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## Research Article

# Extraction, Isolation of Diosgenin from *Dioscorea Alata* Tuber and Development of a Polyherbal Syrup for Ovarian Disorder (PCOD)

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## ABSTRACT

Endocrine disorder is a condition related to hormones, and Polycystic Ovarian Disorder is one of them. This condition predominantly affects women. In females, it disrupts hormonal balance, compromises ovarian function, and causes metabolic irregularities, frequently leading to infertility. In recent years, herbal therapies have gained interest due to their multi-targeted mechanisms and fewer side effects than conventional treatments. This research work aims to extract and isolate diosgenin, which is a steroidal sapogenin, from the tubers of *Dioscorea alata*, and develop a polyherbal syrup formulation for the treatment of PCOD. Diosgenin was extracted using the soxhlet extraction method, followed by acid hydrolysis of saponin glycosides, and further purified with suitable organic solvents. Its identification and purity were confirmed through the UV spectroscopy technique and chromatographic technique. Diosgenin is an important precursor for steroidal hormone synthesis and shows estrogenic, anti-inflammatory, and modulatory effects on ovarian activity. A polyherbal syrup was formulated by combining diosgenin and amla extract distinguish for their role in maintaining hormonal balance and promoting reproductive health. This formulation was assessed for its possible therapeutic effectiveness. The synergistic effects of *Dioscorea alata* and Amla phytoconstituents improve hormonal balance, reduce oxidative stress and support the regulation of menstrual cycles. Overall, this study shows the potential of diosgenin obtained from *Dioscorea alata* tuber and also Amla. It supports the formulation of an effective polyherbal syrup for PCOD treatment. Further pharmacological and clinical studies are required to confirm its safety and effectiveness

## INTRODUCTION

*Dioscorea alata* is an edible tuberous plant that is a member of the Dioscoreaceae family. It is also known as purple yam, winged yam, greater yam or

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water yam. It is widely cultivated around the world in tropical and subtropical regions [1], especially in Asia, Africa, and the Pacific Islands. The plant is prized for its starchy tubers, which are an important food source and rich in carbohydrates. *Dioscorea alata* is well known for its therapeutic qualities, such as anti-inflammatory, antioxidant, and antidiabetic qualities, in addition to its culinary uses. The plant has been shown to have a wide range of bioactive chemicals that add to its many medicinal benefits. Additionally, because it is used in traditional medicine and rituals, the plant has cultural significance in many communities [2][3][4].

**Diosgenin:** Diosgenin is a natural bioactive steroidal sapogenin and it extracted from the tuber

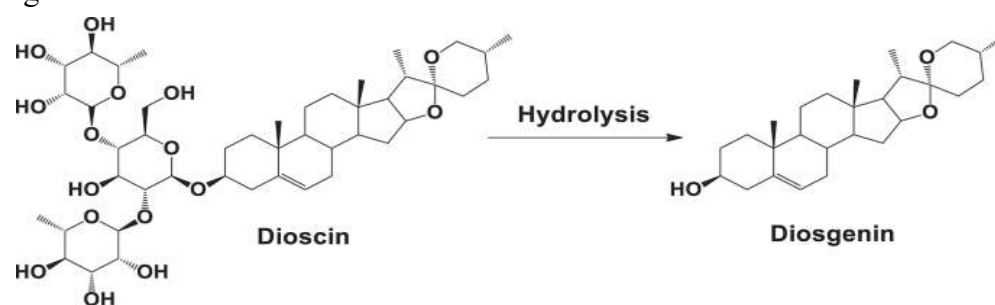


Figure: 1 Chemical structure of Diosgenin (C<sub>27</sub>H<sub>42</sub>O<sub>3</sub>) [9]

**Polycystic ovarian disorder:** Polycystic means many cysts. Polycystic ovarian disorder is a complex disorder of chronic oligoanovulation or oligomenorrhoea and clinical or biochemical hyperandrogenism [10][11]. The condition known as polycystic ovarian disorder is characterised by an imbalance of hormones in women. This condition can cause irregular menstrual cycles, fertility issues, and difficulty in conceiving. Diabetes and heart disease are two major health issues that can develop if proper treatment is not provided. In this condition, many small cysts grow on women ovaries. That's why this condition known as Polycystic ovarian disorder. The cysts cause hormone abnormalities, although they are

not dangerous. Long-term issues can be avoided, and symptoms can be managed with early diagnosis and treatment [12][13].

of *Dioscorea* species. It is a member of the triterpene group. Many pharmaceutical industries are showing interest in the manufacturing of diosgenin [5][8]. It is the aglycone that is produced when saponin dioscin, a substance present in *Dioscorea* species, is hydrolysed [6][8]. After the acidic hydrolysis of glycosides diosgenin is obtained in the form of white precipitate. Then, washed with a suitable organic solvent, obtain pure diosgenin in the form of white or off-white powder. It is a primary and important precursor for the synthesis of oral contraceptives, corticosteroids, sexual hormones, and other steroidal medications [7][8].

not dangerous. Long-term issues can be avoided, and symptoms can be managed with early diagnosis and treatment [12][13].

**Symptoms:** Symptoms may include irregular menstrual cycle, obesity, change in blood pressure, Body mass index, increase androgen level in blood, high glucose in blood, enlarge breasts, and hyperinsulinemia. Ultrasound may show polycysts. PCOS women may have unwanted body or facial hair growth, thinning hair on the scalp, Skin problems like skin darkening and acne. Hyperandrogenism and anovulation interact with insulin resistance in the

PCOD. Ovarian hyperandrogenism is mainly caused by inherent steroidogenic defect of theca cells. Increase level of Lutenising hormone (LH) and insulin amplify the intrinsic abnormality of

theca steroidogenesis. Decrease ratio of Follicle-stimulating hormone (FSH)/ Lutenising hormone (LH) are involved [11][14][15].

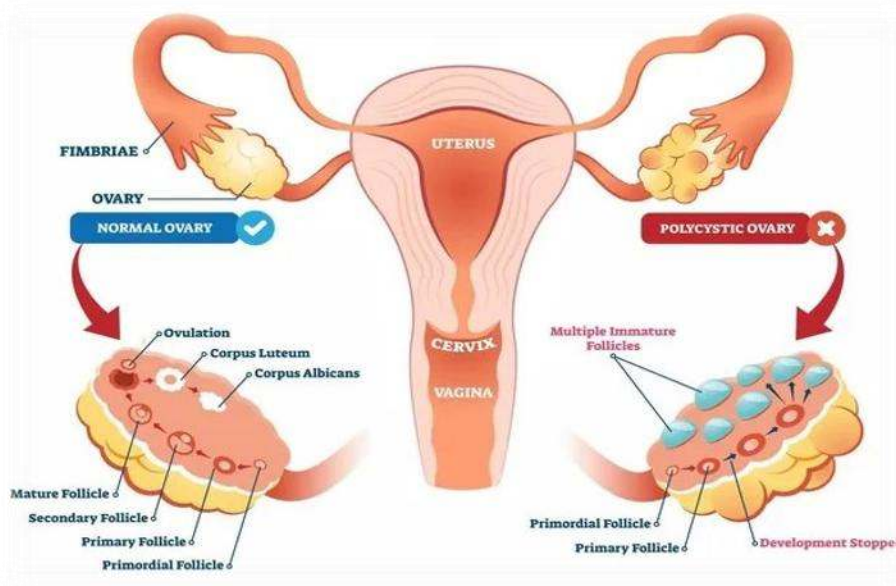


Figure: 2 Polycystic ovary disorder [16]

**Polyherbal Syrup:** Polyherbal formulations, which integrate various medicinal herbs, are recognized for their ability to produce synergistic therapeutic benefits while reducing toxicity. Conventional treatments sometimes give side effects, resulting in a growing interest in herbal treatment or medicines [17][18]. Herbal medications like *Dioscorea alata* (Purple yam) and *Emblica officinalis* (Amla) exhibit notable pharmacological effects, including hormonal regulation, antioxidant activity, and anti-inflammatory properties [19][20][21][22]. Consequently, this study aims to develop a polyherbal syrup formulation for the treatment of Polycystic Ovary Disorder (PCOD) [23][24]. The syrup formulation chosen ease administration and enhances patient compliance.

### Taxonomy of Herbs:

***Dioscorea alata*:** Common name- Water yam, Winged yam, Purple yam

Family: Dioscoreaceae

Genus: Dioscorea

Species: *Dioscorea alata* L. [25][26]

**Phytoconstituents:** Flavonoids, Glycosides, Tannins, Anthraquinones

Major Phytoconstituent: Steroidal compound (Diosgenin).

**Uses:** Anti-inflammatory, Antioxidant, Anti-diabetic, Anti-hypertensive, Anti-cancer [27][28][29][30], Aphrodisiac activity, Anthelmintic and Diuretic activity [31].



Figure: 3 *Dioscorea alata* (A) Plant (B) Tuber

***Phyllanthus emblica*:** Common name- Amla or Indian gooseberry

Family: Phyllanthaceae

Genus: Phyllanthu

Species: *Phyllanthus emblica* L.

**Phytoconstituents:** Tannins (emblicanin A&B), Polyphenols (gallic acid, ellagic acid), Flavonoids (quercetin, rutin), Vitamin C [32][33].

**Uses:** Antioxidant, Anti-inflammatory, Diuretic, Anti-aging, Anti-diabetic, Anti-microbial, Hepatoprotective, Gastroprotective, Chemo preventive properties [33][34][35][36].



Figure: 4 *Phyllanthus emblica* (A) Tree (B) Amla fruit [37]

**Materials And Method:** Polyherbal syrup was prepared by decoction method [38]. *Dioscorea alata* (Purple yam) Tuber and *Phyllanthus emblica* (Amla) fruit was obtained from local market of Rishikesh, Dehradun, Uttarakhand. Both plants specimens have been detected, approved, and authenticated by Botanical Survey of India (BSI), Northern Regional Centre, Kaulagarh Road, Dehradun. *Phyllanthus emblica* was extracted by cold maceration with hydroalcoholic solvent. Then, extract was filtered and concentrated. After

that, use this concentrated extract in polyherbal syrup. *Phyllanthus emblica* extract safe and effective amount is 500-1000 mg/day [39]. *Dioscorea alata* was extracted by Soxhlet apparatus with suitable organic solvents and after the acid hydrolysis process obtained pure form of diosgenin [40][38]. Which is use for Polyherbal syrup formulation. Diosgenin safe and effective amount is 8-50 mg/day [41]. An inverted sugar syrup base is used in the formulation due to its high

performance, stability, and sweetness [42][43][44].

**Extraction method of Diosgenin from *Dioscorea alata* Tuber:** Extraction process was done by using Soxhlet apparatus.



Figure: 4 (A) Tuber



(B) Powder Drug

**Defatting of Sample (Removal of Fats from Sample)**

- 300 g powdered drug was weighed and packed into a soxhlet apparatus.



Figure:5 (A) Defatting



(B) Defatted extract

**Extraction with methanol or ethanol**

- The defatted marc (residue) was placed in a Soxhlet apparatus.

- Then, extraction of the marc (residue) was performed using 95% methanol or ethanol for 6-8 hour at a temperature 60-70° C.
- After that, extract was collected and evaporated on water bath at medium temperature.

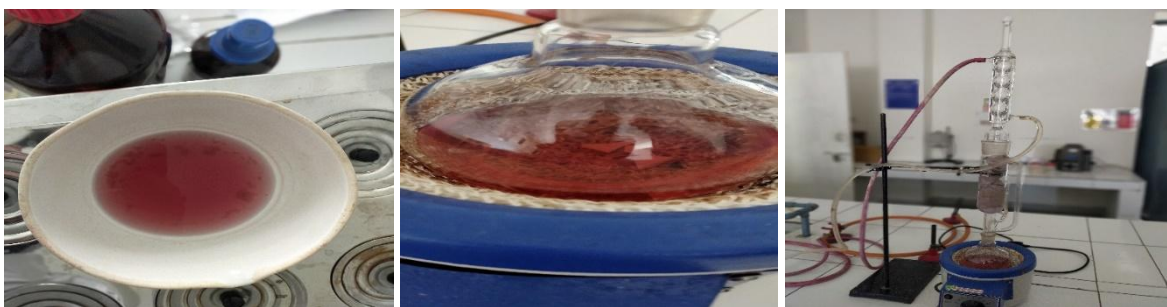


Figure: 6 (A) Extraction

(B) Extract

(C) Evaporation

### Acid Hydrolysis of glycosides (remove sugar moiety)

- The concentrated extract was treated with 2N HCL in a 1:1 ratio.
- Then, the mixture was filtered to obtain the residue.
- The residue was washed with distilled water.
- This step removed the sugar moiety of diosgenin [45].



Figure 7: Acid Hydrolysis

### Isolation of Diosgenin

- The Hydrolysed mixture was transferred into a separating funnel.
- Then, 25 ml of chloroform was added into the separating funnel and shaken properly with venting.
- The mixture was allowed to stand for phase separation. The lower layer was chloroform layer contained diosgenin and upper layer was aq. layer.
- The lower layer was collected, and this same procedure was repeated twice with aqueous layer.
- Then, all the chloroform layers were mixed and evaporated on a water bath.
- Residue was collected and recrystallized with methanol.
- After the recrystallization, pure diosgenin crystals were obtained.
- White or off-white crystals of diosgenin were obtained [40][46][47].

Weight of Diosgenin / Weight of powdered drug x 100 =  $2.4 / 300 \times 100 = 0.8\%$

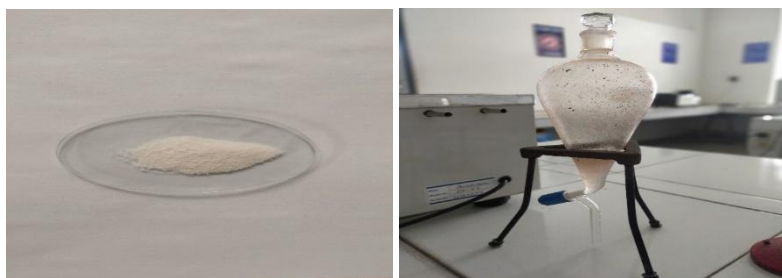


Figure:8 (A) Separation

(B) Diosgenin

**Extraction method of *Phyllanthus emblica*:**  
Extraction of *Phyllanthus emblica* (amla) was done by using the cold maceration method.

- 60 g of amla powder was weighed and the powder was transferred into a clean, dry beaker.
- Then, 250 ml hydroalcoholic solvent (70% ethanol) was added into the beaker and the drug was soaked properly.
- The beaker was covered and kept for 3-7 days at room temperature.

- The mixture was stirred 2-3 times daily for better extraction.
- After, 3-7 days, the mixture was filtered, and the filtrate was evaporated on a water bath at low temp.
- A thick extract was obtained after evaporation.
- The obtained extract was stored in an air tight container for further use [48][49].

% Yield= Weight of amla extract/ Weight of powdered drug x 100  
 $18 / 60 \times 100 = 30\%$



Figure 9: (A) Amla Powder

(B) Cold maceration

(C) Evaporation

**Identification Test for both Drug:**

**Table 1: Test for Diosgenin [50][52][53]**

S.no.	Test	Procedure	Observation	Result
1.	Salkowski Test	Dissolve extract in chloroform. Add conc. Sulfuric acid.	Reddish-brown color	Steroidal compound Present

2.	Keller-Killani Test	Dissolve extract in glacial acetic acid + add few drops of FeCl <sub>3</sub> + add H <sub>2</sub> SO <sub>4</sub>	Brown ring at interface	Glycosides present
3.	Vanillin-Sulfuric acid Test	Add vanillin reagent in extract + conc. H <sub>2</sub> SO <sub>4</sub> , Heat gently.	Pink /Red color	Steroidal sapogenin present
4.	Foam Test	Extract + water, Shake thoroughly.	Foam produce	Saponin present



Figure:10 (1)

(2)

(3)

(4)

Distance travelled by solvent

**Thin Layer Chromatography (TLC):**

Stationary phase- Silica gel G

Mobile phase- Hexane: Ethyl acetate (7:3)

Rf value =  $\frac{\text{Distance travelled by compound}}{\text{Distance travelled by solvent}}$

$$R_f \text{ value} = \frac{3.1}{5} = 0.6$$

Mobile phase- Toluene: Ethyl acetate (7:3) [51]

$$R_f \text{ value} = \frac{2.8}{5} = 0.5$$

**Table 2: Test for Phyllanthus emblica (Amla) [52][53]**

S.NO.	TEST	PROCEDURE	OBSERVATION	RESULT
1.	Test for Tannins	Add few drops of FeCl <sub>3</sub> solution + amla extract	blue/black color	Tannins present
2.	Test for Phenols	Add lead acetate solution + extract	White precipitate	Phenolic compound present
3.	Test for Saponins	Extract + water, Shake thoroughly.	Foam produce	Saponins present

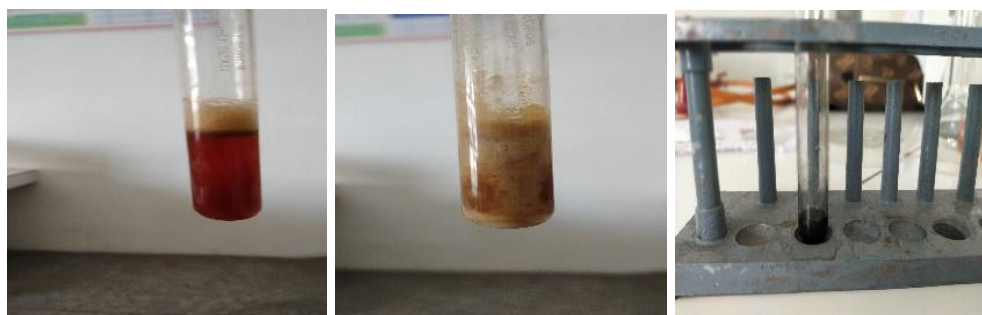


Figure: 11 (1)

(2)

(3)

**Thin Layer Chromatography (TLC):** For the separation of Tannins, Phenols, Flavonoids  
Stationary phase- silica gel G

- For Glycosides, Mobile phase- Chloroform: Methanol (9:1) [55]  
Rf value =  $3.9/7.0 = 0.5$

Mobile phase- Toluene: Ethyl acetate:  
Acetic acid:  
Formic acid (2.3: 4.7: 2.3: 0.6) [54]  
Rf value =  $2.8/7.0 = 0.4$

**Formulation of Polyherbal Syrup:** The Polyherbal syrup was prepared using the following composition for a 200 ml batch.

**Table 3: Composition of Polyherbal Syrup for 200 ml [4][56][57][58][59][60][61][62][64]**

S.no.	Ingredient	Quantity	Function
1.	Diosgenin	0.8 g	Improve hormonal regulation, Anti-androgenic effect, Anti-inflammatory action, Improve insulin resistance, Anti-oxidant activity, Improve ovulation or Fertility enhancement.
2,	Amla extract	10 g	Anti-oxidant activity, Hormonal balance Support, Anti-inflammatory activity, Improve lipid profile, Improve menstrual cycle regulation, Improve liver function.
3.	Citric acid	0.3 g	Inversion of sugar, Prevent crystallization, Maintain pH of syrup.
4.	Glycerin	10 ml	Co-solvent, Viscosity enhancer, Stability enhancer, Preservative support, Sweetening agent.
5.	Sodium benzoate	0.2 g	Preservative
6.	Ethanol	10 ml	Co-solvent
7.	Sucrose 66.7% w/w (Inverted base)	133.4 g	Syrup base
8.	Cardamom extract	2 ml	Flavouring agent
9.	Distilled water	q.s up to 200 ml	Vehicle or solvent

### Preparation method of Polyherbal Syrup:

- 133.4 g of sucrose was weighed and transferred into a beaker.
- Then, 60 ml of distilled water was added into the beaker and the mixture was boiled at a temperature 60-70° C with continuous stirring until the sucrose was dissolved [61].
- After that, 0.3 g citric acid was added to the solution for the inversion of sucrose and solution was heated for 20 min at a temperature 40° C [63].
- Simple syrup (solution) was cooled at room temperature and then filtered
- 0.8 g of diosgenin was weighed and dissolved in 10 ml of ethanol with continuous stirring.
- Then, 10 ml of glycerine was added to the ethanol solution and mixed well.
- 10 g of amla extract was weighed and dissolved in distilled water.
- After that, both solutions were added to the simple syrup with the continuous stirring.
- For flavour, 2 ml cardamom extract was added to the formulation with stirring.
- The final volume made up with distilled water and stored in suitable container.



(A) Diosgenin



(B) Amla extract



(C) Citric acid



(D) Glycerin



(G) Simple syrup (Base)



(E) Ethanol



(F) Sodium benzoate

(H) Cardamom

### Figure 12: Composition of Polyherbal Syrup

#### Evaluation Parameters of Polyherbal Syrup:

The evaluation of polyherbal syrup was evaluated for various parameter [64][65].

- **Organoleptic properties:** The organoleptic properties of the syrup, including appearance, color, odor, and taste, were evaluated.

- **pH Determination:** The pH of formulation was evaluated using a digital pH meter at room temperature. The pH of polyherbal syrup typically ranges from 4.0-6.5 [66]

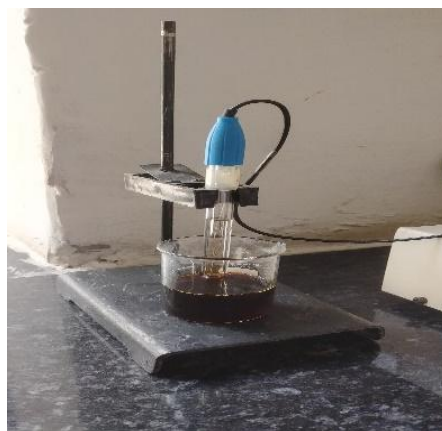


Figure 13: pH meter

- **Viscosity measurement:** Viscosity of the formulation was determined at room temperature using a Brookfield viscometer with spindle no.62 and 100 rpm, with readings taken in triplicate, and the mean value

recorded. The viscosity of polyherbal syrup typically ranges from 100-800 cP [64][67].



Figure 14: Brookfield Viscometer

- **Clarity test:** Homogeneity of the syrup was measured by visual examination in terms of

uniform distribution, absence of phase separation, and lack of particle matter [68].



**Figure 15: Clarity test**

- Pourability test:** The ease of flow was visually assessed by pouring the syrup from a container at a fixed angle. The formulation was examined to ensure uninterrupted, smooth flow of syrup [69].



**Figure 16: Pourability test**

**Table 4: Evaluation Parameter**

S.NO.	PARAMETER	RESULT
1.	Color	Brown
2.	Odour	Pleasant aromatic
3.	Taste	Sweet with slight bitterness
4.	Appearance	Clear, brown liquid without turbidity
5.	pH	5.2
6.	Viscosity	137cP
7.	Clarity Test	No visible particulate matter
8.	Pourability	Smooth flow

### In Vitro study of Polyherbal Syrup

- **KMnO<sub>4</sub> Test for Anti-oxidant activity:** Potassium permanganate (KMnO<sub>4</sub>) is a strong oxidizing agent. Anti-oxidants reduce potassium permanganate, resulting in decolorization of its purple color and it indicates anti-oxidant activity.

#### Procedure:

1. 1ml of sample and 9 ml of distilled water was taken in a beaker.

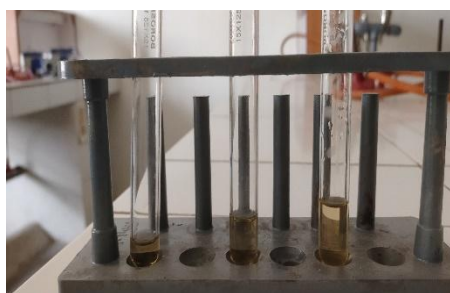


Figure 17: (A) Before

2. The solution was mixed well to obtain the stock solution.
3. Then, different volumes of the stock solution (1ml, 2ml, 3ml) were taken in separate test tubes.
4. 1ml of KMnO<sub>4</sub> solution was added to each test tube.
5. The mixture were gently shaken and observed.
6. The purple color of KMnO<sub>4</sub> was decolorized to light brown/yellowish brown solution, showed antioxidant activity [70][71].



B) After decolorization of KMnO<sub>4</sub> color

- **Anti-inflammatory Activity by Egg Albumin Denaturation method:** Protein denaturation is a well- established model for studying inflammation. Egg albumin undergoes denaturation when heated, leading to its coagulation. The ability of the formulation to reduce heat-induced denaturation demonstrates its anti-inflammatory effects.

#### Procedure:

1. 1ml egg white (albumin) was taken in a test tube, and 1ml of distilled water was added.

2. 1ml of egg white (albumin) was taken in another test tube, and 1ml of the sample (syrup) was added.
3. Both test tube mixtures were mixed well and heated in a water bath at 60-70° C for 10-15 minutes.
4. The mixtures was observed after 10-15 minutes.
5. The sample mixture did not coagulate and remained slightly cloudy.
6. The control (egg + water) mixture showed coagulation [72].

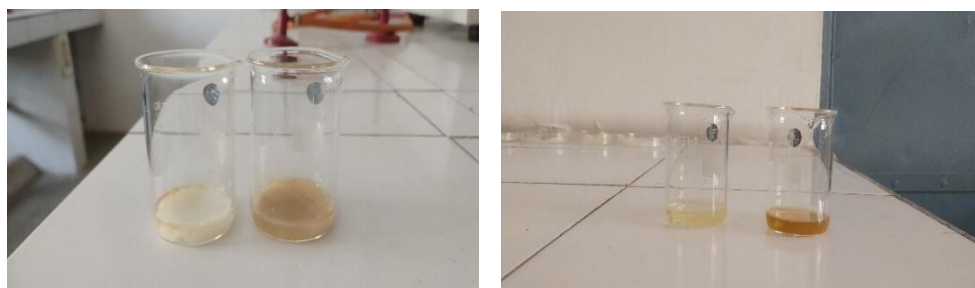


Figure 18: (A) Before heat

(B) After heat



Figure 19: Final formulation- Polyherbal Syrup for Ovarian Disorder

## RESULT:

In this study, Diosgenin was extracted from tubers of *Dioscorea alata*, and extract of *Phyllanthus emblica* (amla) was prepared. Subsequently, a polyherbal syrup was formulated using a suitable method. Various physicochemical parameters were performed for polyherbal syrup. Such as color, odor, taste, viscosity, clarity, and pourability test were evaluated. The formulation was found to be pleasant appearance, uniform consistency, and suitable viscosity range with good pourability. The formulation was found to be pharmaceutically acceptable. The potassium permanganate (KMnO<sub>4</sub>) antioxidant test was performed for polyherbal syrup to determine the antioxidant

activity. In this test, purple color of KMnO<sub>4</sub> solution was decolorized when the syrup was present. It indicates strong antioxidant activity of polyherbal syrup. The egg albumin denaturation method was performed to evaluate anti-inflammatory activity. In this test, the control sample coagulated upon heating. The syrup treated sample showed less coagulation and turbidity compared to the control. This indicates inhibition of protein denaturation. These results demonstrate that the polyherbal syrup has significant anti-inflammatory and anti-oxidant properties.

**DISCUSSION:** PCOD is characterized by inflammation, hormonal imbalance and oxidative stress. In this study, Amla extract (which is rich source of vitamin C and phenolic compounds) and diosgenin (a steroidal saponin) were utilized due to their established medicinal or therapeutic effects. The biological activity of the formulation was enhanced by the successful extraction of diosgenin and amla constituents. The polyherbal syrup stability and suitability for oral administration were validated by the physicochemical analysis. The KMnO<sub>4</sub> test showed antioxidant activity, and it demonstrated the formulation's capacity to counteract free radicals. The presence of phenolics and vitamin C in amla may be responsible for this activity. Similarly, the egg albumin denaturation method demonstrated that the polyherbal syrup prevented protein denaturation, an important mechanism in inflammation. This signifies that diosgenin and other phytoconstituents may be responsible for the formulation's possible anti-inflammatory activity.

Therefore, the synergistic action of amla and diosgenin may help in minimizing the oxidative stress and inflammation. Which are major factors involved in PCOD.

### **CONCLUSION:**

In this study, a polyherbal syrup with amla extract and diosgenin was successfully developed. In vitro, the formulation showed significant antioxidant and anti-inflammatory properties along with acceptable physicochemical characteristics. These results suggest that the developed polyherbal syrup may have therapeutic benefits in PCOD treatment. However, further in vivo and clinical studies are required to confirm its efficacy and safety.

**ACKNOWLEDGEMENT:** I would like to sincerely thank my department head and guide for

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