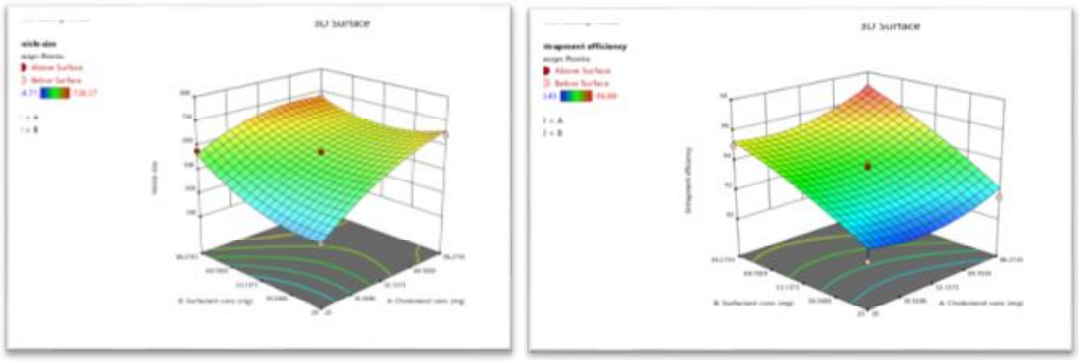

Fig. 5. 3D surface graph for vesicle size (left) and entrapment efficiency (right)of cinnamon niosomes

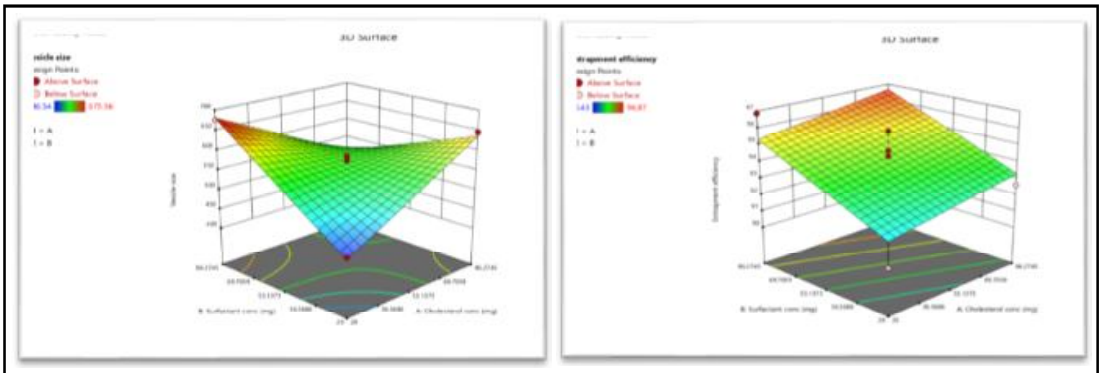

Fig. 63D surface graph for vesicle size (left) and entrapment efficiency (right) of ginger niosomes

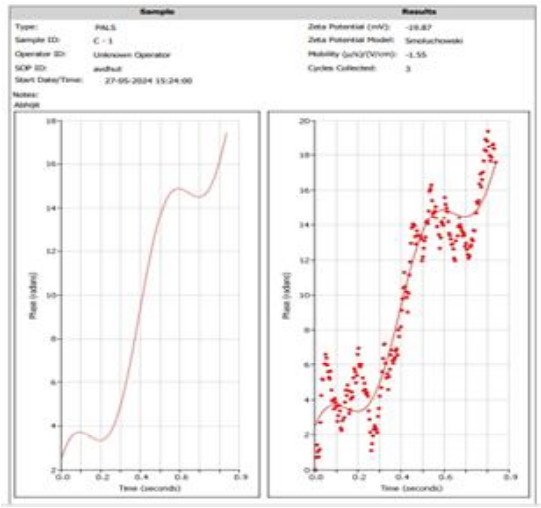

Fig. 7 Zeta potential of niosomal suspension

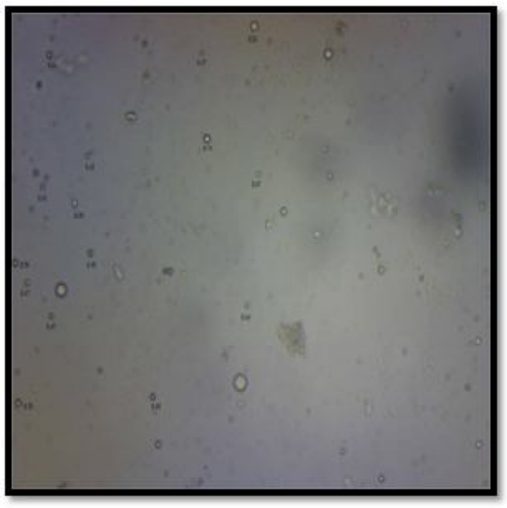

Fig. 8 Microscopic images of niosomal vesicle

Fig. 9. Batches of niosomal suspension

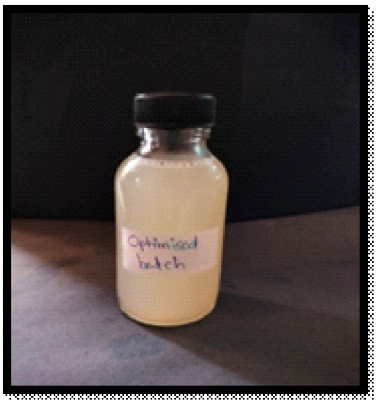

Fig. 10 Optimized batch of the niosomal suspension

diameter. This can be explained on the basis of the structure of the surfactant. Surfactant with larger alkyl chain give larger vesicle size. The size of formed vesicles is determined by the hydrophilic lipophilic balance of the Span employed. Lowering the HLB results in smaller vesicles due to a decrease in surface free energy with increased hydrophobicity. With an increase in the concentration of cholesterol the vesicle size increases, this may be due to the increase in the bending modulus of the vesicular membranesi.e the increase in the total elastic energy of the niosomal population. To reduce free energy in equilibrium, indicate that the system "trends" toward increasing the quantity of large vesicles, as the elastic energy of such vesicles is lower than that of tiny ones (fig. 5 & 6).[9,10]

Effect of variable on percent entrapment :

The percentage of entrapment increases with the cholesterol concentration of the bilayers[11]. Including cholesterol in formulations leads to greater drug entrapment due to larger vesicles and wider lipid bilayers[12] HLB of surfactant has an inverse connection with entrapment efficiency. Increasing the HLB of a surfactant decreases its entrapment efficiency (fig. 5 & 6).[13]

In vitro Drug Release :

Table-4 demonstrate the cumulative % release of *Zingiber officinale* and *Cinnamomum zeylanicum* extracts at different time intervals and the corresponding models checked with. The drug release mechanism was ascertained by fitting the release data to different kinetic models. First-order, zero-order, Higuchi, and Korsmeyer-Peppas models were among those examined. presents the findings. The Korsmeyer-Peppas model exhibited the strongest correlation coefficients (R^2) for both extracts, indicating that a combination of erosion and diffusion processes is involved in the release mechanism.[14]

Characterization of optimized niosomes :

Table-4. In vitro Drug release profile and corresponding models of the niosomal formulations

Time[hr]	Cummulative % release of cinnamon	Cummulative % release of ginger
1	8.1± 0.6	7.8 ±0.5
2	15.1± 0.6	13.7± 0.6
4	22.5± 0.8	21.6± 0.7
6	30.1± 1.0	28.7± 0.8
8	38.2± 1.3	36.8± 1.2
12	47.5± 1.5	45.4 ±1.3
24	61.4± 1.6	59.5± 1.4
48	75.6± 1.8	73.3± 1.6
72	88.3± 1.9	86.7± 1.8

Model	R^2 (cinnamon)	R^2 (Ginger)
Zero order	0.935	0.929
First order	0.911	0.904
higuchi	0.958	0.952
Korsmeyer peppas	0.970	0.965

Table-5. Factors used for design of experiment

Independent variables	Factors	Levels	
X_1	Concentration of surfactant	20	100
X_2	Concentration of cholesterol	20	100
Dependent variables			
Y_1	Vesicle size		
Y_2	% Entrapment		

Zeta potential of optimized niosomes :

The zeta potential is a key factor in the stability of Niosomes as it determines the electrostatic or charge repulsion/attraction between particles. It is a crucial characteristic that influences stability. A negative or neutral value indicates that niosome particles have no charge and the system is stable. The Zeta potential was determined using the Zeta Sizer (Malvern Instrument, UK) as shown in figure 7. The optimized batch of niosome formulation has a zeta potential of -19.0 mV. This negative zeta potential value indicates the stability of niosomes.

Vesicle size of optimized formulation :

The vesicle size of the formulation is examined using a digital microscope. The improved batch of niosomes was diluted with distilled water and viewed under a microscope at 10X magnification. Figure 8 shows that an optimized batch had a particle size of 0.4µm under a digital microscope.

Ether injection technique was used to formulate niosomal suspension. Design expert software is utilized to optimize niosomes. The prepared niosomes were assessed for drug release, vesicle size, and entrapment percentage. 6th batch out of 13 that were formulated was determined to be the best batch for niosomes. According to a design expert the ideal range for particle size is 300 to 700 nanometers. Furthermore, it was discovered that the vesicle size of an optimized batch had a vesicle size range of 400 – 700 nm, indicating its significance. The intended range of values for % entrapment efficiency of drug entrapment ranges from 98.02% to 99.51%. Furthermore, the drug entrapment efficiency value for optimized batch was discovered to be 98.89%, indicating the optimized batch's entrapment efficiency that the batch can accumulate a considerable amount of drug in its vesicles. The optimized batches were subjected to zeta potential analysis, revealing that the niosomal formulations had good stability. Each and every result was appropriate and suitable as per the pre-established standards. The obtained results of the study indicate the possibility and scope for further studies involving the presently studied herbs.

Conflict of interest :

The authors declare that there are no conflicts of interest amongst the authors.

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