CAPITAL UNIVERSITY OF SCIENCE AND TECHNOLOGY, ISLAMABAD

Phytochemical Insights: *Ginkgo biloba* as a Natural Remedy for Polycystic Ovarian Syndrome

by

Aqsa Noor

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in the

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Department of Bioinformatics and Biosciences

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With deepest gratitude, I dedicate this thesis to the most important pillars in my life—my beloved family. To my father, whose strength, wisdom, and steadfast support have guided me through every step of this journey. Your unwavering belief in my abilities and the values you have instilled in me have been a source of constant motivation. Thank you for being my role model and for always encouraging me to aim higher. To my mother, whose unconditional love, patience, and sacrifices have been the heartbeat of my progress. Your nurturing presence, constant prayers, and words of encouragement have lifted me in moments of doubt. Your endless devotion has inspired me more than words can express. To my brother, for always being there with kindness, humor, and reassurance. Whether it was offering a listening ear, lightening the mood, or simply standing by me, your presence made this journey less overwhelming and more meaningful. Without your collective love and support, this thesis would not have been possible. This accomplishment is as much yours as it is mine. And to myself, for the perseverance, late nights, and quiet battles no one saw. This thesis stands not only as a symbol of academic success but also of personal growth, strength, and resilience. I am proud of the journey and the person I have become through it.

CERTIFICATE OF APPROVAL

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(Aqsa Noor)

Abstract

Polycystic Ovary Syndrome (PCOS) is a complex endocrine disorder involving a combination of ovarian dysfunction, hormonal imbalance, anovulation, and metabolic irregularities. A key pathological driver is androgen excess, often accompanied by insulin resistance, obesity, cardiovascular complications, mood disorders, and an elevated risk of type II diabetes and cancer. Due to the limitations and side effects of conventional therapies, there is a growing interest in plant-derived compounds as alternative treatment options. This study explores the therapeutic potential of *Ginkgo biloba*, a medicinal plant renowned for its rich profile of bioactive constituents. Notable compounds include flavonoids, terpenoids (such as ginkgolides and bilobalide), and quercetin—compounds known for their potent antioxidant, anti-inflammatory, neuroprotective, and vasodilatory properties. These phytochemicals were subjected to molecular docking studies targeting key proteins implicated in PCOS pathophysiology: androgen receptor (AR), insulin receptor (IR), and tumor necrosis factor-alpha (TNF-α). The selection of candidate ligands involved a detailed analysis of their Absorption, Distribution, Metabolism, and Excretion (ADME) profiles, toxicity predictions, and molecular binding affinities. Among the tested compounds, quercetin emerged as the lead candidate, displaying superior pharmacokinetic characteristics, strong binding affinity to all three target proteins, and no predicted toxicity. Its performance was further benchmarked against Clomiphene citrate, a standard therapeutic agent for PCOS, revealing quercetin's potential as a safer and more efficacious alternative. This research highlights the promise of *Ginkgo biloba* as a source of natural therapeutic agents for PCOS, providing valuable insights into the use of herbal-origin compounds for managing this multifactorial syndrome. The findings underscore the relevance of plant-based drug discovery in the ongoing quest for safer and more effective PCOS treatments.

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Abbreviations

ADME	Absorption, Distribution, Metabolism, Excretion
ADMET	Absorption, Distribution, Metabolism, Excretion, and Toxicity
AMH	Anti-Müllerian Hormone
AE-PCOS	Androgen Excess and Polycystic Ovarian Syndrome Society
BMI	Body Mass Index
COCs	Combined Oral Contraceptives
CVD	Cardiovascular Diseases
ED	Eating Disorder
EDCs	Endocrine Disrupting Chemicals
EGF	Epidermal Growth Factor
ERs	Estrogen Receptors
ERT	Estrogen Replacement Therapy
FSH	Follicle-Stimulating Hormone
FSHR	Follicle-Stimulating Hormone Receptor
GnRH	Gonadotropin Hormone-Releasing Hormone
GnRHR	Gonadotropin Hormone-Releasing Hormone Receptor
GRAVY	Grand Average of Hydropathicity
HA	Hyperandrogenic Anovulation
HDL	High Density Lipoprotein
HPO	Hypothalamic Pituitary Ovarian
HRT	Hormone Replacement Therapy
LDL	Low Density Lipoprotein
LH	Luteinizing Hormone
LHR	Luteinizing Hormone Receptor

LIPO	Lipophilicity
NICHD	National Institute of Child Health and Human Development
OS	Oxidative Stress
OSI	Oxidative Stress Index
PCOS	Polycystic Ovarian Syndrome
PDB	Protein Data Bank
pI	Isoelectric Point
POLAR	Polarity
QoL	Quality of Life
ROS	Reactive Oxygen Species
T2DM	Type 2 Diabetes Mellitus
TNF-α	Tumor Necrosis Factor-alpha
VDR	Vitamin D Receptor
VLDL	Very Low Density Lipoprotein

Chapter 1

Introduction

Polycystic Ovary Syndrome (PCOS), also known as Stein Leventhal syndrome, affects women of reproductive age in every location and is a common, complicated endocrine condition. It is characterized mostly by high androgen levels and disordered ovarian function. Also, it has many metabolic problems. These elements, when combined, produce various different system and reproductive health issues [1]. With high risks of cardiovascular diseases, insulin resistance, obesity, type 2 diabetes, and endometrial cancer, PCOS is a known cause of anovulatory infertility [2]. While this rate can vary based on ethnic background, lifestyle choices, and diagnostic criteria, the worldwide prevalence of PCOS is roughly 5% to 10% among women of childbearing age. Research indicates that a number of Asian women, rather than Caucasian women, could have earlier symptoms, and the syndrome usually affects people with overweight or obesity [3].

The clinical manifestations of PCOS are diverse, as well as capable of classification into endocrine, metabolic, reproductive dysfunctions. Endocrine abnormalities feature increased androgen levels, changed gonadotropin secretion, and disturbances in estrogen and progesterone balance, leading to irregular or absent ovulation [4]. Metabolic features can include insulin resistance, also elevated insulin levels, plus abnormal lipid profiles, and even persistent low-grade inflammation, all of that heightens the risk for metabolic syndrome and cardiovascular issues [5]. Reproductive signs often involve irregular menstrual cycles, ovulatory dysfunction,

and infertility. Acne, excessive hair growth, and hair thinning are visible signs of androgen excess. Furthermore, many women that have PCOS undergo psychological challenges, which include anxiety, depression, and a diminished quality in life [6].

Personal changes in diet, drugs enhancing insulin sensitivity like metformin, hormonal therapy (e.g. birth control pills and antiandrogens), and ovulation stimulating agents like clomiphene citrate and letrozole are among the typical PCOS therapies [7]. On the other hand, these pharmaceutical treatments are associated with side effects and are often not feasible for every patieny. Therefore, natural, plant-based therapies have been gaining a lot of popularity as they can be very effective in the management of PCOS symptoms and have less harmful effects [8]. It has been reported in the various researches that medicinal plants can be very beneficial in regulating the hormonal, insulin, and oxidative stress pathways. That makes the plants potential substitutes for PCOS treatment. With the recognition of plant-based medicines, an extensive study is conducted to find the influence of the plants on the hormonal, insulin and oxidative stress pathways that ultimately lead to effective PCOS treatment [9].

For a long time traditional Chinese medicine has been using *Ginkgo biloba*, a medicinal herb with a rich phytochemical composition that has attracted the interest of the present health sectors. It is the flavonoids, terpenoids (ginkgolides and bilobalide), quercetin and other bioactive compounds that mainly owe to the herb powerful antioxidant, anti-inflammatory, neuroprotective and vasodilatory activities. This sets the stage for *Ginkgo biloba* to help treat PCOS which is largely the output of oxidative stress, inflammation, and metabolic dysfunction [10].

Ginkgo biloba is known to prevent the significant oxidative stress that forms the basis of PCOS, affecting ovarian function, androgen production, and insulin resistance if the stress gets any worse. The antioxidant function of *Ginkgo biloba*, which is quite outright, will serve these functions well by eliminating undesirable reactive oxygen species (ROS) and in turn enhancing mitochondrial function in

ovarian cells. Also, *Ginkgo biloba* has been able to prove that it is useful in insulin signal improvement, as well as glucose metabolism change, which are quite encouraging for those who resist to insulin and have metabolic problems in PCOS concomitantly [11].

Also, note that *Ginkgo biloba* has been shown to possess the properties affecting the internal system mainly hormone production that may directly or indirectly impact the hypothalamic-pituitary-ovarian (HPO) axis, which is the central regulatory element of the reproductive system and is quite frequently disturbed in PCOS [12]. The fact that *Ginkgo biloba* can reduce cortisol levels and brain neurotransmitter activity may also be of help to regain hormonal balance and rescue the ovary from the impairment of the hormonal system, thus restoring ovulatory function. Over and above, such an impact of the herb on blood flow can be an asset to the ovary by aiding in the process of follicular development and eliminating the chances of getting an ovarian cyst [13]. The use of *Ginkgo biloba* is therefore a necessity in the ongoing quest towards the discovery of new and effective phyto-based PCOS treatments. The synergism of the phyto-compounds and their probable influence on the endocrine system indicates that *Ginkgo biloba* is a likely natural therapeutic agent for PCOS [14].

Another area where *Ginkgo biloba* has shown therapeutic promise is its potential to lower androgen levels, a hallmark of PCOS pathophysiology. Elevated androgens such as testosterone are implicated in hirsutism, acne, and ovulatory disruption. Preclinical studies suggest that the flavonoids in *Ginkgo biloba* may exert anti-androgenic activity, possibly by modulating 5α-reductase enzymes or androgen receptor sensitivity. This makes the herb potentially effective in reducing symptoms like excessive facial hair and acne that commonly distress women with PCOS [10, 14].

In addition, *Ginkgo biloba*'s ability to modulate inflammatory markers such as TNF-α and IL-6 is particularly significant in PCOS, as these pro-inflammatory cytokines are known to be elevated and contribute to insulin resistance and follicular arrest. By reducing this low-grade chronic inflammation, *Ginkgo biloba* not

only improves metabolic outcomes but may also promote healthy ovarian function and regular menstrual cycles, which are often absent in PCOS patients [11]. This dual benefit of metabolic and reproductive improvement further reinforces its potential role as a multi-targeted botanical therapy.

1.1 Problem Statement

Polycystic Ovary Syndrome (PCOS) is a disorder caused by hormonal imbalance that, despite its very common occurrence, has only a few treatment options. *Ginkgo biloba* is mainly known for its potential as a natural substitute because it has antioxidant and endocrine-modulating properties. It is, however, still not clear enough about the phytochemical action of the plant in PCOS. So, the present study is designed to elucidate the question if *Ginkgo biloba* might be a therapeutic herb for PCOS symptoms.

1.2 Aim and Objectives

Through an *in silico* approach, this study aims to investigate the therapeutic potential of *Ginkgo biloba* as a treatment for PCOS. The objectives of the analysis are:

- Identifying bioactive compounds in *Ginkgo biloba* with potential activity against PCOS-related molecular targets.
- Evaluating the pharmacokinetic properties (ADME) and toxicity profile of *Ginkgo biloba* compounds to assess their drug-likeness.
- Conducting molecular docking studies to determine the binding affinity of *Ginkgo biloba* phytochemicals with key proteins involved in PCOS pathophysiology.

Chapter 2

Literature Review

2.1 Polycystic Ovarian Syndrome

Polycystic ovary syndrome (PCOS) is a complicated hormonal condition that targets women who are fertile. PCOS and infertility are proportional as PCOS ovulatory disturbance is the single reason for ovulatory infertility [4]. The manifestations of PCOS are complex and include such processes as the excess production of hormones by the ovaries, high levels of androgens and the absence of ovulation [15]. A number of disorders related to the metabolism like the presence of insulin resistance, an increase in the levels of insulin, disturbances of glucose metabolism, dyslipidemia, obesity, and the high probability of the development of type 2 diabetes mellitus (T2DM) and the metabolic syndrome, blood pressure, and heart disease (CVD) have been seen in individuals concerned [16]. At the same time, the prolonged action of estrogens without progestins is associated with the rise of endometrial hyperplasia and female cancer occurrence such as endometrial, ovarian, and breast cancer [17].

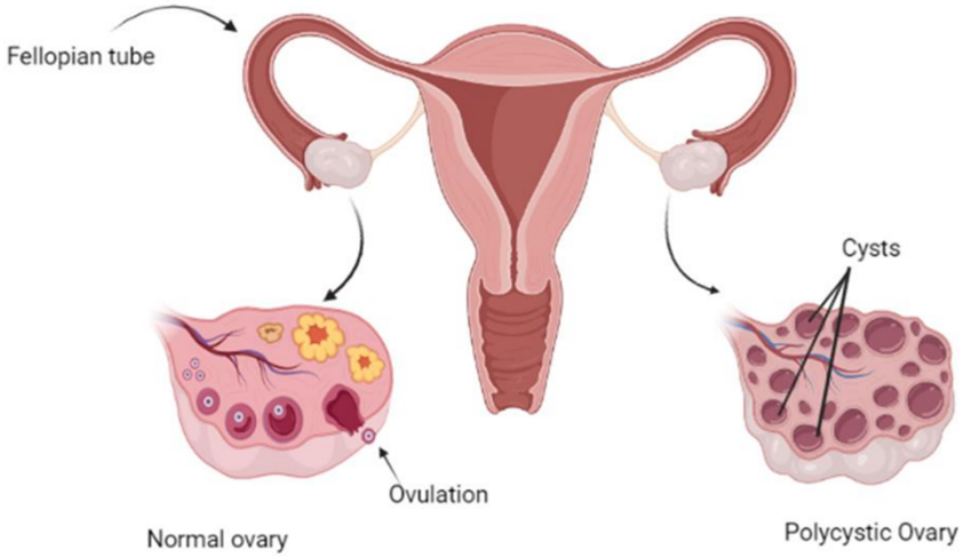


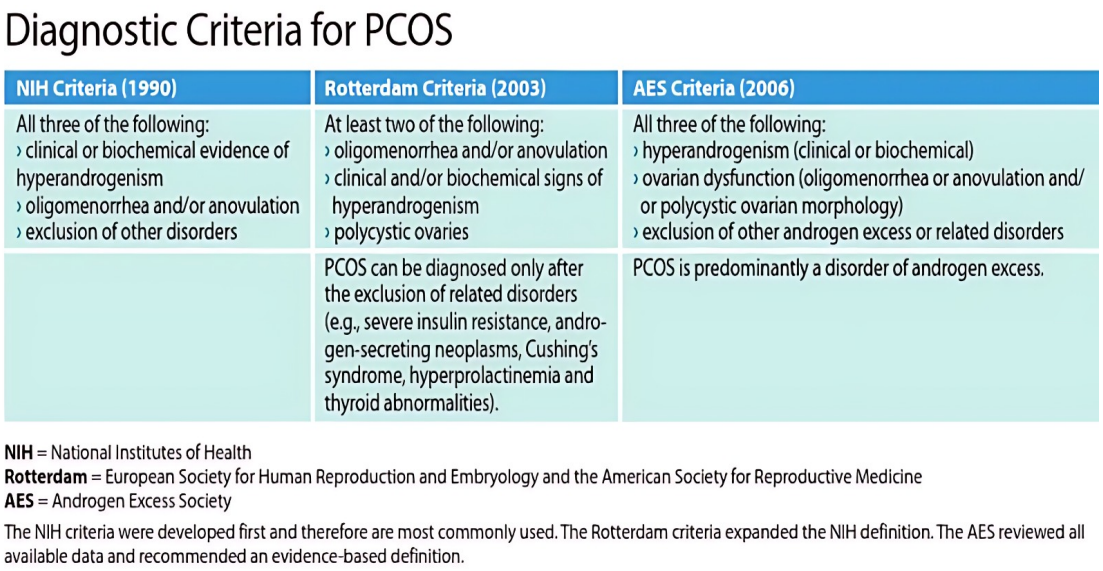
FIGURE 2.1: Visual overview of PCOS. Comparison of a normal ovary and a polycystic ovary: The normal ovary shows follicle development and ovulation, while the polycystic ovary contains multiple cysts and lacks regular ovulation, a hallmark of Polycystic Ovary Syndrome (PCOS) [15].

Polycystic ovary syndrome also has major neuropsychiatric consequences. People suffering from affect have higher incidence of eating problems, bipolar disorder, major depression, generalized anxiety disorder, and bipolar disorder [18]. PCOS has also been linked to lower sex satisfaction and psychosocial deficits, hence further decreasing quality of life (QoL) [19].

2.1.1 Prevalence and Diagnostic Criteria

The widespread of PCOS around the world is not constant (mainly) because of diverse genetic differences, various ecological (e.g. viruses, allergens, toxins, pollutants) influences, divergent types of living, and different ways of identifying the disease in the research. The multiple classification systems of PCOS diagnosis make the prevalence rates high in number, but they still have different diagnostic criteria applied [20]. The first formal diagnostic criteria for Polycystic Ovary Syndrome (PCOS) were established in 1990 by the National Institute of Child Health and Human Development (NICHD) [21]. According to these criteria, a diagnosis of PCOS required the presence of both clinical or biochemical signs of hyperandrogenism and chronic anovulation, while also ruling out other endocrine

disorders such as hyperprolactinemia, congenital adrenal hyperplasia, and Cushing's syndrome [22].



Diagnostic Criteria for PCOS

NIH Criteria (1990)	Rotterdam Criteria (2003)	AES Criteria (2006)
All three of the following: › clinical or biochemical evidence of hyperandrogenism › oligomenorrhea and/or anovulation › exclusion of other disorders	At least two of the following: › oligomenorrhea and/or anovulation › clinical and/or biochemical signs of hyperandrogenism › polycystic ovaries	All three of the following: › hyperandrogenism (clinical or biochemical) › ovarian dysfunction (oligomenorrhea or anovulation and/or polycystic ovarian morphology) › exclusion of other androgen excess or related disorders
	PCOS can be diagnosed only after the exclusion of related disorders (e.g., severe insulin resistance, androgen-secreting neoplasms, Cushing's syndrome, hyperprolactinemia and thyroid abnormalities).	PCOS is predominantly a disorder of androgen excess.

NIH = National Institutes of Health
Rotterdam = European Society for Human Reproduction and Embryology and the American Society for Reproductive Medicine
AES = Androgen Excess Society

The NIH criteria were developed first and therefore are most commonly used. The Rotterdam criteria expanded the NIH definition. The AES reviewed all available data and recommended an evidence-based definition.

FIGURE 2.2: Evolution of diagnostic criteria for Polycystic Ovary Syndrome (PCOS): Timeline showing the NIH criteria (1990), Rotterdam criteria (2003), and Androgen Excess and PCOS Society criteria (2006), highlighting differences in diagnostic emphasis on hyperandrogenism, ovulatory dysfunction, and polycystic ovaries [20–22]

In 2003, the Rotterdam Consensus updated the diagnostic criteria for polycystic ovary syndrome (PCOS), indicating that a diagnosis could be made when at least two of the following three features are present: (i) irregular or absent ovulation, (ii) clinical or biochemical evidence of elevated androgens, and (iii) polycystic ovaries identified via ultrasound. This broader definition led to a notable increase in the number of women diagnosed with PCOS globally. Later, in 2006, the Androgen Excess and Polycystic Ovary Syndrome Society (AE-PCOS) refined these criteria further, emphasizing hyperandrogenism as a central component alongside ovarian dysfunction, while also ensuring exclusion of other causes of androgen excess [23].

The Rotterdam criteria were the foundation stones of the epidemiological studies, which seems to be quite interesting. Based on them, the prevalence rates have been reported. According to an interview including Sir Lankan women 6.3% had the disease and it was conducted with 3,030 women from the 15 to 39 age group. Further, the infection ratio was only 2.2% in the Southern Chinese women and altogether, the Thai women were 5.7% suffering from PCOS [24]. PCOS in Pakistan

has generally been reported to be at a figure of 52% up to 55% which is considered a high disease burden in this area [25]. A study in the UK's Oxford city on the 8% to 26% range of estimates depending on which diagnostic criteria were used was somehow also very open to interpretation [26]. For example, studies in the United States showed PCOS cases occurred in 8% of Black women and 4.8% of Caucasian women. As the Australian group studied the prevalence for a birth cohort, the rates were of 8.7% when counting conducted with NICHD criteria and 11.9% according to the Rotterdam criteria, and still 69% of those with PCOS went undiagnosed [27, 28].

2.1.2 Pathophysiology

The pathophysiology of Polycystic Ovary Syndrome (PCOS) involves complex disruptions across multiple physiological systems. These include abnormalities in steroid hormone biosynthesis, neuroendocrine signaling, ovarian follicle development, metabolic regulation, insulin responsiveness and secretion, adipocyte function, autonomic nervous activity, and inflammatory mediators [29].

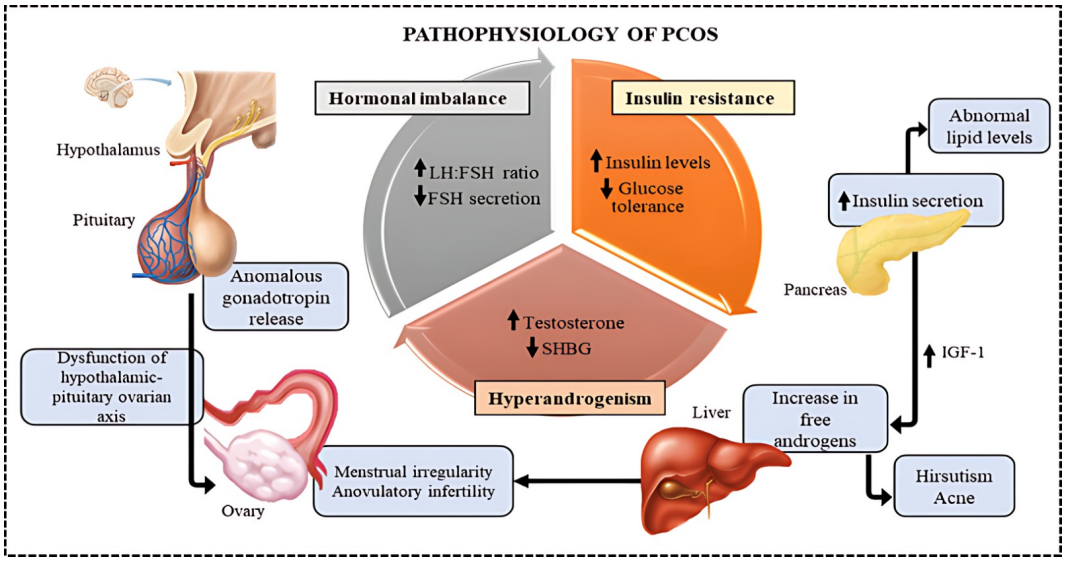


FIGURE 2.3: Polycystic Ovary Syndrome (PCOS) is a multifactorial endocrine disorder marked by hormonal disruptions that lead to irregular menstrual cycles, elevated androgen levels, and the formation of ovarian cysts. Insulin resistance is a central factor, resulting in increased insulin (hyperinsulinemia), which stimulates the ovaries to produce more androgens. This hormonal imbalance disrupts normal ovarian function, causing anovulation and contributing to the development of polycystic ovaries [29].

Key contributors to these physiological disruptions include high carbohydrate intake, elevated insulin and androgen levels, and chronic low-grade inflammation (Figure 2.3) [29].

2.1.2.1 Androgen Overload

Hyperandrogenemia is the hallmark biochemical abnormality in PCOS, manifesting clinically as hirsutism, acne, and hair thinning. Approximately 75–90% of women with oligomenorrhea and PCOS show elevated androgen levels, with severity increasing proportionally. Both the ovaries and adrenal glands contribute to this androgen excess [30].

Free testosterone is often elevated and serves as a biochemical marker for hyperandrogenism. This hormonal imbalance interferes with normal follicle development. At the initial stages of gonadotropin activity, excess androgens encourage the growth of primordial follicles, leading to an increase in the number of antral follicles [31].

The hypothalamus regulates the release of gonadotropins by producing gonadotropin-releasing hormone (GnRH), which signals the anterior pituitary to secrete luteinizing hormone (LH) and follicle-stimulating hormone (FSH). LH targets receptors on ovarian theca cells, prompting them to generate androgens. In contrast, FSH binds to receptors on granulosa cells, enabling the transformation of androgens into estrogens and fostering the growth of ovarian follicles. In polycystic ovary syndrome (PCOS), the normal function of the hypothalamic-pituitary-ovarian (HPO) axis is disrupted, resulting in an imbalance between LH and FSH secretion. This typically manifests as an increased LH to FSH ratio, which is a distinguishing feature of PCOS [32].

The overproduction of LH drives theca cell hyperplasia and enhances the expression of steroidogenic enzymes, contributing to excessive androgen synthesis. These androgens exert their effects by binding to the androgen receptor (AR), a nuclear transcription factor that is expressed in multiple ovarian cell types, including granulosa and theca cells. Over activation of AR signaling in response to elevated

androgen levels disrupts normal folliculogenesis, leading to the arrest of follicular development, particularly at the preantral and antral stages [33].

This impaired maturation process results in the accumulation of immature follicles and the development of multiple cystic structures along the ovarian cortex. Furthermore, increased AR activity perpetuates the expression of genes involved in androgen biosynthesis, amplifying the hyperandrogenic environment characteristic of PCOS [34].

Another potential mechanism involves impaired cortisol metabolism. Reduced activity of 5α-reductase (which synthesizes cortisol) and 11β-hydroxysteroid dehydrogenase type 1 (which reactivates cortisol from cortisone) results in increased peripheral metabolism of cortisol. This suppresses ACTH via negative feedback, allowing testosterone levels to remain elevated while plasma cortisol remains within normal limits [34].

Genetic factors also influence androgen biosynthesis in PCOS, particularly genes in the CYP family, which play vital roles in steroidogenesis and are implicated in the development of hyperandrogenism [35].

2.1.2.2 Excessive Insulin

Insulin is critical for managing glucose levels and lipogenesis, and it also acts as a growth-promoting hormone influencing the metabolism of proteins, fats, and carbohydrates. Insulin receptors are widely expressed across tissues of the HPO axis, where insulin enhances the effects of gonadotropins in steroid-producing organs such as the ovaries and adrenal glands [36].

Hyperinsulinemia promotes androgen excess, partly because insulin mimics LH's action and indirectly enhances GnRH release. Elevated insulin also reduces sex hormone-binding globulin (SHBG) levels, thereby increasing the proportion of unbound, biologically active androgens. This exacerbates symptoms like acne,

excessive hair growth, and scalp hair thinning [37]. Research suggests that improving insulin sensitivity can lead to reduced androgen levels and symptom relief in PCOS [38–40].

2.1.2.3 Oxidative Stress

Oxidative stress (OS) occurs when there is an imbalance between reactive oxygen species (ROS), also known as oxidants, and the body's antioxidant defenses. Free radicals are highly reactive molecules that attempt to stabilize themselves by capturing electrons, often from antioxidants. When antioxidant levels are inadequate, these free radicals can cause damage to cells and tissues. The assessment of oxidative stress involves measuring biomarkers such as the oxidative stress index (OSI), total oxidant status (TOS), and total antioxidant status (TAS), which together provide an indication of the overall balance between oxidants and antioxidants in the body [41].

A substantial increase in oxidative stress markers has been documented in PCOS patients, especially those with concurrent conditions such as insulin resistance, obesity, cardiovascular diseases, and certain cancers [42]. Oxidative stress affects various cellular organelles and molecular pathways. For example, mitochondrial dysfunction-a known factor in PCOS-is linked to increase ROS, reduced mitochondrial respiration, and decreased antioxidant glutathione levels [43].

PCOS is frequently associated with a disrupted redox balance, contributing to metabolic conditions like obesity, hyperinsulinemia, and lipid abnormalities [44]. Obesity, affecting 40–50% of PCOS patients, enhances lipid breakdown, which in turn generates more free radicals and oxidative stress [45]. Elevated glucose levels (hyperglycemia) can increase ROS release from immune cells. These ROS regulate key cellular processes such as proliferation, differentiation, and apoptosis.

However, excessive ROS can activate inflammatory responses, such as increased TNF-α production and stimulation of NF-κB, a transcription factor involved in inflammation [46]. Inflammation can, in turn, drive androgen production by the ovaries, forming a direct link between chronic inflammation and hyperandrogenism

in PCOS [47]. TNF-α also contributes to insulin resistance, impairs follicular quality, and promotes cyst formation in ovarian tissue [48].

To study the effects of oxidative, glycation, and nitrosative stress in PCOS, researchers evaluate both oxidant and antioxidant levels. These biomarkers offer valuable insights into disease mechanisms and therapeutic responses [49]. Such markers are measurable in blood serum and follicular fluid. Their relevance lies not only in understanding disease development but also in guiding treatment strategies for oxidative stress–related disorders [50].

2.1.3 Toxic Environmental Factors

A growing body of research underscores the significant role that environmental toxins play in affecting human health and reproductive outcomes [51, 52]. Endocrine-disrupting chemicals (EDCs)-such as components of tobacco, heavy metals like lead and mercury, pesticides, and industrial pollutants-are capable of interfering with the hormone-regulated systems of the body [53]. These chemicals exert their effects through diverse mechanisms: binding to membrane-bound and nuclear estrogen receptors, interacting with estrogen-related receptors, altering hormone metabolism, and disturbing regulatory feedback loops. EDCs may also impact both genomic and non-genomic pathways, influence neuroendocrine cell function, and cause epigenetic modifications such as DNA methylation or histone changes [54].

Experimental studies suggest that exposure to industrial EDCs can worsen reproductive and metabolic health, potentially intensifying conditions similar to PCOS. In genetically predisposed individuals, these chemicals might initiate PCOS-like manifestations or further disrupt hormonal homeostasis and fertility [55]. Prenatal exposure to such chemicals can disturb hormone signaling, possibly increasing the risk of developing PCOS later in life. For instance, elevated levels of bisphenol-A (BPA) have been observed in PCOS patients, with a positive association between BPA levels and androgens, implying a potential causal role [56]. Adolescent girls

with PCOS have shown higher BPA concentrations compared to their peers, independent of body weight, further strengthening the link between BPA and androgen levels [57]. Exposure to hormone-mimicking chemicals during fetal development may alter the programming of hormone-sensitive tissues, thereby increasing PCOS risk and possibly contributing to transgenerational effects [58].

Although some EDCs-such as phthalic acid esters, BPA, and octylphenol-may not visibly affect PCOS symptoms or insulin resistance, octylphenol in particular has been linked to insulin resistance among PCOS patients [59]. Recently, attention has turned to the association between PCOS and anogenital distance (AGD), a marker of fetal androgen exposure. Research shows that women with PCOS tend to have a greater AGD compared to controls, suggesting intrauterine androgen exposure as a potential factor in PCOS development [58]. Animal studies support this, showing that prenatal testosterone exposure can result in PCOS-like traits. In addition, daughters of women with PCOS have been found to have longer AGD, indicating increased exposure to testosterone in utero [60].

2.1.4 Geo-Epidemiology

The geographical distribution of diseases such as PCOS - referred to as geo - epidemiology - offers crucial insights into how genetic and environmental elements interact [61]. Geographic factors like climate, elevation, terrain, proximity to water bodies, and local flora and fauna may influence PCOS development. These environmental variables can directly impact lifestyle-related risk factors, including water quality, food availability, and nutritional supplementation. Access to these resources is often shaped by local diets, economic conditions, and public health policies. Consequently, geographical location may indirectly affect PCOS risk by influencing body weight and metabolism, thereby affecting the onset and manifestation of PCOS symptoms [62].

Cultural and behavioral norms that differ between regions also contribute to disparities in PCOS prevalence and outcomes. Dietary habits, for instance, can vary

widely and significantly affect obesity rates-a key risk factor for PCOS [63]. Additionally, societal attitudes toward women's health, particularly regarding infertility, may delay diagnosis and treatment in certain cultures. Psychological issues, such as anxiety and depression, are frequently associated with PCOS and may be compounded by social stigma [64, 65].

2.1.5 Psychological Effects in PCOS

A significant proportion of women diagnosed with PCOS experience psychological issues, with studies showing depression rates between 28% and 64% [66, 67] and anxiety affecting 34% to 57% of this population [68]. Additionally, increased risks of social phobia and suicidal tendencies have been reported in PCOS patients [69]. The causes behind these psychological concurrent disorder remain complex. Some theories suggest that the physical symptoms of PCOS contribute to mental health challenges [70, 71], though findings are not always consistent. While some studies link depression and anxiety to acne [72], hirsutism, and higher BMI [73], other research fails to establish these connections.

2.1.5.1 Anxiety and Depression

Women diagnosed with PCOS are reported to have up to a threefold increased risk of experiencing depressive symptoms and are five times more likely to suffer from anxiety than those without the condition [74]. These associations remain significant even when controlling for factors such as hirsutism and BMI [75]. Research suggests that nearly half of PCOS patients exhibit signs of depression [76]. However, it remains unclear whether psychological symptoms are a direct consequence of the disorder itself or arise due to social stigmas linked to PCOS symptoms like infertility and obesity [77]. Moreover, maternal PCOS may increase the risk of psychiatric disorders in children. A 2020 study found that children of mothers with PCOS were more likely to suffer from anxiety, mood and sleep disorders, developmental delays, and autism spectrum disorders-especially among offspring of obese mothers-indicating a possible interaction between PCOS and maternal obesity [78].

2.1.5.2 Eating Disorders

Body dissatisfaction and obesity are recognized as key risk factors for eating disorders (ED) [79], and evidence points to a higher prevalence of ED in women with PCOS [80, 81]. An Australian longitudinal study, which evaluated self-esteem, psychological distress, and eating behavior in PCOS patients, found a significant increase in diagnosed or treated eating disorders. This association persisted even after adjusting for BMI and other confounders [82].

2.1.6 Gut Microbiota Dysbiosis

Although multiple factors-such as genetic predisposition, lifestyle, metabolism, and environment-are implicated in PCOS, its exact etiology remains elusive [83]. In 2012, researchers proposed a potential role of gut microbiota in PCOS development [84]. Since then, studies have found significant shifts in gut microbial composition among PCOS patients, which are often associated with clinical and metabolic aspects of the syndrome (Figure 2.4) [85, 86]. However, the precise mechanisms underlying the gut-PCOS relationship remain poorly defined.

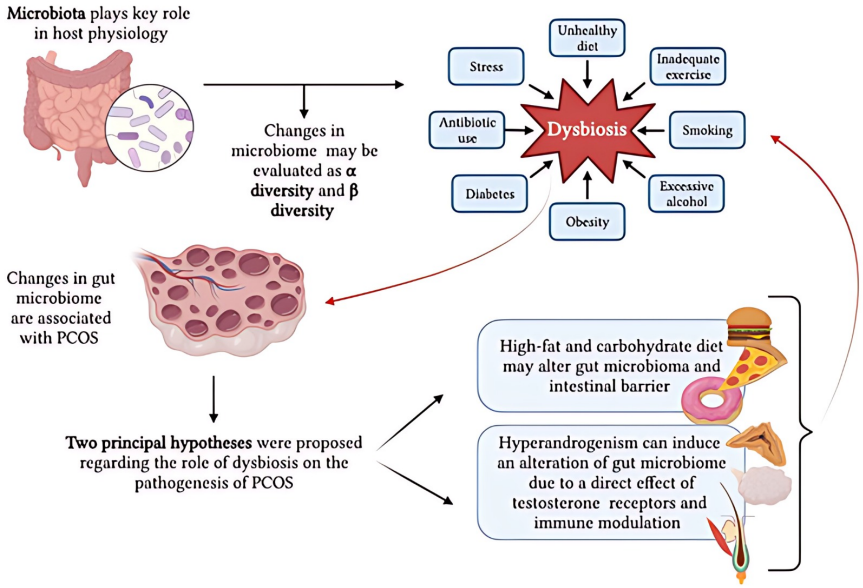


FIGURE 2.4: Role of gut microbiota symbiosis in PCOS: The figure illustrates how factors such as diet, obesity, stress, and lifestyle contribute to dysbiosis, which alters gut microbiome diversity. These changes are linked to PCOS pathogenesis through mechanisms involving high-fat/carbohydrate diets and hyperandrogenism-driven modulation of gut microbiota [87].

Research by Kelley *et al.* demonstrated distinct phylogenetic differences in the gut microbiota of mice treated with letrozole (a PCOS model) compared to controls. The treatment was linked to increased presence of certain bacteria within the Firmicutes and Bacteroidetes phyla, consistent with previous mouse model studies. Additionally, letrozole-treated mice exhibited weight gain, indicating a possible connection between microbiome alterations and increased adiposity [88]. Similar findings were reported in rats, where letrozole administration resulted in increased Prevotella and decreased levels of Ruminococcus, Lactobacillus, and Clostridium [89].

Prevotella and decreased levels of Ruminococcus, Lactobacillus, and Clostridium [89]. A pilot study examining stool samples from 24 women with PCOS and 16 healthy controls identified reduced levels of bacteria from the S24-7 Bacteroidetes and ML615J-28 Tenericutes phyla in PCOS patients [89]. Liu *et al.* observed that the dysbiosis in PCOS women closely resembled that of obese individuals, with more pronounced microbial imbalance in obese PCOS patients. They observed reductions in beneficial bacteria such as *Ruminococcaceae* and *Akkermensia*, alongside an increase in gram-negative bacteria like Escherichia/Shigella and Bacteroides [83].

Additionally, Torres *et al.* noted higher levels of *Bacteroides coprophilus*, *Porphyromonas spp.*, *Faecalibacterium prausnitzii*, and *Blautia spp.* in PCOS patients, while *Roseburia spp.*, *Anaerococcus spp.*, *Odoribacter spp.*, and *Ruminococcus bromii* were lower [90]. In another recent investigation comparing gut microbiota in 38 PCOS patients and 30 healthy women, PCOS patients exhibited reduced levels of Bifidobacterium, Faecalibacterium, and Blautia, but increased abundance of Clostridium and Parabacteroides [91].

2.1.7 Treatment Approaches

PCOS management needs different specialists like gynecologists, endocrinologists, and primary care physicians. Therapeutic interventions should be given based on

the preferences and health issues of the patient such as fertility preservation, menstrual regulation, hyperandrogenic symptom control, and metabolic risk reduction [92]. A detailed metabolic evaluation should be conducted regarding lipids, glucose, and blood pressure status and is a must. Moreover, the assessment of comorbid conditions, such as depression, anxiety, and obstructive sleep apnea is essential, as they are the most frequent among PCOS patients [93].

2.1.7.1 Ovulatory Dysfunction and Infertility

The Lifestyle changes, notably the loss of weight through calorie restriction and scheduled physical exercise, are primary therapies for upgrading both insulin sensitivity and ovulation [94]. Even a 5-10% reduction in body weight has been shown to restore ovulatory cycles and enhance fertility outcomes. Pharmacological ovulation induction is recommended for women who fail to conceive through lifestyle interventions alone. Letrozole, an aromatase inhibitor, has demonstrated superior efficacy over clomiphene citrate in terms of ovulation rates and live birth outcomes. The role of metformin in fertility enhancement remains controversial; while it has been widely used as an insulin-sensitizing agent, meta-analyses indicate that it does not significantly improve live birth rates when compared to standard ovulation induction therapies [95].

2.1.7.2 Menstrual Irregularities

According to the Endocrine Society, hormonal contraceptives-such as birth control pills, skin patches, or vaginal rings-are recommended as the primary treatment for managing irregular periods and symptoms of androgen excess, like acne and hirsutism, in individuals who are not seeking pregnancy [96]. Limited research has indicated that metformin has the potential to reinstate regular menstrual cycles in around 50% to 70% of women diagnosed with PCOS. However, it has been demonstrated that oral contraceptives are more effective than metformin in terms of controlling menstrual cycles and reducing testosterone levels. There is a lack of empirical evidence indicating the superiority of any specific oral contraceptive

in the treatment of PCOS [97]. To prevent endometrial hyperplasia caused by extended periods of anovulation, treatment options include progesterone-based therapies, combined oral contraceptives with progestin, or the use of a levonorgestrel-releasing intrauterine device such as Mirena. When managing irregular menstrual cycles, it is important to consider the patient's comfort and personal preferences in selecting the most appropriate treatment [98].

2.1.7.3 Hyperandrogenic Symptoms (Hirsutism and Acne)

Hirsutism, a distressing dermatological manifestation of PCOS, necessitates prolonged treatment for clinical improvement. First-line therapy consists of COCs (Combined Oral Contraceptives.), which reduce ovarian androgen secretion. In cases of moderate to severe hirsutism, anti-androgen medications like spironolactone (at doses of 100–200 mg per day), finasteride, and flutamide can serve as effective supplementary treatments. Spironolactone, in particular, has been shown to significantly reduce terminal hair growth. Topical eflornithine can be used for facial hirsutism, while permanent hair reduction can be achieved through laser therapy or electrolysis [30].

Acne associated with PCOS is primarily managed with COCs, which reduce sebaceous gland activity. In cases of moderate to severe acne, combination therapy with topical agents (benzoyl peroxide, retinoids, antibiotics) is recommended. Spironolactone is also effective in treatment-resistant androgenic acne due to its anti-androgenic properties [33].

2.1.7.4 Essential Calcium and Vitamin D

Vitamin D plays a vital role in reproductive health, particularly in the maturation of ovarian follicles, luteinization, and overall reproductive function. It influences these processes by regulating Anti-Müllerian Hormone (AMH) signaling, promoting progesterone production in granulosa cells, and increasing the sensitivity of follicles to follicle-stimulating hormone (FSH). Additionally, vitamin D is

involved in glucose metabolism through its interaction with the vitamin D receptor (VDR), which is found in skeletal muscle and pancreatic β-cells. The enzyme 1α-hydroxylase converts 25-hydroxyvitamin D [25(OH)-D] into its active form, 1,25-dihydroxyvitamin D. The presence of a vitamin D response element within the insulin gene promoter further suggests that vitamin D may play an important role in maintaining glucose balance [99].

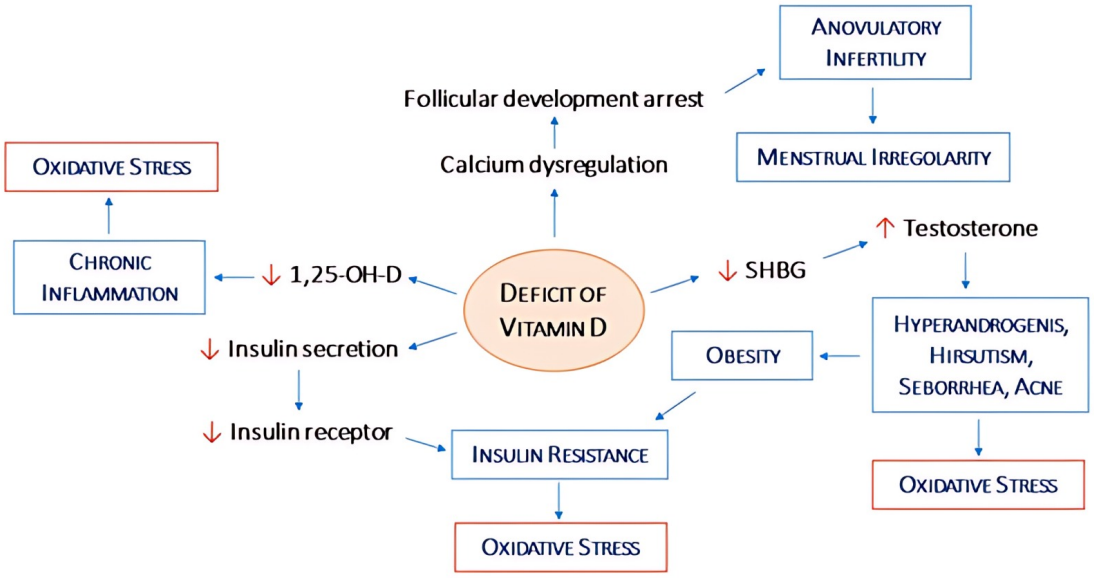


Figure 2.5: The association between between vitamin D deficiency and the onset of insulin resistance, metabolic syndrome, hormonal disturbances, and infertility in PCOS (1,25-OH-D: 1,25-dihydroxyvitamin D; SHBG: Sex Hormone-Binding Globulin) [103].

Low levels of 1,25(OH)D have been implicated in exacerbating several symptoms of PCOS, including ovulatory and menstrual irregularities, insulin resistance, obesity, hyperandrogenism, and infertility, while also increasing cardiovascular disease risk. In PCOS patients with hypovitaminosis D, supplementation can attenuate elevated AMH (Anti-Müllerian Hormone) concentrations and improve inflammatory profiles. Furthermore, the administration of combined vitamin D and calcium supplementation alongside metformin has demonstrated significant improvements in ovulation, menstrual cyclicity, folliculogenesis, and androgenic symptoms [100, 101]. Given that AMH levels are typically elevated in PCOS due to impaired follicular development, Vitamin D therapy offers potential in modulating these markers [102].

TABLE 2.1: Effects of Vitamin D and Calcium Supplementation in PCOS

Parameter Affected	**Observed Effect**	**References**
AMH Levels	Decreased	[100]
Ovulation & Menstrual Cycle	Improved	[100]
Follicular Development	Enhanced	[100]
Insulin Sensitivity	Increased	[100]
Serum Testosterone	Decreased	[100]
Lipid Profile (LDL, VLDL)	Decreased	[100]

Meta-analyses have underscored that calcium and vitamin D supplementation in conjunction with metformin significantly enhances follicular maturation, menstrual regularity, and insulin responsiveness.

Additionally, such interventions are associated with reductions in hirsutism, serum testosterone, thyroglobulin (Tg), total cholesterol, very low-density lipoprotein cholesterol (VLDL-C), and low-density lipoprotein (LDL) levels in women with PCOS [104].

2.1.8 Functional Dynamics of Clomiphene Citrate in the Body

Clomiphene citrate facilitates ovulation by inducing an increase in the frequency of luteinizing hormone (LH) pulses, typically observed within two to three days following administration during the follicular phase. This phenomenon suggests a stimulatory effect on the hypothalamic secretion of gonadotropin-releasing hormone (GnRH) as shown in figure 2.6 [105].

Additionally, clomiphene may augment the sensitivity of pituitary gonadotrophs to GnRH via its partial estrogen agonist properties, leading to elevated levels of gonadotropins and recruitment of multiple follicles. Consequently, this results in heightened estradiol levels in the pre-ovulatory phase and increased progesterone during the luteal phase. Notably, clomiphene-induced ovulatory cycles often feature larger follicular diameters compared to spontaneous cycles [105].

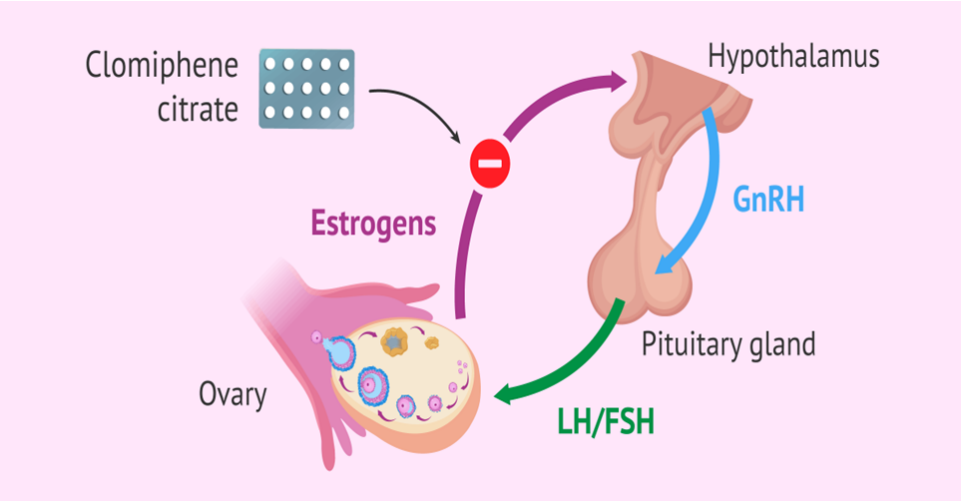


FIGURE 2.6: Mechanism of action of clomiphene citrate: how clomiphene citrate blocks estrogen's negative feedback on the hypothalamus and pituitary, leading to increased GnRH secretion, stimulation of LH and FSH release, enhanced follicular development, and induction of ovulation [105].

Clomiphene also acts directly on the ovary, sensitizing granulosa cells to gonadotropins and enhancing aromatase activity. However, it exerts anti-estrogenic effects on the endometrial lining and cervical mucus, which may be attributed to differential distribution of estrogen receptor subtypes (α and β) across reproductive tissues [106].

2.1.9 Adverse Effects of Clomiphene Citrate

The use of clomiphene citrate is associated with several side effects, most notably the risk of multiple gestations. Twin pregnancies occur in approximately 6–8% of cases, while triplets, quadruplets, and quintuplets are less common but still reported in 1%, 0.3%, and 0.1% of cases, respectively. Additionally, there is a 23% rate of missed abortions, potentially linked to premature oocyte meiosis driven by excessive LH secretion. Mild ovarian hyperstimulation syndrome (OHSS) has been observed in 13% of patients [107].

Minor adverse effects include visual disturbances, hot flashes, nausea, and breast discomfort. Some studies have raised concerns regarding a potential association between prolonged use of ovulation-inducing agents like clomiphene and an elevated risk of ovarian malignancies [107].

2.1.10 Dietary Supplements in PCOS Management

Myo-inositol and D-chiro-inositol are naturally occurring compounds found in foods like fruits and legumes that have shown benefits in managing PCOS, particularly in obese individuals, due to their ability to enhance insulin sensitivity. In women with PCOS, myo-inositol promotes glucose uptake in the ovaries and improves follicle-stimulating hormone (FSH) signaling, while D-chiro-inositol helps reduce androgen production driven by insulin [108, 109]. Research indicates that a 40:1 ratio of myo-inositol to D-chiro-inositol is the most effective formulation for improving ovulation and embryo quality in PCOS patients [110].

Supplementation with 2–4 g/day of myo-inositol effectively reduces hyperandrogenism and dyslipidemia with minimal side effects. Similarly, vitamin D3 at weekly doses of 20,000–50,000 IU improves menstrual regularity, blood glucose profiles, and hirsutism in 30–50% of PCOS women [111, 112]. Omega-3 fatty acids (1,200 mg/day) significantly reduce fasting insulin and glucose levels, though weight loss is not observed [113]. Selenium (200 μg/day) and zinc sulfate (220 mg/day) also contribute to lowering insulin and triglyceride levels [114, 115].

2.1.11 Probiotics, Prebiotics, and Synbiotics in PCOS

Probiotic supplementation has demonstrated notable potential in enhancing metabolic health in individuals with PCOS. For example, Ahmadi *et al.* found that a 12-week course of probiotics containing Lactobacillus acidophilus, *Bifidobacterium bifidum*, and *Lactobacillus casei* led to reductions in fasting blood glucose, insulin levels, triglycerides, and very-low-density lipoprotein cholesterol (VLDL-C). Comparable metabolic improvements have also been observed with the use of multi-strain probiotic formulations, including strains such as *Lactobacillus rhamnosus*, *Bifidobacterium breve*, and *Streptococcus thermophiles* [116–118].

Hormonal modulation has also been observed. A study by Karamalli *et al.* noted that 12-week probiotic supplementation increased SHBG and antioxidant levels

while reducing testosterone in PCOS women [119]. Fecal microbiota transplantation also demonstrated reduced androgen levels and restored menstrual regularity [85].

Prebiotic administration (resistant dextrin, 20 g/day) led to decreased LDL, triglycerides, hsCRP, and increased HDL [120]. Synbiotic beverages containing inulin and Lactobacillus strains have been shown to reduce testosterone, BMI, and waist circumference while improving insulin sensitivity [121–123].

2.1.12 Impact of Physical Activity on Reproductive Function in PCOS

Numerous studies highlight the role of physical activity in improving menstrual frequency and ovulation in PCOS patients [124–126]. Exercise contributes to cycle regulation and transition from anovulatory to ovulatory cycles, demonstrating greater benefits than calorie restriction alone [127].

Lifestyle interventions, particularly for overweight and obese individuals, enhance conception rates and reduce treatment-related complications [128]. While the type of exercise may not be critical, resistance training shows limited reproductive benefits despite improvements in metabolic outcomes [129, 130].

Losing just 5% of body weight can lead to notable improvements in ovulation, lower metabolic risk factors, and enhance the likelihood of conception. Achieving an optimal BMI also minimizes pregnancy-related complications, including hypertension, gestational diabetes, and preterm birth [131, 132].

Targeted weight loss through sustained exercise is particularly beneficial for women undergoing fertility treatments, as it improves drug responsiveness and reduces required dosages [133]. Longer interventions are more effective in promoting sustained weight loss and reproductive improvements.

2.1.13 Estrogen

Estrogen is a vital steroid hormone predominantly produced in the ovaries, central to regulating the menstrual cycle and facilitating female reproductive function (Figure 2.5). Estrogen influences multiple body systems beyond its reproductive role, affecting the skeletal, neuroendocrine, and immune systems in both men and women. Imbalances or changes in estrogen levels have been linked to a range of health conditions, including metabolic disorders, obesity, various cancers, osteoporosis, uterine fibroids, and endometriosis [134, 135].

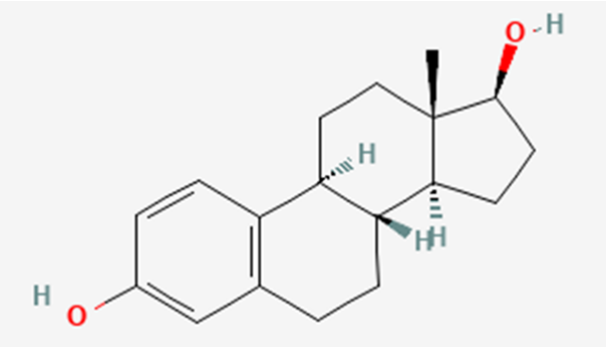


FIGURE 2.7: Chemical structure of Estrogen [136].

Estrogen carries out its biological functions by interacting with two different nuclear receptors, estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ), which are found in a variety of target tissues [137]. These receptors are produced from distinct genes located on separate chromosomes and display unique patterns of expression depending on the tissue type. ERα is mainly found in the uterus, liver, mammary glands, pituitary gland, cervix, hypothalamus, and vagina, while ERβ is predominantly located in the lungs, ovaries, and prostate [138].

In individuals with PCOS, the functioning of estrogen and its receptors is often impaired. Estrogen operates through genomic pathways via ERα and ERβ and through non-genomic mechanisms, including those involving the G-protein-coupled estrogen receptor (G-PER). Disruptions in these signaling pathways can adversely affect cellular functions like ovulation, cell cycle progression, proliferation, migration, and invassion. Selective estrogen receptor modulators (SERMs) like tamoxifen and clomiphene are known to be clinically effective in addressing

subfertility associated with Polycystic Ovary Syndrome (PCOS). However, the full extent of estrogen signaling's involvement in PCOS is still an active area of research. [139].

2.1.14 Progesterone

Progesterone (Figure 2.6) plays a key role in reproductive physiology, including ovulation, luteal phase support, and implantation, in addition to functions within the immune and central nervous systems. First identified in the 1920s through its role in promoting embryo implantation and corpus luteum activity, progesterone has since been recognized ss its broader implications in human health [140, 141].

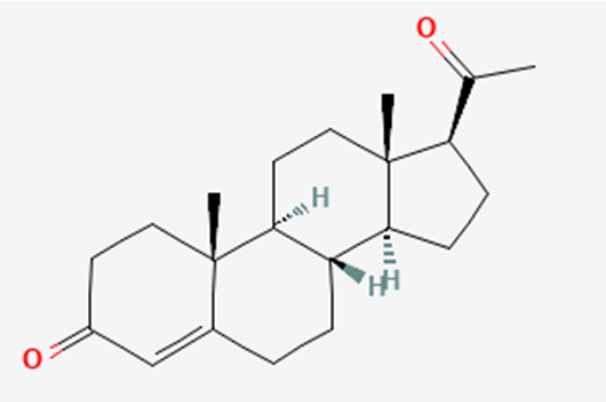


FIGURE 2.8: Chemical structure of Progesterone [136].

Women with PCOS often present with insufficient progesterone levels during the early luteal phase. Alterations in the synthesis of progesterone by granulosa cells in polycystic ovaries may contribute to the hormonal imbalances characteristic of the syndrome. A lack of cyclical progesterone exposure may underlie abnormal gonadotropin and androgen profiles, further linking progesterone deficiency to disrupted hypothalamic-pituitary-ovarian axis regulation [142].

2.1.15 Testosterone

Testosterone (Figure 2.9), while predominantly considered a male sex hormone due to its role in male sexual differentiation and fertility, also serves important

functions in the female body [143, 144]. In embryonic development, testosterone is critical for male differentiation beginning around the seventh week of gestation, triggering the formation of testicular structures and suppression of Müllerian duct development through anti-Müllerian hormone secreted by Sertoli cells [144].

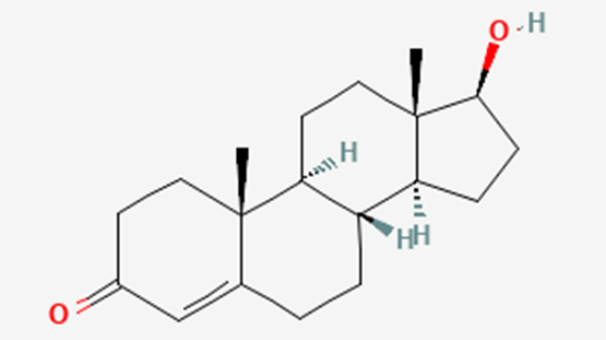


FIGURE 2.9: Chemical structure of Testosterone [136].

In women with PCOS, testosterone levels typically remain at or below 150 ng/dL ($\leq$5.2 nmol/L) [145]. However, increased levels of bioavailable testosterone, reflected by a high free androgen index, have been linked to a greater risk of developing hypertension-particularly in the presence of insulin resistance and obesity, which are common in PCOS. Research indicates that elevated androgen levels in PCOS may play a significant role in increasing cardiovascular and metabolic risk factors [146].

2.1.16 Role of AR, IR, and TNF-α in PCOS Pathophysiology

The pathogenesis of polycystic ovary syndrome (PCOS) involves complex interactions between hormonal, metabolic, and inflammatory pathways. Among the key molecular players implicated are the androgen receptor (AR), insulin receptor (IR), and tumor necrosis factor-alpha (TNF-α), each of which contributes significantly to the disruption of ovarian and systemic homeostasis [147–149].

The androgen receptor (AR) is a type of nuclear receptor responsible for carrying out the actions of androgens like testosterone and dihydrotestosterone (DHT).

In PCOS, hyperandrogenism-often due to increased ovarian and adrenal androgen production-is a hallmark feature. Overexpression or hypersensitivity of AR in ovarian theca and granulosa cells can disrupt follicular development, inhibit ovulation, and contribute to the persistence of immature follicles. Aberrant AR signaling also exacerbates metabolic dysfunction and contributes to insulin resistance [147].

The insulin receptor (IR), a transmembrane tyrosine kinase receptor, regulates glucose uptake and metabolic signaling. In PCOS, there is often a post-receptor defect in insulin signaling that leads to insulin resistance, independent of body weight. Hyperinsulinemia resulting from IR dysfunction enhances ovarian androgen synthesis by upregulating cytochrome P450c17α activity in theca cells and suppressing hepatic production of sex hormone-binding globulin (SHBG), thereby increasing free androgens. This contributes to both reproductive and metabolic abnormalities in PCOS [148].

Tumor necrosis factor-alpha (TNF-α), a major pro-inflammatory cytokine, plays a crucial role in immune system regulation and the development of systemic inflammation. Higher levels of TNF-α observed in individuals with PCOS contribute to chronic low-grade inflammation, insulin resistance, and heightened androgen levels. TNF-α contributes to insulin resistance by activating serine kinases, which interfere with insulin receptor substrate (IRS) function and disrupt normal insulin signaling. Furthermore, TNF-α may directly stimulate ovarian androgen production and disrupt folliculogenesis [148, 149].

Collectively, dysregulation in AR, IR, and TNF-α pathways underscores the multifactorial nature of PCOS, where endocrine, metabolic, and inflammatory components converge to impair ovarian function and systemic metabolic health.

2.1.17 Medicinal Plants

Plants have long been recognized as a source of therapeutic compounds used to treat various human diseases [150]. The interest in medicinal plants continues to grow due to their efficacy and fewer side effects compared to conventional

medicines. These plants are rich in bioactive constituents including alkaloids, flavonoids, and terpenes, many of which have been integrated into modern drug formulations [151–153]. Historically, plants served as essential resources for ancient civilizations in China, India, and Africa. Documentation of their use for medicinal purposes underscores the long-standing role of plant-based remedies in healthcare [154–156].

Medicinal plants such as *Ginkgo*, Ginseng, Garlic, Ginger, and others have demonstrated antioxidant, anti-inflammatory, anti-diabetic, antimicrobial, and hepatoprotective properties [157]. Rising interest in herbal treatments is partly due to concerns about the toxicity of synthetic drugs and the global demand for safer alternatives. However, regional differences in biodiversity and access to medicinal plants influence their availability and use [158].

Modern techniques, including high-throughput screening and bioassay-guided isolation, are now being used to identify and develop active compounds from plants for pharmaceutical applications. In low-resource settings, traditional plant-based medicine remains a cost-effective and accessible healthcare option [152].

Given the vast number of plant species yet to be pharmacologically studied, medicinal plants continue to represent a promising avenue for future therapeutic discoveries [159].

2.1.18 Phytoestrogens

Phytoestrogens are plant based compounds that mimic or modulate estrogenic activity in the body. These compounds are investigated for their potential in reducing the risk of hormone-related disorders, cardiovascular diseases, menopausal symptoms, and osteoporosis. Due to safety concerns surrounding conventional hormone therapies, many women opt for phytoestrogens as natural alternatives to hormone replacement therapy (HRT) and estrogen replacement therapy (ERT) [160].

2.1.18.1 Classification of Phytoestrogens

Phytoestrogens are mainly classified into three categories: isoflavones, coumestans, and lignans (Figure 2.8) [161–164]. Structurally, they are polyphenolic, containing one or more phenolic rings [165].

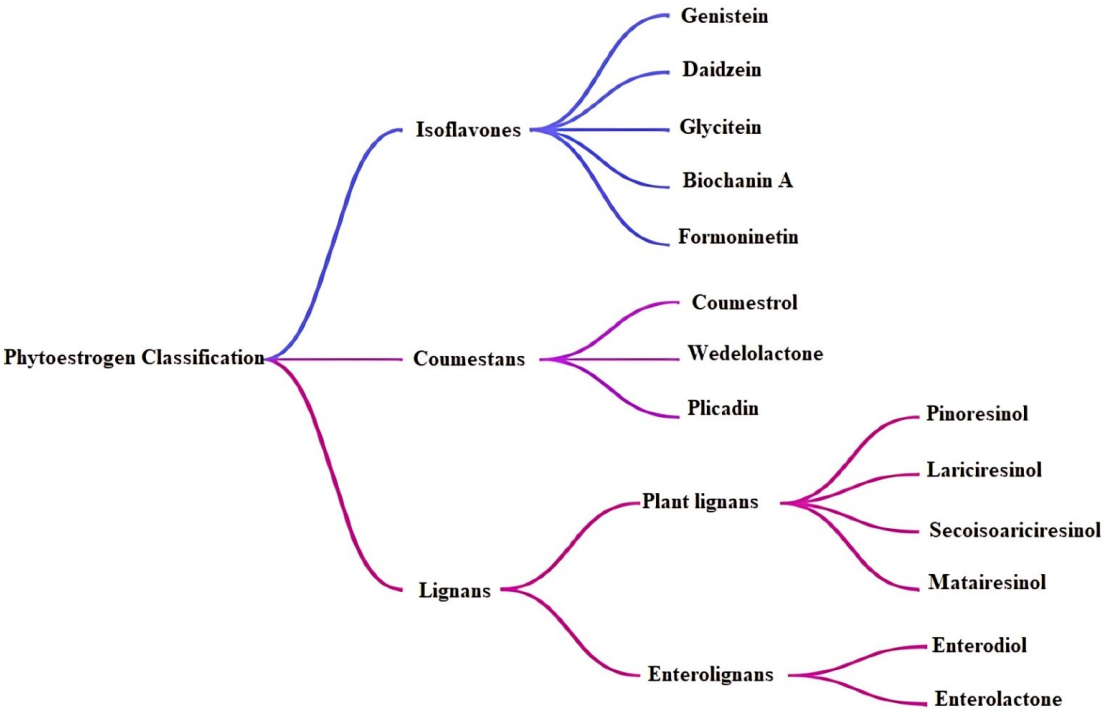


FIGURE 2.10: Major Classes of Phytoestrogens [161–164].

Isoflavones and lignans are the most common dietary phytoestrogens, with soy and other legumes serving as primary sources. Lesser amounts are also found in fruits, vegetables, and grains [166, 167].

2.1.18.2 Cellular and Molecular Actions of Phytoestrogens

Phytoestrogens interact with estrogen receptors through both genomic and non-genomic pathways. They typically exhibit weak estrogenic activity, having a lower binding affinity (1/100 to 1/10,000) than 17-β-estradiol. However, their high serum concentrations allow them to act as both agonists and antagonists [168, 169]. Their efficacy is influenced by circulating estrogen levels and the specific receptor subtype they bind-ERα or ERβ [170].

Genistein, a well-studied isoflavone, shows a significantly higher affinity for ERβ than ERα. Its metabolite, daidzein, has a lower binding affinity, but conversion to equol greatly enhances its estrogenic potential. This selective binding to ERβ is thought to explain the reduced risk of adverse effects often linked to estrogen therapy, such as cardiovascular events or cancer [171–173].

Phytoestrogens are also considered selective estrogen receptor modulators (SERMs) due to their receptor-specific actions [174, 175]. Beyond direct ER binding, they may activate secondary messengers via G-protein-coupled receptors and epidermal growth factor receptor (EGFR) pathways, affecting cellular functions independent of gene transcription [176–178]. Other mechanisms include modulation of MAPK signaling, ion channel activity, and inhibition of enzymes like topoisomerases and tyrosine kinases, contributing to anti-proliferative and antioxidant effects.

2.1.19 The Genus *Ginkgo*

The genus *Ginkgo* is a monotypic genus comprising a single surviving species, *Ginkgo biloba*, which is often regarded as a "living fossil" due to its evolutionary lineage dating back over 200 million years. Native to China, *Ginkgo biloba* has been widely cultivated across East Asia, and later globally, for its medicinal, ornamental, and cultural significance. As the only extant species in the division Ginkgophyta, this species ranks among the Earth's oldest trees. It is widely used in traditional Chinese medicine and has attracted extensive research interest because of its properties that protect the nervous system, combat oxidation, reduce inflammation, and regulate vascular function [182].

2.1.20 *Ginkgo biloba*

Ginkgo biloba is a large deciduous tree belonging to the family Ginkgoaceae. It can grow to heights of 20–35 meters and is easily recognized by its distinctive fan-shaped leaves (Figure 2.11). The tree is highly tolerant of environmental stresses, including pollution, poor soil, and urban conditions, making it a common feature

in city landscapes and traditional herbal gardens. Though it is cultivated globally, its origin is traced to specific forested regions in southeastern China where wild populations still exist [182].

FIGURE 2.11: *Ginkgo biloba* tree with its characteristic fan-shaped leaves. Widely known for its medicinal applications in neurological and circulatory health [182].

2.1.20.1 Phytochemical Constituents

Ginkgo biloba is rich in diverse phytochemical constituents that contribute to its wide range of pharmacological effects. The primary bioactive compounds include flavonoids such as quercetin, kaempferol, isorhamnetin, and rutin, which mainly occur as glycoside derivatives and exhibit antioxidant, anti-inflammatory, and neuroprotective activities. Terpenoids, particularly ginkgolides (A, B, C, J, and M) and bilobalide, are unique to *Ginkgo* and are known for their neuroprotective, anti-inflammatory, and vascular-regulatory properties [185]. Additionally, *Ginkgo* contains organic acids like ferulic acid and gallic acid, which contribute to its antioxidant and anti-tumor effects. Biflavonoids such as ginkgetin and amentoflavone add anti-obesity and antithrombotic benefits. Other constituents include polyprenols, ginkgolic acids, and minor compounds like ginkgotoxin. Standardized extracts, such as EGb 761, typically contain about 24% flavonoid glycosides, 6% terpenoids, and 5–10% organic acids, collectively responsible for the plant's therapeutic potential in treating neurological, cardiovascular, and metabolic disorders [183]. Additionally, various bioactive constituents of *Ginkgo biloba*, including flavonoids,

terpene lactones, quercetin, and ginkgolides, have been reported to provide beneficial impacts on endocrine and metabolic disorders (Figure 2.12) [184].

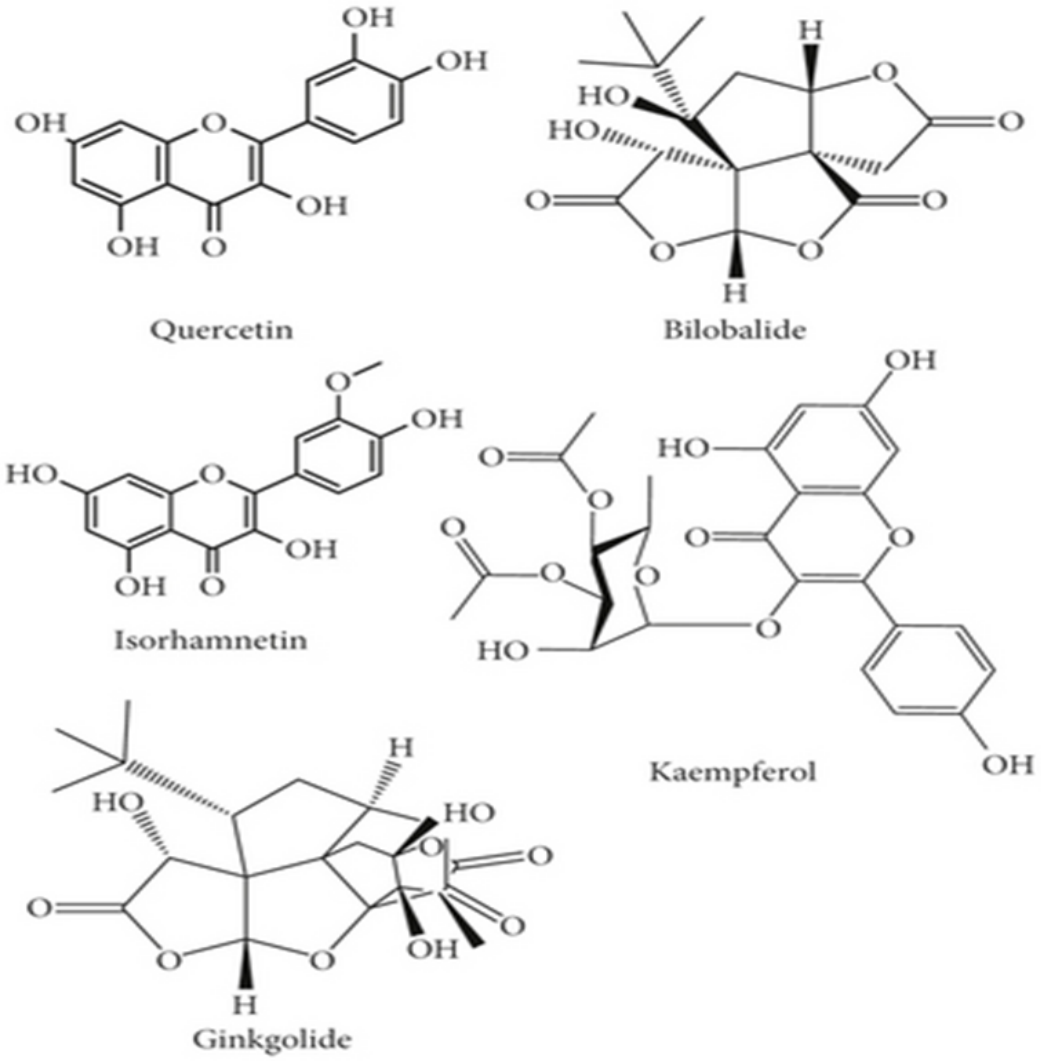


FIGURE 2.12: Major phytochemical constituents of *Ginkgo biloba* including ginkgolides, bilobalide, and various flavonoid glycosides [185].

2.1.20.2 Medicinal Applications

Ginkgo biloba has long been used in traditional medicine systems to enhance cognitive performance, improve memory, and treat circulatory disorders. Extracts from the leaves are widely used in managing conditions such as tinnitus, Alzheimer's disease, dementia, and peripheral vascular disease. *Ginkgo*'s terpene lactones are known to inhibit platelet-activating factor, thereby improving microcirculation and protecting neural tissues [185].

In traditional Chinese medicine, *Ginkgo biloba* seeds (after detoxification) have been employed to treat respiratory conditions such as asthma and bronchitis.

Moreover, the leaf extract has demonstrated potential in reducing anxiety symptoms, improving mood, and managing premenstrual syndrome (PMS). Recent studies also suggest its efficacy in addressing oxidative stress and insulin resistance in PCOS patients [186].

Topically, *Ginkgo* leaf preparations have been used to treat skin inflammation and age-related skin conditions due to their antioxidant-rich profile. Its anti-inflammatory and vasodilatory properties also support wound healing and dermal regeneration [187]. As research advances, *Ginkgo biloba* continues to be investigated for its therapeutic potential across diverse disease states.

Beyond its well-known cognitive perks, *Ginkgo* also has anti-inflammatory properties that may help ease symptoms of premenstrual syndrome (PMS) and irregular menstrual cycles. In traditional medicine, it's been used to address sexual dysfunction by enhancing microcirculation-something particularly helpful for individuals dealing with erectile issues. This improved blood flow is partly thanks to *Ginkgo*'s ability to stimulate the release of nitric oxide, which plays a key role in supporting vascular health [188].

Moreover, there are major studies that have suggested that *Ginkgo biloba* is a lady-healthy herb because of its flavonoid nature that affects the contractility of the smooth muscle cells and it is mostly the reason for the labors support in the traditional cultures.

Also, it was suggested by some scientists that the ability of this plant to protect the human brain against diseases was another cause for the antianxiety and antidepressant effects it revealed, thus, showing how widely medicinal effects it has. According to the therapeutic potential of *Ginkgo biloba* has never stopped making it a weighty subject of scientific research, hereby being vividly shown as the medicine that takes many forms, that is, vascular, neurological, and reproductive influence are among them [185–188].

2.1.21 Computational Approaches in Drug Discovery

In silico drug discovery refers to the use of computational modeling techniques to identify and design novel therapeutic agents. Unlike traditional drug development strategies, which often rely on empirical testing of chemical compounds through extensive laboratory and animal experimentation, *in silico* approaches begin with a mechanistic understanding of biological pathways and molecular interactions.

These computational strategies allow for the rational design of drug candidates by simulating and predicting their behavior within biological systems, thereby improving the efficiency and specificity of drug development processes [189].

The primary aim of drug discovery is to find molecules that can be quickly transformed into effective treatments for a broad spectrum of diseases, including those originating from genetic mutations (endogenous) as well as those resulting from external infectious agents like bacteria, viruses, and parasites (exogenous).

In this context, data mining technologies-particularly in the omics era-have revolutionized target identification by leveraging genomic, transcriptomic, and proteomic datasets to uncover biomarkers and therapeutic targets. The term "target" in biomedical research is broad, encompassing genes, microRNAs, proteins, metabolic pathways, cellular phenotypes, and other molecular entities. Computational biology and bioinformatics tools have become essential for integrating biological knowledge with statistical and computational methodologies to identify, prioritize, and validate potential drug targets [190].

Computational platforms support numerous functions in the drug development pipeline. These methods include virtual screening, de novo drug design, and the forecasting of pharmacokinetic characteristics such as absorption, distribution, metabolism, excretion, and toxicity (ADMET), as illustrated in Figure 2.13. Progress in protein-ligand interaction modeling and structure-based drug design has allowed researchers to shift from time-consuming experimental methods to more streamlined and cost-effective *in silico* techniques [189].

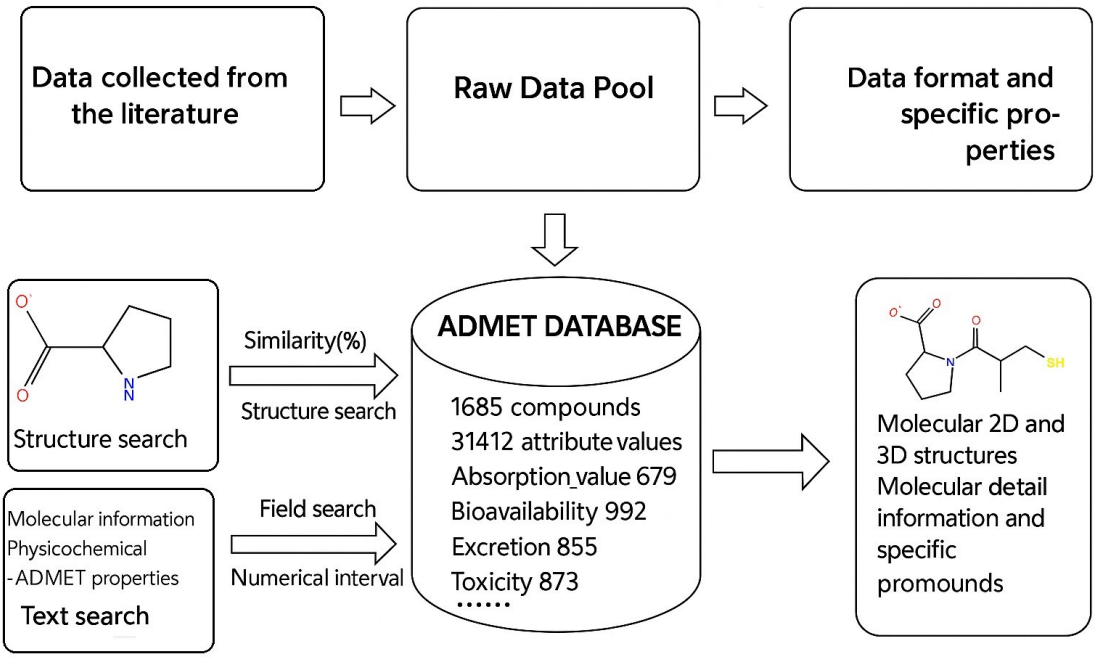


FIGURE 2.13: Computational ADMET Screening Workflow [191]

After identifying a target protein, virtual high-throughput screening is employed to evaluate its interaction with large molecular libraries. Molecules demonstrating favorable binding characteristics are shortlisted for further analysis. Sophisticated algorithms, which combine insights from molecular modeling, structural biology, and chemical informatics, are used to simulate binding behavior between candidate molecules and their targets. Although these simulations are approximate, they help narrow down potential candidates for in vitro or in vivo validation. This computational triaging significantly reduces the need for exhaustive laboratory testing of thousands or millions of compounds, making the drug discovery process faster and more cost-effective [189].

2.1.22 Molecular Docking

Molecular docking is a computational strategy employed to model and predict the interactions between a small molecule (ligand) and a biological macromolecule (usually a protein), at an atomic level. It is particularly useful for identifying how a ligand fits into the active or binding site of a target protein, offering crucial insights into the mechanism of action of potential drug candidates [192].

Docking algorithms attempt to predict both the structural configuration (binding pose) and the binding strength (affinity) between ligands and proteins. These tools serve a wide range of applications in the drug discovery pipeline, including hit identification through virtual screening, structure-activity relationship (SAR) studies, lead optimization, and mechanistic elucidation. Docking is also used to assist crystallographic studies by refining the fit of ligands to electron density maps and to explore the impact of protein mutations on ligand binding [193].

In virtual screening workflows, docking is typically preceded by ligand-based filtering using molecular descriptors or physicochemical properties to enhance library enrichment and reduce redundancy. Techniques such as shape-matching have also shown promise, occasionally performing on par with, or better than, traditional docking methods. Nonetheless, docking remains a critical final step in many virtual screening protocols, as it provides a three-dimensional hypothesis for the binding interaction between a compound and its target, facilitating rational drug design and further experimental validation [193].

Chapter 3

Research Methodology

The methodological framework presented in Figure 3.1 depicts a computational strategy for assessing the therapeutic potential of *Ginkgo biloba* in the treatment of PCOS. This section offers a detailed summary of the systematic procedures used to investigate the binding interactions between *Ginkgo biloba*'s bioactive compounds and crucial target proteins involved in the pathogenesis of PCOS.

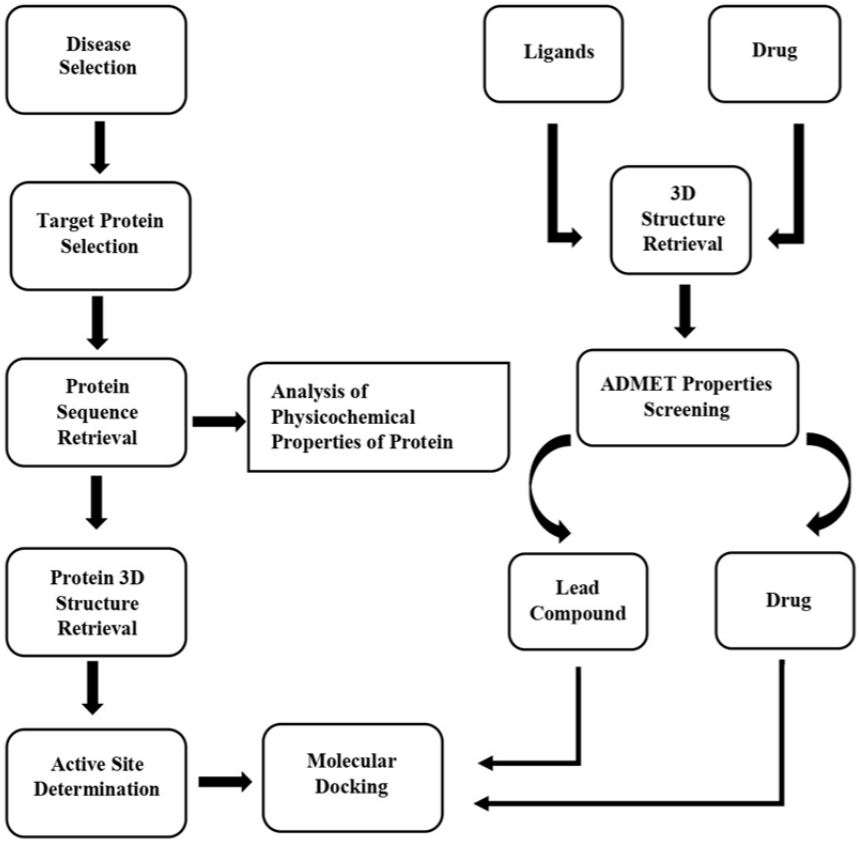


FIGURE 3.1: Conceptualization of Research Plan

The methodology comprises several essential stages, beginning with the selection of the disease, relevant target proteins, potential ligands, and a reference drug. Initially, the amino acid sequences of the chosen target proteins are obtained to assess their physicochemical characteristics and to model or retrieve their three-dimensional (3D) structures. These 3D structures are then used to predict active binding sites and facilitate molecular docking studies. At the same time, the 3D conformations of the selected ligands and the standard drug are collected and evaluated for their ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) profiles. The process concludes with molecular docking analyses of both the standard and lead compounds with the target proteins to evaluate binding affinities and interactions.

3.1 Disease Selection

Polycystic Ovary Syndrome (PCOS) is a widespread endocrine disorder affecting women of reproductive age worldwide. It is commonly marked by elevated insulin levels, increased androgen production, irregular menstrual cycles, and persistent metabolic imbalances [194]. Although synthetic medications like clomiphene, metformin, and spironolactone are frequently used to manage PCOS, they may cause side effects, including the risk of congenital abnormalities. It emphasizes the necessity to further study the root causes of PCOS and to develop more secure treatment alternatives [195].

3.2 Selection of Target Proteins

PCOS involves abnormalities in various hormonal and metabolic systems, including increased androgen production, insulin resistance, and ongoing low-grade inflammation. Therefore, four critical proteins were selected as molecular targets for this study: the androgen receptor (AR), the insulin receptor (IR), and inflammatory markers such as tumor necrosis factor-alpha (TNF-α). The androgen receptor is central to the regulation of androgen activity, which is often hightened

in PCOS. The insulin receptor plays a vital role in glucose metabolism and is frequently implicated in the insulin resistance observed in PCOS patients. Inflammatory cytokines like TNF-α contributes to the persistent low-level inflammation seen in this disorder [147–149]. Targeting these proteins offers a comprehensive approach to understanding and potentially managing PCOS through in silico drug discovery methods.

3.3 Retrieval of Primary Sequences

The primary amino acid sequences of the selected target proteins—Androgen Receptor (AR), Insulin Receptor (IR), and key inflammatory receptors such as TNF-α—were obtained from the UniProt database (https://www.uniprot.org/) in FASTA format. The UniProt accession numbers used for this study are P10275 for AR, P06213 for IR, and P01375 for TNF-α,.These sequences serve as the basis for further structural and functional analyses

3.4 Physicochemical Property Analysis

Understanding the physicochemical characteristics of the target proteins is essential for predicting their stability, solubility, and biological behavior. The ProtParam tool (https://web.expasy.org/protparam/) was employed to calculate key parameters, including molecular weight, theoretical isoelectric point (pI), counts of positively (Arg + Lys) and negatively (Asp + Glu) charged residues, extinction coefficients (with and without cysteine), instability index, aliphatic index, and the grand average of hydropathicity (GRAVY). These properties help assess the biochemical suitability of the proteins for docking and other computational studies.

3.5 Retrieval of 3D Protein Structures

Three-dimensional structures of the androgen receptor, insulin receptor, and TNF-α were retrieved from the Protein Data Bank (PDB) (https://www.rcsb.org/).

The specific PDB IDs used were: 1E3G for AR, 3LOH for IR, and 2AZ5 for TNF-α. These structures, along with their resolution data, provide the foundation for active site identification and molecular docking analysis.

3.6 Retrieval of Ligand and Standard Drug Structures

The three-dimensional (3D) structures of bioactive compounds present in *Ginkgo biloba*, along with an FDA-approved reference drug, were obtained from publicly available chemical databases, including PubChem (pubchem.ncbi.nlm.nih.gov/) and ChemSpider (http://www.chemspider.com/). Five phytochemicals commonly reported in *G. biloba* were selected based on their known pharmacological relevance:

TABLE 3.1: Bioactive Constituents of Ginkgo biloba and Their Therapeutic Effects

Compound	PubChem CID	Reported Activities
Ginkgolide A	9909368	Anti-inflammatory, antioxidant
Ginkgolide B	11973122	Neuroprotective, antioxidant
Bilobalide	73581	Anti-apoptotic, antioxidant
Quercetin	5280343	Anti-androgenic, anti-inflammatory
Kaempferol	5280863	Antioxidant, hormonal balance

Clomiphene citrate, a widely used therapeutic agent in the management of PCOS, was selected as the standard reference drug for comparative evaluation [196].

3.7 ADME Profiling and Toxicity Prediction of Ligands

To assess drug-likeness and pharmacokinetic feasibility, the selected phytochemicals were subjected to ADME (Absorption, Distribution, Metabolism, and Excretion) analysis using the SwissADME tool (http://www.swissadme.ch/). This platform offers detailed information on molecular descriptors, bioavailability scores,

blood-brain barrier permeability, gastrointestinal absorption, and drug-likeness filters. Additionally, toxicity predictions for the ligands were conducted using the ProTox-II server (https://tox-new.charite.de/), which estimates lethal dose (LD_{50}), hepatotoxicity, carcinogenicity, immunotoxicity, and other toxicity endpoints.

3.8 Active Site Identification and Molecular Docking

The potential binding sites of the selected target proteins were identified using the CASTp server (http://sts.bioe.uic.edu/), which analyzes protein surface topography to locate active site pockets. Subsequently, molecular docking simulations were performed using the CB-Dock tool (http://cao.labshare.cn/cb-dock/), which integrates cavity prediction with blind docking to generate binding affinities and poses. Docking interactions between the ligands and target proteins were visualized and analyzed using BIOVIA Discovery Studio Visualizer to understand the nature of ligand-protein binding interactions such as hydrogen bonding, hydrophobic contacts, and π–π stacking [197].

3.9 Identification of the Lead Compound

The identification of the most promising inhibitor was achieved by evaluating binding affinities, interaction profiles, pharmacokinetic properties, and predicted toxicity levels. The compound demonstrating the strongest interaction with key target proteins, favorable ADME characteristics, and minimal toxicity was designated as the lead candidate for potential therapeutic application in PCOS management.

Chapter 4

Results

An in silico study was conducted to evaluate the therapeutic potential of phytochemicals derived from Ginkgo biloba against Polycystic Ovary Syndrome (PCOS), by targeting key molecular pathways involved in its pathophysiology.

4.1 Protein Retrieval

4.1.1 Primary Sequence

The primary sequences of the selected target proteins were retrieved from the UniProt database (https://www.uniprot.org), a widely used and reliable source for high-quality protein sequence data. The selected targets included: Androgen Receptor (AR) – UniProt ID: P10275, Insulin Receptor (INSR) – UniProt ID: P06213, Tumor Necrosis Factor-alpha (TNF-α) – UniProt ID: P01375, and Nuclear factor erythroid 2-related factor 2 (Nrf2) – UniProt ID: Q16236. The retrieved sequences, provided in FASTA format, serve as the foundational data for subsequent structure prediction, active site analysis, and docking simulations. The amino acid lengths of these proteins vary, reflecting differences in their biological roles and structural complexity. Understanding these primary sequences is crucial for accurately modeling protein-ligand interactions and for evaluating the therapeutic binding potential of bioactive compounds from *Ginkgo biloba*.

>sp|P10275|ANDR_HUMAN Androgen receptor

```
MEVQLGLGRVYPRPPSKTYRGAFQNLFQSVREVIQNPGPRHPEAASAAPPGASLLLLQQQ
QQQQQQQQQQQQQQQQQQQQQETSPRQQQQQQGEDGSPQAHRRGPTGYLVLDEEQQPSQPQ
SALECHPERGCVPEPGAAVAASKGLPQQLPAPPDEDDSAAPSTLSLLGPTFPGLSSCSAD
LKDILSEASTMQLLQQQQQEAVSEGSSSGRAREASGAPTSSKDNYLGGTSTISDNAKELC
KAVSVSMGLGVEALEHLSPGEQLRGDCMYAPLLGVPPAVRPTPCAPLAECKGSLLDDSAG
KSTEDTAEYSPFKGGYTKGLEGESLGCSGSAAAGSSGTLELPSTLSLYKSGALDEAAAYQ
SRDYYNFPLALAGPPPPPPPPHPHARIKLENPLDYGSAWAAAAAQCRYGDLASLHGAGAA
GPGSGSPSAAASSSWHTLFTAEEGQLYGPCGGGGGGGGGGGGGGGGGGGGGEAGAVAP
YGYTRPPQGLAGQESDFTAPDVWYPGGMVSRVPYPSPTCVKSEMGPWMDSYSGPYGDMRL
ETARDHVLPIDYYFPPQKTCLICGDEASGCHYGALTCGSCKVFFKRAAEGKQKYLCASRN
DCTIDKFRRKNCPSCRLRKCYEAGMTLGARKLKKLGNLKLQEEGEASSTTSPTEETTQKL
TVSHIEGYECQPIFLNVLEAIEPGVVCAGHDNNQPDSFAALLSSLNELGERQLVHVVKWA
KALPGFRNLHVDDQMAVIQYSWMGLMVFAMGWRSFTNVNSRMLYFAPDLVFNEYRMHKSR
MYSQCVRMRHLSQEFGWLQITPQEFLCMKALLLFSIIPVDGLKNQKFFDELRMNYIKELD
RIIACKRKNPTSCSRRFYQLTKLLDSVQPIARELHQFTFDLLIKSHMVSVDFPEMMAEII
SVQVPKILSGKVKPIYFHTQ
```

FIGURE 4.1: Protein Sequence of Androgen receptor

>sp|P06213|IR_HUMAN Insulin receptor

```
MATGGRRGAAAAPLLVAVAALLLGAAGHLYPGEVCPGMDIRNNLTRLHELENCSVIEGHL
QILLMFKTRPEDFRDLSFPKLIMITDYLLLFRVYGLESLKDLFPNLTVIRGSRLFFNYAL
VIFEMVHLKELGLYNLMNITRGSVRIEKNNELCYLATIDWSRILDSVEDNYIVLNKDDNE
ECGDICPGTAKGKTNCPATVINGQFVERCWTHSHCQKVCPTICKSHGCTAEGLCCHSECL
GNCSQPDDPTKCVACRNFYLDGRCVETCPPPYYHFQDWRCVNFSFCQDLHHKCKNSRRQG
CHQYVIHNNKCIPECPSGYTMNSSNLLCTPCLGPCPKVCHLLEGEKTIDSVTSAQELRGC
TVINGSLIINIRGGNNLAAELEANLGLIEEISGYLKIRRSYALVSLSFFRKLRLIRGETL
EIGNYSFYALDNQNLRQLWDWSKHNLTITQGKLFFHYNPKLCLSEIHKMEEVSGTKGRQE
RNDIALKTNGDQASCENELLKFSYIRTSFDKILLRWEPYWPPDFRDLLGFMLFYKEAPYQ
NVTEFDGQDACGSNSWTVVDIDPPLRSNDPKSQNHPGWLMRGLKPWTQYAIFVKTLVTFS
DERRTYGAKSDIIYVQTDATNPSVPLDPISVSNSSSQIILKWKPPSDPNGNITHYLVFWE
RQAEDSELFELDYCLKGLKLPSRTWSPPFESEDSQKHNQSEYEDSAGECCSCPKTDSQIL
KELEESSFRKTFEDYLHNVVFVPRKTSSGTGAEDPRPSRKRRSLGDVGNVTVAVPTVAAF
PNTSSTSVPTSPEEHRPFEKVVNKESLVISGLRHFTGYRIELQACNQDTPEERCSVAAYV
SARTMPEAKADDIVGPVTHEIFENNVVHLMWQEPKEPNGLIVLYEVSYRRYGDEELHLCV
SRKHFALERGCRLRGLSPGNYSVRIRATSLAGNGSWTEPTYFYVTDYLDVPSNIAKIIIG
PLIFVFLFSVVIGSIYLFLRKRQPDGPLGPLYASSNPEYLSASDVFPCSVYVPDEWEVSR
EKITLLRELGQGSFGMVYEGNARDIIKGEAETRVAVKTVNESASLRERIEFLNEASVMKG
FTCHHVVRLLGVVSKGQPTLVVMELMAHGDLKSYLRSLRPEAENNPGRPPPTLQEMIQMA
AEIADGMAYLNAKKFVHRDLAARNCMVAHDFTVKIGDFGMTRDIYETDYYRKGGKGLLPV
RWMAPESLKDGVFTTSSDMWSFGVVLWEITSLAEQPYQGLSNEQVLKFVMDGGYLDQPDN
CPERVTDLMRMCWQFNPKMRPTFLEIVNLLKDDLHPSFPEVSFFHSEENKAPESEELEME
FEDMENVPLDRSSHCQREEAGGRDGGSSLGFKRSYEEHIPYTHMNGGKKNGRILTLPRSN
PS
```

FIGURE 4.2: Protein Sequence of Insulin receptor

>sp|P01375|TNFA_HUMAN Tumor necrosis factor

```
MSTESMIRDVELAEEALPKKTGGPQGSRRCLFLSLFSFLIVAGATTLFCLLHFGVIGPQR
EEFPRDLSLISPLAQAVRSSSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELR
DNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRE
TPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLDFAESGQVYFGIIAL
```

FIGURE 4.3: Protein Sequence of Tumor necrosis factor

4.1.2 Physicochemical Properties of Target Proteins

The physicochemical properties of the selected target proteins-Androgen Receptor (AR), Insulin Receptor (IR), and Tumor Necrosis Factor-alpha (TNF-α) were analyzed using the ProtParam tool (https://web.expasy.org/protparam/). This computational tool allows for the prediction of essential characteristics based on the protein's amino acid sequence. The evaluated parameters include:

a) Molecular Weight, which influences protein mobility and interaction capacity

b) Theoretical Isoelectric Point (pI), indicating the pH at which the protein carries no net charge

c) Amino Acid Composition, including counts of positively (Arg, Lys) and negatively (Asp, Glu) charged residues

d) Extinction Coefficient, useful for protein quantification via spectrophotometry

e) Instability Index, predicting protein stability in vitro

f) Aliphatic Index, related to thermostability

g) Grand Average of Hydropathicity (GRAVY), which reflects protein solubility and hydrophobicity

Analyzing these features aids in understanding the biochemical behavior, solubility, stability, and interaction potential of each target protein, laying a crucial foundation for successful docking and binding studies with the phytochemicals derived from *Ginkgo biloba*.

TABLE 4.1: Physiochemical Properties of AR, IR, and TNF-α

Physicochemical Properties	**AR**	**INSR**	**TNF-α**
Molecular weight	99187.84	156332.86	25644.42
Theoretical pI	6.00	5.83	6.44
Negatively charged residues (Asp + Glu)	92	176	23
Positively charged residues (Arg + Lys)	81	149	22
Ext. coefficient 1	94795	188876	21680
Ext. coefficient 2	93170	185990	21430
Instability index	56.78	48.27	40.75
Aliphatic index	68.04	80.04	98.37
Gravy	-0.439	-0.359	-0.047

The findings of the physicochemical analysis (Table 4.1) reveal significant insights into the structural and biochemical characteristics of the selected target proteins-Androgen Receptor (AR), Insulin Receptor (IR), and Tumor Necrosis Factor-alpha (TNF-α).

The molecular weights of the proteins are calculated as follows: AR at 99,187.84 Da, IR at 156,332.86 Da, and TNF-α at 25,644.42 Da. These values highlight the size variation among the proteins, with INSR being the largest and TNF-α, the smallest, which may influence their biological roles and interactions with ligands.

The theoretical isoelectric point (pI) values indicate the net charge of the proteins at physiological pH. AR (pI = 6.00) and IR (pI = 5.83) exhibit slightly acidic nature, while TNF-α (pI = 6.44) leans closer to neutrality.

When comparing charged amino acid residues, INSR shows the highest number of both negatively (Asp + Glu = 176) and positively (Arg + Lys = 149) charged residues, indicating a highly charged and potentially interactive surface. TNF-α shows a nearly balanced distribution of negative (23) and positive (22) charges. The extinction coefficients, which estimate absorbance at 280 nm in water and indicate the presence of aromatic residues, are highest in INSR (188,876 M^{-1} cm^{-1}) and lowest in TNF-α (21,680 M^{-1} cm^{-1}). AR has moderate value, supporting its differential aromatic residue composition and potential detectability in spectroscopic analysis.

The instability index reveals that all four proteins have values above 40, indicating potential instability in vitro. AR (56.78) appear less stable than TNF-α (40.75), which is borderline stable. IR, while unstable (48.27), shows relatively better stability among the large proteins.

For thermostability, assessed via the aliphatic index, TNF-α scores highest (98.37), suggesting higher thermal stability, followed by IR (80.04), Nrf2 (77.17), and AR (68.04).

Lastly, the GRAVY (Grand Average of Hydropathicity) score, which reflects the hydrophobic or hydrophilic nature of the protein, is negative for all proteins, indicating an overall hydrophilic character. Among them, TNF-α (-0.047) is the least hydrophilic.

These comprehensive physicochemical profiles are essential for understanding each protein's behavior in biological systems and provide foundational data for molecular docking and drug-likeness assessments in the context of Ginkgo biloba-based therapy for PCOS.

4.1.3 Protein 3D Structure Retrieval

The Protein Data Bank (PDB) is a leading international database that provides reliable and accurate structural information on biological macromolecules. It acts as a foundational resource for various computational and experimental studies, offering high-quality three-dimensional structures essential for drug discovery and molecular docking studies [200]. In this study, the 3D structures of the selected target proteins-Androgen Receptor (AR), Insulin Receptor (INSR), and Tumor Necrosis Factor-alpha (TNF-α) -were retrieved from the PDB database (https://www.rcsb.org/). The respective PDB entry IDs utilized are:

AR: PDB ID 1E3G
IR: PDB ID 4XLV
TNF-α: PDB ID 2AZ5

The use of experimentally determined protein structures ensures the structural integrity required for molecular docking simulations. Importantly, these retrieved models were curated to exclude non-essential components such as water molecules, ligands, and ions, which could interfere with docking accuracy. The cleaned structures were then utilized for downstream computational analysis, including active site prediction and ligand docking (figure 4.4).

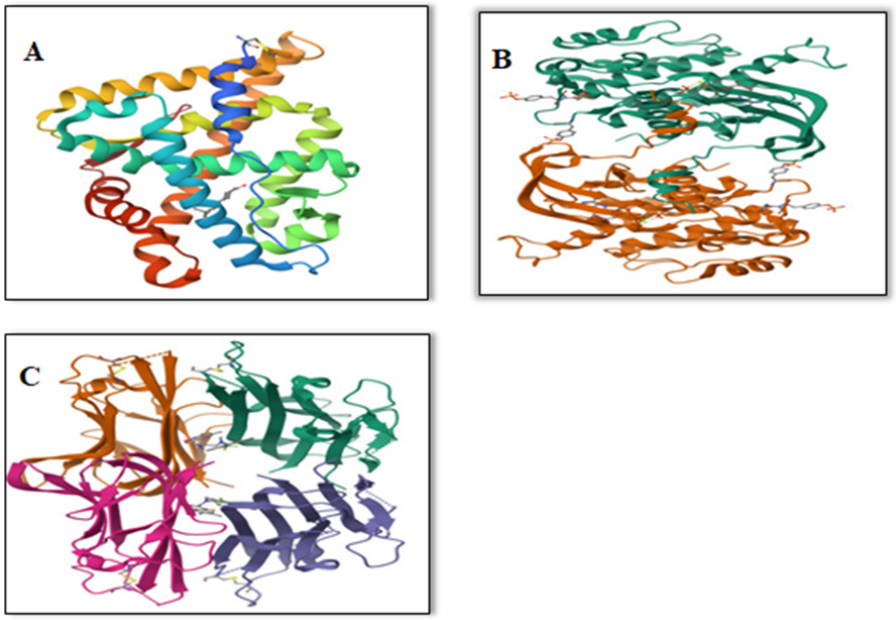


FIGURE 4.4: 3D Structure of Proteins. (A) Protein structure representing AR, (B) Protein structure representing IR, and (C) Protein structure representing TNF-α.

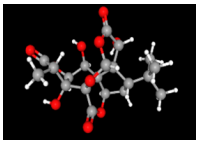
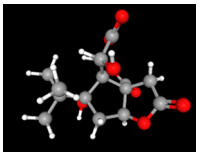
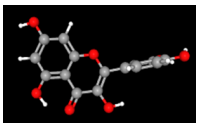
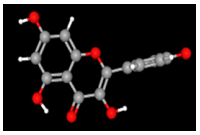
4.2 Ligand Retrieval

4.2.1 Ligand Chemical Structure

Through a comprehensive literature review, five key bioactive compounds were identified from Ginkgo biloba, a medicinal plant traditionally used for various therapeutic purposes. The 3D chemical structures of these phytoconstituents were retrieved using well-established chemical database: PubChem (pubchem.ncbi.nih.gov). PubChem is a widely recognized open-access platform hosted by the National Institutes of Health (NIH), offering extensive data on chemical substances, their structural attributes, and associated biological activities. It serves as a foundational

resource for compound identification, characterization, and download in multiple formats suitable for computational modeling. By leveraging this databases, the 3D structures of *Ginkgo biloba* phytoconstituents were efficiently acquired for use in docking studies. This database approach enabled a holistic view of the ligands' chemical and physical characteristics, laying a solid foundation for their pharmacological evaluation in the context of PCOS. A detailed list of the retrieved compounds is provided in Table 4.2.

TABLE 4.2: Ligand Structures

Database	**Ligand Name / ID**	**Molecular Formula**	**Molecular Weight**	**Ligand Structure**
PubChem	Ginkgolide A / 9909368	$C_{20}H_{24}O_9$	408.4 g/mol	
	Gingkolide B / 11973122	$C_{20}H_{24}O_{10}$	424.4 g/mol	
	Bilobalide / 73581	$C_{15}H_{18}O_8$	326.30 g/mol	
	Quercetin / 5280343	$C_{15}H_{10}O_7$	302.23 g/mol	
	Kaempferol / 5280863	$C_{15}H_{10}O_6$	286.24 g/mol	

4.2.2 ADME Properties

SwissADME (http://www.swissadme.ch/)xt is a widely used web-based platform designed to predict key pharmacokinetic, physicochemical, and drug-likeness properties of chemical compounds. This tool is particularly valuable during the early stages of drug development, allowing researchers to evaluate the pharmacological potential and oral bioavailability of candidate molecules [198]. In this study,

the selected phytoconstituents from Ginkgo biloba were subjected to ADME (Absorption, Distribution, Metabolism, and Excretion) profiling using SwissADME. The goal was to identify bioactive compounds with favorable pharmacokinetic and physicochemical profiles suitable for further therapeutic investigation.

4.2.2.1 Bioavailability Radar

SwissADME provides a bioavailability radar, a visual tool that quickly assesses drug-likeness. This radar evaluates six fundamental physicochemical parameters: lipophilicity (LIPO), size, polarity (POLAR), solubility (INSOLU), saturation (INSATU), and flexibility (FLEX). The pink region in the radar plot represents the optimal physicochemical space for oral bioavailability (Figure 4.5). Compounds whose values fall within this area are considered to have drug-like properties [199].

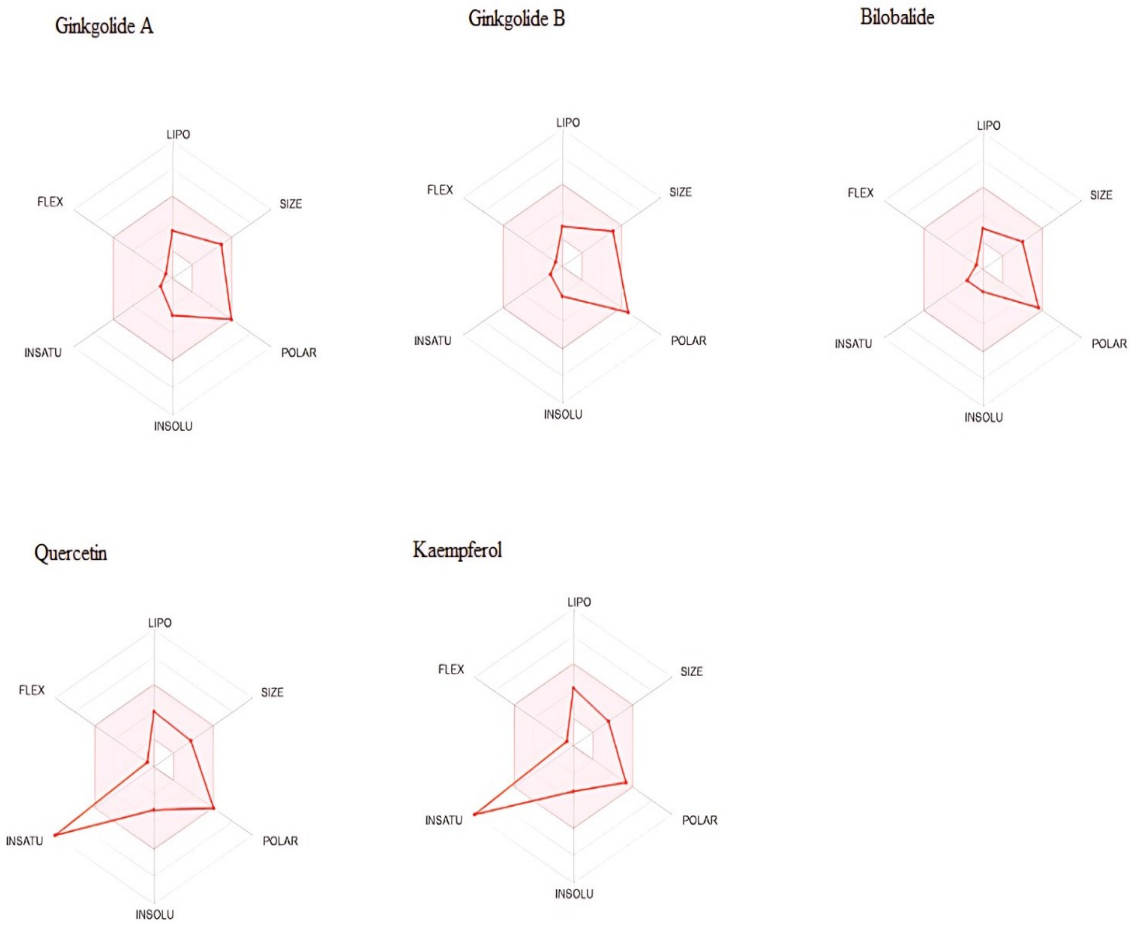


FIGURE 4.5: Bioavailability Radar of Ligands.

4.2.2.2 Physicochemical Properties

Key physicochemical properties for each ligand were computed, including molecular weight, molecular formula, number of heavy atoms, fraction of sp^3 carbons (Csp^3), number of aromatic heavy atoms, rotatable bonds, hydrogen bond donors and acceptors, molar refractivity, and Topological Polar Surface Area (TPSA), as summarized in Table 4.3. These parameters are essential for understanding the molecular features that influence a compound's solubility, permeability, and binding affinity.

TABLE 4.3: Physicochemical Properties of Ligands

	Physicochemical Properties								
Ligands	**Formula**	**MW (g/-mol)**	**No. of HA**	**No. of AHA**	**Fraction Csp3**	**No. RB**	**No. of HBA**	**No. of HBD**	**MR**	**TPSA (Å^2)**
Ginkgolide A	$C_{20}H_{24}O_9$	408.40	29	0	0.85	1	9	2	92.13	128.59
Ginkgolide B	$C_{20}H_{24}O_{10}$	424.40	30	0	0.85	1	10	3	93.29	148.82
Bilobalide	$C_{15}H_{18}O_8$	326.30	23	0	0.80	1	8	2	71.20	119.36
Quercetin	$C_{15}H_{10}O_7$	302.24	22	16	0.00	1	7	5	78.03	131.36
Kaempferol	$C_{15}H_{10}O_6$	286.24	21	16	0.00	1	6	4	76.01	111.13

(MW: Molecular Weight, No. of HA: Number of Heavy Atoms, No. of AHA: Number of Aromatic Heavy Atoms, No. RB: Number of Rotatable Bonds, No. of HBA: Number of Hydrogen Bond Acceptor, No. of HBD: Number of Hydrogen Bond Donor, MR: Molar Refractivity, TPSA: Topological Polar Surface Area).

4.2.2.3 Lipophilicity

Lipophilicity plays a pivotal role in determining the absorption and distribution of compounds in biological systems. SwissADME calculates log P (octanol/water partition coefficient) using five distinct methods: WLOGP, XLOGP3, MLOGP, SILICOS-IT, and iLOGP. Each method employs a different computational strategy:

1. XLOGP3: Knowledge-based and atomistic.
2. WLOGP: Fragmental-based.
3. MLOGP: Topological, based on molecular descriptors.
4. SILICOS-IT: Hybrid of fragmental and topological descriptors.
5. iLOGP: Physics-based, using free energy of solvation (GB/SA model).

A consensus log P value is computed as the average of these five models, offering a more robust estimation of lipophilicity (Table 4.4) [198].

TABLE 4.4: Lipophilicity Properties of Ligands.

	Lipophilicity					
Ligands	**Log Po/w (iLOGP)**	**Log Po/w (XLOGP3)**	**Log Po/w (WLOGP)**	**Log Po/w (MLOGP)**	**Log Po/w (SILICOS-IT)**	**Consensus Log Po/w**
Ginkgolide A	1.12	0.59	-0.34	0.83	0.81	0.60
Ginkgolide B	1.59	-0.38	-1.37	0.06	-0.07	-0.03
Bilobalide	0.81	-0.27	-0.74	0.42	0.57	0.16
Quercetin	1.63	1.54	1.99	-0.56	1.54	1.23
Kaempferol	1.70	1.90	2.28	-0-03	2.03	1.58

4.2.2.4 Water Solubility

Water solubility was predicted using three distinct models: ESOL, Ali, and SILICOS-IT (Table 4.5). These models classify solubility into qualitative and quantitative ranges based on logarithmic solubility values (Log S):

1. Highly soluble: Log S $>$ –2
2. Soluble: –4 $<$ Log S $\leq$ –2
3. Moderately soluble: –6 $<$ Log S $\leq$ –4
4. Poorly soluble: –10 $<$ Log S $\leq$ –6
5. Insoluble: Log S $<$ –10

These classifications help determine the ease with which a compound can dissolve in aqueous environments, a critical factor for oral bioavailability.

Table 4.5: Water Solubility of Ligands

	Water Solubility											
	Solubility (ESOL)				**Solubility (Ali)**				**Solubility (Silicos-IT)**			
Ligands	**Log S (ESOL)**	mg ml^{-1}	mol L^{-1}	**Class (ESOL)**	**Log S (Ali)**	mg ml^{-1}	mol L^{-1}	**Class (Ali)**	**LogS (Silicos-IT)**	mg ml^{-1}	mol L^{-1}	**Class (Silicos-IT)**
Ginkgolide A	-2.68	8.58e-01	2.10e-03	S	-2.86	5.59e-01	1.37e-03	S	-1.59	1.06e+01	2.59e-02	S
Ginkgolide B	-2.17	2.90e+00	6.83e-03	S	-2.28	2.22e+00	5.22e-03	S	-0.77	7.27e+01	1.71e-01	S
Bilobalide	-1.63	7.70e+00	2.36e-02	VS	-1.78	5.45e+00	1.67e-02	VS	-1.12	2.48e+01	7.59e-02	S
Quercetin	-3.16	2.11e-01	6.98e-04	S	-3.91	3.74e-02	1.24e-04	S	-3.24	1.73e-01	5.73e-04	S
Kaempferol	-3.31	1.40e-01	4.90e-04	S	-3.86	3.98e-02	1.39e-04	S	-3.82	4.29e-02	1.50e-04	S

4.2.2.5 BOILED-Egg Model

SwissADME also provides the BOILED-Egg model, which estimates passive absorption in gastrointestinal and brain penetration (Figure 4.6). Compounds falling within the white area of the plot are predicted to exhibit high gastrointestinal absorption, whereas those located in the yellow region (the yolk) are more likely to cross the blood–brain barrier.

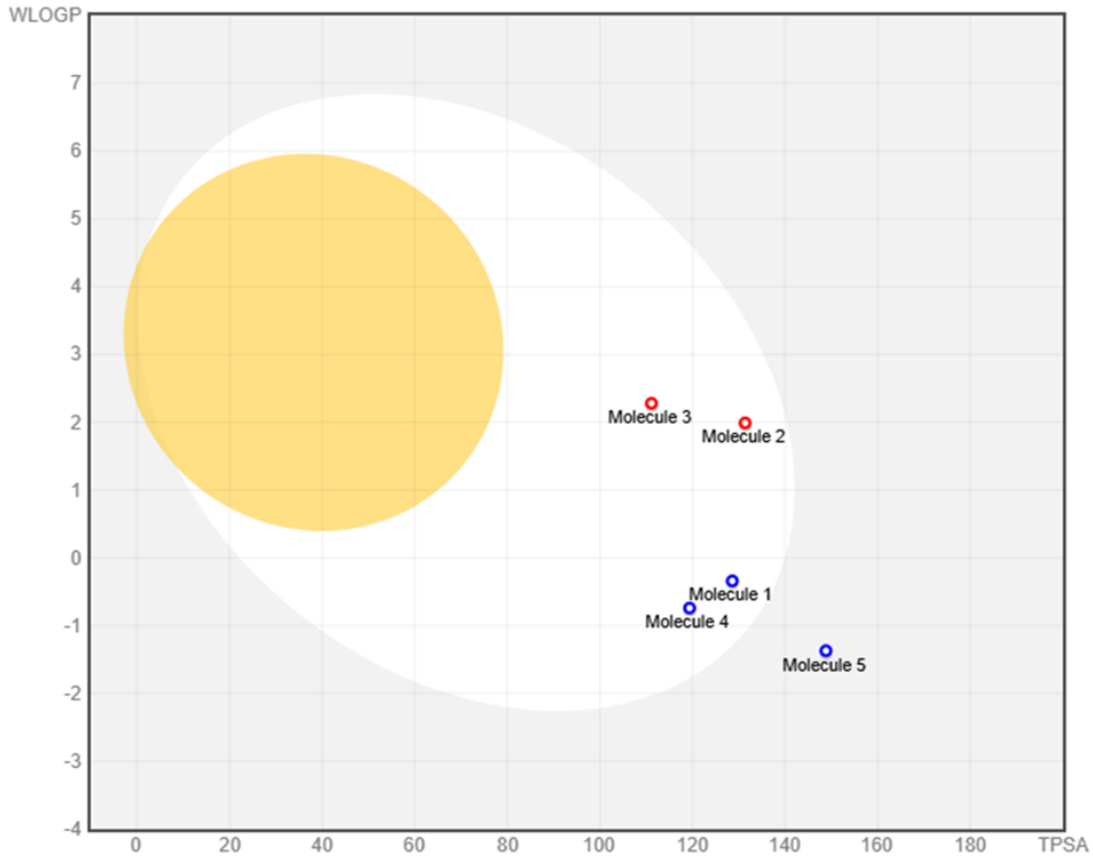


FIGURE 4.6: BOILED-Egg model illustrating passive gastrointestinal absorption (HIA) and blood–brain barrier (BBB) penetration based on WLOGP versus TPSA values. Molecule 1;Ginkgolide A , Molecule 2;Quercetin , Molecule 3; Kaempferol , Molecule 4; Bilobalide, Molecule 5; Ginkgolide B.

The model also identifies whether compounds are P-glycoprotein (P-gp) substrates, which are often effluxed out of the gastrointestinal tract and brain, impacting drug efficacy. Moreover, SwissADME evaluates interactions with major cytochrome P450 isoenzymes (CYP3A4, CYP2C9, CYP2C19, CYP2D6), which are responsible for the metabolism of most drugs in the human body (Table 4.6).

TABLE 4.6: Pharmacokinetics of Ligands.

Ligands	Pharmacokinetics								
	GI absorption	**BBB permeant**	**Pgp substrate**	**CYP1A2 inhibitor**	**CYP2C19 inhibitor**	**CYP2C9 inhibitor**	**CYP2D6 inhibitor**	**CYP3A4 inhibitor**	**Skin Permeation log Kp**
Gingkolide A	↑	×	✓	×	×	×	×	×	-8.37 cm/s
Ginkgolide B	↓	×	✓	×	×	×	×	×	-9.16 cm/s
Bilobalide	↑	×	✓	×	×	×	×	×	-8.48 cm/s
Quercetin	↑	×	×	×	×	×	×	×	-7.05 cm/s
Kaempferol	↑	×	×	✓	×	×	✓	✓	-6.70 cm/s

4.2.2.6 Drug-Likeness

The drug-likeness of each ligand was assessed using five well-established rule-based filters, developed by leading pharmaceutical companies:

1. Lipinski's Rule of Five (Pfizer)
2. Ghose Filter (Amgen)
3. Veber Rule (GSK)
4. Egan Filter (Pharmacia)
5. Muegge Filter (Bayer)

These filters evaluate structural and physicochemical properties-such as molecular weight, lipophilicity, and H-bond capacity-to predict oral bioavailability (Table 4.7). Molecules that comply with most of these filters are more likely to exhibit favorable pharmacokinetics and thus qualify as potential drug candidates [198].

TABLE 4.7: Drug likeness of Ligands.

Ligands	**Number of Violations**					**Bioavailability Scorea**
	Lipinski	**Ghose**	**Veber**	**Egan**	**Muegge**	
Gingkolide A	0	0	0	0	0	0.55
Gingkolide B	0	1	1	1	0	0.55
Bilobalide	0	1	0	0	0	0.55
Quercetin	0	0	0	0	0	0.55
Kaempferol	0	0	0	0	0	0.55

A detailed ADME-based evaluation was conducted on the five selected phyto constituents - Ginkgolide A, Ginkgolide B, Bilobalide, Quercetin, and Kaempferol-to refine the ligand pool for subsequent toxicity analysis and potential drug development. This refinement focused primarily on two critical factors: cytochrome P450 enzyme inhibition and drug-likeness rule violations.

A detailed ADME-based evaluation was conducted on the five selected phytoconstituents-Ginkgolide A, Ginkgolide B, Bilobalide, Quercetin, and Kaempferol-to refine the ligand pool for subsequent toxicity analysis and potential drug development. This refinement focused primarily on two critical factors: cytochrome P450 enzyme inhibition and drug-likeness rule violations.

In contrast, Quercetin, flavonoids, had no violations in any of the drug-likeness filters, demonstrating strong drug-like features from a structural standpoint. Ginkgolide B and Bilobalide exhibited minor violations in drug-likeness filters. Ginkgolide B failed to meet the Ghose, Veber, and Egan rules, while Bilobalide violated only the Ghose filter. However, both compounds complied with Lipinski's Rule of Five and showed no evidence of cytochrome inhibition, which justified their retention. Despite Ginkgolide B's lower gastrointestinal absorption compared to the others, its physicochemical parameters remained within acceptable limits for further consideration.

Ultimately, the refined ligand pool was narrowed down to four candidates-Ginkgolide A, Ginkgolide B, and Bilobalide. Ginkgolide A demonstrated the most favorable profile, with no drug-likeness violations, high gastrointestinal absorption, moderate water solubility, balanced lipophilicity, and no CYP inhibition, making it the most promising candidate. Ginkgolide B, while having some minor rule violations and lower GI absorption, posed no safety concerns related to metabolism. Bilobalide also showed a strong profile with high water solubility, no CYP inhibition, and compliance with most drug-likeness criteria. Due to high GI absorption, good physicochemical parameters, and strong drug-likeness profiles justify the inclusion of Quercetin in further studies Therefore, based on ADME profiling, Kaempferol were excluded from further stages, while Ginkgolide A, Ginkgolide B, Bilobalide and Quercetin were retained for toxicity evaluation and future exploration.

4.2.3 Toxicity Prediction

Toxicity assessment is a critical phase in the drug development process, aimed at identifying and minimizing potential harmful effects associated with candidate

compounds. In this study, the toxicity profiles of the three refined *Ginkgo biloba* phytoconstituents - Ginkgolide A, Ginkgolide B, Bilobalide and Quercetin -were evaluated using the ProTox-II webserver (https://tox-new.charite.de/), a state-of-the-art computational tool for *in silico* toxicity prediction.

ProTox-II offers an integrative approach to toxicity prediction by employing machine learning models and extensive toxicological databases to forecast various toxic endpoints, including hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity, along with the median lethal dose (LD_{50}) and toxicity class. This predictive capability aligns with modern drug development practices, emphasizing early-stage identification of toxicity risks to enhance safety and efficacy.

By applying ProTox-II to Ginkgolide A, Ginkgolide B, Bilobalide and Quercetin-compounds previously refined based on favorable ADME profiles-this analysis ensures that the selected ligands not only exhibit drug-like characteristics but also possess acceptable safety profiles. The use of ProTox-II facilitates data-driven decision-making in the selection and optimization of these Ginkgo biloba phytoconstituents, ultimately contributing to the efficient and responsible development of therapeutic candidates with minimized toxicological risks.

TABLE 4.8: Toxicity of Ligands

Ligands	**Hepato toxicity**	**Carcino genicity**	**Immuno toxicity**	**Muta genicity**	**Cyto toxicity**
Ginkgolide A	×	×	✓	×	×
Ginkgolide B	×	×	✓	×	×
Bilobalide	×	×	✓	×	×
Quercetin	×	×	×	×	×

×: Inactive ✓: Active

In the toxicity assessment of the four studied Ginkgo biloba phytoconstituents-Ginkgolide A, Ginkgolide B, and Bilobalide-three compounds were found to exhibit some degree of toxicity. Specifically, Ginkgolide A, Ginkgolide B and Bilobalide showed immunotoxic potential, indicated by activity in that endpoint. In contrast, Quercetin was inactive across all evaluated toxicity endpoints, including

hepatotoxicity, carcinogenicity, mutagenicity, and cytotoxicity. Given this favorable safety profile, Quercetin was retained for further investigation, while the immunotoxic compounds Ginkgolide B and Bilobalide were excluded from subsequent stages of analysis.

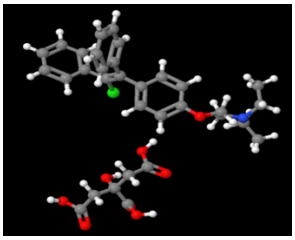
4.3 Standard Drug

4.3.1 Drug Structure Retrieval

The three-dimensional structure of *Clomiphene citrate* was obtained from *ChemSpider* (http://www.chemspider.com/), an open-access platform that offers detailed chemical data (table 4.9). This database provides essential structural and chemical information that aids in accurate molecular modeling.

The use of ChemSpider ensures that the retrieved compound structure is both credible and suitable for computational analyses, thereby supporting further research into Clomiphene citrate's pharmacological properties and potential therapeutic roles.

TABLE 4.9: Standard Drug Structure

Standard Drug	**Molecular Formula**	**Molecular Weight**	**Drug Structure**
Clomiphene citrate	$C_{32}H_{36}ClNO_8$	598.083 Da	

4.3.2 ADMET Properties

The pharmacokinetic evaluation of Clomiphene citrate was conducted using SwissADME (http://www.swissadme.ch/), an open-access computational tool widely

used in drug discovery research. SwissADME enables a detailed assessment of Absorption, Distribution, Metabolism, and Excretion (ADME) characteristics, providing crucial insights for early-stage drug development. This tool offers a comprehensive profile including physicochemical parameters (Table 4.10), lipophilicity (Table 4.11), water solubility (Table 4.12), and pharmacokinetic behavior (Table 4.13), and drug-likeness properties (Table 4.14).

Beyond static descriptors, SwissADME incorporates predictive visual tools such as the Bioavailability Radar (Figure 4.7), iLOGP, and the BOILED-Egg model (Figure 4.8), which assist in forecasting essential drug-like attributes such as oral bioavailability and blood–brain barrier permeability. These advanced features aid researchers in identifying promising candidates with favorable pharmacokinetic profiles.

To complement the ADME analysis, ProTox-II (https://tox-new.charite.de/) was employed for the toxicity prediction of Clomiphene citrate, with results presented in Table 4.15.

This combined approach, using both SwissADME and ProTox-II, supports a thorough understanding of the compound's safety, efficacy, and potential as a therapeutic agent, contributing to a more informed and strategic drug development process.

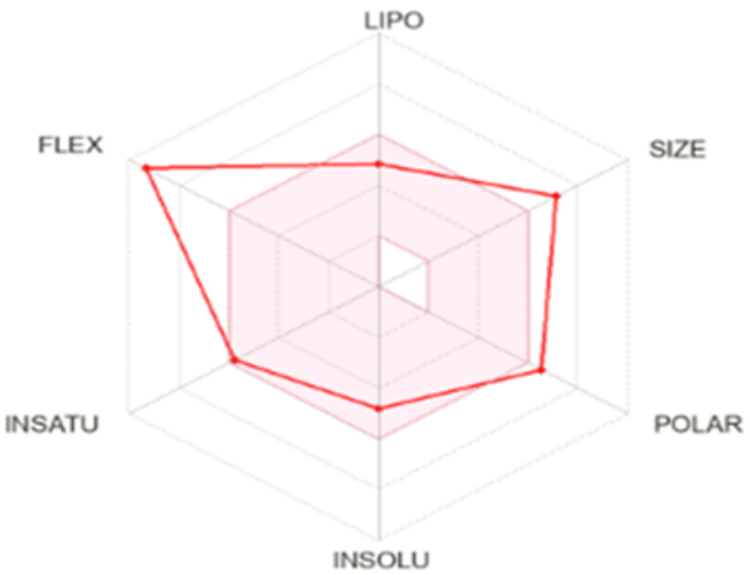


FIGURE 4.7: Bioavailability radar of Clomiphene citrate.

TABLE 4.10: Physicochemical Properties of Clomiphene citrate.

Physicochemical Properties	**Results**
Formula	$C_{32}H_{36}ClNO_8$
Molecular Weight	598.08 g/ mol
Number of Heavy Atoms	42
Number of Aromatic Heavy Atoms	18
Number of Rotatable Bonds	0.28
Fraction Csp3	14
Number of Hydrogen Bond Acceptor	9
Number of Hydrogen Bond Donor	4
Molar Refractivity	161.99
Topological Polar Surface Area	144.60 $Å^2$

TABLE 4.11: Lipophilicity of Clomiphene citrate.

Lipophilicity	**Results**
Log Po/w (iLOGP)	4.53
Log Po/w (XLOGP3)	2.95
Log Po/w (WLOGP)	5.31
Log Po/w (MLOGP)	2.93
Log Po/w (SILICOS-IT)	6.50
Consensus Log Po/w	4.45

TABLE 4.12: Water Solubility of Clomiphene citrate.

Water Solubility	**Results**
Log S (ESOL)	-4.80
Solubility	9.48e-03 mg ml^{-1} ; 1.59e-05 mol L^{-1}
Class	Moderate solubility
Log S (Ali)	-5.65
Solubility	1.34e-03 mg ml^{-1}; 2.24e-06 mol L^{-1}
Class	Moderate solubility
Log S (SILICOS-IT)	-9.52
Solubility	1.79e-07 mg ml^{-1}; 2.99e-10 mol L^{-1}
Class	Poorly solubility

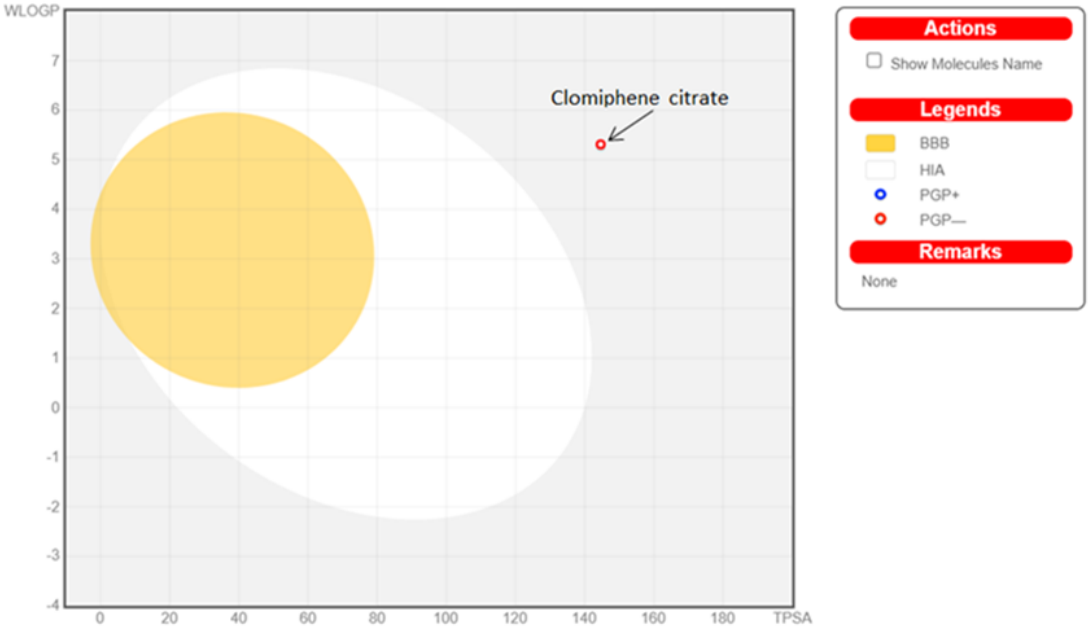


FIGURE 4.8: Boiled Egg representation of Clomiphene citrate.

TABLE 4.13: Pharmacokinetics of Clomiphene citrate.

Pharmacokinetics	**Results**
GI absorption	↓
BBB permeant	×
P-gp substrate	×
CYP1A2 inhibitor	×
CYP2C19 inhibitor	×
CYP2C9 inhibitor	×
CYP2D6 inhibitor	✓
CYP3A4 inhibitor	×
Log Kp (skin permeation)	-7.85 cm/s

TABLE 4.14: Druglikeness of Clomiphene citrate.

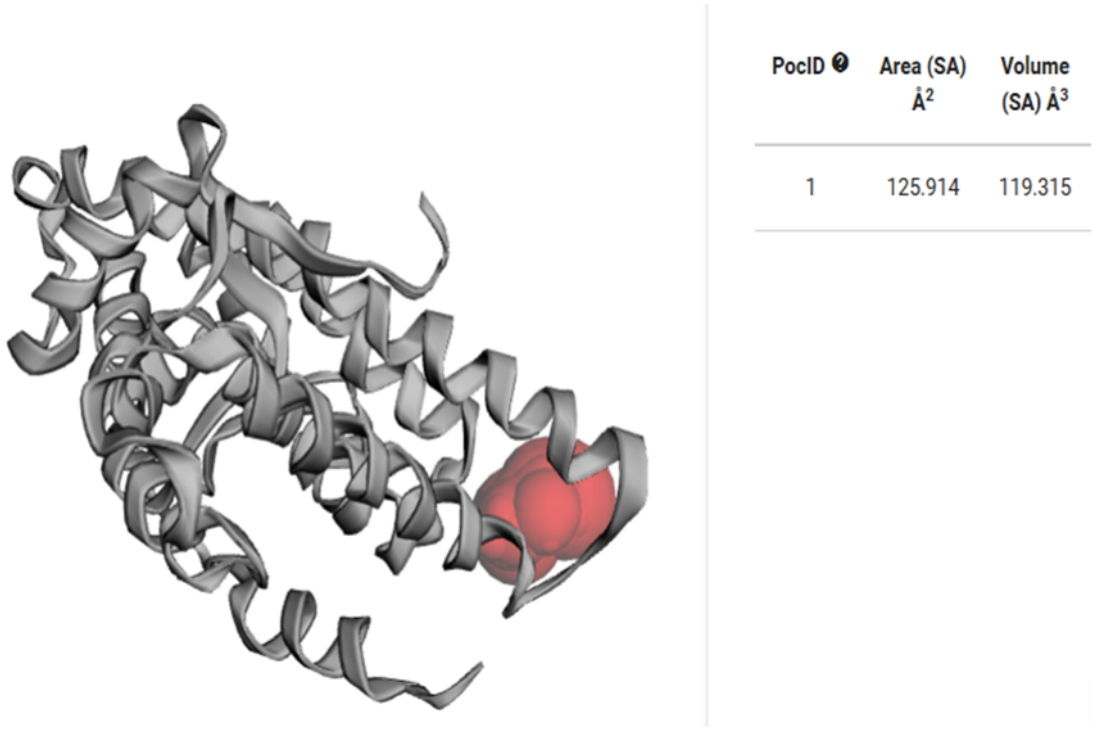
Druglikeness	**Results**
Lipinski	Yes; 1 violation: MW>500
Ghose	No; 3 violations: MW>480, MR>130, #atoms>70
Veber	No; 2 violations: Rotors>10, TPSA>140
Egan	No; 1 violation: TPSA>131.6
Muegge	Yes
Bioavailability Score	0.56

TABLE 4.15: Toxicity of Clomiphene citrate.

Toxicity	**Prediction**
Hepatotoxicity	×
Carcinogenicity	×
Immunotoxicity	✓
Mutagenicity	×
Cytotoxicity	×

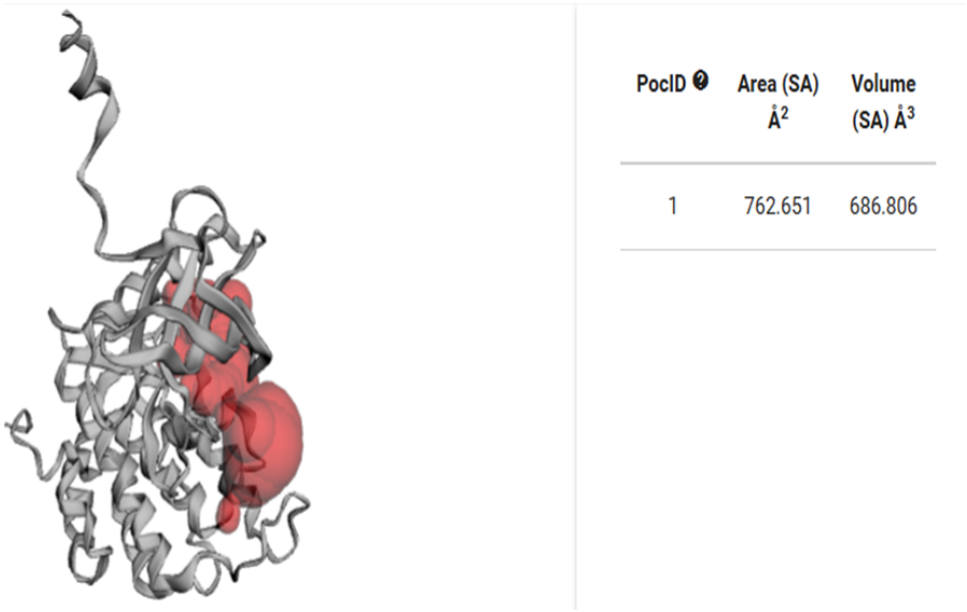
4.4 Active Site Determination

The active sites of the target proteins the androgen receptor (Figure 4.9), the insulin receptor (Figure 4.10), and tumor necrosis factor-alpha (Figure 4.11) have been identified with Castp (http://sts.bioe.uic.edu/).



PocID	Area (SA) Å^2	Volume (SA) Å^3
1	125.914	119.315

FIGURE 4.9: Active site of AR.



PocID	Area (SA) Å^2	Volume (SA) Å^3
1	762.651	686.806

FIGURE 4.10: Active site of IR.

PocID	Area (SA) Å^2	Volume (SA) Å^3
1	2743.256	4514.068

FIGURE 4.11: Active site of TNF-α.

4.5 Molecular Docking

Molecular docking is a key computational technique used to predict how two or more molecules, such as a drug and its target protein, interact to form a stable complex. This method helps estimate the most favorable three-dimensional binding conformations between ligands and receptors. By generating multiple binding

poses, docking algorithms evaluate and rank these interactions using scoring functions, ultimately identifying the most likely biologically relevant configurations [200].

In this study, both the selected phytochemicals and the standard drug were docked against the identified target proteins using CB-Dock (http://cao.labshare.cn/cb-dock/), an automated docking tool that predicts binding sites and performs blind docking. The resulting ligand–protein complexes were further analyzed and visualized using BIOVIA Discovery Studio Visualizer, which provides detailed insights into molecular interactions such as hydrogen bonding, hydrophobic contacts, and binding affinities.

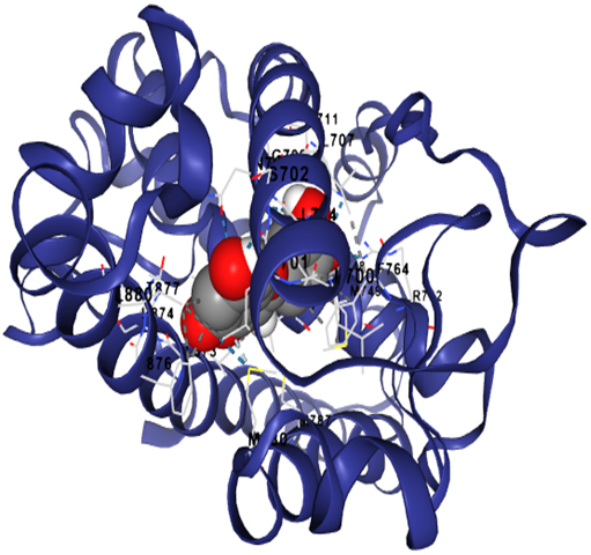
4.5.1 Target Proteins and Ligand

The selected target proteins are AR, IR, and TNF-α that are docked with Quercetin.

The molecular docking results reveal that quercetin exhibits the strongest binding affinity with the androgen receptor at Pocket C2, with a Vina score of -8.8 kcal/-mol. This score suggests a highly favorable interaction, as docking scores below -7.0 kcal/mol are generally considered favorable, while scores below -8.0 kcal/mol indicate very strong binding. Among the identified pockets, Pocket C2 stands out not only for its superior binding affinity but also for its structural suitability, with a cavity volume of 436 $Å^3$, indicating a reasonably spacious and well-defined pocket capable of effectively accommodating the ligand.

Notably, quercetin interacts with several key amino acid residues within Pocket C2, including LEU701, SER702, LEU704, ASN705, LEU707, GLY708, GLN711, TRP741, MET742, MET745, VAL746, PHE747, ALA748, MET749, GLY750, ARG752, TYR763, PHE764, ALA765, MET780, GLN783, MET787, GLU872, LEU873, HIS874, PHE876, THR877, LEU880, VAL889, PHE891, MET895, and ILE899. These residues contribute to a stable and favorable interaction through hydrogen bonding, hydrophobic contacts, and van der Waals interactions.

Other pockets, such as C1 and C4, showed Vina scores of -7.8 and -6.9 kcal/-mol, respectively, suggesting moderate to good interactions. However, Pocket C2 clearly represents the most promising binding site for quercetin on the androgen receptor, based on both energetic and spatial considerations (Figure 4.12).



CurPocket ID	Vina score	Cavity volume (Å^3)	Center (x, y, z)	Docking size (x, y, z)
C2	-8.8	436	0, 31, 5	21, 21, 21
C1	-7.8	830	7, 19, 6	21, 21, 21
C4	-6.9	300	20, 33, 9	21, 21, 21
C3	-6.6	350	-3, 36, 27	21, 21, 21
C5	-5.7	275	-7, 27, 20	21, 21, 21

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FIGURE 4.12: Molecular Docking of AR and Ligand

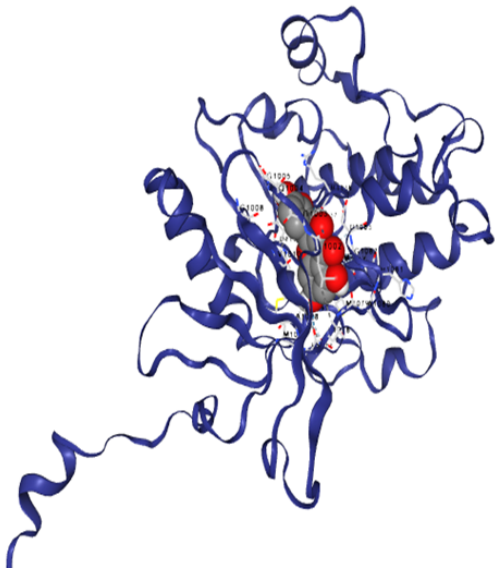
The molecular docking analysis of Insulin Receptor with ligand revealed that Pocket C3 exhibited the strongest binding affinity, with a Vina score of -7.7 kcal/-mol and a cavity volume of 352 Å^3, indicating a well-defined and favorable site for ligand accommodation. However, Pocket C1 closely follows, displaying a Vina score of -7.6 kcal/mol with a significantly larger cavity volume of 797 Å^3. This suggests that while both pockets offer strong interactions, Pocket C3 may provide a more compact and tighter binding environment, whereas Pocket C1 may allow greater ligand flexibility due to its size.

Importantly, Pocket C1 involves interactions with several key residues in Chain A of the target protein, including:HIS1057, HIS1058, ARG1101, THR1105, GLN1107, GLU1108, MET1109, GLN1111, MET1112, GLU1115, ASP1143, PHE1144, THR1145, VAL1146, ASP1266, LEU1267, HIS1268, SER1270, PHE1271, and VAL1274.

These residues represent a diverse combination of polar, charged, and hydrophobic amino acids, indicating the possibility of multiple interaction types-such as

hydrogen bonding (e.g., via GLN, GLU, ARG, HIS), π-π stacking or hydrophobic interactions (e.g., PHE, VAL, MET), and electrostatic interactions (e.g., ASP, GLU, ARG). The presence of aromatic residues like PHE1144 and HIS1057/1058, along with charged residues such as ARG1101 and GLU1108, may enhance ligand stabilization through π-cation or hydrogen bond interactions.

While Pocket C3 remains the top candidate based on binding affinity alone, Pocket C1 is also a highly favorable site, not only due to its strong score but also due to the rich interaction profile provided by its constituent residues. These factors make Pocket C1 a compelling alternative or complementary binding site for the ligand (Figure 4.13).



CurPocket ID	Vina score	Cavity volume (Å^3)	Center (x, y, z)	Docking size (x, y, z)
C3	-7.7	352	28, 4, 66	21, 21, 21
C1	-7.6	797	21, -14, 64	21, 21, 21
C4	-6.8	325	22, 0, 73	21, 21, 21
C5	-6.0	268	21, 16, 51	21, 21, 21
C2	-5.7	765	27, 19, 69	21, 21, 21

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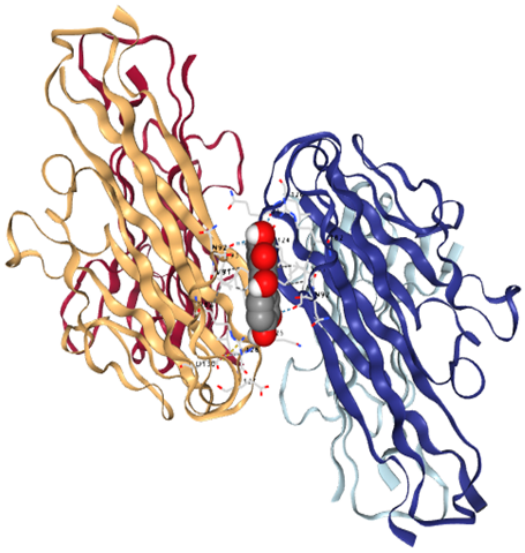
FIGURE 4.13: Molecular docking of IR and Ligand

Molecular docking analysis of TNF-α identified Pocket C1 as the most favorable binding site for the ligand (Figure 12), with a Vina score of -8.5 kcal/mol. This score suggests a very strong binding affinity, surpassing the commonly accepted threshold of -8.0 kcal/mol for highly favorable ligand–protein interactions. In addition to this strong binding energy, Pocket C1 features the largest cavity volume (1263 Å^3) among all identified pockets, indicating a spacious and structurally accommodating binding environment. The ligand interacts with residues from both Chain B and Chain D of the target protein. Specifically, the contact residues in

Chain B include: GLU53, GLY54, LEU55, ARG82, TYR87, VAL91, ASN92, LEU93, LEU94, SER95, PHE124, GLN125, LEU126, GLU127, and ASP130. In Chain D, overlapping residues involved in the interaction include: ARG82, VAL91, ASN92, LEU93, LEU94, SER95, PHE124, GLN125, LEU126, GLU127, and ASP130.

These residues reflect a diverse set of interactions, combining polar (GLU, ASP, ASN, SER, GLN), non-polar (LEU, VAL, PHE, TYR), and charged residues (ARG, GLU, ASP). Such a composition enables the formation of multiple types of non-covalent interactions, including: Conventional hydrogen bonds (e.g., with GLU, ASN, SER, GLN), Electrostatic interactions (e.g., involving ARG82, GLU127, ASP130) and Hydrophobic contacts (e.g., through LEU93, VAL91, PHE124, TYR87). The presence of aromatic residues like TYR87 and PHE124 may also support π-π stacking or π-cation interactions, further stabilizing the ligand within the pocket (Figure 4.14).



CurPocket ID	Vina score	Cavity volume (Å^3)	Center (x, y, z)	Docking size (x, y, z)
C1	-8.5	1263	-5, 82, 28	21, 21, 21
C3	-7.6	244	-17, 72, 36	21, 21, 21
C5	-7.4	232	-12, 69, 18	21, 21, 21
C2	-7.2	268	8, 67, 12	21, 21, 21
C4	-5.4	233	-11, 71, 2	21, 21, 21

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FIGURE 4.14: Molecular docking of TNF-α and Ligand

4.5.2 Target Proteins and Drug Interaction

To investigate the potential therapeutic mechanism of Clomiphene citrate in the management of Polycystic Ovary Syndrome (PCOS), molecular docking was conducted with three key receptor proteins: AR, IR and TNF-α .These receptors play

crucial roles in regulating ovarian function and hormonal balance, both of which are commonly disrupted in PCOS.

Clomiphene citrate, a well-established ovulation-inducing agent, was docked against the active sites of AR, IR and TNF-α using the CB-Dock platform. The docking analysis aimed to predict the binding affinity and molecular interactions between the drug and these targets. The docking scores, along with visual inspection of binding modes using BIOVIA Discovery Studio Visualizer, helped identify key residues involved in ligand-receptor interactions. These findings contribute to understanding the molecular basis of clomiphene citrate's therapeutic efficacy and its relevance in restoring reproductive function in PCOS patients.

The molecular docking analysis of AR identified Pocket C1 as the most favorable binding site for the ligand, with a Vina score of -7.8 kcal/mol and a large cavity volume of 830 $Å^3$, indicating strong binding affinity and sufficient space for ligand accommodation. In contrast, other pockets showed weaker interactions, with C4 (-5.9), C3 (-5.6), C5 (-5.5), and C2 (-3.5) kcal/mol, respectively. The ligand in Pocket C1 interacts with 25 key residues in Chain A: GLU678, ALA679, GLU681, PRO682, GLY683, VAL684, VAL685, GLN711, HIS714, VAL715, LEU744, MET745, ALA748, TRP751, ARG752, THR755, ASN756, TYR763, PHE764, PRO766, PRO801, GLN802, PHE804, LEU805, and LYS808. These residues contribute to various stabilizing interactions, including hydrogen bonding, hydrophobic contacts, electrostatic interactions, and π-π stacking, supporting the stability and specificity of the ligand–protein complex and highlighting Pocket C1 as the most promising site for further investigation (figure 4.15).

Whereas , the docking results of IR identified Pocket C1 as the most favorable binding site, with a Vina score of -6.2 kcal/mol and a cavity volume of 797 $Å^3$, indicating moderate binding affinity and sufficient space for ligand accommodation. Compared to other pockets-C3 (-6.0 kcal/mol), C4 (-5.7 kcal/mol), C2 (-5.6 kcal/mol), and C5 (-5.5 kcal/mol)-Pocket C1 exhibits the best combination of energy and volume, making it the most promising among the five. The ligand in Pocket C1 interacts with key residues in Chain A, including PRO1099, ARG1101, PRO1102, THR1105, GLN1107, GLU1108, GLN1111,

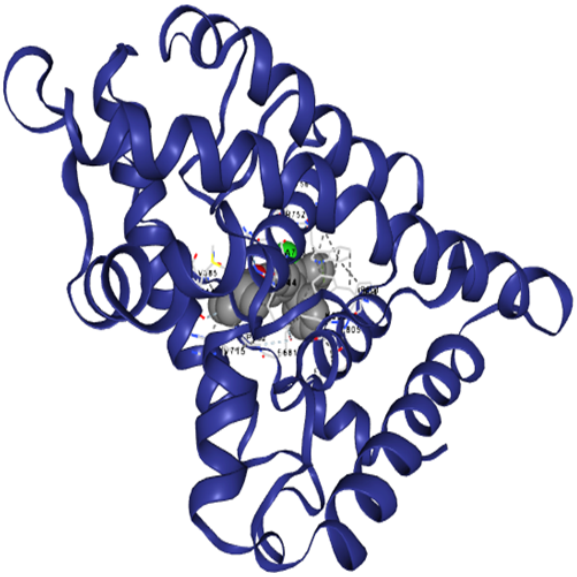
MET1112, GLU1115, ASP1143, PHE1144, THR1145, VAL1146, ASP1265, ASP1 266, LEU1267, HIS1268, PRO1269, and SER1270.

These residues facilitate a variety of interactions such as hydrogen bonding (GLN1 107, GLU1108, SER1270), electrostatic interactions (ARG1101, ASP1143, ASP1265 / 1266), and hydrophobic contacts (MET1112, PHE1144, VAL1146). The presence of polar, charged, and non-polar residues suggests a well-balanced and stable ligand–protein interface, further supporting Pocket C1 as a key target site for future structural or functional investigations (figure 4.16).

In contrast, the molecular docking analysis of TNF-α revealed that Pocket C3 exhibits the strongest binding affinity among all identified sites, with a Vina score of -7.6 kcal/mol and a cavity volume of 244 $Å^3$, indicating a compact yet energetically favorable binding pocket. Although Pocket C1 has a significantly larger cavity (1263 $Å^3$), its binding score is slightly weaker (-7.1 kcal/mol), suggesting that Pocket C3 may offer a more specific and tighter binding environment. The ligand in Pocket C3 interacts with residues from multiple chains of the target protein.

In Chain A, the interacting residues include HIS15, LEU57, ILE58, TYR59, SER60, GLN61, LEU63, PRO117, TYR119, LEU120, GLY121, GLY122, GLN149, TYR151, ILE155 and LEU157. In Chain B, interactions are formed with LEU57, TYR59, SER60, GLN61, LEU94, SER95, ALA96, TYR119, LEU120, GLY121, GLY122, VAL123, TYR151, ILE155, and LEU157, while Chain D contributes residues such as GLY54, LEU55, LEU57, GLY122, VAL123, GLN125, and LEU157. These residues represent a combination of polar (SER, GLN, HIS), hydrophobic (LEU, ILE, VAL, PRO), and aromatic (TYR) amino acids, supporting a range of stabilizing interactions such as hydrogen bonding, hydrophobic effects, and π–π stacking.

The convergence of similar contact residues across three chains suggests a conserved and well-structured binding pocket, making Pocket C3 the most promising site for stable ligand–protein interaction and a potential target for therapeutic development (figure 4.17).



CurPocket ID	Vina score	Cavity volume (Å^3)	Center (x, y, z)	Docking size (x, y, z)
⊙C1	-7.8	830	7, 19, 6	24, 24, 24
○C4	-5.9	300	20, 33, 9	24, 24, 24
○C3	-5.6	350	-3, 36, 27	24, 24, 24
○C5	-5.5	275	-7, 27, 20	24, 24, 24
○C2	-3.5	436	0, 31, 5	24, 24, 24

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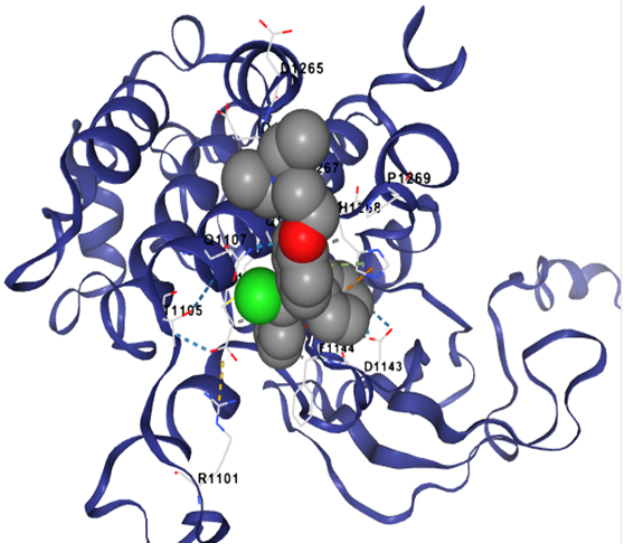
FIGURE 4.15: Molecular docking of AR and Clomiphene citrate.



CurPocket ID	Vina score	Cavity volume (Å^3)	Center (x, y, z)	Docking size (x, y, z)
⊙C1	-6.2	797	21, -14, 64	24, 24, 24
○C3	-6.0	352	28, 4, 66	24, 24, 24
○C4	-5.7	325	22, 0, 73	24, 24, 24
○C2	-5.6	765	27, 19, 69	24, 24, 24
○C5	-5.5	268	21, 16, 51	24, 24, 24

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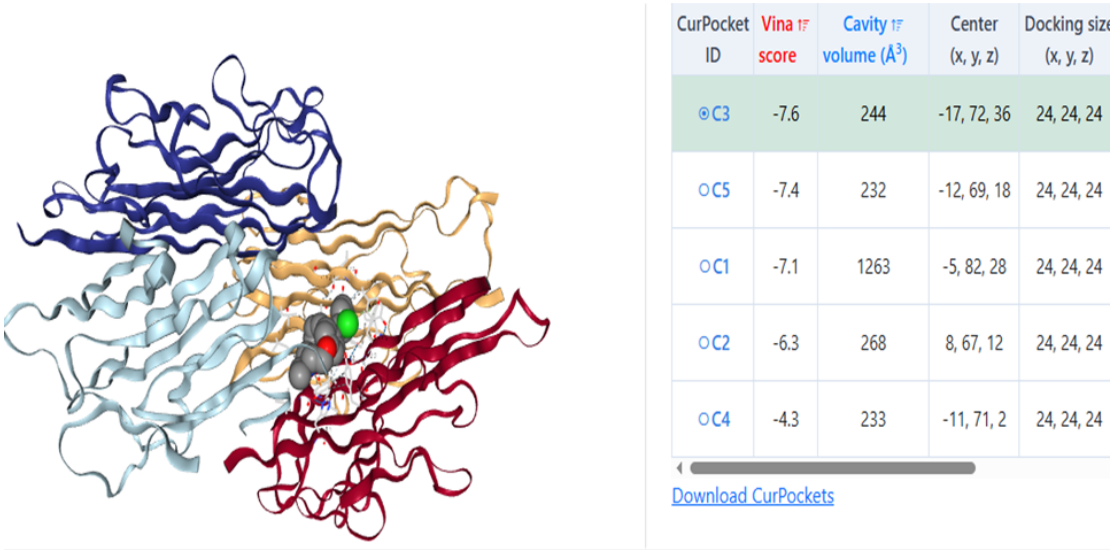
FIGURE 4.16: Molecular docking of IR and Clomiphene citrate.



CurPocket ID	Vina score	Cavity volume ($Å^3$)	Center (x, y, z)	Docking size (x, y, z)
C3	-7.6	244	-17, 72, 36	24, 24, 24
C5	-7.4	232	-12, 69, 18	24, 24, 24
C1	-7.1	1263	-5, 82, 28	24, 24, 24
C2	-6.3	268	8, 67, 12	24, 24, 24
C4	-4.3	233	-11, 71, 2	24, 24, 24

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FIGURE 4.17: Molecular docking of TNF-α and Clomiphene citrate.

4.6 Lead Compound

The ligand quercetin has emerged as the lead compound in this study. It demonstrates favorable pharmacokinetic characteristics alongside a lack of predicted toxicity, reinforcing its potential for safe therapeutic use. Furthermore, molecular docking analyses confirm that quercetin exhibits strong binding affinity to the active sites of the androgen receptor (AR), insulin receptor (IR), and tumor necrosis factor-alpha (TNF-α), suggesting effective molecular interaction and target modulation.

Quercetin's pharmacokinetic profile indicates promising behavior in terms of absorption, distribution, metabolism, and excretion (ADME), which are essential parameters for drug efficacy and bioavailability. The absence of toxicity further supports its suitability for clinical development, minimizing the risk of adverse effects in therapeutic applications.

The robust molecular interactions observed between quercetin and the active binding sites of AR, IR, and TNF-α underscore its potential role in regulating pathways associated with hormonal signaling, insulin sensitivity, and inflammation. These findings highlight quercetin's relevance in the context of complex metabolic and endocrine disorders.

In summary, quercetin stands out as a promising lead compound due to its favorable pharmacokinetic profile, non-toxic nature, and strong binding affinity to AR, IR, and TNF-α, making it a noteworthy candidate for further exploration in drug development and related biomedical research.

Chapter 5

Conclusion and Recommendations

5.1 Conclusion

This study has provided meaningful insights into the therapeutic potential of *Ginkgo biloba* in the management of Polycystic Ovary Syndrome (PCOS), utilizing an *in silico* approach. The primary goal was to assess the interaction between *Ginkgo biloba* bioactives and key PCOS-associated proteins—Androgen Receptor (AR), Insulin Receptor (IR), and Tumor Necrosis Factor-alpha (TNF-α)—to explore novel treatment possibilities. The objectives focused on identifying active phytoconstituents, evaluating their pharmacokinetic and toxicity profiles, analyzing their binding conformations with target proteins, and benchmarking them against a reference drug to propose a lead candidate.

Through computational screening, the flavonoid quercetin, a major constituent of *Ginkgo biloba*, was identified as the lead compound. Quercetin demonstrated strong binding affinities to AR, IR, and TNF-α, alongside favorable pharmacokinetic characteristics and an acceptable safety profile. This fulfills the objective of identifying a potent bioactive compound with potential therapeutic effects against PCOS.

Detailed molecular docking analysis revealed quercetin's effective and distinct binding interactions at the active sites of the selected proteins. These interactions suggest its ability to modulate androgen activity, improve insulin signaling, and attenuate inflammatory responses—key aspects of PCOS pathophysiology. This addresses the second objective of evaluating binding conformations with relevant targets.

Comparative evaluation with the reference drug, Clomiphene citrate, revealed that quercetin offers a unique pharmacological profile, positioning it as a promising natural alternative. While Clomiphene is a well-established treatment for ovulatory dysfunction, quercetin's multi-targeted activity may offer a more holistic therapeutic approach. This meets the third objective of comparison and lead compound selection.

Despite the promising results, the study acknowledges certain limitations. Computational predictions, while informative, require further validation. Future research should include in vitro and in vivo studies, clinical assessments, and optimization of quercetin derivatives for enhanced efficacy. Exploring combination therapies, mechanism-based studies, and patient stratification could further strengthen the clinical applicability of these findings.

In comparison with other herbal candidates studied for PCOS—such as Asparagus racemosus and Asparagus officinalis, which have shown encouraging outcomes in enhancing ovulatory function and hormonal balance—*Ginkgo biloba* adds value as a rich source of flavonoids with proven antioxidant and anti-inflammatory actions. Given its distinct molecular interactions and systemic benefits, *Ginkgo biloba* holds strong potential as part of an integrative strategy for PCOS management.

In conclusion, this thesis not only highlights the therapeutic promise of *Ginkgo biloba* and its key constituent quercetin but also establishes a foundation for future research aimed at developing effective, plant-based alternatives for PCOS treatment. The insights gained here contribute meaningfully to the growing body of evidence supporting natural compounds as complementary tools in addressing complex endocrine disorders.

5.2 Future Prospective

This study lays a strong foundation for future research exploring the therapeutic potential of *Ginkgo biloba* in the management of Polycystic Ovary Syndrome (PCOS). While the current *in silico* findings are encouraging, they represent an initial step in a broader research trajectory. Expanding molecular docking analyses to include additional PCOS-relevant targets—such as inflammatory cytokines, oxidative stress markers, and steroidogenic enzymes—could provide a more comprehensive understanding of *Ginkgo biloba*'s therapeutic scope.

Future work should also focus on in vitro and in vivo validation of the lead compound quercetin, confirming its efficacy, bioavailability, and safety in biological systems. Optimizing quercetin's pharmacological properties through structural modifications or nanoformulations may enhance its therapeutic potential. Moreover, combining quercetin with existing PCOS treatments could offer synergistic effects and reduce side effects associated with conventional drugs. Investigating the molecular mechanisms through which quercetin influences hormonal balance, insulin signaling, and inflammation would deepen mechanistic understanding and support personalized medicine strategies. Tailoring interventions based on individual metabolic or genetic profiles may increase treatment success.

Interdisciplinary collaboration—integrating molecular biology, pharmacology, gynecology, and bioinformatics—will be essential to translate these findings into practical healthcare applications. Ethical considerations, particularly in clinical research and botanical drug development, must remain a priority to ensure responsible and transparent scientific progress. Ultimately, continued research on *Ginkgo biloba* and its bioactive constituents like quercetin could reshape current PCOS management strategies, contributing to safer, more effective, and holistic treatment options.

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