

RESEARCH ARTICLE

Development and Evaluation of Polyherbal Phyto-phospholipid Complexes (Phytosomes) for PCOD Treatment

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ABSTRACT

Polycystic ovary disorder (PCOD) is a common endocrine condition affecting women of reproductive age that is distinguished by hyperandrogenism, ovulatory failure, and polycystic ovaries. It is still not entirely apparent what causes polycystic ovary syndrome (PCOS), and the treatments that are currently available tend to focus more on alleviating symptoms than on addressing the underlying causes. The bioavailability and effectiveness of bioactive chemicals can be improved by the use of phytosomes, which are sophisticated delivery methods. Phytosomes for the treatment of polycystic ovary syndrome (PCOS) are the primary focus of this study. Materials such as milk thistle (*Silybum marianum*), curcumin (*Curcuma longa*), and *Ginkgo biloba* were utilized with the purpose of highlighting the potential benefits of these substances in the treatment of PCOD. A fourier-transform infrared (FTIR) analysis was carried out, and the results of the *ex-vivo* analysis confirmed that the formulation PS3 with a ratio of 2:1 exhibited a greater release profile.

Keywords: Polycystic ovary disorder, Phytosomes, Milk thistle, Bioavailability, Insulin resistance, Oxidative stress, Anti-inflammatory, Herbal extracts.

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INTRODUCTION

A considerable number of women of reproductive age all over the world are affected by polycystic ovary disorder (PCOD), which is a complicated hormonal condition. Approximately 6 to 10% of women of reproductive age around the world are affected by it. According to the findings of a few studies, the prevalence could be as high as 15 to 20% when utilizing more comprehensive diagnostic criteria. PCOD, which is characterized by the presence of numerous cysts on the ovaries, is a condition that induces disruptions in the normal menstrual cycle and can result in a variety of symptoms. These symptoms include excessive menstrual bleeding, irregular periods, and anovulation, which is the absence of ovulation. There is no established explanation for PCOD, although it is thought to be caused by a confluence of factors, including those related to lifestyle, environment, and genetics. Insulin resistance is a typical trait that contributes to increased

testosterone production. At the core of PCOD is the presence of hormonal imbalances, namely excessive levels of androgens (male hormones) and luteinizing hormone (LH), as well as frequently low levels of follicle-stimulating hormone (FSH), which hinders ovulation. A poor diet, a lack of physical activity, and obesity are all lifestyle variables that contribute to the worsening of insulin resistance and hormonal abnormalities. Women who have PCOD frequently have increased levels of inflammatory markers, which can further contribute to insulin resistance and ovarian dysfunction. Inflammation is also found to be involved in this condition.¹ There is a possibility that environmental variables, like as exposure to substances that affect the endocrine system, also have a role. The formation and continued presence of PCOD is a multidimensional illness that calls for an all-encompassing care strategy. This syndrome is caused by the interaction of a number of variables, including genetic, hormonal, metabolic, and environmental factors. The

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treatment consists of an all-encompassing and multi-pronged approach that is specifically designed to manage symptoms and correct the hormonal imbalances that are at the root of the condition. Modifications to one's lifestyle are an essential component in the management of PCOD. These modifications include a balanced diet that is abundant in whole grains, lean proteins, fruits, and vegetables while reducing processed carbs. This is done in order to enhance insulin sensitivity and assist in weight control. In order to improve insulin sensitivity, maintain a healthy weight, and regulate menstrual cycles, it is essential to engage in regular physical activity. This activity should include both aerobic workouts and strength training. It is possible for individuals who are overweight or obese to experience significant relief from symptoms and an improvement in their hormonal profiles with even a moderate weight loss of 5 to 10%.

A phytosome is a herbal formulation that contains the active compounds of a plant extract that have formed a bond with phospholipids, often phosphatidylcholine, in order to enhance the bioavailability and absorption of the formulation. Phospholipids, often phosphatidylcholine, are attached to the active components that are present in extracts of herbs in order to produce these substances. This complexation method makes it simpler for the active ingredient compounds to pass through biological membranes and be absorbed.² It does this by increasing the solubility of the compounds in lipid and aqueous environments. Therefore, in contrast to traditional herbal extracts, phytosomal compositions have the potential to result in higher quantities of the active components in the blood. The phospholipid coating protects the active components from the enzymes and gastric acids that are produced by the digestive tract. This helps to ensure that the active chemicals continue to retain their potency until they are able to enter circulation.

The leaves of the *Ginkgo* tree are the source of the herb known as *Ginkgo biloba*. It is generally known that the phytosome derived from *G. biloba* has vasodilatory properties, which increase the flow of blood through the body. In patients with PCOD, increasing circulation can be advantageous, particularly in terms of maintaining ovarian function and general reproductive health. *G. biloba* has a strong antioxidant capacity, which aids in lowering oxidative stress, which is a typical problem in polycystic ovary syndrome in patients.^{1,3} Insulin resistance can be made worse by oxidative stress, which also has the capacity to alter endocrine function and may contribute to the maintenance of hormonal equilibrium. Curcumin is the principal active ingredient of turmeric (*Curcuma longa*). In order to improve the bioavailability and therapeutic efficiency of curcumin, an improved formulation called curcumin phytosome has been manufactured. There is evidence that it can increase insulin sensitivity by altering the pathways that are involved in insulin signaling. Increased insulin sensitivity can assist in the regulation of blood glucose levels, the reduction of hyperinsulinemia, and the consequent reduction of androgen production, which is frequently associated with polycystic ovary syndrome (PCOS).

Indirectly lowering testosterone levels, curcumin can help improve symptoms such as hirsutism (excessive hair growth), acne, and irregular menstrual periods. This is accomplished *via* reducing insulin resistance and inflammation, which are both contributors to the condition. A better hormonal balance can also improve fertility in people who have PCOS. Milk thistle, also known as *Silybum marianum*, is a flowering herb that has been discovered to contain a high concentration of silymarin and to possess hepatoprotective and antioxidant effects.³ Research has demonstrated that it can enhance insulin sensitivity, which in turn can assist in the regulation of blood glucose levels and the reduction of insulin levels. As a result of this improvement, androgen levels may drop, and symptoms of PCOD, such as acne and hirsutism, may become less severe.

MATERIALS AND METHODS

Materials

Milk thistle extract and curcumin extract were obtained from India Mart. *G. biloba* extract was purchased from NutriJa. N-hexane and ethanol were obtained from Sigma Aldrich. Every chemical utilized was analytical grade, and it was utilized exactly as it was delivered.

Flavonoid Screening

To a volume of 2 mL of extract, two to three drops of sodium hydroxide were applied. Flavonoids were present because the substance initially had a dark yellow color, but once a few drops of diluted hydrochloric acid were added, the color progressively disappeared, suggesting that flavonoids were present.

Tannins Screening

About 200 mg of extract and 10 mL of distilled water were introduced into a water bath and brought to a boil. After being filtered, the filtrate was combined with a ferric chloride solution that had a weight-to-volume ratio of 5%.⁴ The observation of a solution that was a dark green color served as evidence of the presence of tannins.

Terpenoid Screening

The Salkowski test was utilized in order to identify terpenoids. In order to create a layer, 5 mL of extract was combined with 2 mL of chloroform, and then 3 mL of strong sulfuric acid was added. For the purpose of demonstrating that the presence of terpenoids was there, the interface was colored in a way that was reddish-brown in appearance.⁵

Carbohydrate Screening (Barfoed's test)

A test tube was filled with 1-mL of each of the extract aqueous solution and Barfoed's reagent, and the mixture was heated in a water bath for 2 minutes. The presence of the crimson precipitate indicates the presence of carbs.

Partition Coefficient

About 10 mg of the herbal extract combination were dispersed and agitated individually for 30 to 40 minutes in a 20 mL (1:1) mixture of octanol and buffer with a pH of 7.4.⁶ After some time had passed, the water and oil layers were separated, and a

Table 1: The design of the phytosome formulation

Formulation	Phytosomal extract (gm)	Soy lecithin (gm)	Extract: Soy lecithin (w/w)
PS1	1	1	1:1
PS2	1	2	1:2
PS3	2	1	2:1
PS4	2	2	2:2
PS5	3	1	3:1

UV-vis spectro-photometer was utilized in order to determine the amount of extract that was dissolved in each phase. With the assistance of the following formula, the partition coefficient (K) of the drug was determined when it was calculated.

$K = \text{concentration of drug in organic phase} / \text{Conc of drug in aqueous phase}$

Fourier-transform Infrared Spectroscopy

The wavelength range of fourier-transform infrared spectroscopy (FTIR) for the analysis of phytosomes of milk thistle, curcumin extract, and *G. biloba* is typically in the mid-infrared region, spanning from approximately 4000 to 400 cm^{-1} . The identification of characteristic peaks that are associated with the many different functional groups that are present in phytosome complexes is made possible by this range being used.⁷ The findings obtained from the infrared spectrophotometer model number 234 manufactured by Perkin Elmer were recorded.

Rotary Evaporation Technique for Herbal Extracts Loaded Phytosomes

The appropriate quantity of herbal extracts and soy lecithin were mixed along with 20 mL of organic solvents, such as acetone, in a flask with a rotating round bottom. The mixture was agitated for three hours at a temperature ranging from 40 to 45°C. N-hexane was applied to the sample in a very thin layer, and a magnetic stirrer was used to provide continuous stirring of the mixture (Table 1).⁸ During the process of collecting and storing the precipitate that was produced at room temperature, a container made of amber-colored glass was utilized.

Particle Size Determination

An electric field was used to measure zeta potential using electrophoretic mobility. Zeta potential was measured at 25°C using the same apparatus after dilution in a 1 mM NaCl solution.

Percentage Yield

The prepared phytosomes underwent complete drying and precise weighing. This weight was subsequently divided by the combined weight of the drugs and nonvolatile recipients.

Determination of Entrapment Efficiency

About 50 mL of distilled water was used to distribute 100 mg of dried phytosomes. After two hours of stirring, the liquid was filtered using 45-micron-pore Whatman filter paper. It was then appropriately diluted and analyzed spectrophotometrically at

λ_{max} 273. The standard calibration curve was used to determine the amount of medication that was entrapped.

Scanning Electron Microscopy

Using a JSM-6490LV made by JOEL, Japan, morphological features were examined using scanning electron microscopy (SEM) at NIPER. Spread out over a circular aluminum stub that had been previously treated with silver glue to improve electron conductivity. The phytosomes were then positioned inside the viewing region of the apparatus. Micrographs were obtained and they were analyzed under a SEM at different magnifications. Using a secondary electron detector, the samples were examined under low vacuum.

Ex-vivo Studies

A Franz diffusion cell with a surface area of 0.75 cm^2 was utilized in order to enable the ex vivo release of phytosome extracts. An egg membrane was present, which served to divide the donor chamber from the receptor chamber. To simulate physiological circumstances, a phosphate buffer with a pH of 7.4 was maintained at $37 \pm 10^\circ\text{C}$. Additionally, a magnetic bead was used to continually mix the phosphate buffer inside the receptor compartment.⁹ It was determined that one milliliter of phytosomal suspension was sufficient to fill the donor chamber. Using a UV-vis spectrophotometer at various wavelengths and comparing the results to a suitable blank at 273 nm, the concentration of the extracts was measured at various times and at varied wavelengths.¹⁰

RESULTS AND DISCUSSIONS

Qualitative Phytochemical Screening

Qualitative phytochemical screening revealed that a combination of milk thistle extract, curcumin extract, and *G. biloba* included flavonoids, tannin, terpenoids, and carbs. Tanning agents, flavonoids, and polyphenols were discovered to be present in curcumin. Flavonoids were discovered in milk thistle, while *G. biloba* was shown to contain flavonoids, polysaccharides, and terpenoids alongside flavonoids. In spite of the fact that a mixture of extracts demonstrated a lower solubility in water (12.08 mg/mL) compared to the pH 7.4 buffer (19.03 mg/mL), it was soluble in ethanol nonetheless. When a number of different herbal extracts were put through their paces, the partition coefficient was shown to be 0.887.

Evaluation of Phytosomes

The lowest particle size was correlated with a low phospholipid content. Zeta potential creates an energy barrier that is repulsive and stops particles from aggregating, making it an essential physical feature for vesicle stability prediction. All suspended particles resist one another and are not likely to cluster whether they have strong negative or positive zeta potentials. All phytosomes had zeta potentials between -20.4 and -29.6 mV. Their charge greatly influences the stability of vesicles and their interactions with skin vesicles. It was discovered that elevating the phospholipid ratio improved the phytosomes' entrapment effectiveness and percentage yield.

The phytosomal formulation PS4, created using the rotary evaporator technique with a 2:2 herbal extract-phospholipid ratio, exhibited the highest entrapment efficiency at 65.91%. In contrast, PS5, with a 3:1 ratio, showed the lowest entrapment efficiency (Figure 1). The entrapment efficiency of phytosomes is influenced by the phospholipid content. Initially, increasing the phospholipid concentration improved entrapment efficiency. Larger particle sizes are undesired for high-quality phytosomes, nevertheless, as more phospholipid concentration was added beyond a certain point. Because the phytochemical and the charged phospholipid's polar head interact to enhance phytochemical conjugation with the polar heads on the vessel's inner and outer walls, the increased efficiency may be explained by this interaction.

SEM of phytosomes revealed the presence of spheroid or irregular shapes with rough surface morphology. SEM provided crucial insights into the solid-state characteristics and surface morphology of the complexes (Figure 2). Active compounds can appear highly crystalline under SEM; however, these structured crystals disappear after complexation. When diluted in distilled water and gently shaken, SEM showed that phytosomes possess vesicle-like morphologies.

FTIR Study

For the purpose of structural investigation, FTIR is an effective technique that provides a wide range of functional groups in addition to distinct band numbers, positions, forms, and intensity characteristics.¹¹ A comparison of the spectroscopy of a crude medicine is one method that can be utilized to

validate the growth of phytosomes (Table 2). Flavonolignans, more specifically silymarin, are the primary components of milk thistle, also known as *S. marianum*. Milk thistle exhibits distinguishable peaks in its FTIR spectrum. These peaks include a broad peak around 3400 to 3200 cm^{-1} , which corresponds to O-H stretching vibrations that are indicative of hydroxyl groups; a sharp peak near 1730 cm^{-1} , which is indicative of C=O stretching vibrations of ester groups; and peaks around 1600 to 1400 cm^{-1} , which are attributed to aromatic C=C stretching vibrations originating from flavonoid structures. Furthermore, the presence of ether connections is shown by the appearance of C-O-C stretching vibrations in the region of 1200 to 1000 cm^{-1} during the experiment. Curcumin Extract, which is derived from *C. longa*, contains a high concentration of curcuminoids, with curcumin being the primary component. A large peak at 3500 to 3200 cm^{-1} for O-H stretching, a peak near 1620 to 1600 cm^{-1} for C=O stretching of the conjugated ketone group, and strong peaks around 1500 to 1400 cm^{-1} for aromatic C=C stretching are all typical peaks that may be observed in the fourier transform infrared spectrum of curcumin. At around 1270 to 1200 cm^{-1} , it is obvious that C-O-C stretching vibrations are present, which is a reflection of the methoxy groups that are present in curcumin. There are several different flavonoids and terpenoids that can be found in *G. biloba*. A number of significant peaks can be observed in its Fourier transform infrared spectrum. These include broad O-H stretching vibrations around 3400 to 3200 cm^{-1} , which are indicative of flavonoids containing hydroxyl groups; sharp C=O stretching peaks near 1730 cm^{-1} , which are associated with esters and ketones; and multiple peaks in the range of 1600 to 1400 cm^{-1} , which are attributed to aromatic C=C stretching vibrations. It is common for flavonoid glycosides and

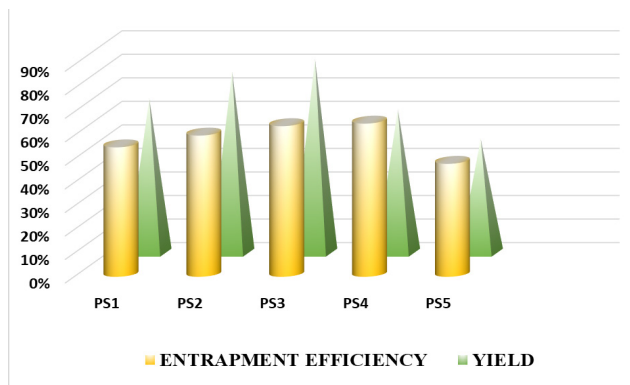


Figure 1: Entrapment efficiency and yield of phytosomes

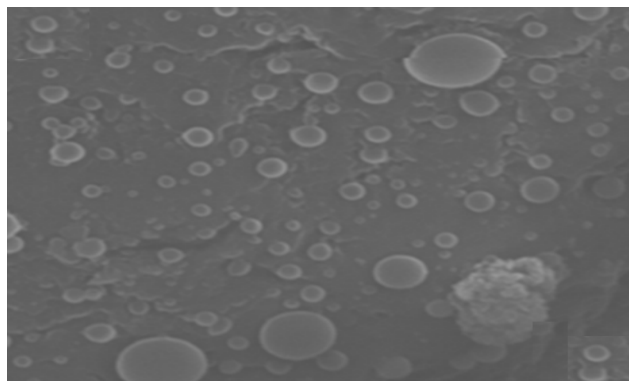


Figure 2: SEM image of PS3

Table 2: FTIR peaks of milk thistle, curcumin extract and *G. biloba*

Functional group	Milk thistle	Curcumin extract (cm^{-1})	<i>G. biloba</i>
C-H	2852	-	1450–1400
O-H	3500–3200	3363	3511–3179
C=C	17289	1624	1694
C-O	1289–1226	1325	-
C=O	1722	1729–1725	1700

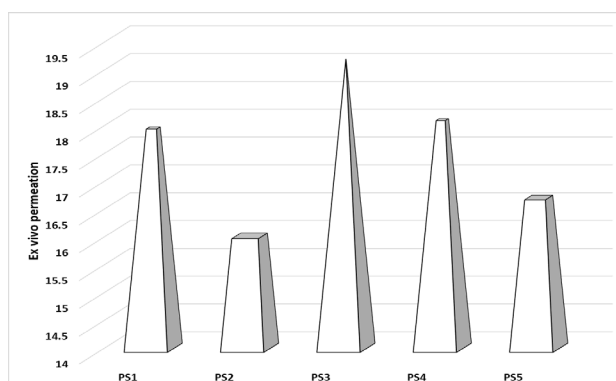


Figure 3: Ex-vivo release pattern of phytosomes

terpenoid esters to exhibit C-O-C stretching vibrations, which are represented as peaks in the range of 1200 to 1000 cm^{-1} .

Ex-vivo Studies

The evaluation of the permeation of phytosome formulations and herbal extracts in phosphate buffer (pH 7.4) is illustrated in Figure 3. An evaluation and comparison of the enhancement in the phytosome release profile in dissolving media for the release of herbal extract over a period of up to five hours was carried out with the use of ultraviolet analysis. The release of herbal extracts, on the other hand, was able to explain the PS3 phytosome's distinctive characteristic of prolonged release. This was demonstrated by the fact that the release of herbal extracts continuously grew until it reached 98.07% after 5 hours. The increased wettability and solubility of the herbal extract may be related to the changes in phytosome release rates.¹² This is because the enhanced phytosomes have greater wettability. As to the assessment, one of the reasons behind the herbal extract's low water solubility was its partly amorphous state, which also caused the dissolution rate to accelerate to five hours. The dispersion of herbal extracts grew more efficient in a way that depended on time and dosage. Herbal extracts are also more easily able to pass across the membrane due to the interaction of phospholipids with it. Excess phospholipids block the phytosomes' ability to release the extract, causing a prolonged release pattern at high chemical concentrations. When phytosomes are combined at a ratio of around 2:1 with phospholipid and herbal extract, their effectiveness is greatly increased.

CONCLUSION

Phytosomes, an improved delivery technology that improves the bioavailability and therapeutic efficacy of herbal extracts, have demonstrated encouraging results in the treatment of PCOD. The incorporation of bioactive chemicals derived from plants such as milk thistle, curcumin, and *G. biloba* into phytosomes has the potential to greatly enhance the levels of absorption and efficiency of these compounds. These plants' phytosomes have the potential to be used as a treatment for PCOS, according to the screening and other investigations. From the results of the FTIR study, it was discovered that phytosomes had a range of medical benefits, including anti-inflammatory, anti-cancer, and antioxidant properties. PS3 was discovered to have the highest drug release. The enhanced distribution and absorption of bioactive substances such as silymarin, curcumin, and elements of *G. biloba* can address important pathological characteristics of PCOS, such as insulin resistance, hormonal imbalance, inflammation, and oxidative stress.

REFERENCES

1. Kasim SF, Saadoon SJ. The Correlation of BMI and Age to Some Hormonal Indices in Iraqi Women with the Polycystic Ovarian Syndrome. *International Journal of Drug Delivery Technology*. 2022;12(2):829-833. <https://doi.org/10.25258/ijddt.12.2.64>
2. McCartney CR, and Marshall JC. Clinical practice. Polycystic ovary syndrome. *N Engl J Med*. 2016; 375:54–64. <https://doi.org/10.1056/nejmcp1514916>
3. Conway GS, Honour JW, and Jacobs HS. Heterogeneity of the polycystic ovary syndrome: clinical, endocrine and ultrasound features in 556 patients. *Clin Endocrinol (Oxf)*. 1989; 30(4):459-70. <https://doi.org/10.1111/j.1365-2265.1989.tb00446.x>
4. Tiwari R, Tiwari G, Sharma S, Ramachandran V. An Exploration of herbal extracts loaded phyto-phospholipid complexes (Phytosomes) against polycystic ovarian syndrome: Formulation considerations. *Pharmaceutical Nanotechnology*. 2023;11(1):44-55. <https://doi.org/10.2174/2211738510666220919125434>
5. Anjana R, Kumar S, Sharma H, Khar R. Phytosome drug delivery of natural products: A promising technique for enhancing bioavailability. *International Journal of Drug Delivery Technology*. 2017;7(03):157-65. <https://doi.org/10.25258/ijddt.v7i03.9559>
6. Cuomo J, Appendino G, Dern AS, Schneider E, McKinnon TP, et al. Comparative absorption of a standardized curcuminoid mixture and its lecithin formulation. *J Nat Prod*. 2011;74: 664-669. <https://doi.org/10.1021/np1007262>
7. Anjana R, Kumar S, Sharma H, Khar R. Phytosome drug delivery of natural products: A promising technique for enhancing bioavailability. *International Journal of Drug Delivery Technology*. 2017;7(03):157-65. <https://doi.org/10.25258/ijddt.v7i03.9559>
8. Saumya D, Kumar DM, Anamika. Antibacterial and Antifungal Potential of Berberine Isolated from Stem of *Berberis aristata* DC. *International Journal of Drug Delivery Technology*. 2023;13(2):454-460. <https://doi.org/10.25258/ijddt.13.2.01>
9. Hussein H, Abid AJ, Alsaadi ZH. Screening for Antibacterial and Antibiofilm Activity of Some Plant Extract and Chlorhexidine against *Streptococcus mutans*. *International Journal of Drug Delivery Technology*. 2022;12(2):610-615. <https://doi.org/10.25258/ijddt.v7i03.9554>
10. Joshi, A. PCOD (Polycystic Ovarian Disease): A Review Article. *ECS Transactions*. 2022;107(1):15973. <https://doi.org/10.1149/10701.15973ecst>
11. Burger, C. W., Korsen, T., Van Kessel, H., Van Dop, P. A., Caron, F. J. M., & Schoemaker, J. Pulsatile luteinizing hormone patterns in the follicular phase of the menstrual cycle, polycystic ovarian disease (PCOD) and non-PCOD secondary amenorrhea. *The Journal of Clinical Endocrinology & Metabolism*. 1985;61(6):1126-32. <https://doi.org/10.1210/jcem-61-6-1126>
12. Shenoy, P. B., & Brundha, M. P. Awareness of polycystic ovarian disease among females of age group 18-30 years. *Journal of Pharmaceutical Sciences and Research*. 2016;8(8):813. <https://doi.org/10.52458/9789391842789.2022.ch14.eb.asu>