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Formulation and Evaluation of Polyherbal Capsules for the Treatment of Poly Cystic Ovarian Syndrome (Pcos)

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ABSTRACT:

Poly Cystic Ovarian Syndrome (PCOS) is a gynecological disorder with increasing presence in the women belonging to the reproductive age group with about 75% occurrence. With various studies, PCOS has shown impact on metabolic functioning also which was earlier only limited to reproductive functioning of women. The Objective of this study is to formulate herbal capsules using the herbal extract prepared by different herbs for the treatment of Poly Cystic Ovarian Syndrome. The capsules were formulated and then further evaluated. The collected herbs were first extracted using ethanol solvent and then further the extract was mixed with several excipients in different ratios based on increasing the ratio of the disintegrating agent to get fine granules. These granules were further made into the final end product which is the polyherbal capsules. These capsules were evaluated and tested. All the performed tests were evaluated and compiled. The performed tests showed all the parameters were in compliance as per the standard pharmacopoeias. The current research study was based on the role of medicinal herbs, namely, Bauhinia variegata, Emblica officinalis, Terminalia belerica, Terminalia chebula, Commiphora wightii, Cinnamon Cassia, Tribulus Terrestris, Hypericum perforatum, Commiphora molmol, Nigella sativa in the treatment of Poly Cystic Ovarian Syndrome. The study includes all the Phytochemical, Pharmacognostic, Rheological evaluation of the herbs and preparation of the end product polyherbal capsules. Also, the prepared formulation complies with all the standards as per Indian Pharmacopoeia and Ayurvedic Formulary.

Keywords: Poly cystic ovarian syndrome, polyherbal capsules, infertility, toxicity studies.

1. Introduction

Poly Cystic Ovarian Syndrome (PCOS) is a gynecological disorder with increasing prevalence, and an ovulatory infertility is a big concern which is almost 75% in women. PCOS now has shown an impact on metabolic functioning of the syndrome with recent researches, but earlier, PCOS was limited only as a reproductive or gynecological disorder. [1] PCOS occurs in women of the reproductive age group or the child-bearing age and is found to affect mostly the endocrine system. Ovaries tend to enlarge in the medical condition of PCOS with the accumulation of a large amount of fluid in it. Several clinical conditions of PCOS include irregular menstrual cycle, hair growth, the problem of acne, obesity, in the long-run problem of infertility, etc. The follicular growth is impaired during the ovarian cycle, which leads to cysts formation as follicles remain in contact with the ovary the underlying condition is due to hormonal imbalance. The anterior pituitary secretes luteinizing hormone (LH) & follicle-stimulating hormone (FSH), which is the major underlying cause of the disease. [2]

The major challenge of women suffering from PCOS includes irregular and impaired estrogen, androgen metabolism, and, also production of androgen control is impaired. The premenopausal and women of reproductive age group are most commonly affected by the disease. The major focus is on normalizing the functions of the ovary. [3]
The Gonadotropin-releasing hormone is secreted by the hypothalamus, and the LH & FSH is secreted by the gonadotrophs in return. The major functions of LH & FSH include reproductive, growth, and, menstrual regulation. This imbalance between the LH, FSH, and their ratio of increased serum concentration is the underlying cause of the diseased condition in women of the reproductive age group. [4]
Both environmental, as well as hereditary factors are the underlying factors for PCOS. Lack of proper physical exercise, obesity which might further cause insulin resistance comes under the environmental factors. Several diagnostic parameters of PCOS include Cushing's syndrome, secreting tumors of adrenal androgen and ovarian, in some cases hypothyroidism, etc. [5] It is believed, slight changes in the lifestyle including proper exercise, walking, weight management might help in the management of the disease by regulating several problems symptoms of PCOS like weight gain, the regularity of the menstrual cycle, hair growth in excess, etc. [6] For the treatment of PCOS, various methods and procedures have been used like changing or modifying the lifestyle, several surgeries, use of Vitamin D, induction of ovulation, etc. [7,8] Various synthetic drugs such as metformin for insulin resistance during PCOS have been prescribed to women; Clomiphene Citrate too was used for ovulation stimulation. But, these drugs showed several side-effects. Therefore, the role of herbal drugs has increased in the treatment of the disease. Various studies suggest that herbs have been useful in the treatment of PCOS. The steroidogenic property is seen in the case of herbs and provides several benefits with the treatment of PCOS. [9] One of the major benefits of using herbs in combination is that it provides a synergistic or potentiating effect. [10] The herbs selected for the study are Bauhinia variegata, Emblica officinalis, Terminalia belerica, Terminalia chebula, Commiphora wightii, Cinnamon Cassia, Tribulus Terrestris, Hypericum perforatum, Commiphora molmol, and, Nigella sativa. As per a study, the use of Ayurvedic herbs such as Bauhinia variegata (Kanchnar Guggul) is an effective way for the treatment of PCOS. The herb shows no adverse drug reaction or risks and can be used to treat hormonal imbalance, minimize the ovaries with polycystic appearance, menstrual irregularities, the problem of acne, and pain improvement. [11] The combination of medicinal herbs, namely, Terminalia chebula (Harad), Emblica officinalis (Amla), and Terminalia bellirica (Baheda) is collectively called Triphala. These are the most common Ayurvedic preparations with

significant effect. The plant part with great medicinal significance is the fruit. Due to the presence of vitamin C and anti-oxidant effects in fruits like Amla, these are considered useful in treating the condition of menorrhagia. [12] Due to the scavenging of free radicals, Amla also helps in detoxifying as well as helps to improve the hormonal imbalance in the body. [10] The next drug used for the study is Commiphora wightii (Guggul), and as per a study, the ethanolic extract of Guggul is used as a potent medicinal herb to the treatment of PCOS. Commiphora wightii plays a significant role in normalizing hormone levels and reducing ovarian follicle abnormalities. [13] Cinnamon Cassia is another herb used for research. Insulin resistance is one of the common factors gathered by the researchers in the pathogenesis of PCOS which leads to hyperinsulinemia. Cinnamon cassia is seen to have a sensitizing effect on PCOS-induced hyperinsulinemia. Also, the study states that Cinnamon has a vital role in improving the menstrual cycle and effective in the treatment of PCOS. [14] Another medicinal herb selected for the study is Tribulus terrestris. As per a research study, the ethanolic extract of Tribulus terrestris in a pre-clinical PCOS study showed the effect on body mass, synthesis of androgen, etc. The treatment of Tribulus terrestris extract showed a positive effect on the growth of healthy follicles and minimizing the cystic follicles in number. Thus, Tribulus terrestris has a significant on women with PCOS. It has a role in improving lipid, estrogenic, insulin-sensitizing, and hyperandrogenic profile. [1] Hypericum perforatum or St. John wort's is another medicinal plant used in PCOS to modulate the serotonin levels in the body. Mood swings or depression are the signs of illness due to PCOS. Thus, Hypericum perforatum modulates the levels of serotonin. [15] St. John Wort is useful in treating depression or mood swings during PCOS. [16] Commiphora molmol or Myrrh is another medicinal plant chosen for the research. Myrrh is used in PCOS as an Amenorrhea agent, generally given with iron compounds so as to induce menstruation. Another role in PCOS is as a Menorrhagia, which helps to prevent excessive loss of blood during the menstrual cycle. Myrrh also helps in painful or irregular menstruation. [17] The medicinal herb, Nigella sativa or Kalaunji, is used in the study as it is used in PCOS to decrease menstrual irregularities in women. The severity of menstrual bleeding also tends to stabilize by the use of Nigella sativa. The herb acts on oligomenorrhea or irregular menstrual bleeding which is a common symptom of PCOS by the anti-oxidant and anti-inflammatory mechanisms. [18]

2. Methods and Materials

Selection and Collection of Plant Material:

The bark of Bauhinia variegata, fruit of Emblica officinalis, seeds of Terminalia bellerica, Terminalia chebula, Tribulus Terrestris, and Nigella sativa, resin of Commiphora wightii, Commiphora molmol, sticks of Cinnamon Cassia and seeds of flowers of Hypericum perforatum, were procured from the botanical garden of Pranveer Singh Institute of Technology (PSIT), Kanpur.

They were dried and grounded properly in a mixer grinder. Further, it was passed through sieve number 60. The polyherbal powder was then mixed in a fixed ratio with a maximum of Commiphora wightii and the remaining in the total amount of Commiphora wightii was mixed.

Table 1 List of Plant Parts Used.

S.No.	Plant part used
1.	Bark of Bauhinia variegate

2.	Fruit part of Emblica officinalis
3.	Seeds of Terminalia bellirica
4.	Seeds of Terminalia chebula
5.	Resin of Commiphora wightii
6.	Sticks of Cinnamon Cassia
7.	Seeds of Tribulus Terrestris
8.	Flowers of Hypericum perforatum
9.	Resin of Commiphora molmol
10.	Seeds of Nigella sativa

Figure 1 Bark of Bauhinia variegata

Figure 2 Fruit of Emblica officinalis

Figure 3 Seeds of Terminalia belerica

Figure 4 Seeds of Terminalia chebula

Figure 5 Resin of Commiphora wightii

Figure 6 Sticks of Cinnamon cassia

Figure 7 Seeds of Tribulus terrestris

Figure 8 Resin of Commiphora molmol

Figure 9 Seeds of Nigella sativa

Figure 10 Flower of Hypericum perforatum

Preparation of the Polyherbal Powder (PHP)

PHP was prepared by mixing the selected 10 herbs in a particular ratio. The ratio selected for the preparation of PHP was in the ratio of 2.5:1.0:0.10:0.10:0.10:0.5:0.83:0.83:0.5:0.5 which

includes herbs in the order Guggul, Kanchanar, Harad, Baheda, Amla, Dalchini, Gokhru, Myrrh, Kalaunji, and St. John's wort respectively.
The selected herbs were first properly washed, and shade dried and then they were blend into a fine powder using mixer grinder. The obtained PHP was then made to get fine and homogenous particles by using sieve of 60 mesh size.

Determination of Drug Excipients Interaction: [20]
The drug excipients interaction testing was done through the isothermal method. About 100 mg of the PHP was taken and separately excipients including lactose, starch, talc, magnesium stearate, sodium benzoate, microcrystalline cellulose, and sodium starch glycolate were weighed individually, 100 mg each. Sample 1 was PHP; sample (2-7) was the individual excipients and combination of drug- excipients samples (8-14) was in the combination of PHP and excipient combination.

The samples were placed in clean glass vials along with ultra pure water 10 µL with the help of micropipette was added separately into each glass vials and mixed using capillary without touching the sample. The glass vials, were labeled, and sealed properly to avoid any contamination and kept in the hot air oven for a period of 4 weeks at 40-50°C. After the end of the fourth week, the samples were analyzed using FTIR. The bands were studied and the drug- excipient interaction was studied.

Preparation of the extract by Soxhelation Method [21]
The powdered herbs were extracted using ethanol solvent by the Soxhelation process. The herbs were extracted in the fixed ratio and extracted for the active constituents. For the process of soxhelation, about 200 grams of powdered herbs were extracted in about 800 ml of ethanol. The process was carried out at around 40-50℃. The obtained extract was concentrated with the rotary evaporator till the extract gets concentrated.
Preparation of the Granules [22]
The method used for the preparation of the granules was Wet Granulation Method. The extract was weighed 500 mg and mixed with 500 mg of excipients. The excipients selected include lactose, talc, magnesium stearate, starch, microcrystalline cellulose, sodium benzoate, and sodium starch glycolate.
The extract was first mixed with lactose to form a mass which is less sticky in nature. After that, other excipients were added in successive order till the extract starts forming a uniform mass. Starch, then talc in very less quantity (2-3%), magnesium stearate, cellulose, sodium benzoate, and sodium starch glycolate at the last. The concentration of sodium starch glycolate was varied in the formulation (1%-5%).
Sodium starch glycolate is used as a super disintegrant in the formulation, which helps in faster disintegration of the granules. The granules were then dried in desiccator to remove moisture. The granules were then sieved through sieve no. 44 and then sieve no. 60 to get even finer particles. The prepared granules were then tested as per the flow properties and other factors like microscopy, pH, moisture content, reaction of granules with different reagents, drug interaction test, and Differential scanning colorimetry.

Table 2 Formulation of granules in different concentrations

S. No.	Extract (500 mg)	Lactose (mg)	Starch (mg)	Talc (mg)	Mag. Stearate (mg)	Sodium Benzoate (mg)	Cellulose (MCC) (mg)	Sodium starch glycolate (mg)
F1	500	300	100	30	30	0.5	40	--

F2	500	290	110	25	40	0.5	25	10
F3	500	300	100	25	25	0.5	30	20
F4	500	280	100	30	40	0.5	20	30
F5	500	260	100	30	35	0.5	35	40
F6	500	270	100	25	35	0.5	20	50

Figure 11 Prepared granules

Evaluation of the prepared granules: [22]

Determination of the flow properties of the prepared granules:

The prepared granules were prepared in bulk for the determination of the flow properties. The granules were tested for Bulk density, Carr's index, Tapped density, Angle of repose, and Hausner's ratio.

Bulk density: It was estimated for flow of the granules by weighing it 25 grams and placed in 100 ml of graduated cylinder without disturbing to get the exact space covered by granules. Bulk density can be calculated by: Bulk density= weight of granules (gm)/ volume occupied by the powder in the measuring cylinder (ml)

Tapped density: The tapped density of the prepared granules was calculated by placing 25 grams of granules in 100 ml of measuring cylinder and tapping the cylinder 2 inches above the ground till no deviation in measurement is seen. It is calculated by: Tapped Density= weight of the prepared granules (gm)/ space occupied by the measuring cylinder after tapping (ml). [23]

The Carr's Index:

It is the abbreviation used for the calculation of compressibility of the prepared granules. Tapped density and bulk density are the two parameters used for the calculation.

Formula for Carr's Index is = (bulk Density- tapped Density/ Tapped Density) * 100.

Hausner's Ratio:

Hauser's ratio is the property used to define how fine the granules are in terms of Flowability. It can be calculated by:

Hauser's ratio= Tapped density/ Bulk density [24]

Angle of Repose: The property for granules was calculated after placing a funnel at a fixed height of 10 mm from the ground and paper was placed beneath it. Pouring of granules done using funnel and the height and diameter of the circle formed on plain paper were noted. The formula for angle of repose is:

Angle of repose (θ) = tan-1 h/r; where, h is observed height, r is the radius formed by the base of cone, and tan ɵ is height. [25]

Moisture content evaluation: [26]
The moisture content of the prepared granules was evaluated by placing 10 grams of the granules into a petri dish and kept in oven for drying at 100-105 ºC for about 4-5 hours till no change in weight is observed. The weigh was taken periodically to check the difference of the weight. The percentage of it is given as: % moisture content= [(weight initial- weigh final/weight of granules taken]* 100)

pH determination: The pH for the granules was calculated using pH meter. The pH meter was calibrated by using capsules of pH 4 and 7. Then around 1gram of the granules was properly dissolved in 100 ml demineralised water with the help using sonicator. The pH was measured for same.

Granules disintegration test: The disintegration test of the granules was done to check if the granules disintegrate in as given set of time. The test was performed in a digital disintegration assembly. The granules were weighed and put into the disintegrating tubes covered with the disc. The temperature was maintained to room temperature 37±5ºC. The time for granules to permeate through the mesh was observed and noted.

Microscopical Evaluation of the granules: [27] The structure of the prepared granules was observed under the compound microscope for the evaluation of the granule structure. The prepared granules were placed on the glass slide and several staining dyes like Phloroglucinol in combination with conc. HCl was used to observe the granule structure. Glycerin was also used with saffranine or any other staining dye to observe the structure distinctly. Other staining dyes used include Iodine solution, and Sudan III. The slide was covered using cover slip without applying much pressure and in proper light, the slide was observed using the microscope.

Assessment of physical nature of granules by using different reagents: [28] The granules were assessed based on their physical nature by testing them with different reagents. The change in color of the granules after a time interval of 5, 10, 15, and 30 minutes was observed. The reagents used for the estimation include n-hexane, iodine solution, chloroform, ferric chloride, acetone, and methanol. The results were observed and noted.

Formulation of the capsules [28]
The prepared granules were further converted to capsule formulation. The capsules include equal amount of extract (500mg) and excipients. The granules were filled into the herbal capsule shells of size 000 by using hand operated capsule filling machine with accuracy. The prepared capsules were then further evaluated based on various evaluation parameters like weight variation, drug uniformity content, disintegration, dissolution, thermal evaluation of the formulation by Differential scanning colorimetry (DSC).

Figure 12 Prepared herbal capsules

Evaluation of the prepared capsules:
The capsules were evaluated based on weight variation, drug uniformity content, disintegration, dissolution, thermal evaluation of the formulation by Differential scanning colorimetry.

Maximum wavelength (λ_{max}) estimation: [28]
Exactly measured 10 mg of the herbal extract was mixed properly in 10 ml of the phosphate buffer with pH 6.8. The prepared stock mixture is 1000 µg/ml in concentration. The solution was further diluted to 100 µg/ml solution. The wavelength determination was done was done using Ultra violet spectrophotometer in the range between 200-400 nm.

Preparation of buffer pH 6.8: [29]
The phosphate buffer preparation is done by disodium hydrogen phosphate and potassium dihydrogen phosphate to produce 1000ml of the buffer. 28.80grams of disodium hydrogen phosphate and potassium dihydrogen phosphate comprise of 11.45 grams. The solutions were mixed thoroughly with the help of sonicator till mixing is done. The prepared buffer was checked on pH meter and filtered.

Preparation of the calibration curve for the polyherbal formulation: [28]
The curve standard was initiated mixing 10 mg of the herbal extract in 10ml of phosphate buffer with pH 6.8. The prepared concentration of the stock solution is 1000 µg/ml. It was again diluted as 100µg/ml, 200µg/ml, 300µg/ml, 400µg/ml, 500µg/ml. The calibration of UV spectrophotometer was done by running the initial baseline by placing the phosphate buffer in both the cuvette; after the calibration, the prepared dilutions were placed serially in one cuvette. The maximum wavelength of 275.20 nm was observed and the absorbance was noted. The process was continued interday and intraday to get accurate readings.

Determination of Weight Variation of Capsules:
The weight variation test is done to establish the uniformity of weight in the capsules. The weight of the prepared capsules must fall in the particular range to avoid deviations. The process includes selecting exact twenty capsules from the batch randomly and the average of these twenty capsules is compared to the weight of the individual capsule. This process is done as per Indian Pharmacopoeia, 2010. The deviation in the weight should not fall under 90% and should not exceed 110% of the amount calculated. The limit should not exceed ±5% for capsule more than 300mg.

Disintegration test:

The disintegrating test of the capsules was performed on the disintegration apparatus. The test is generally done on the conventional dosage forms. The disintegration rate is defined as the rate or time with which the prepared formulation breaks or disintegrates into the smaller particles and reaches the site of action, the gastrointestinal tract. The disintegration test was performed on the randomly selected six capsules from the lot. The required conditions are 1000ml of beaker and 37±5 ºC temperature of the apparatus. Each capsule was kept in the six tubes of the assembly separately with dissolution medium. The time taken by the particles to cross the mesh at a particular time is the disintegration time.

Dissolution test/ Invitro Release [30]
The dissolution test of the capsules was done as per USP type-I apparatus that is, rotating basket type assembly. The assembly was set at 37±5 ºC and at rpm of 100± 5. The capsule was weighed and then kept in the basket then fitted to assembly. 1000ml beaker in the assembly was poured with 900 ml of the phosphate buffer with pH 6.8. The sample was withdrawn at an interval of 10 mins. 2ml of the sample was withdrawn and then volume was made up with 2ml of buffer in the assembly. The volume of the sample was diluted so that it can be observed under ultra violet spectrophotometer. The sample was observed under UV, and absorbance was noted. This absorbance was then calculated for the concentration and % drug release. The standard calibrated curve was used for the determination.

Drug content uniformity analysis:
The test for drug content was done as per the specifications mentioned in Indian Pharmacopoeia, 2007. About twenty capsules were taken and emptied its content in mortar pestle. Accurately weighed 500 mg of the granules were mixed with the phosphate buffer pH 6.8. Ultraviolet spectrophotometer was used for the estimation of drug content in each capsule at 275.20 nm wavelength.

Formulation characterization
Prepared formulation FTIR analysis [31]
The prepared six types of granules were analyzed by FTIR analysis. The drug and excipients showed no signs of drug interaction. The Perkin Elmer FTIR spectrum was used to scan the range of 4000 to 400 $cm^{-1.}$

Differential Scanning colorimetry [32]
The sample which can be analyzed based on the changes in temperature is called differential scanning colorimetry. The breakdown of heat gives information about the various processes. DSC (SDT Q600) model has been used for the estimation. The estimation has been done in the nitrogen based environment.

Toxicity Studies [33]
Acute toxicity study (OECD Guidelines)
The 14 day study was accomplished by with the aqueous extract of the prepared polyherbal extract. The testing of the herbal extract was done on female rats in dose of 500, 1000mg/kg. The dosing was done with compliance of Organization for Economic Development (OECD) guidelines. OECD Guideline 425 was followed. The female Wistar rats were weighed and the extract was dissolved in distilled water as per the body weight. The extract was given orally to the rats. The rats were observed for any irritation for the first 15 minutes and monitored for the starting 4 hours. The next 48 hours the animals were monitored on daily basis. The study was continued for 14 days in which the rats were monitored for any changes like irritation in

skin, nervous system, eyes, etc. The changes in body weight were also noted after the end of every week.

Sub-acute toxicity study

The 28 day animal toxicity study was done as per OECD Guidelines number. 407. Three female rats in groups were selected for perusal of evaluation. They got dose of 750mg/kg and 2000mg/kg of the extract dissolved in distilled water; whereas group III female rat received distilled water only. The rats were given dose orally with the syringe used for feeding. The body measurements of rats were done at the end of every week. After 28 days completion of dosing, the rats were sacrificed. The biochemical and histological parameters were tested. Blood sample and all the vital organs like heart, ovaries, liver, intestine, and kidney were used for examining the histological changes.

Stability Study: [34]

The stability study is done so that the prepared formulation remains stable over the period of time under different conditions. The stability study is done so as to determine the shelf life of the formulation and notice any kind of changes that occurs in the formulation or the properties of the prepared formulation. The prepared capsules were stored in B.O.D incubator for a period of 30 days at 40-45°C.

3. Results and Discussion

Determination of Drug Excipient Interaction

The Infrared spectroscopy of pure herbs and its combination with the excipients showed that the herbs are very much compatible with the excipient mixture. The wavelengths fall in the range of 4000 cm^{-1} to 400 cm^{-1} in the spectroscopy evaluation. The drug-excipient compatibility study was performed for 4 weeks and it showed no significant changes in the peak of the mixtures containing herb- excipients.

The FT-IR spectra of PHP and the combination with excipient showed compatibility studies for over four weeks. No change in physical behavior or change in the location of the herb-excipient mixture was seen. The mixture was found to be stable and lacks any physical or chemical changes.

Table 3 The mixture of polyherbal powder and excipients and observed changes in 4 weeks

S.No.	Samples	Week
		1 2 3 4
1	Polyherbal powder (100mg)	No change
2	Polyherbal powder+ Lactose	No change
3	Polyherbal powder+ Starch	No change
4	Polyherbal powder+ Talc	No change
5	Polyherbal powder+ Magnesium stearate	No change
6	Polyherbal powder+ Sodium benzoate	No change
7	Polyherbal powder+ Microcrystalline cellulose	No change
8	Polyherbal powder+ Sodium starch glycolate	No change
The sample contains 100 mg Polyherbal powder+ 100 mg excipients		

Table 4 Presence of functional group in IR data

Peak seen(YES/NO)

Wavelength no.	Functional group	PHP	PHP + Lactose	PHP + Starch	PHP+ Talc	PHP+ Magnesium stearate	PHP+ Sodium benzoate	PHP+ Microcrystalline cellulose	PHP+Sodium starch glycolate
3600-3200	-O-H	YES	YES	YES	YES	YES	YES	YES	YES
2935-2865	-C-H	YES	YES	YES	YES	YES	YES	YES	YES
1500-1650	-C=C	YES	YES	YES	YES	YES	YES	YES	YES
1300-1600	-N-O_2	YES	YES	YES	YES	YES	YES	YES	YES
1000-1100	=C-O	YES	YES	YES	YES	YES	YES	YES	YES
668-770	=C-H	YES	YES	YES	YES	YES	YES	YES	YES

FT-IR Graphs for the determination of herb-excipient interaction:

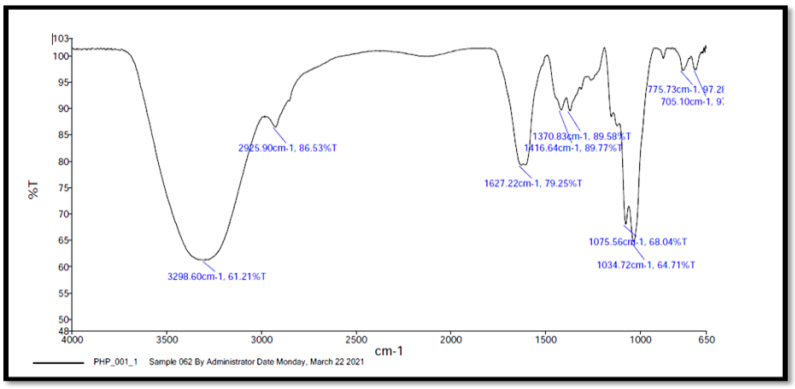

Figure 13 FT-IR of Polyherbal Powder

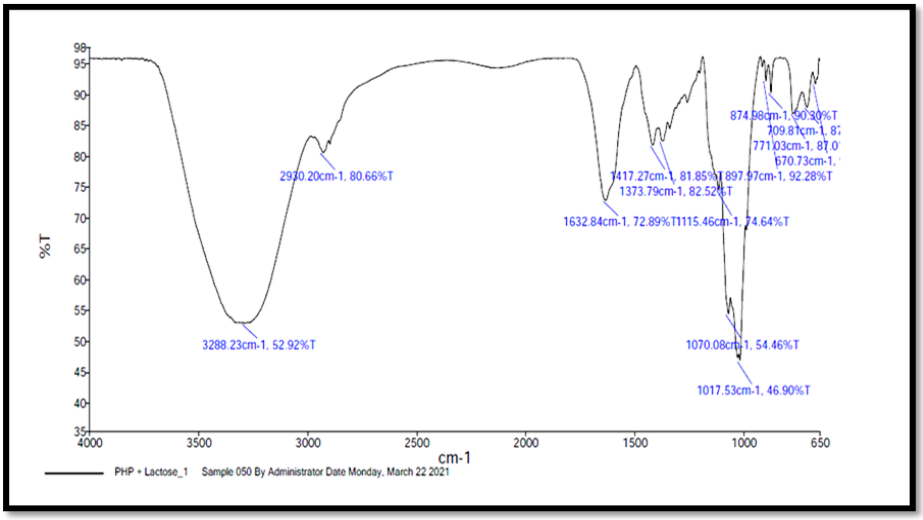

Figure 14 FT-IR of Polyherbal powder+ Lactose

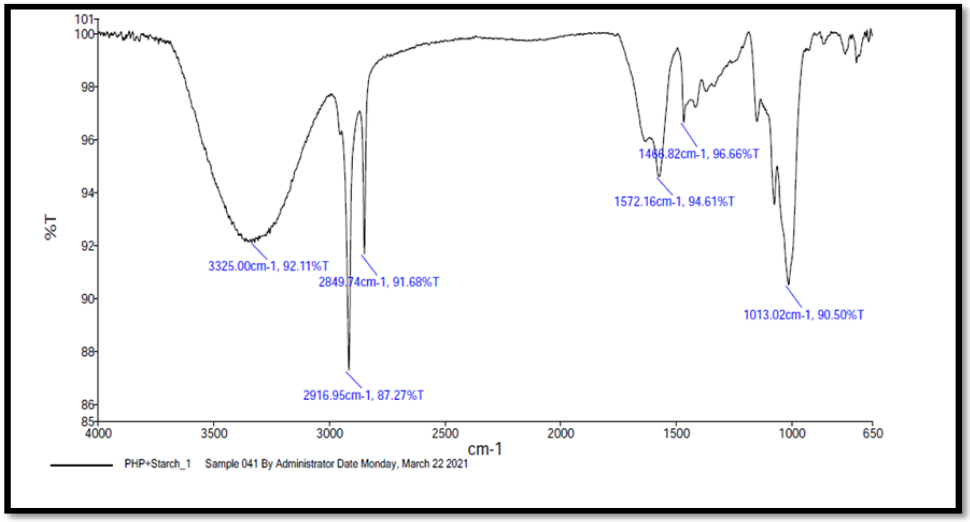

Figure 15 FT-IR of Polyherbal powder+ Starch

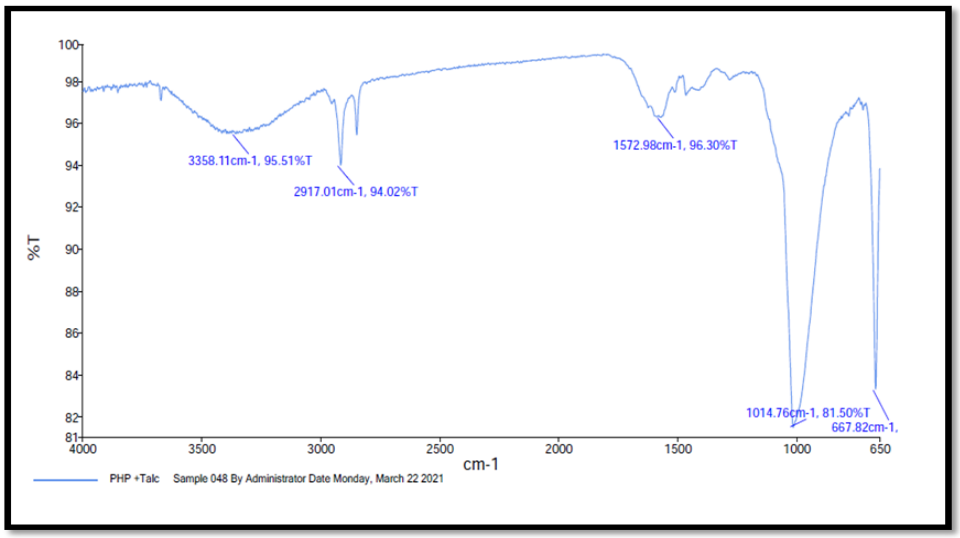

Figure 16 FT-IR of Polyherbal powder+ Talc

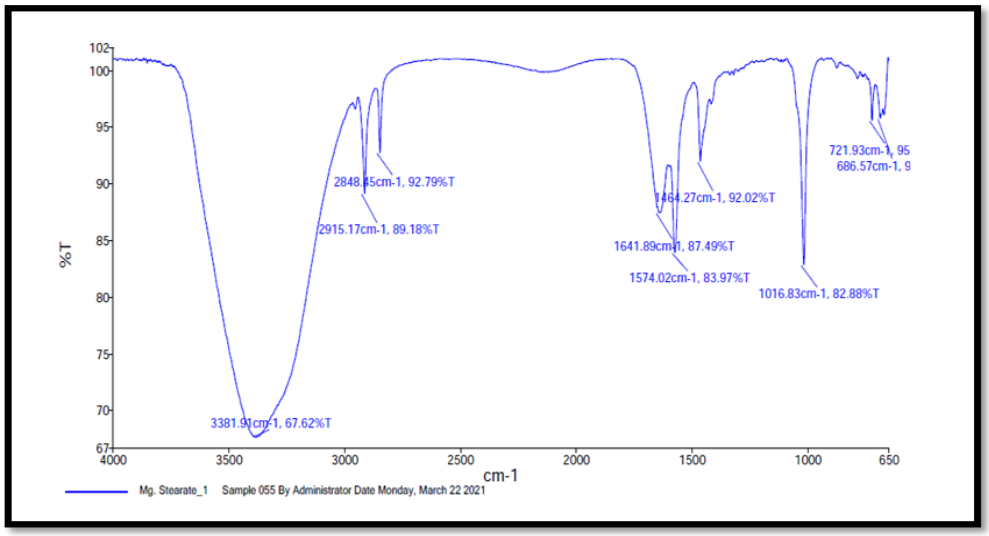

Figure 17 FT-IR of Polyherbal powder+ Magnesium stearate

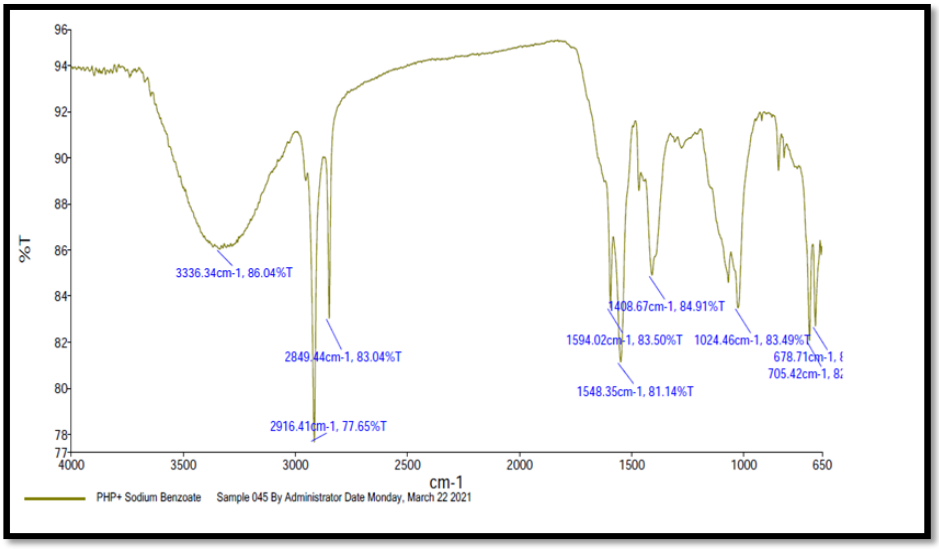

Figure 18 FT-IR of Polyherbal powder+ Sodium benzoate

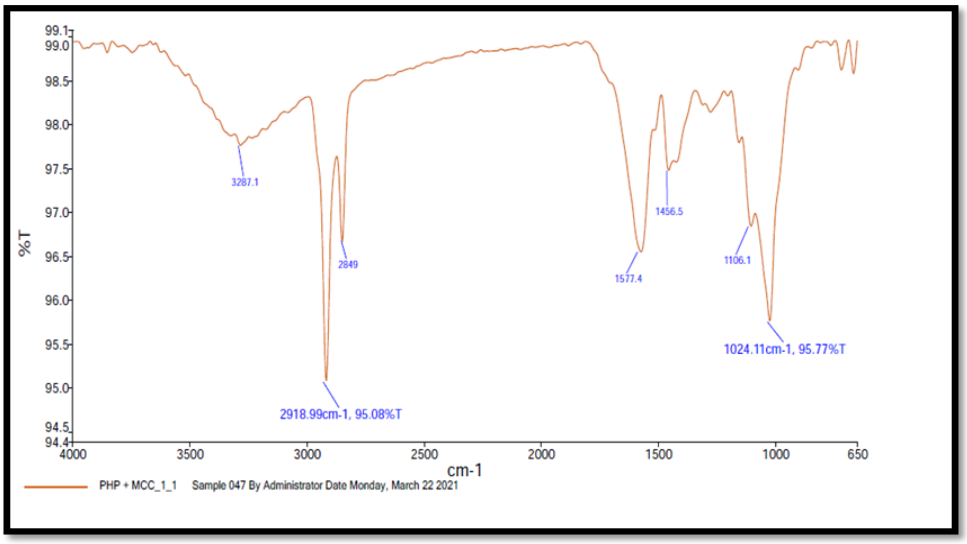

Figure 19 FT-IR of Polyherbal powder+ Microcrystalline cellulose

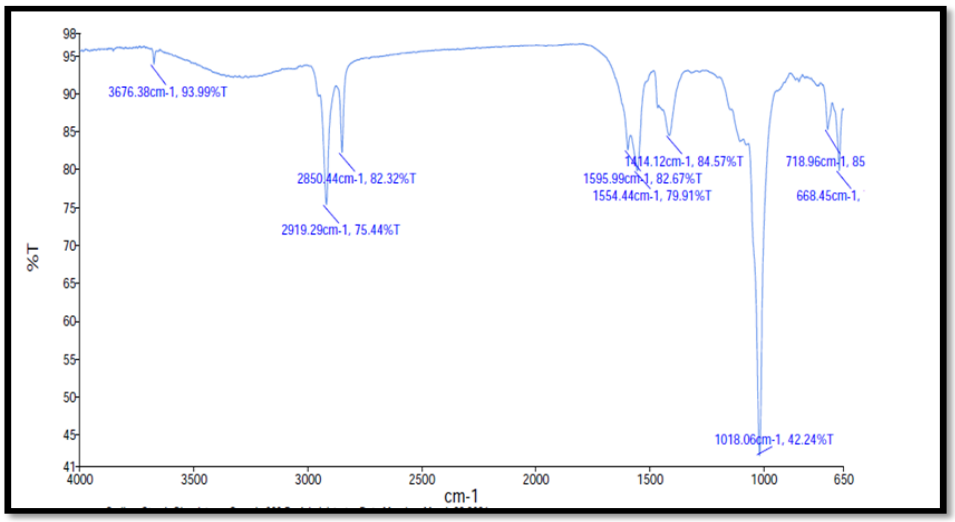

Figure 20 FT-IR of Polyherbal powder+ Sodium starch glycolate

Evaluation of the prepared granules

Rheological parameters (flow properties) of the granules:

The results for flow property of granules show that except for F1, all the other granules (F2-F6) shows excellent flow properties. The F1 shows fair flow properties. Results of rheological parameters of the granules are tabulated in Table no. 5.

Table 5 Evaluation of flow properties of granules

Rheological Property	F1	F2	F3	F4	F5	F6
Bulk Density (gm/Ml)	0.5	0.52	0.53	0.54	0.55	0.6
Tapped Density (gm/Ml)	0.6	0.57	0.57	0.59	0.58	0.57
Carr's Index (%)	16.66	8.77	9.25	8.47	8.62	5
Hausner's Ratio	1.2	1.09	1.07	1.09	1.09	1.08
Angle of Repose	28.6±0.5°	27±0.5°	27.3±0.5°	26±0.5°	25.8±o.5°	25.6±0.5°

Moisture content evaluation of the granules:

Table 6 shows moisture content percentage for the prepared granules. The result shows that the moisture content was in the range and the granules were stable.

Table 6 % Moisture content in granules

Prepared granules	% Moisture content
F1	5.3%
F2	7.2%
F3	5.8%
F4	6.9%
F5	8%
F6	3.3%

pH determination of granules:

The pH for all the six formulations of granules was determined. Table No. 7 depicts the results of pH of granules.

Table 7 pH of Granules

Prepared granules	pH
F1	6.15
F2	6.3
F3	6.2
F4	6.4
F5	6.2
F6	6.5

Disintegration test of Granules:

Table 8 Disintegration of granules

Prepared granules	Disintegration time
F1	23±2 mins 15 seconds
F2	19±2mins 30 mins
F3	18±2 mins 40 seconds
F4	18±1 min 10 seconds
F5	17±2mins 30 seconds
F6	16±2 mins 10 seconds

Evaluation of microscopical properties of granules:

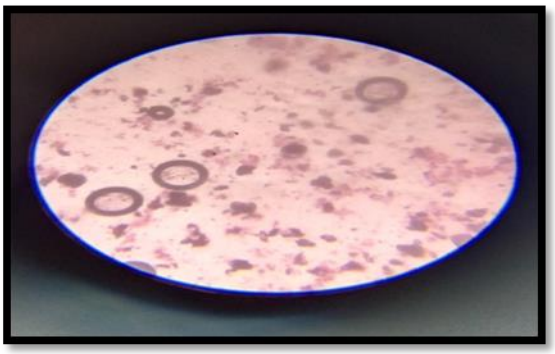

Figure 21 Microscopy slide Granules

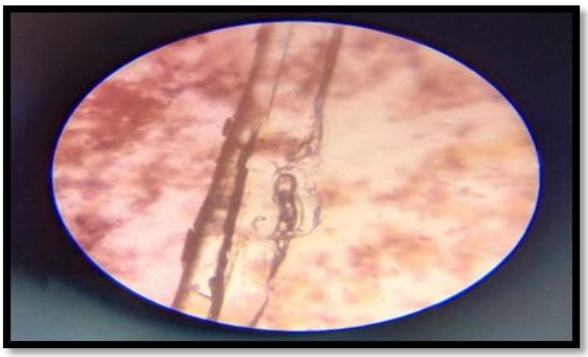

Figure 22 Microscopy slide of Fiber present

Assessment of physical nature of granules using different reagents:

Table 9 Assessment of physical nature of granules using different reagents

Name of reagent	Spontaneous color change	Color change after 5 mins	Color change after 10 mins	Color change after 30 mins
Iodine solution	Reddish brown	Red	Reddish yellow	No change
Chloroform	Pale yellow	No alteration	No alteration	No alteration
Hexane	Transparent	No alteration	No alteration	No alteration
Methanol	Turbid yellow	Pale yellow	Pale yellow	No change
Acetone	transparent	Transparent	No change	No change
Ferric chloride	Brownish yellow	Brown	Brownish yellow	No change
Lead acetate	White precipitate	No change	No change	No change

Evaluation of the finished product (Polyherbal Cpaules):

Maximum wavelength λ max estimation:

The λ max was scanned from the range of 200 to 400 for getting the maximum absorbance. The maximum wavelength of 275.20 nm was recorded.

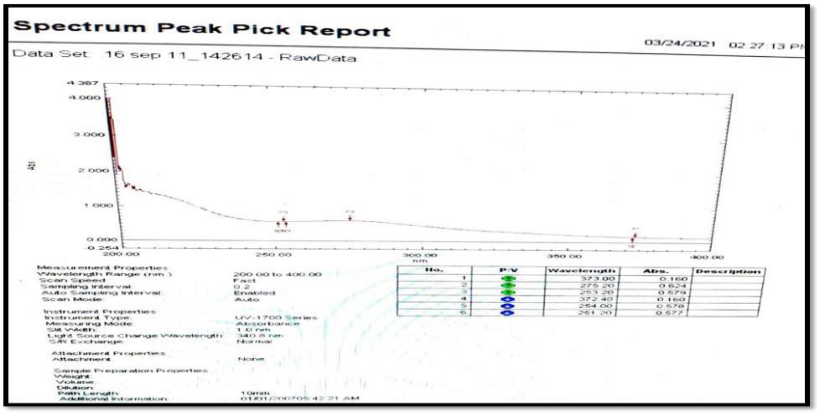

Figure 23 λ max determination

Preparation of the calibration curve:

The calibration curve was prepared by dissolving the herbal extract in phosphate buffer pH 6.8 which was further diluted to 100, 200, 300, 400, 500 µg/ml.

Table 10 Calibration curve readings

S.No.	Concentration in (µg/ml)	Absorbance
1.0	100	0.310
2.0	200	0.590
3.0	300	0.877
4.0	400	1.131
5.0	500	1.387

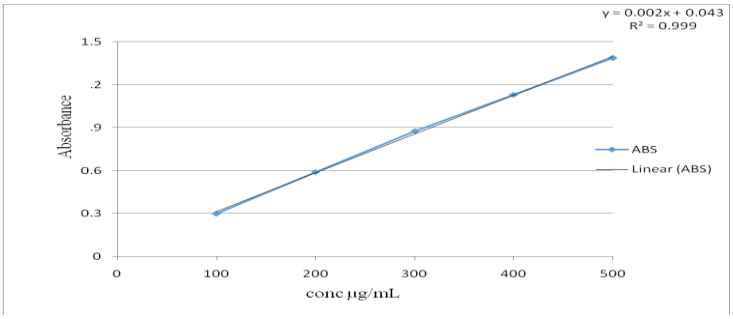

Figure 24 Calibration curve of Phosphate buffer pH (6.8)

Determination of weight variation of capsules:

The result is shown in the table no.10 below which shows that the weight variation of the capsules was not more than ±5% as per Indian pharmacopeia.

Table 11 Weight variation test of capsules

Formulation number	Weight variation result (mg)
F1	998±0.4

F2	997±0.4
F3	996±0.4
F4	999±0.2
F5	998±0.4
F6	997±0.3

Disintegration test of capsules:

Table 12 Disintegration time of capsules

Formulation number	Disintegration time of capsules
F1	14±0.4
F2	14±0.3
F3	13±0.4
F4	12±0.3
F5	11±0.2
F6	10±0.5

Invitro/ Dissolution study of capsules:

The invitro release of the prepared capsules (F1-F6) was tested for the invitro release. The dissolution test apparatus was used for the same. The table below shows the time of dissolution and % drug release.

Table 13 Invitro % release of drug

Time (minutes)	F1	F2	F3	F4	F5	F6
	% drug release	% drug release	% drug release	% drug release	% drug release	% drug release
10	4.5	9.9	17.1	19.8	27.9	32.4
20	5.4	10.8	18.9	25.2	29.7	36.9
30	7.2	12.6	23.4	27.9	32.4	40.7
40	10.6	16.8	25.2	28.6	34.2	43.2
50	14	20.1	28.8	34.2	37.8	46.8
60	20	28	30.6	36.9	41.4	50.4
70	24	31	34.2	41.3	44.1	53.1
80	28.6	35	36.9	43.9	47.7	57.6
90	32	38	42.5	47	49.5	62.1
100	34	42	48.9	49.4	52.2	64.8
110	41	44	51.4	51.2	54.9	67.5
120	45	48	53	55.8	60.8	70.2
130	48	52	55	60.6	65	74
140	52	55	60	64	71	74.9
150	58	62	64	73	76	78.7
160	63	65	70.7	75	80	85.5
170	67	70.6	78	81.1	84	88.2
180	70	74	81	85.9	90	95.8

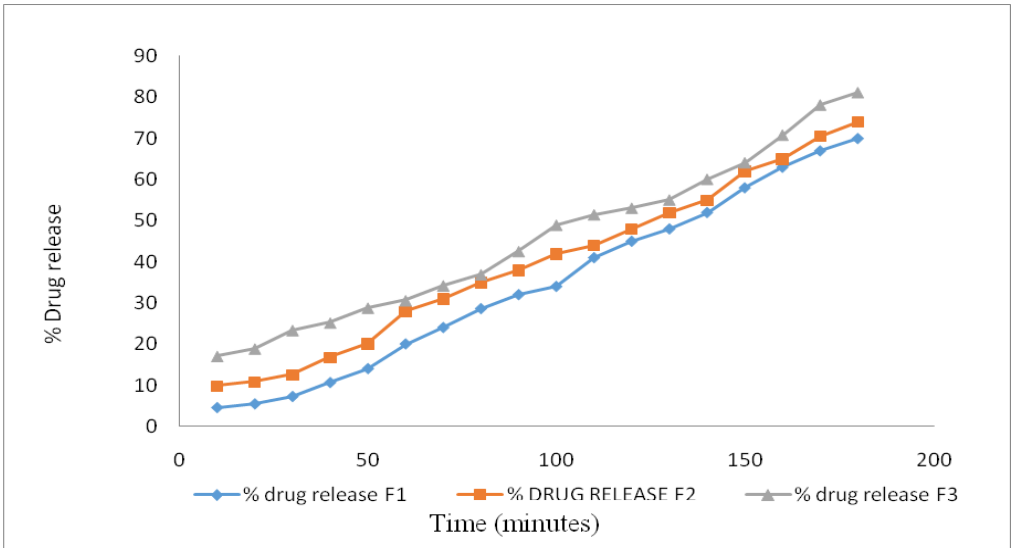

Figure 24 % Drug release for F1-F3

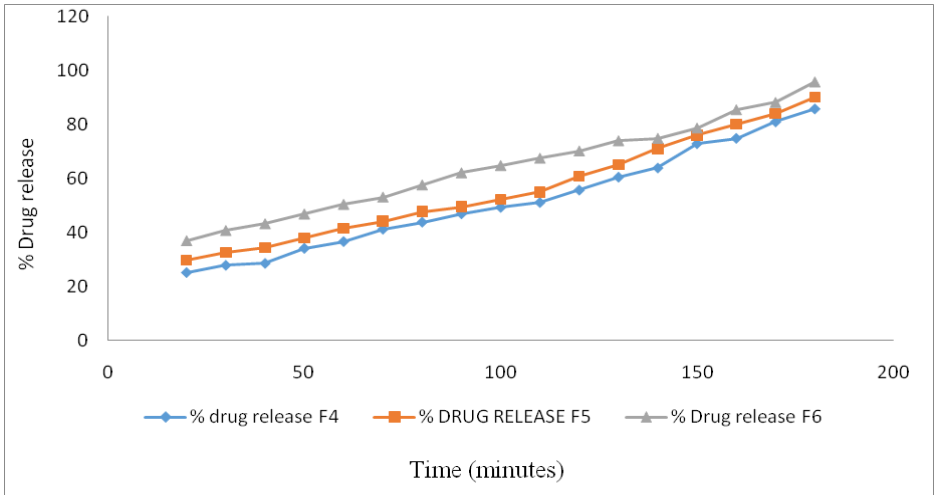

Figure 25 % Drug release for F4-F6

Drug content Uniformity analysis

Table 14 Drug uniformity % in capsules

Formulation number	Drug content %
F1	96.6±0.2%
F2	97.3±0.3%
F3	98±0.2%
F4	96.8±0.4%
F5	97.2±0.2%
F6	98.5±0.3%

FT-IR analysis of the prepared formulation:

The prepared F1-F6 formulation was tested for FT-IR.

The FT-IR of the prepared formulation show a strong –C-H of 2900-2935cm-1 was observed and other peaks which were present in polyherbal powder. The presence of all the functional groups shows no interaction in the formulation.

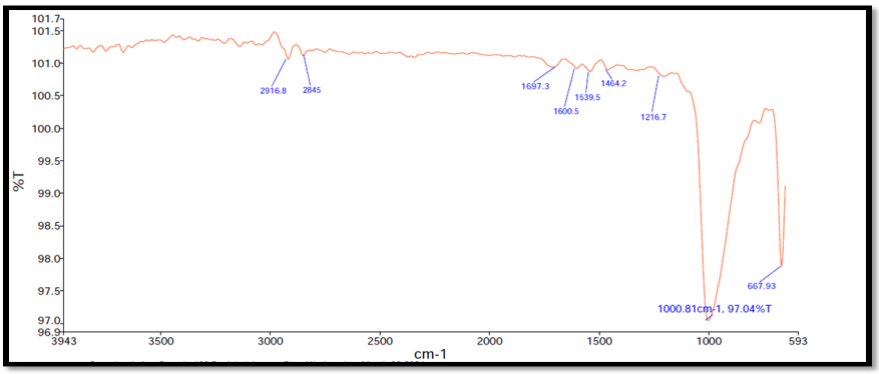

Figure 26 FT-IR of F1

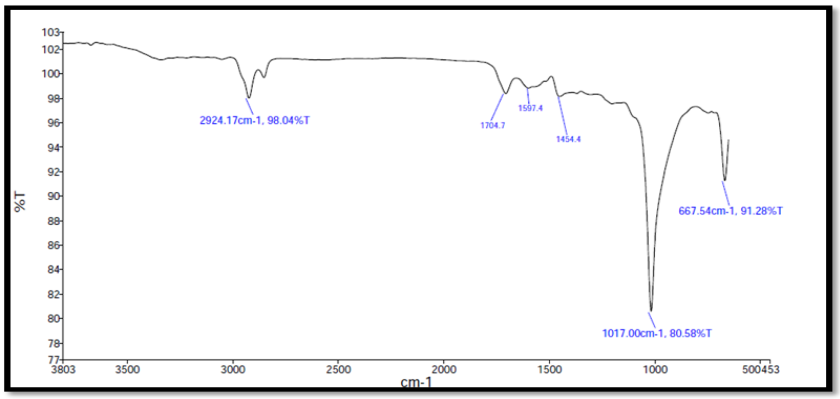

Figure 27 FT-IR of F2

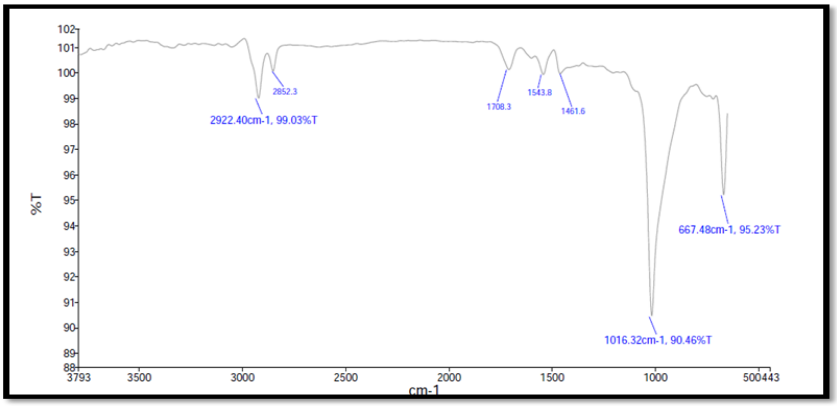

Figure 28 FT-IR of F3

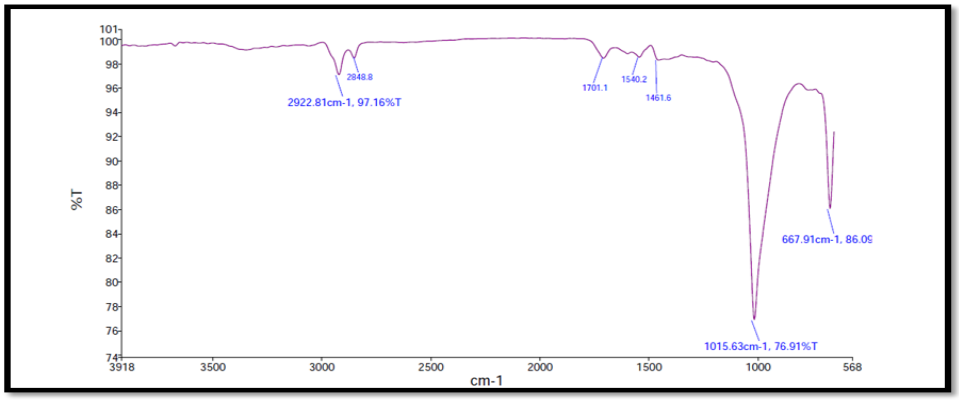

Figure 29 FT-IR of F4

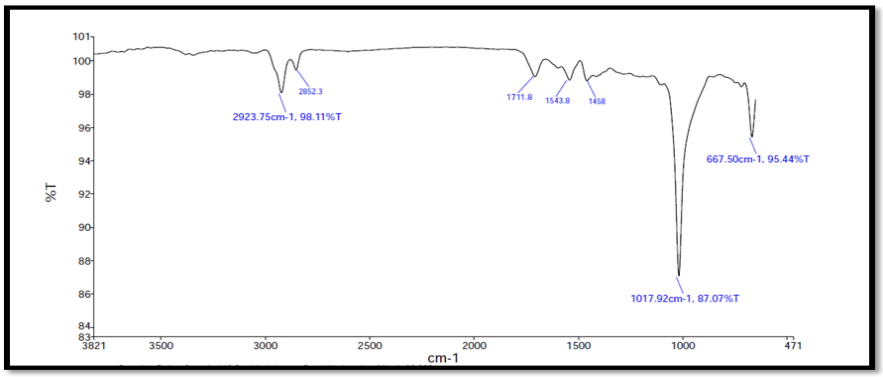

Figure 30 FT-IR of F5

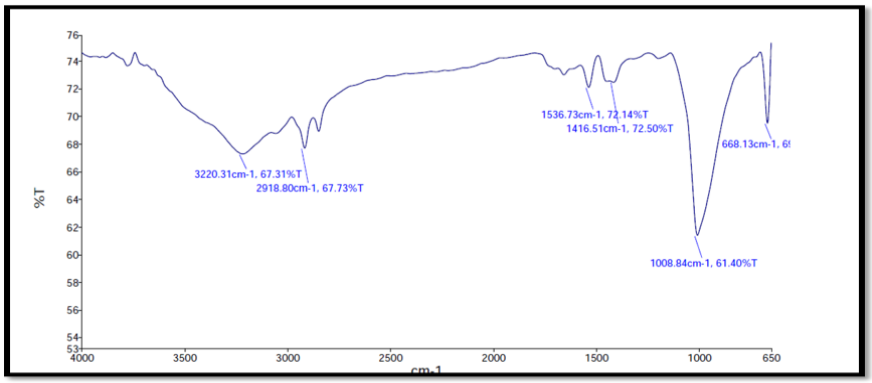

Figure 31 FT-IR of F6

DSC characterization of the prepared formulation:

The Differential scanning colorimetry was evaluated for the prepared polyherbal formulation. 146.85℃ and 205.92℃ were the observed melting points.

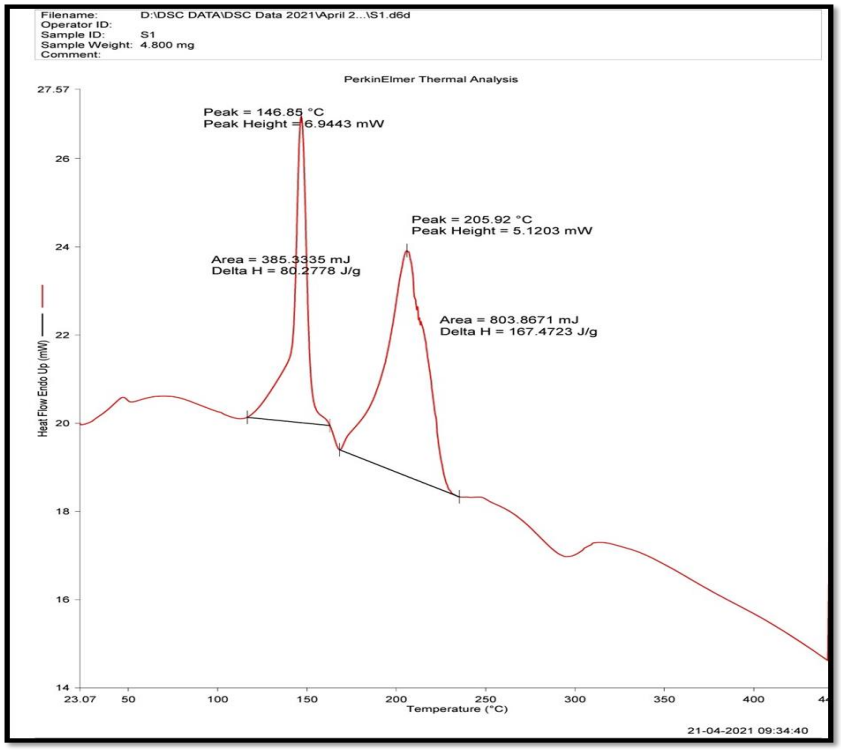

Figure 32 DSC of the prepared formulation

Toxicity study

Acute toxicity study:

The studies in rats were done in dose of 500mg/kg and 1000mg/kg. The female rats did not show any side effects or reactions. The rats were safe and it caused no harm or death of the rats. The acute toxicity was done for 14 days and showed no signs of toxicity in rats.

Sub acute toxicity:

The rats were treated with 750mg/kg and 2000mg/kg of the dose. The female rats showed no allergies or any reaction. They survived for 28 days without any signs of toxicity. After completing of dosing, rats were succumbed to sacrifice and their histology and biochemical parameters were noted. Both the parameters showed no signs of toxicity, making the herbal extract safe and potent. The complete CBC profile as well as effect on testosterone and estrogen was seen in the biochemical reports.

Figure 33 Rat dissection

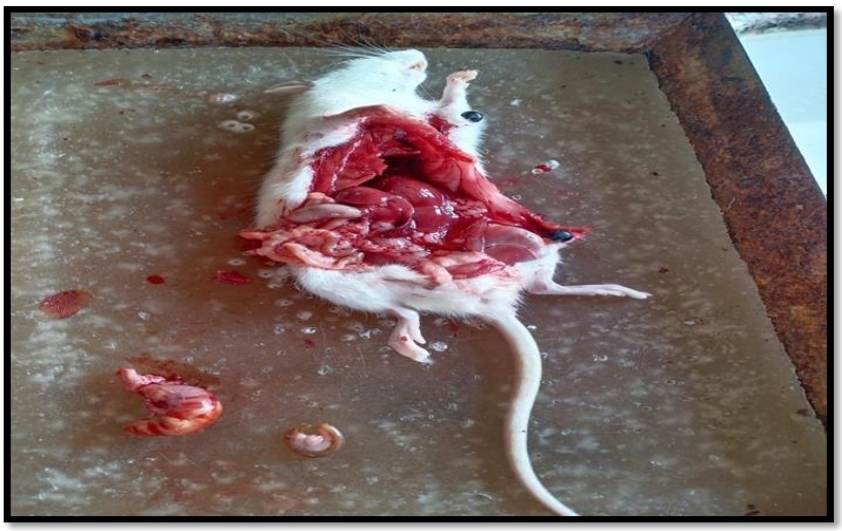

Figure 34 Rat dissection and separation of organs

Histopathology of the female rat organs:

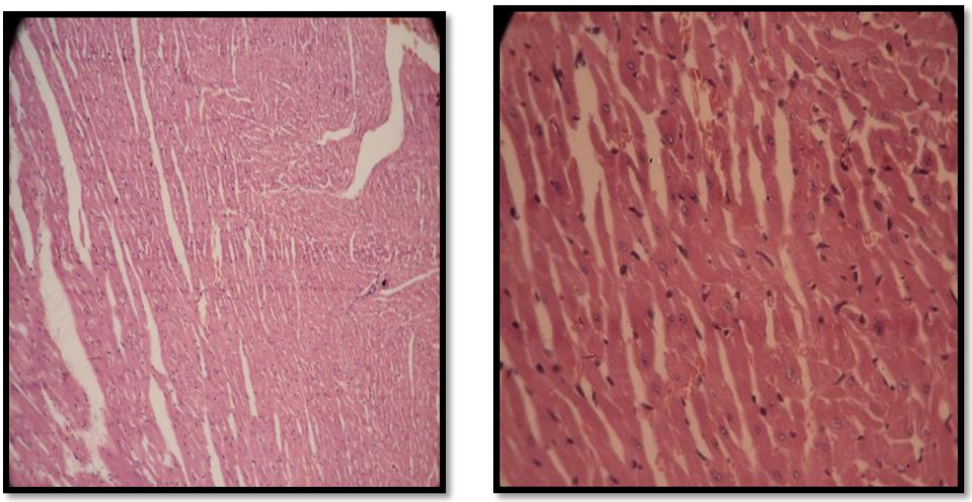

Figure 35 and 36 Histology images of rat heart at 10X and 40X respectively

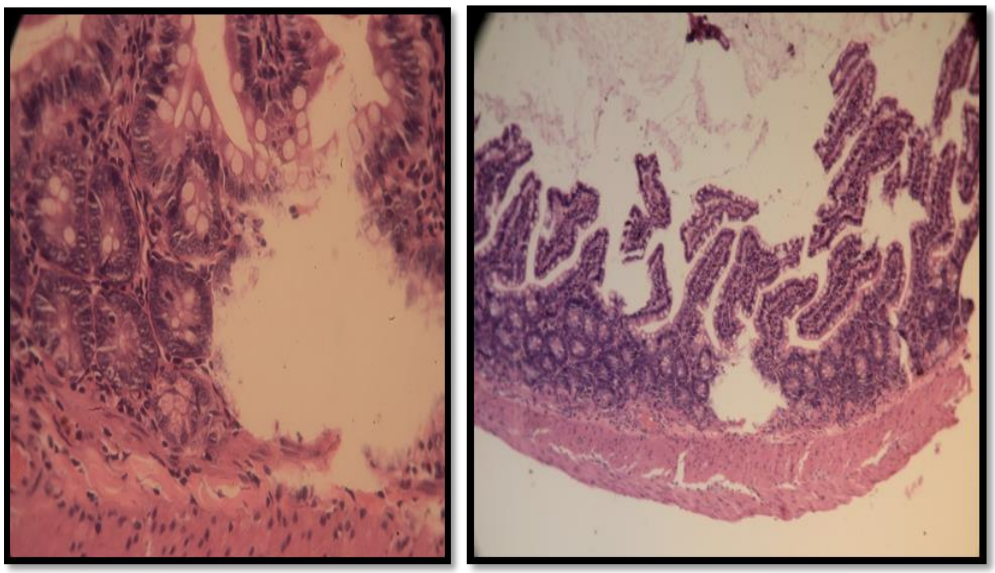

Figure 37 and 38 Histology images of rat intestine at 10X and 40X respectively

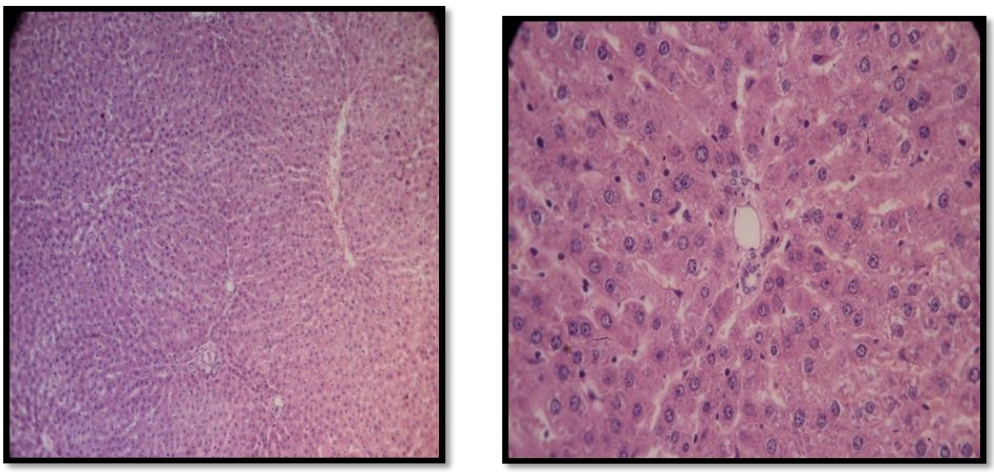

Figure 39 and 40 Histology images of rat liver at 10X and 40X respectively

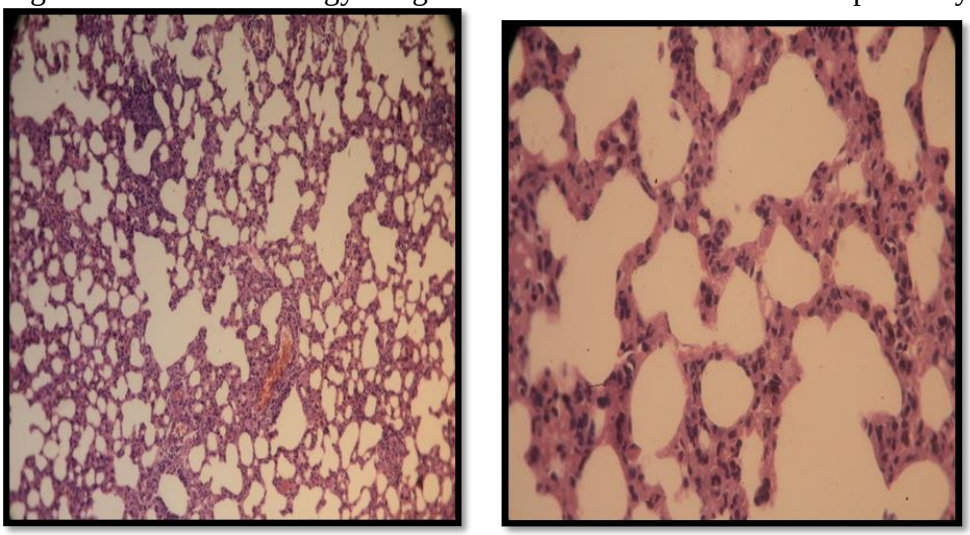

Figure 41 and 42 Histology images of rat lungs at 10X and 40X respectively

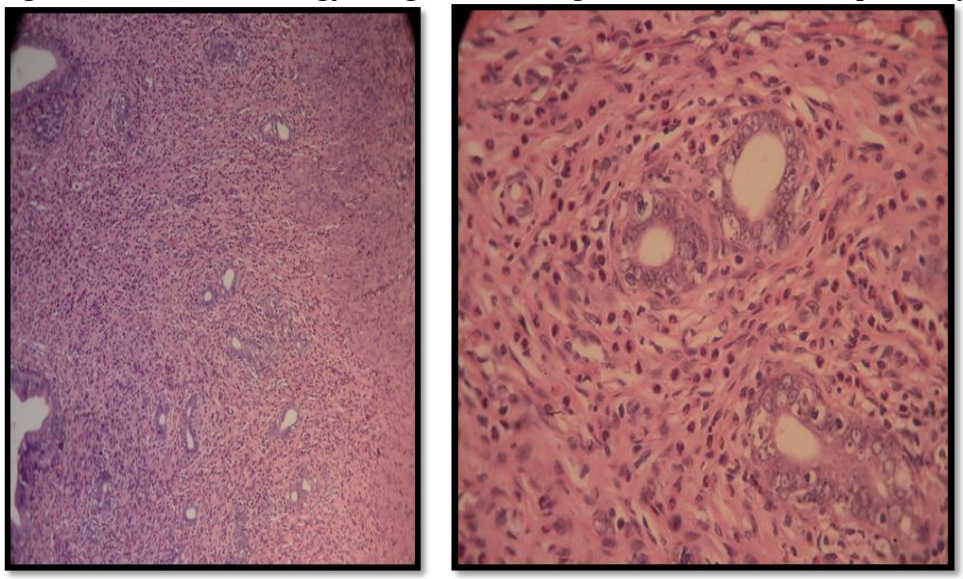

Figure 43 and 44 Histology images of rat ovaries at 10X and 40X respectively

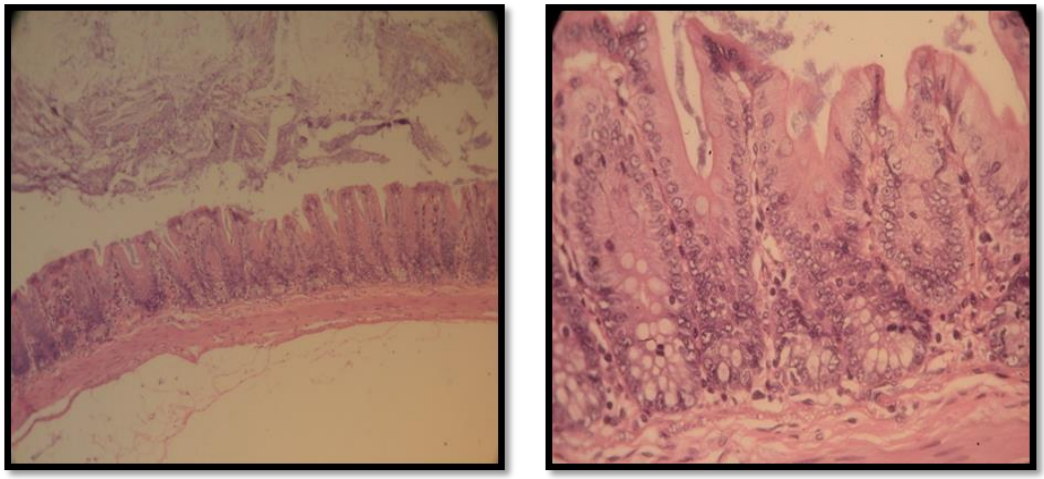

Figure 45 and 46 Histology images of rat stomach at 10X and 40X respectively

Stability study:
The prepared formulation didn't show any much decrease in the percentage content of the formulation after 1 month after storing at 40℃-45℃. The % drug content is given in table below:

Table 15 Stability study

Formulation	Storage condition	7 days	15 days	30 days
F6	40℃-45℃.	98.5±0.2	98.5±0.2	98.4±0.1

There are several studies related to herbal projects which show that herbal treatment has better effect in treating Poly cystic ovarian syndrome in women. The menstrual cycle which is irregular and other hormonal issues can be treated with herbal remedies. The reason for the selection for development of polyherbal capsules was less or least toxicity when compared to allopathic form of medicines. The selected herbs complement each other and have synergistic action for the treatment of the disease.
The prepared capsules showed faster rate of disintegration due to the presence of disintegrant in the formulation. The conventional capsules usually disintegrate in 10 or more minutes of time. But, the prepared herbal capsules took lesser time to disintegrate as seen in case of formulation F6. The formulation or the prepared extract showed no signs of toxicity when tested during the toxicity studies both acute and sub-acute. Thus, the prepared formulation is potent and safe to use for the treatment of PCOS in women.

4. Conclusion

The use of traditional medicines is believed to be used by majority of population in the developing countries, because of no side-effects. Herbal formulations are believed to produce synergistic or potentiating effect on each other. The selected herbs are a boon for the treatment of Polycystic Ovarian Syndrome without producing any side-effects. The herbs in the form of powder, extract and granules were seen to comply with the standards. Thus, the herbs have potent role in the treatment of PCOS.
The current research study was based on the role of medicinal herbs, namely, Bauhinia variegata, Emblica officinalis, Terminalia belerica, Terminalia chebula, Commiphora wightii, Cinnamon Cassia, Tribulus Terrestris, Hypericum perforatum, Commiphora molmol, Nigella sativa in the treatment of Poly Cystic Ovarian Syndrome. The study includes all the Phytochemical, Pharmacognostic, Rheological evaluation of the herbs. These were further evaluated based on the polyherbal extract and formulation of the granules. Also, the prepared formulation complies with all the standards as per Indian Pharmacopoeia and Ayurvedic Formulary.
The various in vivo and in vitro studies can be carried forward for the better understanding pharmacokinetic and pharmacodynamic effects of the herbal medicine.

5. Acknowledgement

I would sincerely like to thank the Director of the Institute of Pharmacy, Dr. A.K. Rai for all the guidance and providing all the facilities to conduct this research. I am also thankful to Pranveer Singh Institute of Technology (PSIT) for providing well equipped labs and working environment.

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