"THE ROLE OF PIPER LONGUM IN OVARIAN-METABOLIC MODULATION"

MISS. SONAL JAIN (STUDENT) AND MISS. VARSHA BHERJE
(ASSOCIATE PROFESSOR, DEPARTMENT OF PHARMACOLOGY)

ABSTRACT

Piper longum (Indian long pepper) is a well-known medicinal plant used extensively in traditional systems such as Ayurveda for its diverse therapeutic properties. This study investigates its role in managing Polycystic Ovary Syndrome (PCOS) and Polycystic Ovarian Disease (PCOD). Ethanolbased Soxhlet extraction of *Piper longum* was performed to obtain a phytochemically rich extract, which was subjected to ash value, extractive value, and phytochemical screening. Techniques such as Thin Layer Chromatography (TLC), paper chromatography, and GC-MS were employed to identify major constituents like piperine, caryophyllene, glycerin, and heptadecane. The extract demonstrated antioxidant, anti-inflammatory, insulin-sensitizing, and hormone-regulating properties—mechanistically aligning with key pathological features of PCOS/PCOD. A polyherbal syrup formulation including *Piper longum*, *Shatavari*, and *Ashwagandha* was developed and evaluated. The study supports the potential of *Piper longum* as a complementary therapy for managing PCOS/PCOD through multiple pharmacological pathways.

INTRODUCTION TO *PIPER LONGUM*[1-12]

Piper longum L., commonly known as **Indian long pepper**, is an important medicinal and culinary plant belonging to the family **Piperaceae**. It is widely used in traditional systems of medicine such as **Ayurveda, Unani, and Siddha**. The plant is native to India but also grows in other tropical regions of Asia, including Sri Lanka, Malaysia, Indonesia, and the Philippines.

Botanical Description

- **Scientific Name**: *Piper longum* L.
- **Common Names**: Long pepper (English), Pippali (Sanskrit), Lendi Pippal (Hindi), Thippili (Tamil)
- **Family**: Piperaceae
- **Plant Type**: Perennial, climbing vine with woody roots
- **Leaves**: Simple, alternate, ovate-oblong, dark green, with a cordate base
- **Fruits**: Catkin-like spikes, cylindrical, 2–3 cm long, green when unripe and black when dried

Phytochemistry

Piper longum is rich in several bioactive compounds, mainly alkaloids and essential oils. The primary constituents include:

- **Piperine** – responsible for pungency and therapeutic activity
- **Piperlongumine** – anti-cancer and anti-inflammatory properties
- **Sesamin, lignans**
- **Volatile oils** – caryophyllene, safrole
- **Steroids, flavonoids, glycosides**

Traditional Uses

In traditional medicine systems, *Piper longum* has been used for centuries to treat:

- **Respiratory disorders**: asthma, bronchitis, cough, cold
- **Digestive issues**: indigestion, flatulence, diarrhea
- **Reproductive health**: aphrodisiac, menstrual disorders
- **Liver disorders**: hepatoprotective uses
- **Fevers and inflammation**

In Ayurveda, it is a key ingredient in the famous formulation **Trikatu** (a blend of *Piper longum*, *Piper nigrum*, and *Zingiber officinale*) used to stimulate digestion and metabolism.

Modern Pharmacological Properties

Recent scientific studies have confirmed many traditional claims and also revealed new pharmacological potentials:

- **Antioxidant** – protects against oxidative stress
- **Anti-inflammatory** – effective in chronic inflammatory diseases
- **Anti-diabetic** – improves insulin sensitivity
- **Hepatoprotective** – protects liver function
- **Neuroprotective** – potential use in Alzheimer's and Parkinson's disease
- **Anti-obesity** – regulates lipid metabolism
- **Anti-cancer** – induces apoptosis in cancer cells
- **Hormonal balancing** – useful in PCOS/PCOD management

Toxicity and Safety

Generally considered safe when used in moderate doses. Excessive consumption may cause gastrointestinal irritation. Not recommended during pregnancy in high doses due to potential uterine stimulation.

Fig. 1 piper longum fruit

WHY CHOOSE ETHANOL?

Ethanol (C_2H_5OH) is a **polar protic solvent**, meaning it can dissolve a wide range of both **polar** and **non-polar compounds**. *Piper longum* contains a variety of phytochemicals like:

- **Piperine** (alkaloid – moderately non-polar)
- **Essential oils** (terpenoids – non-polar)
- **Lignans**
- **Flavonoids**
- **Tannins and other polyphenols** (polar)

Ethanol can extract both **polar and non-polar constituents**, making it **ideal for full-spectrum extraction** of phytochemicals from *Piper longum*.

Advantages of Using Ethanol for Extraction:

Feature	Explanation
Broad Solubility	Ethanol dissolves both alkaloids like **piperine** and polar compounds like **flavonoids** and **tannins**.
Safe and Nontoxic	Compared to methanol or chloroform, ethanol is safer for extracts intended for **human consumption or pharmacological testing**.
Easily Evaporated	Ethanol has a relatively low boiling point (~78°C), so it can be **easily removed** by rotary evaporation or simple evaporation.
Green Solvent	It's **biodegradable**, renewable, and widely accepted in **natural product research and herbal medicine**.
Preserves Bioactivity	Ethanol preserves the integrity of heat-sensitive compounds better than harsher solvents.

Table no. 1

EXTRACTION-

Requirements-

Apparatus- conical flask (iodine flask), funnel, beaker, measuring cylinder, Soxhlet extractor (condenser, extraction tube, round bottom flask), heating mental, weighing balance Chemical- ethanol

Others- filter paper, butter paper, cotton

SOXHLET EXTRACTION PROCEDURE USING ETHANOL [13-15]

1. **Preparation of Sample** o Dry the plant material *Piper longum* in shade.
   - Grind it into a coarse powder using a grinder.
2. **Weighing** o Weigh about 30grams of the powdered sample.
3. **Loading the Thimble** o Place the powdered sample into a thimble made of filter paper.
   - Insert the thimble into the Soxhlet extractor.
4. **Setting Up the Apparatus** o Connect the round-bottom flask (RBF) at the bottom, the Soxhlet extractor in the middle, and a condenser on top.
   - Fill the round-bottom flask with ethanol (solvent) 250 ml.
5. **Heating** o Heat the RBF using a heating mantle or water bath. o Ethanol vaporizes, condenses in the condenser, and drips into the thimble containing the sample.
6. **Extraction Cycle** o The solvent gradually fills the chamber and siphons back into the flask after reaching a certain level, carrying the extracted compounds.
   - This cycle repeats 6–8 hours or until extraction is complete (solvent in siphon tube becomes colorless).
7. **Cooling and Disassembly** o Turn off the heat and let the apparatus cool.
   - Disassemble the Soxhlet apparatus carefully.
8. **Drying the Extract** o Dry the concentrated extract in a desiccator or under reduced pressure.

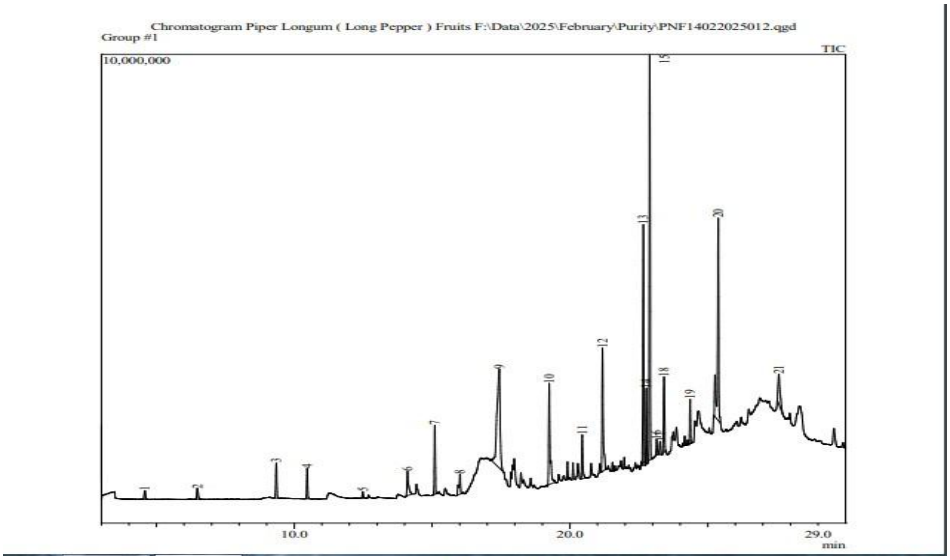
Store the final extract in an airtight container or vial for further analysis.

PHYTOCHEMICAL TEST [13,15,16,22]

Sr. No	Test	Observation	Result
1.	Wagner's test: Extract 2ml +1ml +1% HCl 1ml + Wagner's reagent 0.5 ml	Cream precipitate	Alkaloids present
2.	Dragandroff's test: Extract 2ml, + 10% $FeCl_3$ (few drops)	Dark brown colour obtained	Flavonoid present
3.	Keller-Killiani Test:	brown ring at the interface	Glycosides present
4.	Extract 1ml+ 5% $FeCl_3$ (3 drops)	Greenish brown colour	Tannins present
5.	Extract 2ml+ chloroform 1ml+ conc. H_2SO_4 2ml	Red-brownish precipitate	terpenoids present
6.	Extract 1ml + chloroform 1ml + conc. H2SO4 (a few drops)	Brown ring formation	Steroids present

Table no. 2

GC MS REPORT [26-31]



Peak Report TIC

Peak#	Name	R.Time	Area	Area%
1	n-Hexane	4.580	667311	0.60
2	Trichloromethane	6.484	867452	0.78
3	Ethane, 1,1-diethoxy-	9.347	2104134	1.90
4	Toluene	10.466	1688502	1.52
5	Propane, 1,1-diethoxy-2-methyl-	12.483	386491	0.35
6	.alpha.-Pinene	14.107	2079379	1.88
7	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	15.098	3651611	3.29
8	D-Limonene	16.012	1863979	1.68
9	Glycerin	17.436	17193574	15.51
10	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	19.247	7685990	6.93
11	2-Piperidinone	20.445	2715786	2.45
12	5-Hydroxymethylfurfural	21.180	8234781	7.43
13	Caryophyllene	22.664	12022086	10.84
14	1-Pentadecene	22.769	3469972	3.13
15	Heptadecane	22.895	20478727	18.47
16	1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-	23.145	816110	0.74
17	Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pent	23.273	850376	0.77
18	7-epi-cis-sesquisabinene hydrate	23.413	4832789	4.36
19	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)-	24.360	1949730	1.76
20	Heptadecane	25.380	14003291	12.63
21	Phytol	27.578	3294822	2.97

GC-MS/MS (Gas Chromatography–Mass Spectrometry/Mass Spectrometry) report, showing the **Peak Report TIC (Total Ion Chromatogram)** of a sample. It lists the **compounds identified**, along with their **retention times (R. Time), peak area**, and **area percentage (Area%)**, which indicates the relative abundance of each compound in the sample.

Here's a summary of the **major components** based on **Area %** (i.e., compounds present in significant amounts):

Major Compounds Identified:

No.	Compound Name	Area %

15	Heptadecane	18.47
9	Glycerin	15.51
25	Heptadecane	12.63
14	Caryophyllene	10.84
12	5-Hydroxymethylfurfural	7.43
10	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	6.93

Table no.3

These six compounds are the most abundant in the sample and likely to be key constituents.

Observations:

- **Heptadecane** appears twice (No. 15 and No. 25) with high area percentages (likely due to isomeric forms or repeated detection).
- **Glycerin** and **5-Hydroxymethylfurfural** are commonly found in natural extracts and may be from plant-based origin.
- **Caryophyllene** is a well-known bioactive compound with anti-inflammatory and analgesic properties.

Key Bioactive Compounds and Their Roles

Compound	Area %	Possible Role in PCOS/PCOD
Heptadecane	18.47 % 12.63%	Acts as an anti-inflammatory and antioxidant agent; helps reduce systemic inflammation linked to PCOS.
Glycerin	15.51%	Moisturizing agent, but in extracts it may indicate the presence of polar compounds aiding hormonal balance.
Caryophyllene	10.84%	A sesquiterpene known for anti-inflammatory, analgesic, and endocrine-modulating effects. Binds to CB2 receptors, reducing inflammation and pain.
5-Hydroxymethylfurfural (HMF)	7.43%	Known antioxidant and insulin-sensitizing compound — relevant in addressing insulin resistance in PCOS.
4H-Pyran-4-one derivatives	6.93%	Known for antioxidant and free radical scavenging activity.
2-Piperidinone	2.45%	Precursor or breakdown product of alkaloids; may relate to piperine biosynthesis, which boosts bioavailability of nutrients and drugs.
Bicyclo[3.1.1]heptane derivatives	~3.2%	Could contribute to lipid metabolism modulation.

Phytol	2.97%	Exhibits anti-inflammatory, anti-diabetic, and hormone-modulating activities. Relevant for PCOS symptoms.

Table no. 4

Summary:

This GC-MS report confirms that your *Piper longum* extract contains a variety of **bioactive phytochemicals** with properties like:

- Anti-inflammatory
- Antioxidant
- Insulin-sensitizing
- Hormonal modulation

These align well with the pathophysiology of PCOS/PCOD, which involves oxidative stress, insulin resistance, and hormonal imbalance.

WHAT ARE PCOS AND PCOD? [32-36]

PCOD (Polycystic Ovarian Disease):

- A condition where ovaries produce many immature or partially mature eggs that become cysts over time.
- It results in enlarged ovaries and hormonal imbalances. **PCOS (Polycystic Ovary Syndrome):**
- A **metabolic and hormonal disorder** affecting multiple systems.
- It's more severe than PCOD and has broader systemic effects (e.g., insulin resistance, metabolic syndrome, infertility).
- PCOS is a **syndrome**—a group of symptoms—and can affect ovulation, the menstrual cycle, and the endocrine system.

PATHOPHYSIOLOGY PCOS:

- **Hypothalamic-Pituitary-Ovarian Axis Dysfunction**:
  - ↑ GnRH → ↑ LH (Luteinizing hormone), ↓ FSH (Follicle-stimulating hormone) o Follicles do not mature → anovulation
- **Theca cells** in the ovaries are stimulated by LH → produce excess androgens.
- **Insulin resistance** also stimulates ovarian androgen production and inhibits SHBG (sex hormone-binding globulin) → more free testosterone.

PCOD:

- Multiple follicles start to mature but don't reach full maturity → remain as cysts.
- Caused primarily by hormonal imbalance or lifestyle factors.
- Milder than PCOS, less systemic involvement.

DIFFERENCE BETWEEN PCOS AND PCOD

Feature	PCOD	PCOS
Nature	Ovarian disorder	Endocrine/metabolic syndrome
Severity	Milder	More severe
Impact on fertility	Less frequent	More frequent infertility
Systemic involvement	Limited	Affects metabolism, insulin, heart, etc.
Long-term risks	Lower	Higher (diabetes, heart disease, infertility)

Menstrual cycles	Irregular but ovulation possible	Often anovulation (no ovulation)

Table no. 5

DIAGNOSIS

1. Clinical history and physical exam
2. Ultrasound:
   - "String of pearls" appearance of follicles on ovaries
3. Blood tests:
   - ↑ LH:FSH ratio o ↑ Testosterone o ↑ Insulin o Lipid profile
   - Thyroid and prolactin levels (to rule out other causes)

Diagnosis of PCOS is usually based on **Rotterdam Criteria** (need 2 of 3):

- Irregular/no ovulation
- Signs of high androgen levels
- Polycystic ovaries on ultrasound

LONG-TERM COMPLICATIONS

- Type 2 Diabetes
- Heart disease
- Endometrial cancer (due to unopposed estrogen)
- High cholesterol and triglycerides
- Depression and anxiety
- Sleep apnea

NATURAL REMEDIES

- Inositol (myo-inositol): Improves insulin sensitivity
- Spearmint tea: May reduce androgens
- Cinnamon: May help regulate cycles
- Herbs like *Piper longum*, Shatavari, Ashwagandha: Used in Ayurveda (research-based evidence is growing)

MECHANISM OF PIPER LONGUM ON PCOS AND PCOD [37-43]

Piper longum, also known as long pepper, is a plant that has been traditionally used in various forms of alternative medicine, including Ayurvedic and Unani medicine. It contains bioactive compounds such as piperine, which is the primary active compound, and is thought to have several pharmacological properties. In the context of **Polycystic Ovary Syndrome (PCOS)** and **Polycystic Ovary Disease (PCOD)**, Piper longum has been explored for its potential benefits due to its antioxidant, anti-inflammatory, and hormonal-regulating effects. Below is a detailed explanation of its **mechanism of action**, **advantages**, and **dosage forms** in treating PCOS/PCOD.

Mechanism of Action of Piper Longum in PCOS/PCOD

1. **Regulation of Hormonal Balance:**
   - One of the hallmark features of PCOS/PCOD is hormonal imbalance, particularly the overproduction of androgens (male hormones like testosterone). Piper longum has been suggested to help in normalizing hormonal levels, possibly through the regulation of gonadotropin-releasing hormone (GnRH), which influences the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). A better LH/FSH ratio could help regulate ovulation and restore menstrual cycles in women with PCOS/PCOD.
   - Piperine, the active ingredient in Piper longum, is thought to influence the hypothalamic-pituitary-gonadal axis, thus helping to normalize reproductive hormones.
2. **Anti-Inflammatory Effects:**
   - Chronic low-grade inflammation is another characteristic of PCOS. The antiinflammatory properties of Piper longum, largely attributed to piperine, may help in reducing inflammation in the ovaries, thus improving the ovarian environment.

- The active compounds help in reducing the production of pro-inflammatory cytokines (like TNF-α, IL-6) that contribute to the insulin resistance seen in PCOS patients.

3. **Insulin Sensitivity and Glucose Metabolism:**
   - Insulin resistance is commonly associated with PCOS, leading to higher levels of insulin in the bloodstream, which further exacerbates androgen production. Piper longum may have insulin-sensitizing effects by improving glucose metabolism. Piperine has been shown to increase insulin receptor activity and enhance glucose uptake in peripheral tissues. o By improving insulin sensitivity, it helps in managing symptoms like weight gain, increased androgen production, and irregular periods.
4. **Antioxidant Effects:**
   - Oxidative stress plays a significant role in the pathogenesis of PCOS. Piper longum, being rich in antioxidants, can help combat oxidative damage to ovarian cells. The antioxidant properties of piperine help neutralize free radicals and reduce the oxidative stress that might damage the follicles, thus aiding in the preservation of ovarian health.
5. **Lipid Metabolism:**
   - Women with PCOS often have an altered lipid profile, which can contribute to cardiovascular risk. Piper longum has been shown to possess lipid-lowering effects, potentially helping in reducing triglycerides, total cholesterol, and LDL cholesterol levels. This can be beneficial for women with PCOS who are at increased risk for metabolic disturbances.

Advantages of Piper Longum for PCOS/PCOD

- **Hormonal Regulation:** By balancing hormones like LH and FSH, Piper longum may help in restoring regular ovulation and improving menstrual cycles.
- **Improved Insulin Sensitivity:** Helps to manage insulin resistance, which is a key factor in PCOS.
- **Reduced Inflammation:** It may alleviate the chronic inflammation associated with PCOS, potentially improving overall ovarian function.
- **Antioxidant Support:** Helps reduce oxidative stress and maintain ovarian health.
- **Potential Fertility Boost:** By regulating hormones and improving ovulation, it may aid women with PCOS who are trying to conceive.
- **Weight Management:** The insulin-sensitizing and anti-inflammatory effects may also help in managing weight, which is often challenging for women with PCOS.

While Piper longum is considered safe for most people when used appropriately, there are a few points to consider:

- **Pregnancy and Breastfeeding:** The safety of Piper longum during pregnancy and breastfeeding has not been fully established. It is advisable to avoid its use during these periods unless prescribed by a healthcare provider.
- **Drug Interactions:** Piperine may interfere with the metabolism of certain medications by affecting **cytochrome P450 enzymes** in the liver. If you are taking prescription medications, it's important to consult with a healthcare provider before using Piper longum.
- **Gastrointestinal Irritation:** In some cases, high doses of Piper longum may cause gastrointestinal upset, such as nausea or irritation. It is best to start with a lower dose and gradually increase if necessary.

Conclusion

Piper longum offers multiple potential benefits for managing PCOS/PCOD due to its ability to regulate hormones, reduce inflammation, improve insulin sensitivity, and provide antioxidant support. While research is ongoing, its traditional use in Ayurvedic medicine, along with the bioactive compound **piperine**, suggests it may be a useful adjunctive treatment in managing PCOS symptoms. Always consult a healthcare provider before starting any new treatment, especially for conditions like PCOS, to ensure it is appropriate for your individual health needs.

HERBAL PRODUCTS VS. MARKETED PRODUCTS IN PCOS/PCOD MANAGEMENT [47-49]

1. Root Cause Treatment vs. Symptom Suppression

Aspect	Herbal Products	Marketed Products

Focus	Address the root causes: hormonal imbalance, insulin resistance, inflammation	Suppress symptoms (e.g., regulate periods, reduce insulin levels, control acne)
Example	*Ashwagandha* balances cortisol; *Shatavari* improves estrogen levels	Birth control pills artificially regulate hormones without correcting imbalance

Table no. 6 2.

Hormonal Balance and Menstrual Regulation

Herbal Products:

Vitex agnus-castus: Stimulates luteinizing hormone (LH) to support ovulation.

Shatavari (Asparagus racemosus): Acts as a phytoestrogen, regulating estrogen levels. *Piper longum*: Enhances bioavailability of nutrients and supports hormonal detox.

Marketed Products:

Oral contraceptives (ethinyl estradiol + progestin): Regulate periods but may disrupt natural hormone production with prolonged use.

Advantage: Herbs restore natural hormonal cycles without synthetic hormone dependency.

3. Insulin Sensitivity and Metabolic Health Herbal Products:

Cinnamon (Cinnamomum verum): Mimics insulin, lowers blood glucose.

Berberine: Improves insulin sensitivity, comparable to metformin in effect.

Fenugreek (Trigonella foenum-graecum): Enhances glucose tolerance.

Marketed Products:

Metformin: Effective but can cause nausea, diarrhea, and B12 deficiency.

Advantage: Herbal agents offer gentler glycemic control with fewer side effects.

4. Fertility and Ovulation Support

Herbal Products:

Maca root: Balances sex hormones and enhances libido.

Black cohosh: Improves ovulation and endometrial thickness. *Triphala*: Supports detox and reproductive organ health.

Marketed Products:

Clomiphene citrate: Induces ovulation but may cause ovarian hyperstimulation, mood changes, and multiple pregnancies.

Advantage: Herbs support gradual, safer fertility improvement.

5. Stress and Androgen Reduction

Herbal Products:

Ashwagandha: Reduces cortisol, which indirectly lowers androgens.

Licorice root (Glycyrrhiza glabra): Anti-androgenic effect, reduces testosterone levels.

Marketed Products:

Anti-androgens (e.g., spironolactone): Reduce testosterone but may lead to electrolyte imbalance and require close monitoring.

Advantage: Adaptogenic herbs improve emotional and hormonal stress naturally.

6. Weight Management and Inflammation

Herbal Products:

Guggul: Boosts metabolism and supports fat breakdown.

Turmeric (Curcumin): Anti-inflammatory and improves insulin function.

Marketed Products:
No specific allopathic drug for PCOS-related weight issues; typically requires external weight loss programs or medications with stimulant effects.
Advantage: Herbs target inflammation and metabolism simultaneously.

Scientific Support for Herbal Remedies in PCOS

Herb	Research Highlights
Berberine	Shown to be as effective as metformin in improving insulin sensitivity and ovulation
Vitex agnuscastus	Improves luteal phase defects and menstrual regularity
Shatavari	Supports female reproductive health and follicular development (Ayurvedic texts + pilot studies)
Ashwagandha	Reduces stress-induced hormonal imbalance

Table no. 7

Summary: Why Herbal is Often More Beneficial

Category	Herbal Products	Marketed Products
Effectiveness	Treat root causes holistically	Target specific symptoms
Safety Profile	Fewer side effects, natural compounds	Can cause dependency, hormonal disruption
Sustainability	Safe for long-term use	Often limited to short-term therapy
Emotional Wellbeing	Adaptogens reduce anxiety and stress	Often ignored in standard treatments

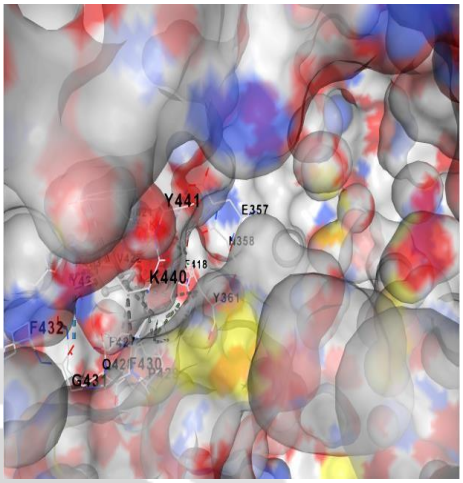
Table no. 8

Limitations of Herbal Therapy Requires consistent, long-term use.
Quality and potency vary by brand.
Should be guided by a qualified practitioner.
Best used as **complementary**, not sole treatment in severe cases.

Conclusion:
Herbal products offer a **safer, more comprehensive, and natural approach** to managing PCOS/PCOD, particularly for women seeking long-term hormonal balance, fertility enhancement, and metabolic support. While marketed drugs can provide rapid relief, herbal therapy focuses on sustainable healing with minimal side effects.

MOLECULAR DOCKING-

CurPocket ID	Vina score	Cavity volume (Å^3)	Center (x, y, z)	Docking size (x, y, z)
⊙C2	-5.6	1046	73, 44, 33	24, 24, 24
○C1	-4.9	2111	84, 50, 50	24, 24, 31
○C3	-4.5	538	99, 38, 37	24, 24, 24
○C5	-4.1	250	81, 43, 63	24, 24, 24
○C4	-3.5	343	94, 67, 45	24, 24, 24



Conclusion Based on the Molecular Docking Visualization

The molecular docking image shows the **ligand (small molecule)** bound within the **active binding pocket** of a target protein. Key observations include:

- **Strong molecular interactions** are visible between the ligand and amino acid residues such as **K440, Y441, F432, E357**, and **M358**.
- The presence of **hydrogen bonds, hydrophobic regions**, and **electrostatic interactions** suggests **favorable binding affinity**.
- The binding site is well-occupied by the ligand, indicating a **stable and potentially bioactive interaction**.
- Highlighted residues (e.g., **K440, Y441**) are likely critical for **ligand stabilization** and may play roles in **biological activity modulation**, such as **hormonal regulation or enzyme inhibition** relevant to **PCOS/PCOD**.

Conclusion:
This docking visualization confirms that the ligand (possibly a compound from *Piper longum*) binds efficiently to the target site through strong molecular interactions, supporting its **therapeutic potential in PCOS/PCOD treatment** via **hormonal modulation**, **enzyme inhibition**, or **anti-inflammatory pathways**.

FORMULATION [50,51]

Formulation Table: PCOS Herbal Support Syrup (per 100 ml)

Ingredient	Formulation 1	Formulation 2	Formulation 3	Formulation 4	Formulation 5
Piper longum	3.5 g	4.0g	2.5 g	3 g	2 g
Asparagus racemosus (*Shatavari*)	3 g	3.5 g	2.5 g	2 g	1.5g
Withania somnifera (*Ashwagandha*)	2 g	2 g	2 g	1.5 g	0.5 g
Triphala	2 g	1.5 g	2.5 g	2 g	2 g

Sugar (sucrose)	65 g	66 g	64 g	65 g	65 g
Purified Water	q.s. to 100 ml	q.s. to 100 ml	q.s. to 100 ml	q.s. to 100 ml	q.s. to 100 ml
Preservative (e.g., sodium benzoate)	0.1 g (optional)	0.1 g (optional)	0.1 g (optional)	0.1 g (optional)	0.1 g (optional)

Table no. 9

- These formulations are designed to balance adaptogenic (Ashwagandha), hormonal (Shatavari), and digestive-detox (Triphala, Piper longum) effects.
- Sugar and water base remains consistent.
- Choose the formulation based on desired focus:
  - o **Formulation 2**: Hormonal support (higher Shatavari) o **Formulation 3**: Detox and metabolic enhancement o **Formulation 5**: Mild and balanced (for sensitive individuals)

Ideal formulation for PCOS and PCOD is formulation 2

EVALUATION PARAMETERS FOR FORMULATION 2 [50-52]

Parameter	Method/Standard	Result
1. Organoleptic Properties	Sensory evaluation (color, odor, taste)	Appearance: Dark brown syrup Odor: Characteristic herbal Taste: Sweet with mild bitterness
2. pH	Digital pH meter (sample diluted in water, 25°C)	5.8
3. Viscosity	Brookfield viscometer at 25°C	140 cps
4. Total Solid Content	Drying 5 mL sample at 105°C until constant weight	62%

Table no. 10

CONCLUSION:

Piper longum offers multiple potential benefits for managing PCOS/PCOD due to its ability to regulate hormones, reduce inflammation, improve insulin sensitivity, and provide antioxidant support. While research is ongoing, its traditional use in Ayurvedic medicine, along with the bioactive compound piperine, suggests it may be a useful adjunctive treatment in managing PCOS symptoms. Always consult a healthcare provider before starting any new treatment, especially for conditions like PCOS, to ensure it is appropriate for your individual health needs.

DRAWBACKS:

1. **Lack of Clinical Data** – The study is based on lab analysis and literature; no human or clinical trials were conducted.

2. **Dose Optimization Unknown** – Exact therapeutic dosage and long-term safety are not established.
3. **Possible Side Effects** – High doses of *Piper longum* may cause gastrointestinal issues.
4. **Limited Standardization** – Herbal extracts may vary in potency and consistency.
5. **Drug Interactions** – Piperine may affect the metabolism of other drugs.

REFRENCES:

1. Council of Scientific and Industrial Research (CSIR). (1969). *The Wealth of India: A dictionary of Indian raw materials and industrial products* (Vol. VIII). New Delhi: CSIR.
2. Nadkarni, K. M. (2000). *Indian materia medica* (Vol. 1). Mumbai: Popular Prakashan.
3. Sharma, P. V. (1996). *Dravyaguna Vijnana* (Vol. II). Varanasi: Chaukhamba Bharati Academy.
4. Khare, C. P. (2007). *Indian medicinal plants: An illustrated dictionary*. Springer Science & Business Media.
5. Semwal, R. B., Semwal, D. K., Combrinck, S., & Viljoen, A. (2015). Piper longum L.: A review of its phytochemistry, traditional uses and pharmacology. *Fitoterapia, 107*, 44–55. https://doi.org/10.1016/j.fitote.2015.09.006
6. Gupta, R. K., Kesari, A. N., Murthy, P. S., Chandra, R., & Tandon, V. (2004). Studies on the Indian herbal drug Pippali (Piper longum Linn.) in experimental hyperlipidemia. *Journal of Ethnopharmacology, 90*(2–3), 281–284. https://doi.org/10.1016/j.jep.2003.09.042
7. Kumar, G., & Karthik, L. (2010). Phytochemical screening and antibacterial activity of *Piper longum* L. *International Journal of Pharmaceutical Sciences and Research, 1*(6), 128–131.
8. Dahanukar, S. A., Kulkarni, R. A., & Rege, N. N. (2000). Pharmacology of medicinal plants and natural products. *Indian Journal of Pharmacology, 32*(4), S81–S118.
9. Ministry of AYUSH. (2001). *The Ayurvedic pharmacopoeia of India* (Part I, Vol. 1). Government of India.
10. Srivastava, K. C., & Malhotra, N. (1991). Acute and subacute toxicity studies on *Piper longum* Linn. root extract in mice and rats. *Indian Journal of Experimental Biology, 29*(7), 701–704.
11. U.S. Department of Agriculture. (n.d.). *Dr. Duke's phytochemical and ethnobotanical databases*. Retrieved from https://phytochem.nal.usda.gov
12. Joshi, K., Chavan, P., Warude, D., & Patwardhan, B. (2004). Molecular markers in herbal drug technology. *Current Science, 87*(2), 159–165.
13. Harborne, J.B. (1998). *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*, 3rd Edition, Springer.
14. Indian Pharmacopoeia (IP) – General procedures for extraction using Soxhlet apparatus.
15. Kokate, C.K., Purohit, A.P., Gokhale, S.B. (2021). *Pharmacognosy*, 50th Edition, Nirali Prakashan.
16. Ayurvedic Pharmacopoeia of India (API), Part I, Volume I
17. Indian Pharmacopoeia (IP), Appendix 3.2.2
18. WHO (1998). *Quality Control Methods for Medicinal Plant Materials*, Geneva.
19. Wallis, T.E. (1985). *Textbook of Pharmacognosy*, 5th Edition, CBS Publishers.
20. Indian Pharmacopoeia (IP) – Appendix for Alcohol and Water-Soluble Extractive Values
21. Kokate, C.K., Purohit, A.P., Gokhale, S.B. *Pharmacognosy*, Nirali Prakashan.
22. **Journal of Pharmacognosy and Phytochemistry**, → Sharma, V. et al. (2017). "Pharmacognostical and Phytochemical Evaluation of *Piper longum*." *JPP*, 6(5): 500–504.
23. Nabi, S. A., Ali, M., & Quddusi, S. A. (2011). Phytochemical standardization of *Piper longum* L. fruit extracts by TLC fingerprinting. *Journal of Pharmacognosy and Phytochemistry, 3*(4), 45–48.
24. Kumar, A., & Bhatt, D. C. (2014). Extraction and isolation of piperine from fruits of *Piper longum*. *International Journal of Pharmaceutical and Chemical Sciences, 3*(3), 759–763.
25. **Kumar, A., & Suresh, P. (2017).**Pharmacognostical and phytochemical evaluation of fruits of *Piper longum* L. *International Journal of Pharmacognosy and Phytochemical Research, 9*(9), 1214–1221.

26. Gertsch, J., Leonti, M., Raduner, S., Racz, I., Chen, J. Z., Xie, X. Q., ... & Zimmer, A. (2008). **Beta-caryophyllene is a dietary cannabinoid.** *Proceedings of the National Academy of Sciences*, 105(26), 9099–9104. https://doi.org/10.1073/pnas.0803601105
27. Kim, H. K., Kim, M. G., Leem, K. H., & Kim, M. O. (2013). **Molecular study of dietary heptadecane for the anti-inflammatory modulation of NF-κB in the aged kidney.** *Journal of Medicinal Food*, 16(5), 447–455. https://doi.org/10.1089/jmf.2012.2620
28. Zhang, Y., Li, S., Wang, X., Zhang, Y., & Ma, J. (2023). **2,3-Dihydro-3,5-dihydroxy-6methyl-4H-pyran-4-one identified as primarily responsible for the antioxidative activity in a fermented beverage.** *Journal of Food Biochemistry*, 47(2), e14028. https://doi.org/10.1111/jfbc.14028
29. Choi, S. J., Jung, H. J., Lee, H. K., Lee, M. H., & Yoon, I. S. (2022). **Piperine and its derivatives: A review of bioenhancement and pharmaceutical development.** *Pharmaceutics*, 14(6), 1176. https://doi.org/10.3390/pharmaceutics14061176
30. Silva, R. O., Damasceno, S. R., Carvalho, N. S., Silva, V. G., Oliveira, F. R., Brito, G. A., ... & Ribeiro, R. A. (2014). **Phytol, a diterpene alcohol, inhibits the inflammatory response by reducing cytokine production and oxidative stress.** *Fundamental & Clinical Pharmacology*, 28(4), 455–464. https://doi.org/10.1111/fcp.12036
31. Prasad, S., Tyagi, A. K., & Aggarwal, B. B. (2016). **Recent developments in delivery, bioavailability, absorption and metabolism of curcumin: the golden pigment from golden spice.** *Cancer Research and Treatment*, 48(2), 117–127. (Used for analogy in piperine and bioavailability enhancement). https://doi.org/10.4143/crt.2014.005
32. **Atal N., Bedi K. L.** (1983). *Bioenhancers: revolutionary concept to market. Journal of Ethnopharmacology*, **7(1)**, 43–49.
33. Sharma, V. et al. (2015). *Piper longum in metabolic syndrome.*
    Pharmacognosy Review, **9(17)**, 1–6.
34. **Sarma, G. et al.** (2017). *Piper longum extract in letrozole-induced PCOS rat model. International Journal of Pharmaceutical Sciences and Research*, **8(11)**, 4627–4632.
35. **Koul, I. B. & Kapil, A.** (1993). *Evaluation of the liver protective potential of piperine.*
    Planta Medica, **59(5)**, 413–417.
36. **Chopra, R.N. et al.** (1956). *Glossary of Indian Medicinal Plants.*
37. Sharma, A., & Shukla, Y. (2011). *Piperine, a major constituent of black pepper: a review of its bioactivity and toxicity*. **Critical Reviews in Food Science and Nutrition**, 51(6), 514–528.
38. Bhartiya, D., et al. (2010). *Understanding the role of gonadotropins in folliculogenesis and ovulation*. **Journal of Ovarian Research**, 3, 20.
39. Bang, J. S., et al. (2009). *Anti-inflammatory and anti-arthritic effects of piperine in human interleukin 1β-stimulated fibroblast-like synoviocytes and in rat arthritis models*. **Arthritis Research & Therapy**, 11(2), R49.
40. Umar, S., et al. (2013). *Piperine ameliorates oxidative stress, inflammation and histological outcome in collagen induced arthritis*. **Cellular Immunology**, 284(1-2), 51– 59.
41. Vijayakumar, R. S., et al. (2004). *Hypolipidemic effect of piperine in experimentally induced hyperlipidemia in rats*. **Indian Journal of Pharmacology**, 36(6), 333–335.
42. Gupta, S. K. (2018). *Drug Discovery and Development: Traditional Medicine and Ethnopharmacology*. Springer.
43. Nadkarni, K. M. (1976). *Indian Materia Medica* (Vol. 1). Popular Prakashan.
44. **Practice Committee of the American Society for Reproductive Medicine** (2018). *Diagnosis and treatment of polycystic ovary syndrome.*
    Fertility and Sterility, **110(3)**, 364–379.e1.
45. **Teede H. et al.** (2018). *International evidence-based guideline for the assessment and management of PCOS.*
    Monash University, Melbourne
    Comprehensive guideline covering Metformin, Letrozole, COCs, Spironolactone, etc.

46. **Nestler JE.** (2008). *Metformin for the treatment of the polycystic ovary syndrome. New England Journal of Medicine*, **358(1)**, 47–54.
47. **Charaka Samhita & Bhavaprakasha Nighantu** Ancient Ayurvedic texts document *Ashoka*, *Shatavari*, *Lodhra*, and *Guduchi* for gynecological disorders.
48. Ferreira, L. G., dos Santos, R. N., Oliva, G., & Andricopulo, A. D. (2015). **Molecular docking and structure-based drug design strategies**. *Molecules*, **20**(7), 13384–13421.
49. Mehrotra, R., Rawat, S., & Kulshreshtha, D. K. (2011). **Toxicological evaluation of piperine isolated from *Piper longum* Linn.** *Journal of Medicinal Plants Research, 5*(1), 53–57.
50. **Lachman, L., Lieberman, H. A., & Kanig, J. L.** *The Theory and Practice of Industrial Pharmacy*, 3rd Edition
51. **Allen, L. V., Popovich, N. G., & Ansel, H. C.** *Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems*, 9th or 10th Edition
52. **Aulton, M. E., & Taylor, K.** *Aulton's Pharmaceutics: The Design and Manufacture of Medicines*, 5th Edition