RESEARCH ARTICLE

Development and Assessment of Phytosomes for the Treatment of Polycystic Ovarian Syndrome

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ABSTRACT

Phytosomes, a novel drug delivery system, have gained attention for their potential in the treatment of polycystic ovarian syndrome (PCOS). Polycystic ovaries, unpredictable menstrual cycles, and hormonal imbalances are the hallmarks of PCOS, a frequent endocrine disorder affecting women of reproductive age. The main aim is to prepare and evaluate phytosomes for the treatment of PCOS. The materials used were Guggul from *Commiphora mukul*, berberine from *Berberis vulgaris* and green tea from *Camellia sinensis*. All the phytosomal formulations are prepared in ratios and further evaluated. Phytosomal screening confirms the presence of required phytosomes like alkaloids, tannins and saponins that are effective in treating PCOS. Fourier-transform infrared (FTIR) evaluation was performed and *ex-vivo* and sub-acute toxicity evaluation confirm that formulation, PY4: Soy lecithin at a 3:1 extract ratio exhibited a greater release profile without any harm. As per the findings of this study, employing optimized phytosome-based medication delivery as bio-enhancers may enhance the bioavailability of herbal extract.

Keywords: Extract, Phytochemical screening, FTIR, *Ex-vivo* release, Sub-acute toxicity.

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INTRODUCTION

Polycystic ovarian syndrome (PCOS) is the most common endocrine condition influencing women in their reproductive years, which can affect up to 15% of women, based on the diagnostic standards used. An excess of androgens, or male sex hormones that are the ovaries in women with PCOS, produce this hormone, which is normally found in trace amounts in women. The multiple tiny cysts (fluid-filled sacs) that develop in the ovaries are referred to as PCOS. Period irregularities, such as uncommon or extended menstrual cycles, can affect women with PCOS.[1] An organ called the placenta forms while a woman is pregnant and is vital to the developing fetus's supply of nourishment and oxygen. It also cleans the fetus's blood of waste materials. The umbilical cord connects the placenta to the fetus, and it adheres to the uterine wall. According to research, placental function could vary in women with PCOS from those without the condition. For instance, some studies suggest that placental insufficiency may be more common in PCOS-affected women.[2] This can result in adverse effects like fetal macrosomia (large birth weight) or intrauterine growth restriction (IUGR). Furthermore, insulin resistance and high androgen levels – hormone imbalances linked to PCOS – may affect placental function and increase the risk of pregnancy complications. The signs of PCOS include hypergonadotropism, hirsutism, uncomfortable and irregular menstrual cycles, amenorrhea, multiple ovarian cysts, and anovulation. These conditions are typically associated with infertility. Anxiety, depression, and a lower quality of life are also linked to PCOS. Due to the high rate of contraindications in PCOS women, unsuccessful therapy in some cases, and the possibility of major side effects, the use of current PCOS treatments is restricted. PCOS is a complicated hormonal condition that necessitates a multimodal approach to therapies. The goals of PCOS management are to lessen symptoms, lower the chance of related problems, and enhance the general quality of life. Medication, lifestyle changes, and, occasionally, fertility therapies are all part of therapies. Although conventional therapies and lifestyle changes are usually the cornerstones of PCOS medical care, many people look for complementary or alternative strategies, such as herbal treatments. Current discussions about global health have focused plenty of emphasis on herbal treatments. Conversely, herbal medicines are fighting for acceptance as legitimate drugs with a distinct identity.[3]

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Phytosomes are herbal formulations that contain the active ingredients of a plant extract bond with phospholipids, usually phosphatidylcholine, to improve bioavailability and absorption. They are created by attaching phospholipids, usually phosphatidylcholine, to the active ingredients found in extracts of herbs.[3] By improving the active ingredient compounds' solubility in lipid and water environments, this complexation procedure makes it easier for them to pass through biological membranes and be absorbed. Therefore, in comparison to conventional herbal extracts, phytosomal compositions may result in increased blood levels of the active ingredients. The active ingredients are shielded from the digestive tract's enzymes and gastric acids by the phospholipid coating, which supports and maintains their effectiveness till they penetrate circulation.[4]

Commiphora mukul, a small tree or shrub, guggulu, is a member of the Burseraceae family. Because of its antimicrobial, anti-inflammatory, and antiseptic qualities, it has pharmacological advantages, including relief from pain, wound cleansing, and recovery. A plant-based health supplement called guggulu is utilized to lower elevated blood cholesterol. It has been utilized as a low-cholesterol level agent in India for a long time.[5] A substance called berberine, which is present in the plant *Berberis vulgaris*, has been investigated for probable impacts on the breakdown of glucose and response to insulin. Berberine could assist women with PCOS with their insulin resistance and menstrual consistency, according to certain investigations. Berberine has been developed into phytosomal formulations to improve its bioavailability and absorption, which may increase its efficacy in treating PCOS signs.

Green tea obtained from the *Camellia sinensis* plant contains polyphenols, along with epigallocatechin gallate (EGCG), having anti-inflammatory and antioxidant effects. According to certain research, green tea extract could encourage PCOS-affected women's insulin sensitivity and levels of androgen. Green tea extract has been established into phytosomal formulations to increase its bioavailability as well as its curative properties.

MATERIALS AND METHODS

Materials

Herbal extracts of *C. mukul*, *C. sinensis* and *B. vulgaris* were purchased from IndiaMart. N-hexane and ethanol were obtained from Sigma Aldrich. Every chemical utilized was analytical grade, and it was utilized exactly as it was delivered.

Alkaloidal Screening

In a boiling water bath, 200 mg of herbal extract and 5 mL of 2N HCl were cooked. It was cooled, then filtered and divided into two similar portions. Dragendorff's reagent was applied to one part and Mayer's reagent to the other one. The precipitates' turbidity indicated the existence of alkaloids.[4,6]

Tannins Screening

A water bath was used to boil 200 mg of extract along with distilled water (10 mL). After filtering it, the filtrate was mixed with a 5% w/v solution of ferric chloride. The observation of a darkish green solution demonstrated tannins.

Saponins Screening

In a test tube, 20 mg of herbal extract and 5 mL of filtered water were combined, and the mixture was brought to a boil with the use of a water bath. The formation of a thick, stable foam detected saponins.

Carbohydrate Screening (Barfoed's test)

About 1-mL of the aqueous solution of extract and 1-mL of Barfoed's reagent were mixed together and heated for 2 minutes in a water bath in a test tube. The red precipitate indicates the presence of carbohydrates.

Partition Coefficient

About 10 mg of the herbal extract mixture were stirred individually for 30 to 40 minutes in a 20 mL (1:1) mixture of n-octanol and buffer (pH 7.4) in order to calculate the partition coefficient. Following some time, the water and oil layers were separated, and a UV-vis spectrophotometer was used to measure the amount of extract dissolved in each phase.[5,6] The partition coefficient (K) of the drug was calculated with the help of the following formula.

Fourier-transform Infrared Spectroscopy Evaluation

Fourier-transform infrared spectroscopy (FTIR) analysis was performed to identify the existence of a functional group. The results from Perkin Elmer Model No. 234 infrared spectrophotometer were documented, and images of all compositions were provided in the 4000 to 400 cm^{-1} wavelength range.[7]

Preparation of Phytosomes

The necessary amount of soy lecithin (Table 1) and herbal extracts (40–45°C) were mixed with 20 mL of organic solvents, like acetone, in a revolving round-bottom flask. The mixture was then swirled for three hours. N-hexane was applied thinly to the sample, and it was continuously stirred with a magnetic stirrer. An amber-colored glass container was used to collect and store the precipitate that resulted at room temperature.[8]

Ex-vivo Studies

Ex vivo releases of extracts from phytosomes were achieved using a Franz diffusion cell with an area of 0.75 cm^2. There was an egg membrane separating the donor and receptor chambers. pH 7.4 was kept at 37 ± 2°C to mimic physiological conditions and was continuously stirred by a magnetic bead inside the receptor compartment. The donor chamber was filled up with one milliliter of phytosomal suspension.[9,10] The concentration of the extracts was observed at different times using a UV-Vis spectrophotometer at different wavelengths in comparison to a suitable blank at 292 nm.

Sub-acute toxicity study

All standards for animal species, housing and feeding requirements, preparation, and dose administration must be complied with by the OECD Guidelines. Several groups of experimental animals get the test material orally once a day in

progressive dosages; each group receives one dose level for a total of 28 days. For every dosage level, ten Wistar female rats weighing 150 ± 20 kg are to be chosen. Any current toxicity was taken into consideration while choosing the dosage level. The greatest dosage level selected was meant to have a toxic impact, not cause death or excruciating pain. The following metrics were used to observe the animal: body weight, hematology, and histopathology.[11-17]

RESULTS AND DISCUSSION

Qualitative Phytochemical Screening

By using qualitative phytochemical screening, it was discovered that a blend of herbal extracts from *C. sinensis, B. vulgaris, and C. mukul* contained alkaloids, flavonoids, saponins, glycosides, phenolic compounds and carbohydrates. Alkaloids and glycosides were found in the phytosomes from green tea and guggul. Alkaloids and glycosides have been demonstrated to have anti-inflammatory effects in PCOS. While a combination of extracts showed less solubility in water (12.63 mg/mL) than in pH 7.4 buffer (18.29 mg/mL), it was soluble in ethanol.[11] After testing a variety of herbal extracts, the partition coefficient was found to be 0.879.

FTIR Study

For structural analysis, FTIR is a powerful method that yields a variety of functional groups alongwith unique band no, locations, shapes, and intensity characteristics. One way to verify the development of phytosomes is to compare the spectroscopy of a crude drug. As indicated in Table 2, FTIR analyses were performed to examine the functional groups of *C. sinensis, B. vulgaris, and C. mukul* crude and herbal extracts. In *C. sinensis*, stretching of C-O and C=O were noted at 1383 and 1704 cm^{-1}. At 3246 cm^{-1} a new C-H peak was found. At 1049 cm^{-1}, in the higher frequency band of *B. vulgaris*, C–O stretching was found. The positions of the C=C and C-H peaks were found to be 1701 and 796 to 3110 cm^{-1}, respectively. In *C. mukul*, C-O stretching has been reported at 1208 cm^{-1}, respectively. Stretching of C=C and C-H was found at 1876 and 3015 to 3103 cm^{-1}. Moreover, there was no discernible variation regarding the infrared spectra of the extracts. A thorough analysis of all notable peaks and their corresponding wavenumbers demonstrated the absence of any notable changes during the extraction process. However, the strength of the distinguishing peaks of the extracts of *C. mukul*, *B. vulgaris*, and *C. sinensis* was diminished due to the solvents used in the extraction process.

Table 1: Formulation design of phytosomes

Phytosomes	*Extract of herb (gm)*	*Soy Lecithin (gm)*	*Extract: Soy Lecithin (w/w)*
PY1	1	1	1:1
PY2	1	2	1:2
PY3	2	1	2:1
PY4	3	1	3:1
PY5	2	2	2:2

Table 2: FTIR peaks of polyherbal extracts of *C. sinensis, B. vulgaris, and C. mukul*

Functional group	*C. sinensis (cm^{-1})*	*B. vulgaris (cm^{-1})*	*C. mukul (cm^{-1})*
C-H	3246	796–3110	3015–3103
C=C	-	1701	1876
C-O	1383	1049	1208
C=O	1704	-	-

Ex-vivo Studies

The evaluation between phytosome formulations and herbal extract permeation in phosphate buffer (pH 7.4) is shown in Figure 1. The enhancement in the phytosome release profile in dissolving media for the release of herbal extract for up to five hrs was evaluated and compared using UV analysis. On the other hand, the PY4 phytosome's unique feature of prolonged-release was explained by the release of herbal extracts, which increased steadily until it reached 97.98% after 5 hours. Changes in phytosome release rates may be related to the enhanced phytosome's increased wettability and solubility of the herbal extract (Table 3). The partially amorphous state of phytosomes contributed to the herbal extract's poor aqueous solubility and accelerated the dissolution rate to 5 hours, as per the evaluation. The dispersion of herbal extracts improved in a time- and dose-dependent manner.[12] Herbal extracts can also more easily pass through the membrane due to the interaction of phospholipids with it. Excessive phospholipid concentrations inhibit the extract's release from phytosomes,

Table 3: *Ex-vivo* release profile of phytosomes

Time (hours)	*PY1*	*PY2*	*PY3*	*PY4*	*PY5*
0	0	0	0	0	0
0.5	77.5	77.08	82.9	97.98	65.59
1	67.97	65.06	73.93	83.22	37.47
2	57.46	53.9	62.66	71.95	29.97
3	41.4	32.65	48.7	63.04	57.1
4	27.09	19.08	29.73	44.99	49.17
5	18.05	15.97	18.9	20.59	17.3

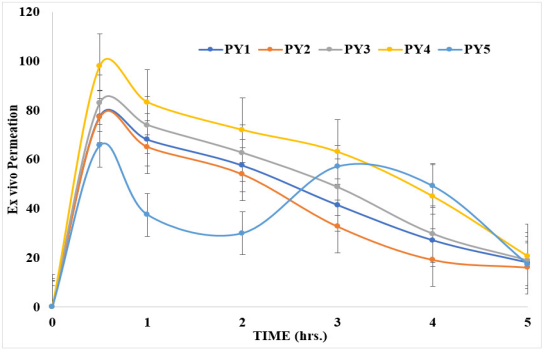

Figure 1: *Ex-vivo* study of phytosomes (PY1 -PY5)

Sub-acute toxicity study

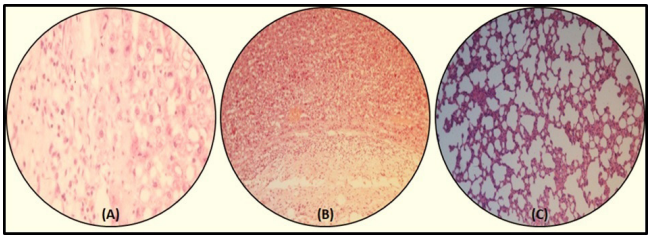

Figure 2: Histopathological images of (A) Kidney, (B) Liver, (C) Lungs at 10X magnification after the sacrifice of the rat.

causing a prolonged release pattern at elevated levels. When phospholipid and herbal extract are combined in a 3:1 ratio, phytosome efficacy is greatly increased.

Following the sacrifice of rat and organ separation for histology, the investigation was carried out for 28 days, and no toxicity was discovered (Figure 2).

CONCLUSION

Phytosomes have great potential for successfully treating ovarian cysts, and further research on them for therapeutic uses is possible in the future. The results show that phytosomes derived from herbal extracts of *C. sinensis, B. vulgaris*, and *C. mukul* increase bioavailability through improved drug release through increased permeability of the biological membrane. Without causing any unfavorable side effects, the phytosomes formulations containing extracts might be an effective dosage for reducing the symptoms of ovarian cysts.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The studies were approved by the Animal Ethics Committee of Pranveer Singh Institute of Technology, India, under registration number 1273/PO/Re/S/09/CPCSEA.

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