FORMULATION, EVALUATION AND STANDARDIZATION OF GUDUCHI, LINSEED, CINNAMON – BASED GUMMIES FOR THE TREATMENT OF PCOD

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ABSTRACT:

Polycystic Ovarian Disease (PCOD) is a PCOD is a multifactorial endocrine illness that affects an extensive percentage of women who are of reproductive age. It is characterized by ovarian cysts, insulin resistance, hormonal imbalance, and irregular menstrual cycles. Conventional therapies often lead to side effects and limited long-term efficacy, prompting the exploration of safe, natural alternatives. The aim of this project is to formulate, evaluate and standardize herbal gummies which include the herbs cinnamon (*Cinnamomum verum*), guduchi (*Tinospora cordifolia*), and linseed (*Linum usitatissimum*), which have been studied for their anti-inflammatory, antioxidant, hormone-regulating, and insulin-sensitizing properties. In order to improve bioavailability, palatability, and patient compliance, gummies were selected as the delivery method. The formulation process involved optimization of herbal extract concentrations, selection of suitable gelling agents and excipients, followed by comprehensive evaluation for organoleptic properties, texture, stability, and in vitro efficacy. Standardization was achieved using modern analytical methods to ensure batch-to-batch consistency and quality control. The resulting polyherbal gummy provides a promising, patient-friendly nutraceutical intervention for the holistic and comprehensive management of PCOD symptoms.

INTRODUCTION:

Polycystic Ovarian Disease (PCOD) is a common endocrine disorder affecting women's reproductive age, which is characterized by hormonal imbalances, irregular menstrual cycles, insulin resistance and the ovary occupied by multiple ovarian cysts. Current pharmacological treatments, while effective also associated with side effects and don't address the root causes of the disorder. As a result, there is growing interest in alternative and complementary therapies that utilize natural ingredients with medicinal properties. [1, 2]

Guduchi (*Tinospora cordifolia*), Linseed (*Linum usitatissimum*), and Cinnamon (*Cinnamomum verum*) are traditionally used in Ayurvedic and herbal medicine because it having potent antioxidant, anti-inflammatory, insulin-sensitizing and hormone-regulating effects. Guduchi is prominent for its adaptogenic and immune-boosting properties, linseed is rich sources of lignans and omega-3 fatty acids that support hormonal balance and cinnamon has demonstrated efficacy in improving insulin sensitivity and regulating menstrual cycles.[3]The objective of this study is to formulate and standardize an innovative gummy formulation that contains extracts of guduchi, linseed, and cinnamon. The formulation process will involve evaluating physicochemical parameters, sensory attributes, and stability, along with standardization to ensure consistency, efficacy, and safety. This novel approach aims to provide a holistic, natural, and patient-friendly option for the dietary management of PCOD.

MATERIALS AND METHODS

Collection of Plant Material:

Crude drug powder was purchased from "Population Siddha Pharmacy". Various parameters evaluated powders such as powder microscopy, ash value, moisture content, and extractive value.

PRELIMINARY STUDIES

Organoleptic Characteristics:

An organoleptic evaluation was carried out by visual observation of powdered crude drugs obtained through colour, odour, and taste.

MICROSCOPICAL EVALUATION

Powder microscopy: Take a clean slide and place a crude drug powder over the slide. Add a drop of phloroglucinol and one drop of Concentrated HCl. Add 1 drop of glycerine and then place a cover slip over the slide. Observe under the Binocular microscope. [4, 6, 8]

QUALITATIVE TEST

Determination Ash value: To determine the amount of ash, a clean, empty crucible was heated to 600°C for an hour in a Bunsen burner, cooled in a desiccator, and its weight was recorded (W1). 1g of sample was taken in the crucible (W2). The sample was ignited over a burner with the help of a blowpipe until it was charred. Then the crucible was placed in a bunsen burner at 550°C for 2-4 hours. The appearance of gray-white ash denotes complete oxidation of all organic matter in the sample. After the ashing furnace was switched off. The crucible was cooled and weighed (W3). The above-mentioned procedure is the same for all crude drugs ash value determination. [7, 9]

Determination of moisture content by loss on drying: The percentage of moisture content was determined by using the oven drying method. Two grams of the sample were accurately measured in a dry, clean Petri dish (W1). For two hours, the Petri dish was heated at 100–105°C until its weight remained steady. The Petri dish was then allowed to cool by using desiccator. It was weighed cooling (W2) after cooling. The percentage of moisture content was calculated by using the formula. [7]

Determination of Extractive value: Weigh about 5g of powdered crude drug. Air dries the drug and macerate with 100ml of water in a stoppered flask. Shake frequently for first 6 hrs and allow standing for 24 hrs. Filter rapidly by filter paper. Collect the filtrate. In a shallow dish with a tarred bottom, evaporate 25 ml of the filtrate until it is completely dry. Dry at 105˚C, weigh. Finally, the extractive value percentage was calculated. [5]

PROCEDURE FOR EXTRACTION

The fine powders (Guduchi, Linseed, and Cinnamon) undergo the Soxhlet extraction method by using hydroalcoholic solvent (70% Ethanol and 30% Water) for two successive days (48hrs), the temperature should not exceed more than 100°C. The dark green colour hydroalcoholic extract was collected, and filtered, and the filtrate was subjected to concentrate by using a heating Mandel. Finally, the dried extract was stored in a desicator. [10, 11, 12, 13, 14, 15]

PHYTOCHEMICAL SCREENING

Test for alkaloids

Dragendroff's test:

To 5 mL of the extract, a few drops of Dragendroff's reagent were added to form an orange-colored precipitate. [16, 17]

Wagner's test:

To 5 mL of the extract, a few drops of Wagner's reagent were added to form a reddish-brown colored precipitate. [16, 17]

Test for flavonoids

Shinoda test:

Few magnesium ribbons are dipped and conc. HCl was added into to the 3ml extract over them and observed for the formation of a magenta (brick red) colour indicating the presence of flavonoids.[16, 17]

Test for proteins

Millon's test:

To 3 mL of the extract, a few drops of Millon's reagent were added to form a red colour.[16, 17]

Test for carbohydrates

Molisch's test:

To the small amount of the extract is added to a few drops of Molisch's reagent. Then addition of conc. H_2SO_4 on the sides of the test tube. After letting the mixture remain for two minutes, 5ml of distilled water were added to dilute it. Two layers came together to form a reddish-violet color. It indicates the presence of carbohydrates. [16, 17]

Fehling's test:

5ml of Fehling's solutions (A and B) were added to the extract, which was then placed in a boiling water bath. Red or yellow precipitate formation is an indication that reducing sugar is present. [16, 17]

Test for tannins

Gold beater's skin test:

A fraction of the extract was dissolved in water and then it was subjected to a water bath at 37°C for 1 hour and treated with ferric chloride solution and observed for the formation of dark green colour. [16]

Test for sterols

Liebermann-Burchard test:

A few drops of chloroform, acetic anhydride, and H_2SO_4 were added to a small amount of the extract and along the sides of the test tube to observe the formation of a dark red or pink colour. [16]

Test for glycosides

Keller-Kiliani test:

A few drops of ferric chloride solution were added to 5 mL of the extract and mixed. Following that, a solution of sulphuric acid and ferric chloride was added. It formed two layers, one showing reddish brown, while the upper layer turned bluish-green, which indicates the presence of glycosides. [17]

Test for phenols

Ferric chloride test:

A fraction of the extract was treated with 5% ferric chloride solution and observed for the formation of a deep blue or black colour. [17]

Test for saponins

Foam test:

A few drops of distilled water were added to a small amount of the extract, and it was rapidly agitated until a persistent froth was visible. [18]

Test for terpenoids

Chloroform test:

In 5 ml of the extract added few drops of chloroform and conc. H2SO4 was added carefully along the sides of the test tube to form a layer and observed for the presence of a reddish-brown color. [18]

Detection of Emodins

Borntrager's test:

Two ml of NH4OH and 3 ml of Benzene were added to the extract. The appearance of red color indicates the presence of emodins. [18]

Detection of amino acids

Ninhydrin Test:

To the extract, 0.25% w/v ninhydrin reagent was added and boiled for a few minutes. The presence of amino acids is indicated by the production of a blue color. [18]

PROCEDURE FOR GUMMY BASE FORMULATION:

- Agar powder, acting as a gelatin substitute, was dissolved in water to make a gel base.
- Jaggery was added to the gel base as a sweetening agent, ensuring the palatability of the gummies.
- To improve flavour, citric acid was added, which improved the gummies' flavour profile.
- A small amount of olive oil was used as a coating agent to prevent sticking during preparation.
- The above mixture was heated and stirred until all ingredients were thoroughly combined
- Once the mixture reached a uniform consistency, it was poured into a mould, forming the desired gummy shape.
- The filled mould was allowed to be set at room temperature, allowing the gummies to solidify and take shape.

- The finished product was prepared for final assessment after the gummies were carefully taken out of the mold after solidification. [3]

Table 1: Composition of Gummies

S.NO	INGREDIENTS	FORMULATION
1.	Agar	2g
2.	Jaggery	2g
3.	Citric Acid	0.08g
4.	Sodium Benzoate	0.2g
5.	Water	20ml

PROCEDURE FOR PCOD GUMMIES:

- Agar powder, acting as a gelatin substitute, was dissolved in water to make a gel base.
- Jaggery was added to the gel base as a sweetening agent, ensuring the palatability of the gummies.
- Herbal extracts of Guduchi, Cinnamon, and flaxseed, obtained through Soxhlet extraction for efficient extraction of the bioactive compound, were added to the gel base.
- To improve flavour, citric acid was added, which improved the gummies' flavour profile.
- A small amount of olive oil was used as a coating agent to prevent sticking during preparation.
- The above mixture was heated and stirred until all ingredients were thoroughly combined, ensuring a uniform distribution of herbal extracts and other components.
- Once the mixture reached a uniform consistency, it was poured into a mould, forming the desired gummy shape.
- The filled mould was allowed to be set at room temperature, allowing the gummies to solidify and take shape.
- The finished product was prepared for final assessment after the gummies were carefully taken out of the mold after solidification. [3]

Table 2: Composition of PCOD Gummies

S.NO	INGREDIENTS	FORMULATION
1.	Guduchi extract	0.3g
2.	Linseed Extract	0.3g
3.	Cinnamon Extract	0.4g
4.	Agar	2g
5.	Jaggery	2g
6.	Citric Acid	0.08g
7.	Sodium Benzoate	0.2g
8.	Water	20ml

STANDARDIZATION OF THE GUMMIES

Organoleptic Evaluation

An organoleptic evaluation was carried out by visual observation of the gummies' preparation, which was obtained through shape, color, and smell.[20]

Thickness

The thickness of a gummy was measured using a vernier caliper. [19]

PH OF GUMMIES

Preparation of buffer solution:

- Dissolving in a phosphate buffer tablet at pH in 100 ml dissolved water.

Calibration of pH meter:

- Set a pH meter to a neutral pH by using a buffer solution.

Determination of pH of gummies:

- Add 1g of gummy in 2 ml of distilled water and melt it at a suitable temperature.[20]

WEIGHT VARIATION

Sample selection:

- A total of 10 gummies were selected.

Weight measurement:

- Weigh each gummy individually using a weighing balance. The weight of all 10 gummies was then determined.

Calculation of weight variation:

- Calculate the weight variation of each gummy by comparing it to a reference weight (usually the average weight of the sample). [19, 20]

IN VITRO CYTOTOXICITY – DIRECT METHOD (AS PER ISO 10993:5)-MTT ASSAY

1. Cell seeding: Each well of 96-well plates containing a full medium was seeded with 30,000 cells. For 24 hours, the plates were incubated at 37°C in an environment containing 5% CO2.

2. Treatment: The cells were incubated for 24 hours at 37°C with 5% CO2 after being treated with different doses of the test samples.

3. MTT Addition: Each well received 10 µl of MTT solution, which was made by dissolving 5 mg of MTT in 1 ml of PBS.

4. MTT Incubation: 3 to 4 hrs at 37°C with 5% CO2 were spent incubating the plates.

5. Supernatant Removal: The supernatant was cautiously extracted from every well after the MTT incubation.

6. Formazan Dissolution: To dissolve the formazan crystals, 200 µl of DMSO was applied to each well. To ensure total disintegration, the plates were gently shook for ten to fifteen minutes.

7. Optical Density Measurement: The optical density (OD) of each well was measured at 570 nm using an ELISA reader (Mindray MR-96, Mindray and Nanshan, China).

8. Microscopy: Cell images were acquired using an Olympus fluorescent microscope (CKX53, OLYMPUS and Japan). [21, 22, 23]

RESULT AND DISCUSSION

Table 3: Organoleptic Evaluation

S.NO	CRUDE DRUGS	COLOUR	ODOUR	TASTE
1.	Guduchi	Green	Unpleasant	Bitter
2.	Linseed	Light brown	Characteristic	Mildly nutty
3.	Cinnamon	Tan brown	Aromatic	Mild sweet

Table 4: Powder Microscopy of Guduchi

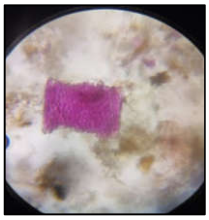


Figure 22: Pitted vessels

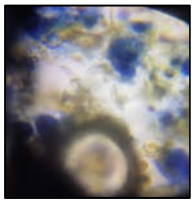


Figure 23: Stone cells

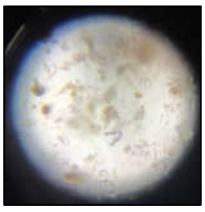


Figure 24: Parenchymatous cells with prismatic crystals

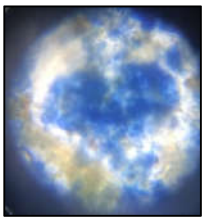
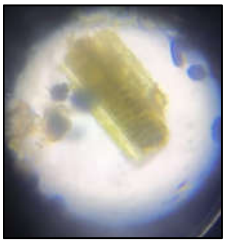


Figure 25: Simple starch with hila

Table 5: Powder Microscopy of Linseed



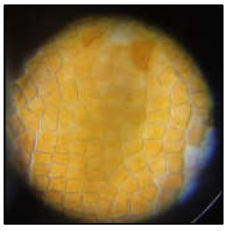


Figure 27: Fragment of hypodermis

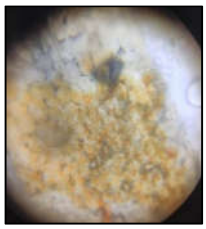
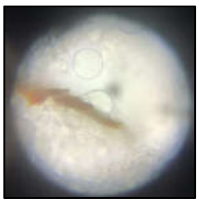
Figure 26: Fibres	
 **Figure 28: Parenchyma with fixed oil**	 **Figure 29: Oil globules with lignified fibers**

Table 6: Powder Microscopy of Cinnamon

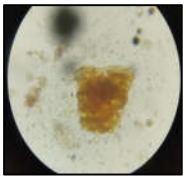
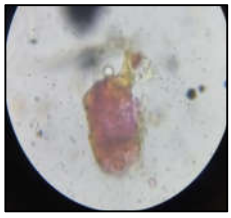
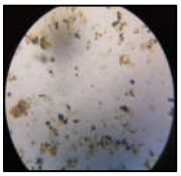
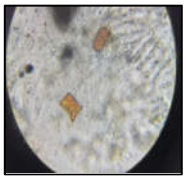
 **Figure 30: Cork cells**	 **Figure 31: Lignified fibres**
 **Figure 32:Starch grains**	 **Figure 33: Calcium Oxalate Crystals**

Table 7: Ash Value

S.NO	CRUDE DRUGS	ASH VALUE
1.	Guduchi	10% W/W
2.	Linseed	13% W/W
3.	Cinnamon	12% W/W

Table 8: Extractive Value

S.NO	SOLVENTS	Guduchi	Linseed	Cinnamon
1.	**Ethanol**	3% W/V	34% W/V	13% W/V
2.	**Chloroform**	3% W/V	36% W/V	1% W/V
3.	**Dichloromethane**	1% W/V	35% W/V	3% W/V
4.	**n-Hexane**	1%W/V	29% W/V	3% W/V
5.	**Water**	12%W/V	6% W/V	12% W/V

Table 9: Phytochemical Screening

S.NO	PHYTOCHEMICAL SCREENING	Guduchi	Linseed	Cinnamon
1	**Test for Alkaloids**			
	a)Dragendroff's test	+	+	+
	b) Wagner's test	+	+	+
2	**Test for Flavonoids**			
	a) Shinoda test	+	+	+
3	**Test for Proteins**			
	a) Millen's test	+	+	+
4	**Test for Carbohydrate**			
	a) Molish test	+	+	+
	b) Fehling's test	+	+	+
5	**Test for Tannins**			
	a) gold beater's skin test	+	+	+
6	**Test for Sterols**			
	a) Liberman-Burchard test	+	+	+
7	**Test for Glycosides**			
	a) Keller Killani test	+	-	+
8	**Test for Phenols**			
	a) Ferric chloride test	+	-	+
9	**Test for Saponins**			
	a) Foam test	+	+	+

10	**Test for Terpenoids**			
	a) Chloroform test	+	+	+
11	**Test for Emodins**			
	a) Borntrager's test	+	+	-
12	**Test for Amino acids**			
	a) Ninhydrin test	+	+	-

Note:

- **(+)** indicates the presence of compounds
- **(-)** indicates absence of compounds

STANDARDIZATION OF THE GUMMIES FORMULATION

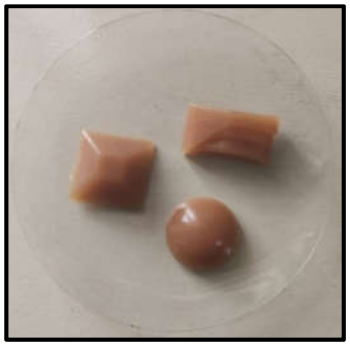


Figure 34: Gummies

Table 10: Organoleptic Evaluation

Color	Chocolate brown
Odour	Pleasant
Shape	Round
Taste	Agreeable taste

Table 11: Thickness of the Gummies

S.NO	THICKNESS OF THE GUMMIES		MEAN VALUE
1	**I TRAIL**	3.55 mm	3.47 mm
2	**II TRAIL**	3.32 mm	
3	**III TRAIL**	3.55 mm	

pH of Gummies:

The pH level of the gummy sample was measured using a digital pH meter. The results indicate that the gummies have a slightly acidic nature, with an average pH value of **4.9.**

***IN VITRO*CYTOTOXICITY – DIRECT METHOD (AS PER ISO 10993:5)-MTT ASSAY**

Table 12: Evaluation criteria

S. No	Grade (%)	Reactivity
1	0	None
2	1-20	Slight
3	21-50	Mild
4	51-70	Moderate
5	>71	Severe

Table 13: Cytotoxicity value

Concentration (µM)	Sample: PHG (Poly Herbal Gummies)	
	Cytotoxicity (%)	Cytotoxic reactivity
Control	1.7755	Slight
100	12.9632	Slight
200	26.1948	Mild
300	32.3024	Mild
400	42.8065	Mild
500	54.4118	Moderate

Table 14: Theca Cells (Tcs), MTT Assay-PHG:

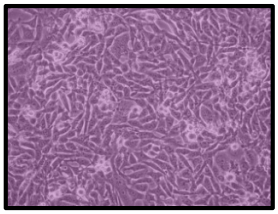


Figure 38: Control

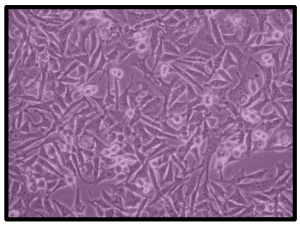


Figure39: 100 µm

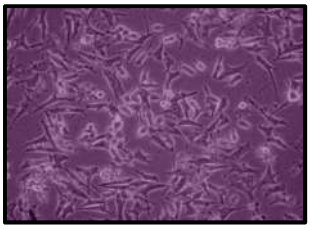


Figure 40: 200µm

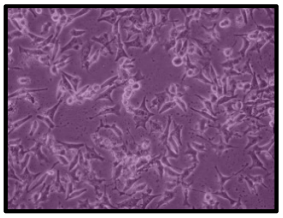


Figure 41: 300µm

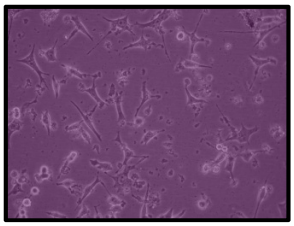


Figure 42: 400µm

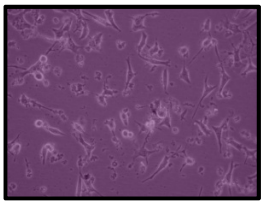


Figure 43: 500µm

Bar chart for *IN VITRO* Studies:

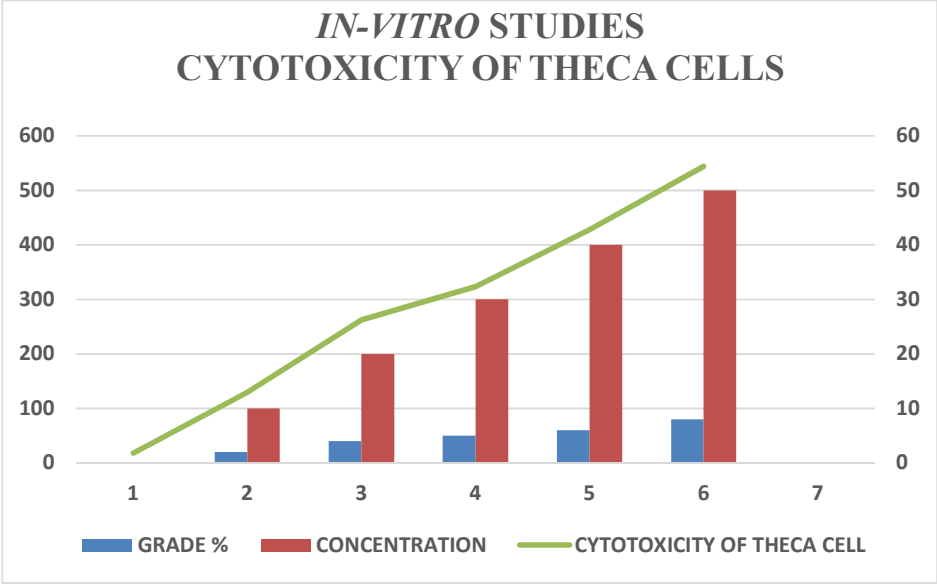


Figure 44: Bar chart for *IN VITRO* STUDIES

While increasing the concentration of the gummy, the cytotoxicity of the theca cells also increases simultaneously.

SUMMARY:

The aim and objective of presenting and evaluating herbal-based gummies for treating PCOD. It should kill theca cells. So, our research study focused on selecting pharmacologically potent herbal drugs (Guduchi, Linseed, and Cinnamon) and was subjected to suitable extraction.

Then the gummies mould array was fabricated, by the help of mould array the gummies were successfully prepared using one ratio of polymer. This gummies type comes under the category of dissolving gummies in the mouth. This type of popular candies with a chewy texture. Gummies are frequently simpler to swallow and more palatable.

The basic standardization parameters like physical examination. Gummies formulation, pH, weight variation, and percentage moisture content, were done for the formulation. And, it was subjected to further study. In vitro cytotoxicity study was done using theca cells with five different concentrations (100µm, 200µm, 300µm, 400µm, 500µm) and one control, so it gives good action against PCOD.

CONCLUSION AND FUTURE PROSPECT:

This is an attempt to develop herbal-based gummies for the treatment of PCOD with a combination of herbal extracts, from the past year, the department of multivitamins, as well as traditional molecules, has led to the gummies-based product development. The arrival of various commercial gummy products is highly anticipated. Gummies may extent a remarkable impact on clinical medicine and cosmetics over the upcoming future. For this formulation, we evaluated weight variation, pH, physical appearance, thickness, and MTT assay.

REFFERENCE:

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