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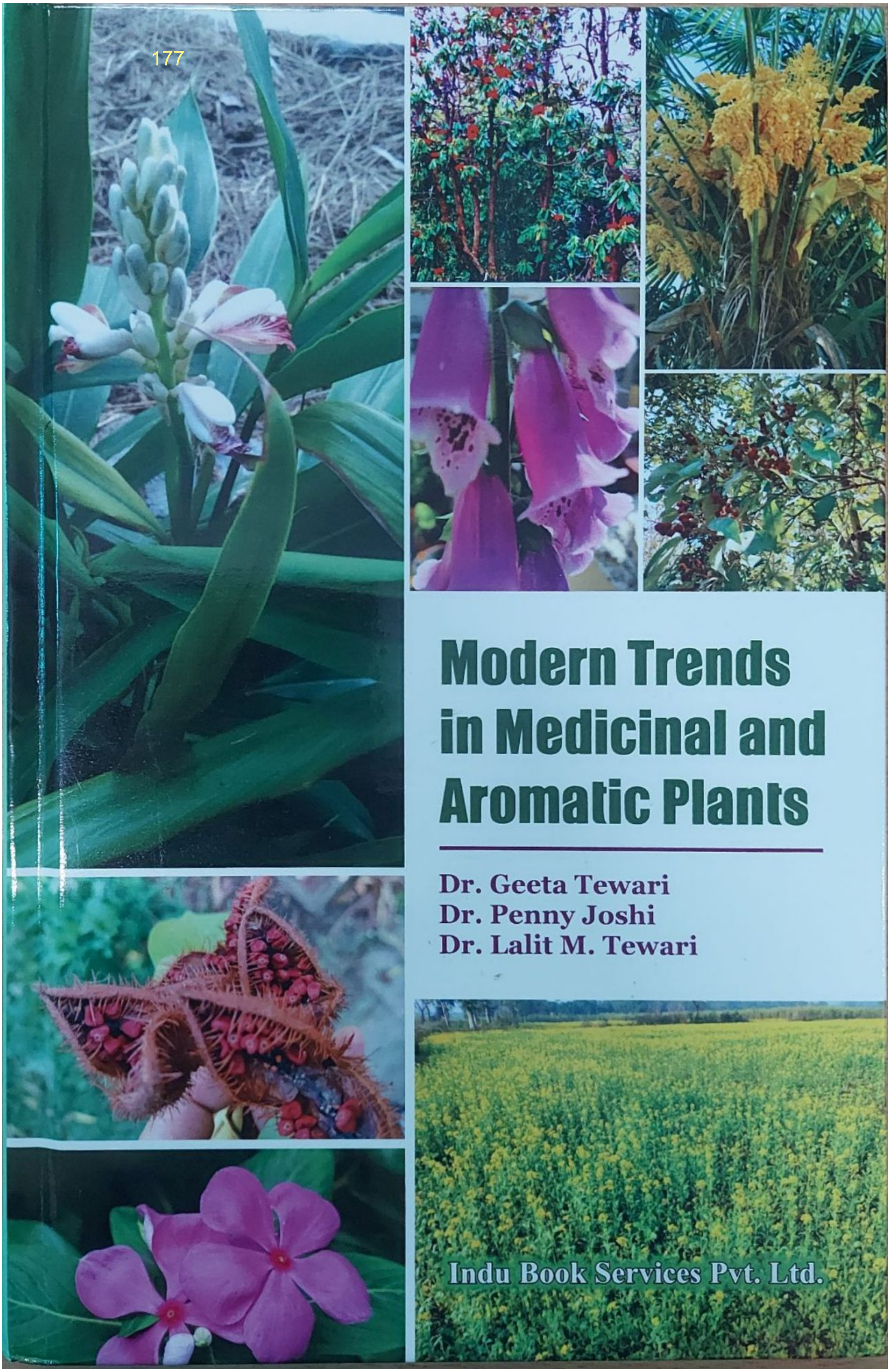
Modern Trends
in Medicinal and
Aromatic Plants
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# Contents

## Chapter 4

# *In-vitro* and *in-vivo* Anti-Polycystic Ovarian Syndrome Activity of Medicinal Plants

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**ABSTRACT**

Many of the medical systems have employed the usage of medicinal plants in the treatment of polycystic ovarian syndrome (PCOS), globally. It is a prevalent endocrine metabolic condition characterised by menstrual abnormalities, polycystic ovaries, anovulation, insulin resistance, hyperandrogenism, hirsutism and obesity. Many medicinal plants like *Urtica dioica, Cinnamomum zeylanicum, Sylibum marianum, Asparagus racemosus, Curcuma longa, Thuja occidentalis, Emblica officinalis, Zingiber officinalis, Saraca indica, Gymnema sylvestre, Tinospora cordifolia,* etc. have been significant in the treatment of PCOS and its associated symptoms like menstrual abnormalities, insulin resistance, obesity, infertility, and endometrial cancer. This review systematically analyses the *in-vivo* and *in-vitro* studies carried out on medicinal plants to explore their possible roles in the treatment of polycystic ovarian syndrome.

**Keywords:** Medicinal Plants, *In-vivo*, *In-vitro*, PCOS, Menstruation, Anovulation

## 4.1 INTRODUCTION

### *4.1.1 Polycystic ovarian syndrome*

Dr Irving Freiler Stein and Dr Michael Leventhal first discovered polycystic ovarian syndrome in 1935 which was known as the Stein-Leventhal syndrome for several years.

PCOS also called hyperandrogenic anovulation (HA), is a group of symptoms that women experience because of cerrtain hormonal imbalances (Goodrazi *et al.*, 1998). It is a well known gynaecological disorder among the reproductive females. It involves insulin resistance, infertility, irregular menstruation, anovulation, hyperandrogenism, acne, hirsutism, obesity, and ovarian cysts (Figure 4.1) (Dahlgren and Janson, 1994). PCOS is most common among women within the age group of 18 to 44 years. Typically, symptoms appear in late adolescence or the early twenties. Not all PCOS patients are identified with all the associated symptoms. Variations in symptoms are recognizable from mild to severe. Global prevalence estimates of PCOS are highly variable, ranging from 3-26% of afflicted female worldwide. In India, around 5-10% of women are affected by PCOS (Azziz *et al.*, 2009; Bharathi *et al.*, 2017).

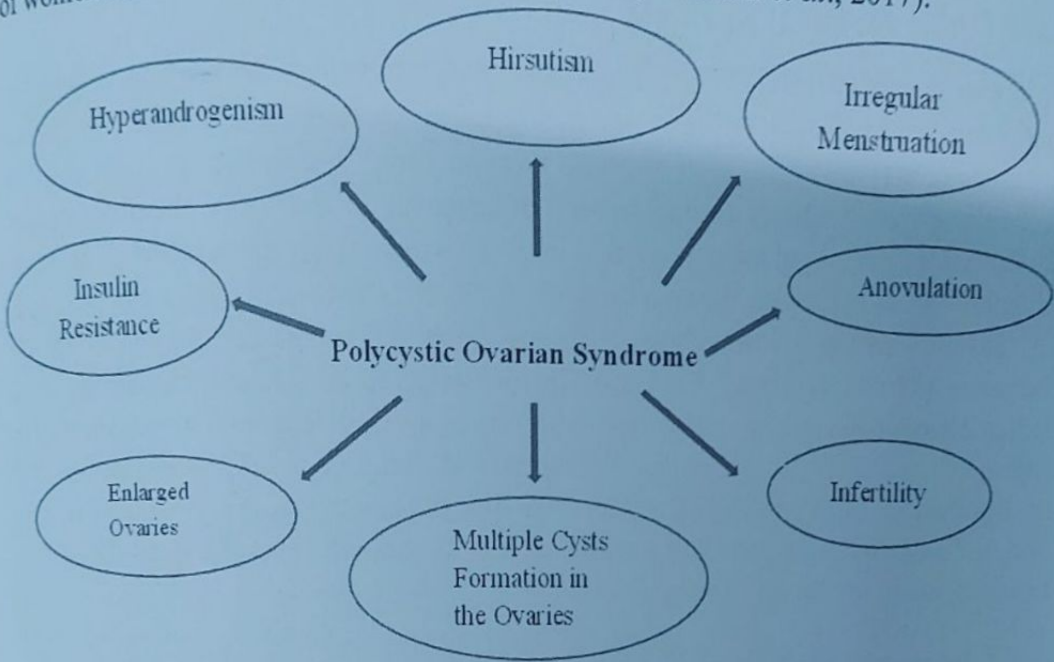


**Figure 4.1: Characteristics of polycystic ovarian syndrome (Louwers and Laven, 2020)**

Presence of PCOS is confirmed if any two or three of the following criteria are met: i) oligo-/an-ovulation; ii) clinical/biochemical sign of hyperandrogenism; and iii) polycystic ovary. This was agreed upon by the Rotterdam Consensus Conference, the European Society for Human Reproduction and Embryology (ESHRE), and the American Society for Reproduction Medicine (ASRM) in 2003. Recently, these diagnostic criteria for adolescent girls and perimenopausal/menopausal women have been proposed (Rotterdam, 2004).

Since PCOS affects multiple physiological systems, different organs must be analysed in order to clarify the disease etiology (Noroozzadeh *et al.*, 2017). In 2010, World Health Organisation (WHO) estimated 116 million women to be afflicted with PCOS, worldwide (Kabel, 2016). PCOS is a chronic disorder that often manifests during or shortly after puberty. According to reports, women with PCOS have thrice the risk of developing endometrial cancer than women without PCOS (Ness *et al.*, 2000) and the risk is still higher in obese women. Obesity is marked in approximately 50% of individuals with PCOS. Insulin resistance or hyperinsulinemia is observed in more than 70% of PCOS cases which is responsible for dyslipidaemia and inflammatory PCOS, as it increases more androgen secretions due to disruption of the luteinizing hormone (Messer *et al.*, 2012; Lauretta *et al.*, 2016). Additionally, PCOS is linked to impaired beta cell function and a decrease in mass of beta cells, even in the absence of insulin resistance. Therefore, impaired cell proliferation and inflammatory cascades are hallmarks of PCOS related pancreatic disturbances (Rojas *et al.*, 2014). Reactive oxygen species and antioxidants are in equilibrium in normal individuals but when imbalanced, they aid in the development of oxidative stress (Agrawal *et al.*, 2005). There is a higher risk of cardiovascular disease in PCOS affected women due to increased oxidative stress (Sabuncu *et al.*, 2001).

### *4.1.2 Pathophysiology of PCOS*

The exact pathophysiology of polycystic ovarian syndrome remains unknown. However, it is reported that the luteinising hormone (LH) and follicle stimulating hormone (FSH) are secreted in response to gonadotropin releasing hormone. Normal follicular growth requires a small amount of intraovarian androgen. The first stimulus for follicular development is provided by FSH which also promotes the conversion of androgen to estrogen in granulosa cells by activating the aromatase enzymes. LH initiates oocyte maturation by inducing theca cell production. PCOS condition increases LH and decreases FSH levels which leads to an increase in androgen and reduced aromatase enzyme. This results in immature follicle development. Excessive androgen in PCOS is related to abdominal fat, leading to hyperinsulinemia and dyslipidaemia. Hyperinsulinemia decreases sex hormone binding globulin (SHBG) and increases androgen synthesis in theca cells, which elevates the quantity of free testosterone in the blood (Balen, 2004).

### *4.1.3 Pharmacotherapy of PCOS*

#### *4.1.3.1 For hyperandrogenism*

A biochemical condition known as hyperandrogenism is characterised by hirsutism, or alopecia and acne induced by peripheral androgen receptor hypersensitivity and high level of circulating androgen concentrations (Chang *et al.*, 2005).

The first line of treatment for hyperandrogenism is hormonal therapy; hirsute women who do not wish to get pregnant frequently utilise estro-progestins, such as combined oral contraceptives (COCs), as the first line of treatment for hyperandrogenism. Progestins and estrogens inhibit the release of LH, which in turn reduces the production of ovarian androgens. Estrogens also enhance the production of SHBG in the liver, which lowers the plasmatic level of free androgens (Vrbikova *et al.*, 2005). The reduced androgen levels lower hyperandrogenism and reflect additional activities throughout COCs (De leo *et al.*, 2007).

Levonorgestrel, one of the estro- progestin compounds, has lately been suggested as one of the progestogens with lower thrombogenicity and no androgenic effects.

The third generation progestins Norgestimate, Desogestrel and Gestodene are antiandrogenic progestins that work by blocking the 5- alpha reductase activity of the androgen receptor or antagonising androgen receptor (Lidegaard *et al*., 2001; Rapkin and Winer, 2007).

The most effective drugs for the management of hirsutism are antiandrogens, which can be divided into two categories: androgen receptor blockers like flutamide, spironolactone and 5- alpha reductase inhibitors like finasteride. Antiandrogens can be used in mild to moderate cases of hirsutism and after 6 months of COCs or when COCs fail. Insulin sensitising medications like rosiglitazone, pioglitazone and metformin act by improving insulin sensitivity with a subsequent decrease in serum androgen concentrations (Luorno and Nestler, 2001).

#### *4.1.3.2 For menstrual disorder*

In oligomenorrheic PCOS women, progestins are used to treat menstrual abnormalities. Menstrual problems are improved by thiazolidinediones such as rosiglitazone and pioglitazone. In addition to obese and insulin resistant PCOS patients, metformin is the most significant and effective insulin sensitising medication utilised to treat oligomenorrhea in PCOS women with normal glucose tolerance. Also, N-acetyl-cysteine is used to treat irregular menstruation because it decreases the oxidative stress implicated in the pathophysiology of PCOS (Fulghesu *et al*., 2002; Morley *et al*., 2017).

#### *4.1.3.3 For infertility*

Clomiphene citrate helps to restore ovulation by inhibiting estrogen receptors at the hypothalamic level, lowering estrogen negative feedback, and enhancing endogenous FSH-LH secretion. Furthermore, metformin, rosiglitazone, and insulin sensitising medications are used to treat infertility, and to restore monofollicular ovulation, and reduced multiple pregnancy rate in PCOS affected women (Ortega *et al*., 2005; Morley *et al*., 2017).

With around 47,000 plant species, several kinds of medicinal plants, and traditional plant-based knowledge amongst the numerous ethnic groups, India is one of the world's mega diversity centres. India has been a centre of ethnomedicinal richness since Vedic times. It has utilised copious medicinal species to produce a variety of pharmaceuticals and medicinal solutions to treat a wide range of illnesses. Different types of diseases and disorders can be treated by administering appropriate dosage of drugs prepared from various parts of plants.

Traditional medicine is the right application of indigenous knowledge for safeguarding the health of people all over the world. The traditional knowledge system is beneficial to various many industries, including agriculture, animal husbandry, ethnic veterinary medicine, natural resource management, primary health care, preventive medicine, psychosocial care, and community development (Maikhuri *et al*., 1998).

## 4.2 IN-VIVO AND IN-VITRO METHODS TO EVALUATE ANTI-PCOS ACTIVITY

### *4.2.1 In-vivo methods*

#### *4.2.1.1 Androgen Induced Models of PCOS*

The most common symptom of polycystic ovarian syndrome is hyperandrogenism. The aetiology of PCOS is that it develops later in life as a result of early exposure to excessive levels of androgens. It was demonstrated more than 30 years ago that elevated amounts of circulating androgens in rodents affected the maturation of ovarian follicles and cyst formation (Parker and Mahesh, 1976). Daily injection or subcutaneous implants of either dehydroepiandrosterone, testosterone propionate, or 5α-dihydrotestosterone are utilized to generate an acute PCOS syndrome in rats (Singh, 2005).

#### *4.2.1.2 Dehydroepiandrosterone (DHEA) induced PCOS*

Dehydroepiandrosterone is the first androgen that becomes active in the female peripubertal period. Roy *et al*. (1962) was the first to use DHEA to develop PCOS in rats. Peripubertal rats, who are about 22 days old, are administered DHEA daily for up to 20 – 27 days at a dose of 6 mg/100 g body weight dissolved in 0.2 ml sesame oil. Rats become acyclic and anovulatory after treatment with DHEA (Mahesh and Greenblatt, 1962; Knudsen *et al*., 1975; Parker and Mahesh, 1976).

#### *4.2.1.3 Testosterone propionate (TP) induced PCOS*

Testosterone induces the development of polycystic ovaries in young female rats (Ota *et al*., 1983, Belooskey *et al*., 2004). In this method, 21 days old rats are injected daily for 35 days with TP dissolved in propylene glycol at a dose of 1 mg/100 g body weight (Belooskey *et al*., 2004).

#### *4.2.1.4 5α-Dihydrotestosterone (DHT) induced PCOS*

5α-dihydrotestosterone (DHT), a nonaromatizable androgen, has been shown to cause ovarian and metabolic abnormalities in rodents. It causes cystic ovaries and irregular cyclicity, therefore it is utilised to depict the female normo-androgenic phenotype of PCOS. Three-week-old rats when were implanted subcutaneously with 90 days of continuous release pellets of 7.5 mg DHT (83 mg daily dosage) show plasma DHT concentrations 1.7-fold higher in treated animals than those in the control group. After 11–13 weeks of DHT therapy, polycystic ovaries and abnormal ovarian cyclicity are observed (Manneras *et al*., 2007).

#### *4.2.1.5 Estradiol valerate (EV) induced PCOS*

Long-acting estrogen i.e. estradiol valerate (EV) produces GnRH dysregulation in the hypothalamus and pituitary, leading in to inappropriate luteinizing hormone release and storage. PCOS is assumed to have been mostly influenced by luteinizing hormone. A single dosage of EV (2 mg) to the young adult cycling rat results in polycystic ovaries and anovulation within 8 weeks (Brawer *et al.*, 1978; Brawer *et al.*, 1986).

#### *4.2.1.6 Aromatase inhibitor induced model of PCOS*

Testosterone and androstenedione are converted into estrogen and estrone, respectively, via aromatase enzyme. Many human tissues, including the placenta, ovary and testis, contain these enzymes (Corbin *et al.*, 1999). Reduced ovarian aromatase activity is one of the pathophysiologic causes for PCOS development (Diamanti, 2008).

#### *4.2.1.7 Letrozole induced PCOS*

Letrozole, a non-steroidal aromatase inhibitor, increases testosterone levels and reduces estrogen levels by preventing androgens from being converted to estrogen in the ovary. Excess testosterone in the ovaries of rats receiving letrozole is likely to cause polycystic ovaries. Lower levels of estrogen result in increased levels of LH, which further increases the release of testosterone from theca cells by reducing the pituitary's inhibitory feedback on LH synthesis. Female rats (6-week-old) when administered with letrozole orally for 21 or 36 days at dosages of 0.1, 0.5, and 1.0 mg/kg per day, turn acyclic and exhibit histological and biochemical traits similar to those in humans with PCOS (Ajika *et al.*, 1972).

## 4.3 *IN-VITRO* METHODS

### *4.3.1 GLUT4 Expression Study*

In polycystic ovarian syndrome, this assay is used to assess insulin sensitivity. There are two types of GLUT4 expression studies:

#### 4.3.1.1 Cell culture and treatment

In this method, differentiated C2C12 myotubes ($1.2 \times 10^5$ cells/well in 6 well plate, obtained from ATCC) in Dulbecco's Modified Eagle Medium (DMEM) high glucose media are treated with 500 μM palmitate for 24 hours. The cells are then washed in sterile phosphate buffer solution and incubated with the sample at nontoxic concentration with and without 100 nM insulin for 24 hours at 37°C with 5% $CO_2$. Post incubation, the medium is withdrawn, and the adhering cells are subjected to total RNA isolation for further analysis (Rosenbaum *et al.*, 1993).

#### 4.3.1.2 Gene expression

In this method RNA is isolated from the treated myotubes. On an agarose gel, the extracted RNA is measured. Reverse transcriptase is used to synthesizes first strand cDNA from total RNA and random primers. With 2 μl of cDNA and 10 μl of SYBR green supermix, the PCR amplifies the target sample in a 20 μl reaction volume. The levels of GLUT4 expression are standardized to 18s rRNA expression, and the control is normalized to 1 (Rosenbaum *et al.*, 1993).

### 4.3.2 Yeast androgen bioassay

In the RIKILT yeast androgen bioassay, the human androgen receptor (hAR) and yeast enhanced green fluorescent protein (yEGFP), which is generated in responses to androgens,. For testing androgenic activity, 200 mL of yeast suspension is grown in minimal medium with l-leucine (MM/L) and combined with 2 mL of undiluted and diluted sample and 2 mL of 5- dihydrotestosterone at final concentrations of 3 nM and 100 nM (positive controls) for ensuring the proper run of the assay (Bovee *et al.*, 2009).

## 4.4 *IN-VIVO* AND *IN-VITRO* ANTI POLYCYSTIC OVARIAN SYNDROME ACTIVITY OF MEDICINAL PLANTS

### *4.4.1 In-vivo Anti Polycystic Ovarian Syndrome Activity of Medicinal Plants*

#### *4.4.1.1 Urtica dioica*

*Urtica dioica* (Urticaceae) is used as a traditional as well as modern medicine for treatment of PCOS. In an investigation conducted in women suffering from PCOS with hyperandrogenism, it was found that the dried extract of *Urtica dioica* roots were effective in the reduction of androgen level as it blocks sex hormone binding globulin (SHBG). This treatment was effective in normalizing acne and menstrual cycle (Samad *et al.*, 2015).

#### *4.4.1.2 Asparagus racemosus*

It has a long history of use in the Indian ayurvedic medical system. It is useful in the treatment of neurological disorders, cancer, ulcers, diarrhoea, and immunomodulatory activities and has a variety of pharmacological effects. Shatavari is enriched with vitamins A, B1, B2, C, E, folic acid, etc. It is also abundant in minerals including calcium, magnesium, phosphorus and iron. These components make this herb a great antioxidant that safeguards a female's reproductive systems. Due to its antidiabetic properties, it also aids in enhancing insulin sensitivity. Additionally, it also helps in increasing fertility. Due to its phytoestrogens, it aids in encouraging healthy ovarian follicle formation, controls menstruation cycle and revitalises the female reproductive system (Kumar *et al.*, 2008).

#### *4.4.1.3 Punica granatum*

*Punica granatum* is commonly known as pomegranate and belongs to the family Punicaceae. This fruit has a variety of medicinal properties. The active constituents of the fruit are folic acid, vitamins (B2, C, and B1), sugars, pantothenic acid, and organic acids. Saturated and unsaturated fatty acids are reported to be present in the seeds of *P. granatum*. Using a control and PCOS group, adult female rats are used to study the impact of pomegranate extract on the management of PCOS. In the experimental group, the level of free testosterone, serum estrogen, and androstenedione hormone are all observed. The study reveals that pomegranate extract has a preventive impact against PCOS associated hormonal abnormalities. According to the study, consumption of the extract may reduce PCOS related difficulties (Hossein *et al.*, 2015).

### *4.4.1.4 Curcuma longa*

*Curcuma longa* (turmeric) of the family Zingiberaceae is used in Indian herbal medicine because it has vast number of medicinal properties. It is also used for various commercial purposes. Curcumin, a water insoluble, low molecular weight and polyphenolic curcuminoid derivative found in the rhizomes of *C. longa,* is the main component of the plant. Curcumin has various pharmacological effects like antihyperlipidemic, antidepressant, anti-inflammatory and estrogenic effects. A study was done to compare the effects of curcumin and clomiphene citrate in female rats with PCOS induced by letrozole-aromatase inhibitors. Progesterone and estradiol serum levels declined in the PCOS induced group. In rats with letrozole induced PCOS, curcumin restored the hormone and lipid profile, antioxidant and glycaemic state, as well as ovarian morphology. Reduced progesterone levels are another sign of an anovulatory cycle, and curcumin can successfully induce ovulation. According to the study, the result may be linked to its numerous pharmacological actions, which may help in managing PCOS symptoms, prevent ovarian cell malfunction, and improve fertility. According to the studies, curcumin's effects are comparable to those of clomiphene citrate (Reddy *et al*., 2016).

### *4.4.1.5 Mimosa pudica*

*Mimosa pudica* from the family Leguminosae, is a shrub. It has been used traditionally to treat a variety of female reproductive system related diseases. It has been reported that *M. pudica* is used for severe menstrual blood loss, leucorrhoea and uterine bleeding. In rats with letrozole induced PCOS, *M. pudica* aqueous slurry was found to reduce hyperandrogenism induced biochemical changes as well as histopathological changes in ovaries (Jadhav *et al*., 2013).

### *4.4.1.6 Cinnamomum zeylanicum*

According to the reports, *Cinnamomum zeylanicum* contains various flavonoids, polyphenols, and procyanidin that control the insulin stimulated glucose uptake and glycogen formation. In a pilot study, Wang *et al*. (2007), examined the results of the oral glucose tolerance test to see how cinnamon extract affected PCOS women. This study found that patients with PCOS had increased insulin sensitivity and lowered oral glucose tolerance after taking cinnamon extract. Another study was conducted on 66 women with PCOS. Participants were divided into two groups randomly; one group served as the intervention group and received 1.5 g of cinnamon powder daily in three separate doses for three months, while the other group served as the control group and received a placebo. Cinnamon decreased the fasting insulin level and insulin resistance in PCOS patients (Hajimonfarednejad *et al*., 2018).

### *4.4.1.7 Silybum marianum*

It is reported that silymarin has various pharmacological properties like antioxidant, anti-inflammatory, hepatoprotective and cardioprotective. Nuclear factor kappa light chain enhancer of activated B cells (NF-kB) activation is strongly inhibited by it. By raising cellular glutathione, it aids in the body's elimination of free radicals and prevents the peroxidation of lipids (Kayedpoor *et al.*, 2017). Silymarin inhibits angiogenesis, which lowers follicular cell proliferation and lowers the generation of testosterone while raising progesterone levels in the corpus luteum. Silymarin decreases testosterone levels, and increases the production of SHBG protein. It also lowers the cyst count. Silymarin affects glucose 6-phosphate, inhibits gluconeogenesis, lowers blood sugar, and alleviates PCOS symptoms as a result. Silymarin lowers blood glucose levels by reducing oxidative stress. It also reduces inflammation in PCOS by blocking the enzymes cyclooxygenase-2 (COX-2) and lipoxygenase. Nebuini *et al.* (2015) examined the impact of silymarin on estradiol valerate induced PCOS rats. In this experiment, silymarin was administered to rats for 14 days at doses of 20 mg/kg, 50 mg/kg, 100 mg/kg and 300 mg/kg. Abdominal size, body weight, the number of cysts, and their size were all shown to decrease in the group given silymarin at 300 mg/kg, which may have been the result of its anti-inflammatory characteristics. Due to the development of corpus luteum in the ovary, different doses of silymarin showed beneficial effects, including a decrease in estradiol, testosterone and LH and a considerable increase in FSH and progesterone hormones. In the follicular sheath, silymarin decreased swelling and collagen, ultimately resulting in the reduction of the thickness of the layer (Nebiuni *et al.*, 2015).

The effects of a fixed combination of *Berberis aristata* and *S. marianum* on sugar and lipid profile were reported in a meta-analysis. Low density lipoprotein, cholesterol, and plasma glucose levels were all reduced by *S. marianum* (Toth *et al.*, 2020). Another study reported that, silibinin a polyphenolic flavonoid extracted from the plant *S. marianum*, significantly downregulated insulin receptor expression in the Alzheimer's group compared to the healthy group (Liu *et al.*, 2019).

#### 4.4.1.8 *Cucurbita maxima*

Synthetic estrogen is now widely used to treat women's infertility and hormonal issues, pumpkin seeds contain phytoestrogens with estrogenic action. Hormone replacement therapy includes progesterone therapy, estrogen therapy, and occasionally a combination of the two. The main estrogen that the ovaries generate and which can remove excess androgen is estradiol. Pumpkin seeds also contain omega-3-fatty acids that can regulate high cholesterol and high insulin levels found in PCOS. Using adult female rats, researchers examined the impact of hydroalcoholic extract of pumpkin seeds on estrogen levels and renal indicators. The estrogen included in the hydroalcoholic extract of pumpkin seeds has the least detrimental impact on renal tissue and can play a significant role in ovulation. The study suggests that it may be an effective substitute for synthetic estrogen and menstrual cycle regulators, which may be helpful in the management and prevention of PCOS (Jahromi and Jahromi, 2019).

#### 4.4.1.9 *Tephrosia purpurea*

*Tephrosia purpurea*, also referred to as wild indigo, is a member of the Fabaceae family. Traditionally it is used in both inflammatory illnesses and several reproductive disorders in females. An investigation conducted on letrozole induced PCOS rats showed that the improvement of rat ovulation may be influenced by *T. purpurea* extract. The effects of *T. purpurea* considerably decreased the endocrinological and biochemical abnormalities brought on by hyperandrogenism as well as the histological changes in the ovary and did not produce any changes in the follicle stimulating hormone and luteinizing hormone (Thakar and Anuradha, 2014).

#### 4.4.1.10 *Linum usitatissimum*

Flax seed is traditionally used as a medicine to treat various diseases and disorders. It is one of the richest sources of omega-3-fatty acid and dietary lignin. It contains various pharmacologically active constituents like alpha-linolenic acid, d-xylose, L-rhamnose, L-galactose and d-galacturonic acid. A case study was conducted on PCOS women for a period of four months. A significant decrease in androgen level and hirsutism was observed in participants who consumed flaxseed. A significant decrease in body mass index, insulin, total serum testosterone and free serum testosterone level was reported (Jelodar *et al.*, 2018).

#### 4.4.1.11 *Thuja occidentalis*

*Thuja occidentalis* L. (Cupressaceae) is also known as cedar. It is used as a folk remedy to cure rheumatism, amenorrhea, cystitis, uterine carcinomas, and as an abortifacient and contraceptives. Thujone, isothujone, fenchone, sabinenes, and α-pinene are the major constituents of cedar essential oil (Biswas *et al.*, 2011), along with the diterpenes beyerene and rimuene (Tsiri *et al.*, 2009). Studies have revealed that thujone is responsible for its healing effects. The essential oil of *T. occidentalis* and its main component, α-thujone, were found to be helpful in PCOS in a study carried out in rats with letrozole induced PCOS afflicted rats. In *T. occidentalis* and α-thujone treated groups, estradiol and progesterone levels were found to be considerably increased, but LH levels, testosterone levels, glucose concentration and low-density lipoprotein cholesterol are reduced markedly (Akkol *et al.*, 2015).

#### 4.4.1.12 *Tribulus terrestris*

*Tribulus terrestris* from the family Zygophyllaceae plays an important role in folk medicine. Main phytochemical of this plant is steroidal saponin and it contains 2,4-dichlorophenoxyacetic acid (2,4-D), glyphosate, and dicamba. It was reported to be effective in the improvement of ovulation in rats with estradiol valerate induced PCOS. In rats with PCOS, *T. terrestris* normalized estrous cyclicity, steroidal hormone levels and regulated ovarian follicular development. So, it was found to be beneficial in treating PCOS (Saiyed *et al.*, 2016).

#### 4.4.1.13 *Emblica officinalis*

*Emblica officinalis* is a rich source of vitamin C and is an antioxidant. It is often referred to as Indian gooseberry. It reduces both inflammation and weight are reduced by it. This fruit contains fibres that aid in maintaining a healthy digestive tract. It strengthens the immune system as well (Khanage *et al.*, 2019).

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#### 4.4.1.14 *Mentha spicata*

*Mentha* is a medicinal plant of the Lamiaceae family. The impact of peppermint tea on the testosterone levels of hirsute women was studied. During the follicular phase of the menstrual cycle, the intervention group in this study got a cup of peppermint tea made with 5 g of dried *Mentha* leaves and 250 mL of boiling water, five days a week. The levels of free testosterone and triglycerides were significantly low in the intervention group, whereas the levels of LH, FSH, and prostaglandin E2 were significantly higher. DHEA and total testosterone levels did not significantly decline. In a different clinical study, the intervention group got chamomile or peppermint tea twice daily for 30 days, which included one menstrual cycle. According to the study's findings, drinking peppermint tea significantly reduced testosterone levels and hirsutism, while raising LH and FSH levels. Another study examined the impact of herbal supplements containing *Mentha*, *Zingiber*, cinnamon in PCOS patients both with and without clomiphene citrate. The antioxidant levels, glycaemic management, menstrual regulation, and pregnancy rate were all found to be significantly impacted by these supplements (Akdogan *et al.,* 2007; Grant, 2010).

#### 7.3.1.15 *Ficus deltoidea*

The effects of an ethanolic extract of *F. deltoidea* leaves on the reproductive organs of letrozole induced PCOS in Sprague dawley rats were studied by Suhaimi *et al.* in 2017. Different concentrations of clomiphene citrate, employed as a standard, were administered to the rats. At the conclusion of the therapy period, the uterus and ovaries were collected and weighed. Preparation of uterine and ovarian tissue was done for histopathological analysis. According to the findings of pathological investigations, the group treated with the extract had less cystic follicles than the group that received no therapy. *F. deltoidea* reduced ovarian wet weight and increased uterine wet weight increased in PCOS females (Suhaimi *et al*., 2017).

#### *4.4.1.16* **Asphaltum punjabianum (Shilajit)**

Shilajit's anti-inflammatory qualities are well -known. It also works as an immunity booster and rejuvenates the female reproductive system (Walale and Khandane, 2018).

#### *4.4.1.17* **Saraca indica**

Anti-inflammatory chemicals are found in Ashoka. It helps to mend the endometrium and heal the harm that inflammation has caused to its sensitive lining. It can also help in painful periods, internal bleeding, haemorrhoids, menometrorrhagia, amenorrhoea, menorrhagia caused by uterine fibroids, leucorrhoea, and acne in women. It also works to keep oestrogen levels in check. It possesses antibacterial and antifungal characteristics that aid in guarding against bacterial and fungal infections of the urinary system. *S. indica* methanolic extract at a dose of 200 mg/kg in female rats was discovered to have anti-estrogenic properties because of its phytoestrogen components (Shahid *et al*., 2015).

## 4.5 *IN-VITRO* ACTIVITY OF MEDICINAL PLANTS FOR TREATMENT OF POLYCYSTIC OVARIAN SYNDROME AND ITS ASSOCIATED SYMPTOMS

### *4.5.1 In-vitro study using yeast androgen bioassay*

The human androgen receptor and yeast enhanced green fluorescent protein, which is produced in response to androgens, both are expressed in yeast cells used in the RIKILT yeast androgen bioassay. *Nardostachys jatamansi* DC and *Tribulus terrestris* L. showed antiandrogenic efficacy in yeast androgen bioassay (Palakkil *et al.*, 2015).

### *4.5.2 In-vitro studies on glucose uptake*

*Euonymus alatus*, which contains kaempferol and quercetin, have been demonstrated to promote increase insulin stimulated glucose uptake in mature 3T3-L1 adipocytes, (Fang *et al.*, 2008). *Momordica charantia* aqueous extract showed potent cell mending properties and promoted insulin production in HIT-T15 hamster pancreatic α-cells (Xiang *et al.*, 2007). *Aegle marmelos* and *Syzygium cumini* methanolic extracts enhanced glucose transport in L6 myotubes, in a PI3 Kinase dependent manner (Anandhrajan *et al.*, 2006). The glucose transport activity in *Pterocarpus marsupium* methanolic extract showed was PPAR mediated PI3 kinase dependent, whereas that of *P. marsupium* isoflavone was PPAR mediated but PI3 kinase independent (Anandhrajan *et al.*, 2005).

### *4.5.3 In-vitro studies using insulin-secreting cell lines*

In a number of beta cell lines, insulin release was boosted by an alcoholic *Gymnema sylvestre* extract in a dose dependent manner. Permeation of the beta cell plasma membrane and non-channel dependent $Ca^{2+}$influx into the beta cells causes the release of insulin (Persaud *et al.*, 1999). Insulinotropic activity was produced in HIT-T15 cells as a result of the insulin secretagogue present in *Tinospora crispa* extract, which increased cytosolic $Ca^{2+}$ absorption from external medium while reducing $Ca^{2+}$efflux from the cytosol (Noor and Stephen, 1998). At low glucose concentrations, incubation of glucose responsive BRIN-BD11 cells with aqueous extract of *Medicago sativa* (Lucerene) resulted in dose dependent stimulation of insulin production (Gray and Flatt, 1997). In clonal BRIN-BD11 pancreatic beta cells, an aqueous extract of *Viscum album* (mistletoe) increased insulin secretion in a dose dependent manner (Gray and Flatt, 1999).

There are various medicinal plants which have potent effect against polycystic ovarian syndrome and its associated symptoms (Table 4.1).

**Table 4.1** List of Medicinal Plants used in Polycystic Ovarian Syndrome and its Associated Symptoms

| Botanical name & family | Active constituents | Study design and tested material | Outcomes | Other uses | References |
|---|---|---|---|---|---|
| *Punica granatum* L. Lythraceae | Ellagitannin, Punicalagin, ellagic acid | Animal | Improvement in testosterone, androstenedione and estrogen level in the treated groups | Parasites, dysentery, diarrhoea and haemorrhoids | Hossein *et al*. (2015) |
| *Gymnema sylvestre* Asclepiadaceae | Gymnemic acid, tartaric acid, gurmarin, glucose, stigmasterol, betaine, and choline | Animal | Regulation of blood glucose level, improving the elevated triglycerides level | Diabetes | Yadav *et al*. (2020) |
| *Tribulus terrestris* Zygophyllaceae | Steroidal saponins, flavonoids, alkaloids, and lignan amides | Animal | Improvement of ovulation in rats, regularized ovarian follicular growth, normalized estrous cyclicity and steroidal hormonal levels | It has diuretic, aphrodisiac, antiurolithiatic, antidiabetic, central nervous system, anticarcinogenic activities. | Saiyed *et al*. (2016) |
| *Cinnamomum zeylanicum* Lauraceae | Cinnamic acid | PCOS women | Improvement in insulin sensitivity and menstrual cyclicity | Gastrointestinal disorders, diarrhoea and bacterial infection | Wang *et al*. (2007) |
| *Asparagus racemosus* Asparagaceae | Shatavarin I–IV, quercetin, rutin | Animal | Regularization of Ovarian follicular growth, menstrual cycle, and helps in combating hyperinsulinemia | Used in Neuronal disorders, ulcer, diarrhoea, ageing, tumours, inflammation, and dyspepsia | Kumar *et al.* (2008) |
| *Urtica dioica* Urticaceae | Caffeic acid, chlorogenic acid | Animal | Normalization of menstrual cycle, improves insulin sensitivity | Prostatic hyperplasia, cardiovascular disorder, depression | Samad *et al.* (2015) |

| | | | | | |
|---|---|---|---|---|---|
| *Curcuma longa* Zingiberaceae | Curcumin | Animal | Reduction in fasting glucose level, glycosylated haemoglobin level, normalized lipid and hormonal profile | Cancer, diabetes, inflammation and also used as food additive | Reddy *et al*. (2016) |
| *Mimosa pudica* Leguminosae | Neoxanthin, viola xanthin, lutein, lycopene, carotenes, tocopherol | Animal | Reduction in elevated androgen level | Leucorrhoea, menorrhagia and dysfunctional uterine bleeding | Jadhav *et al*. (2013) |
| *Silybum marianum* Asteraceae | Silybin, isosilybin, silychristin, and silydianin | Animal | Estradiol, testosterone, and LH levels all decreased | Chronic liver disease and liver cirrhosis | Kayedpoor *et al*. (2017) |
| *Cucurbita maxima* Cucurbitaceae | vanillic acid, syringic acid, p-coumaric acid and ferulic acid | Animal | Improvement of ovulation in rats, regularization of menstrual abnormalities | Intestinal infections and kidney problems and to expel tapeworms | Jahromi and Jahromi (2019) |
| *Tephrosia purpurea* Fabaceae | Flavonoids, chalcones, rotenoids | Animal | Improvement of ovulation in rats | Inflammatory disorders and reproductive disorders | Thakar *et al*. (2014) |
| *Linum usitatissimum* Linaceae | Linolenic acid, linoleic acid, lignans, alkaloids, cyanogenic glycosides | PCOS Women | Significant reduction in body mass index, insulin, total serum testosterone and free serum testosterone level | Cardiovascular disease, diabetes, cancer, arthritis, neurological disorders | Jelodar *et al*. (2018) |
| *Thuja occidentalis* Cupressaceae | Fenchone, sabinenes, α-thujone, α-pinene and isothujone | Animal | Decreased levels of glucose, LDL-C, and total serum testosterone | Rheumatism, amenorrhoea, cystitis, and uterine carcinomas | Biswas *et al*., (2011), Akkol *et al*. (2015) |

| | | | | | |
|---|---|---|---|---|---|
| *Mentha spicata* Lamiaceae | Carvone, limonene, 1,8-cineole. β-pinene | PCOS women | LH, FSH, and prostaglandin E2 levels show significantly increased while free testosterone and triglyceride levels show a decreased | Cold, cough, asthma, fever, obesity, jaundice and digestive problem | Akdogan *et al.* (2007) |
| *Ficus deltoidea* Moraceae | Alkaloids, sterols, flavonoids, saponins, proteins | Animal | Reduction in number of cystic follicles | Relieve headache, fever, and toothache | Suhaimi *et al.* (2017) |
| *Asphaltum punjabianum* | Fulvic acid | PCOS women | Helps in prevention of iron shortage | Antioxidant, anti-inflammato ry, and memory enhancer | Walale *et al.* (2018) |
| *Saraca indica* Fabaceae | Tannins, flavonoids, phytosterols, alkanes, esters, fatty acids and carbohydrates | Animals | Antiestrogenic effect | Uterine fibroids, leucorrhoea, fungal and bacterial diseases | Shahid *et al.* (2015) |
| *Foeniculum vulgare* Apiaceae | Trans-anethole and estragole, alpha-phellandrene, limonene, fenchone, and alpha-pinene | Animal | Increased concentration of FSH levels and decrease in LH and testosterone levels | Gastrointestinal disorders | Karampoor *et al.* (2014) |
| *Camellia sinensis* Theaceae | gallic acid, *p*-coumaric acid and caffeic acid | Animal | Significant decrease in serum LH levels, body and ovarian weight, insulin resistance | Cancer, cardiovascular disorders | Ghafurniyan *et al.* (2015) |
| *Aloe barbadensis* Asphodelaceae | Lignin, saponins, salicylic acids and amino acids and beta-carotene | Animal | Improved glucose intolerance by lowering triglyceride and LDL-C levels. | Anti-inflammatory, antimicrobial, antioxidant | Desai *et al.* (2012) |
| *Glycyrrhiza glabra* Fabaceae | Glycyrrhizin, glycyrrhetinic acid, isoliquiritin, isoflavones | Animal | Improvement in the adverse effects of hyper androgenesis-m due to PCOS in female mice's fertility | Anti-demulcent, antiulcer, anticancer, anti-inflammatory, antidiabetic, | Ahmadi and Mostafavi (2015) |

## 4.6 CONCLUSION

An alternative and less expensive treatment for polycystic ovarian syndrome is emerging in the form of herbal medicines. Many medicinal plants like *Urtica dioica, Cinnamomum zeylanicum, Sylibum marianum, Asparagus racemosus, Curcuma longa, Thuja occidentalis, Emblica officinalis, Zingiber officinalis, Saraca indica, Gymnema sylvestre and Tinospora cordifolia* have proved effective in the treatment of PCOS and its associated symptoms like menstrual abnormalities, insulin resistance, obesity, infertility and endometrial cancer. Herbal therapies are used in the treatment of PCOS and related symptoms. Medicinal plants continue to have a beneficial anti-PCOS impact in both modern and traditional treatment. Current pharmaceutical methods for PCOS are not optimal due to their adverse effects, reduced responsiveness after continuous uses, and high cost. As a result, there is a need to find newer anti-PCOS medications. Many medicinal herbs contain anti-PCOS properties to varying degrees. Some herbs such as *Gymnema sylvestre* leaf extract and *Momordica charantia* fruit juice, have shown potential anti-PCOS activity and play essential roles in beta cell regeneration. *Syzigium cumini* seeds have been found to have antioxidant and antihyperglycemic properties. PCOS related anti-diabetic action of *Trigonella foenumgraecum* seeds has been demonstrated. All of these findings suggest that bioactive compounds derived from Indian medicinal plants with anti-PCOS capabilities should be investigated for their use and pharmacological activities directed towards mitigating PCOS. For analysing the pharmacological activity of these herbs or their active ingredients in the treatment of PCOS, more research is needed so that newer effective formulations for PCOS are available in the market.

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