CAPITAL UNIVERSITY OF SCIENCE AND TECHNOLOGY, ISLAMABAD

Comparative *Insilico* Analysis of Fenugreek and Flaxseed for Drug Development in Polycystic Ovary Syndrome

by

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Dedicated to ALLAH Almighty, Hazrat Muhammad (PBUH), my parents, my brothers Shahyan Tariq and Sharaiz Hussain, my respected teachers for their encouragement, guidance, motivation during my research work and supporting me spiritually throughout my life.

CERTIFICATE OF APPROVAL

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Abstract

The aim of this study was to evaluate the in-silico potential of different ligands obtained from flaxseed and fenugreek which successfully act as a therapeutic agent against PCOS. Therefore, we focused on proteins and ligands, which play a significant role in structural drug design against polycystic ovarian syndrome (PCOS). It is associated with many metabolic disorders and is being characterized by many stalled follicles in the ovaries, anovulation and a hormonal imbalance. PCOS is often associated with androgen excess and a cluster of other morbidities. These morbidities may include insulin resistance, type II diabetes, obesity, cancer, cardiovascular disease and Depression etc. There are many herbal remedies that are used today to treat polycystic ovary. *Trigonella foenum-graecum* (Fenugreek) is an annual plant of family *Fabaceae*. The flaxseed is another commonly found seed usually known as *Linum usitatissiumum* which is rich in linolenic acid and carbohydrates. These plants are rich source of bioactive compounds. The need of the hour is to identify natural potent inhibitors or plant-based drugs essential for the treatment of PCOS. LHB Protein was selected as the target protein primarily causing PCOS and alkaloids, cadmium, lignans, linatine, linoleic acid, cyanogenic glycosides, 4-Hydroxyisoleucine, diosgenin, galactomannan, quercetin, scopoletin, trigonelline were selected as a ligand for the current study. Molecular Docking was used to estimate the strength of a bond between a ligand and a target protein through a special scoring function and to determine the correct structure of the ligand within the target binding site. The 2D structure of the target protein and the ligands was taken as the input for docking. The docking was performed using LHB protein and ligands alkaloids, cadmium, lignans, linatine, linoleic acid, cyanogenic glycosides, 4-Hydroxyisoleucine, diosgenin, galactomannan, quercetin, scopoletin, trigonelline. The best ligands were selected on the basis of best docking score, log p value, hydrogen bond acceptor, hydrogen bond donor and molecular weight. Linatine, linoleic acid, Quercetin, Scopoletin, Trigonelline show the best physiochemical properties. The selection of most efficient PCOS drug was based on the physiochemical and ADMET properties along mechanism of action with side effects. Therefore, Letrozole was selected as a best PCOS drug as compare to

other drugs such as clomiphene's citrate and metformin. The comparison between Linatine, linoleic acid, Quercetin, Scopoletin, Trigonelline and drug Letrozole help us to identify the better treatment for PCOS diseases. Comparison was being performed through parameters like; ADMET properties and physiochemical properties. So, it is determined that Quercetin is bioactive compound which shows us better result over Letrozole according to comparison. All the software's and tools used in the current research study are reliable and authentic.

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Abbreviations

ACTH : Adrenocorticotropic hormone

AhR : Aryl hydrocarbon receptor

ALA : Alpha linolic acid

AMPK: Adenosine monophosphate activated kinase

CC : Clomiphene Citrate

CG : Cyanogenic glycosides

CM: Complementary Medicine

COX : Cytokines cyclooxygenase

CVD : Cardiovascular disease

DM : Diabetes militias

DS : Discovery studio

FSH : Follicle stimulating hormone

GPH : Glycoprotein Hormone

GWAS: Genome wide association

HPO : Hypothalamus Pituitary ovarian

IR : Insulin resistance

IGFBP: Insulin like growth factor Binding protein

LH : luteinizing hormone

L/HBW: Low/ High Birth weight

LHB : Luteinizing hormone beta subunit

MBDs: Methyl -CpG -Binding protein

OS : Oxidative stress

OV : Ovulatory dysfunction

PCOS: Polycystic ovary syndrome

PPAR: Peroxisome proliferate activated receptor

PDB: Protein databank

PD : Patch dock

PUFA: Polysaturated fatty acid

SDG : Secoisolariciresinol diglycoside

SHBG : Sex hormone binding globulin

SNPs : Single nucleotide polymorphism

VEGF : Vascular Endothelial growth factor

WOI : Window of Implantation

Chapter 1

Introduction

In women polycystic ovarian syndrome (PCOS) is most chronic endocrinopathy, which affect 5-7% of women of reproductive age. Persistent anovulation and Hyperandrogenism are the characteristics of PCOS and its morbidities might such as high blood sugar, IR, T2D mellitus at an early stage, dyslipidemia, cardiovascular disease and infertility [1]. Ovulatory disfunction (that contains menstrual dysfunction), hyperandrogenism (i.e. Indication of excess male hormone or androgen effect for e.g. clinically such as hirsutism or biochemically such as hyperandrogenemia or excess levels of androgen) and polycystic ovarian morphology PCOMS an excessive number of preantral follicles in the ovaries) are all features of syndrome [2]. Another symptom of polycystic ovarian syndrome, which is usually related to obesity and IR and causes hyperinsulinemia conditions in 30% to 80% of lean women and 80% of obese women is a high LH/FSH ratio [3] progression of PCOS [4]. The underlying causes of PCOS are yet unclear because the disorder is diverse by nature. There is significant evidence that the syndrome has a significant genetic component but environmental factors, particularly diet, are also essential [5]. Metabolic disorder including insulin resistance (IR) and obesity are intimately related to PCOS. The majority of PCOS patient have Obesity or overweight, IR and compensatory hyperinsulinemia. The majority of skinny women with PCOS also exhibit the IR and hyperinsulinemia metabolic traits, which should be emphasized. In addition to cell dysfunction, hyperinsulinemia

raises the chance of getting type 2 diabetes (T2D), hypertension, dyslipidemia and cardiovascular conditions. Another important factor in emergence of several phenotypic characteristics of PCOS is hyperinsulinemia [6].

PCOS can cause wide range of problems in women because of harmful impact on the reproductive, endocrine, metabolic and psychological systems [7]. Pathophysiology of PCOS is not fully understood. In Recent studies the abnormal adrenocortical function elevated sympathetic nerve activity and abnormalities with the hypothalamus pituitary ovarian axis all contribute to the Hyperandrogenism is associated with elevated blood levels of free testosterone, a key hormone influencing the pathophysiology of PCOS. These sophisticated disorders analyzed and improve elements are degraded. Genetics, neuroendocrine lifestyle/environment, obesity and some other risk factors all contribute to occurrence of polycystic syndrome [8]. Numerous studies has shown that PCOS may be influences by environmental factors, prenatal hormone imbalance, lifestyle, decisions, and genetic abnormalities [9]. The gonadotropic releasing hormone is produced or secreted by luteinizing hormone LH and FSH. Smaller doses of intraovarian androgen are utilized for normal follicular growth. Testosterone is converted to oestrogen in granulose cells more quickly as a result of FSHs activation of the aromatase enzyme and follicular development. LH causes oocyte maturation by promoting theca cell development. LH levels increase while FSH levels decrease when PCOS is present which causes an increase in androgen production, a reduction in the amount of aromatase enzyme, and the development of immature follicles. Hyperinsulinemia and dyslipidemia are related to abdominal fat that is connected to high androgen in PCOS to elevate testosterone levels in blood, hyperinsulinemia increases androgen production in the theca cells while lowering sex hormone binding globulin (SHBG) [10].

Despite the fact that the precise origin of PCOS is uncertain, genetics may play a crucial role. Some ideas suggest that women with PCOS may have acquired the syndrome. Main problem is imbalance of hormone which occur when ovaries produce higher androgens than normal. Excess level of androgens in women affect the development and release of egg during ovulation. Additionally, PCOS women

over produce insulin and this insulin appears to raise the androgen level [11]. Cyst formation in PCOS is due to stopped follicular expansion as a result of disturbed steroidogenesis and a high androstenedione to estradiol ratio.CYP11A1 catalyze the rate limiting step of ovarian steroidogenesis, which include concerting cholesterol into pregnenolone [12].

Several medication including as metformin, clomiphene citrate, glucocorticoids and aromatase inhibitors like anastrozole can be used to treat PCOS. Numerous negative effect such as nausea discomfort in the abdomen and vaginal bleeding have been reported. Because of these certain civilizations now have more intriguing, priceless and convenient ways to apply herbal remedies for testing or even recovering from illness. There are many different types of natural substances that may be found in plants some of which have little estrogenic or antiestrogenic action. These chemicals sometime referred to as phytoestrogen include a number of isoflavonoides, flavonoids, stilbenes and lignans. The two dietary phytoestrogen that have been the subject of greatest investigation are soy isoflavones and flaxseed lignans [13].

A detail genetic history, the exclusion of other hyperandrogenic causes and the requisite laboratory tests should all be done in order to identified PCOS in adolescents. There aren't many controlled clinical trials hence the efficacy of the treatment is in controversy. Therapeutic options include dietary adjustments, oral contraceptives, and insulin sanitizers as example. Long term follow up is crucial to evaluate whether these strategies are successful in influencing the natural history of the reproductive and metabolic mechanism [14]. There have been numerous different therapeutic strategies adopted to control PCOS. The use of medication such as clomiphene citrate, tamoxifen, aromatase inhibitors, glucocorticoids, metformin, and performing laparoscopic ovarian drilling surgery are all possible forms of treatment. However, there is still a great deal of dispute over PCOS treatment option [15]. It is believed to be challenging to Identify PCOS in adolescent, premenopausal and postmenopausal women sine the precise features of PCOS and their combination as diagnostic criteria have not been identifying. Infertility is only increased by PCOS when oligoovulation or anovulation occur according to

revised recommendations. It is still unclear how PCOS patient who have normal ovulatory activity reproduce. Cohort studies tend to show that women with PCOS have the same reproductive potential as women without PCOS toward the end of their reproductive lifetimes [16]. Estrogen has a major role in the hormonal function of all vertebrates. Only steroidal compounds can be identified in animal estrogens with 17 estradiol acting as the majority of species primary physiological oestrogen. Numerous plants generate chemical called phytoestrogens that act as estrogens in animals. Depending on how precisely the term estrogenic activity is applied there will be different standards for what qualifies as a phytoestrogen. Even though no one set of tests can be used to evaluate all potential estrogens, evaluation of an oestrogen must take into consideration both oestrogen receptor binding and biological availability in the test species. To determine phytosterogenic activity it is therefore important to combine in vitro and in vivo experiments that produce dose response correlations [17].

Women with PCOS appear to be protected from metabolic and hormonal abnormalities by a family of plant derived compounds known as phytoestrogens [18]. The oestrogen receptor is more efficiently associated with phytoestrogens. This property allows them to mediate estrogen-like effects on ER-expressing cells, however less effectively than mammalian estrogens. Phytoestrogens can have both estrogenic and antiestrogenic effects, depending on the amount of endogenous oestrogen present. Antioxidant properties, prevention of aromatase, inhibition of protein tyrosine kinases, competition with estradiol for the nuclear type II estrogen-binding site, stimulation of SHGB production (=decrease the relative volume of free estradiol), and inhibition of angiogenesis appear to be factors that may affect their potential anticarcinogenic potency [19].

Plants contain natural compound called phytoestrogens that resemble oestrogen. Phytoestrogens can bind to oestrogen receptors in the reproductive organs with a substantially lower affinity than naturally occurring estrogens but they are usually non-steroidal compounds and can either simulate or counteract the impacts of endogenous estrogens [20]. Phytoestrogens including flavonoids, isoflavones, coumestans, and lignans are found in a wide variety of plants. These chemicals can all

interact with the aryl hydrocarbon receptor, peroxisome proliferator-activated receptor (PPAR), and oestrogen receptor (AhR). Phytoestrogens display a number of noteworthy effects, including upregulation of the pro-inflammatory cytokines cyclooxygenase (COX)-2 and inducible nitric oxide synthase, Downregulation of these cytokines, Activation of antioxidant genes through KEAP, and Inhibition of adipogenesis by Increasing the Signaling Cascades of Adenosine Monophosphate Activated Kinase (AMPK) [21].

Phytoestrogens refer to a variety of non-steroidal compounds, including prenylated flavonoids, isoflavones, coumestans and lignans. They are present in a wide variety of plants. These substances are able to bind to the aryl hydrocarbon receptor, the PPAR family of receptors, the oestrogen receptors alpha and beta, and the peroxisome proliferator-activated receptor (PPAR) family of receptors (AhR).There is abundant evidence that phytoestrogens may alleviate the symptoms of the metabolic syndrome. Women have utilized complementary medicine (CM) at rates ranging from 26% to 91% over the past decade, which is an increase over the preceding decade. Herbal medicine is a well-liked component of CM. Herbal treatments are acknowledged to have pharmacologically active ingredients that have physiological effects on a woman's endocrinology. They have also been favorably associated to a diminution in the prevalence of breast cancer, osteoporosis, and cardiovascular disease [22]. India is the main consumer of fenugreek for both culinary and medicinal uses. In manufacturing soups and pan cakes, fenugreek seeds are also utilized in relatively higher amounts as a spice and flavoring. It is a stomach stimulant that the traditional Indian medical system has recognized to really be effective against anorexia [23].

Fenugreek (*Trigonella foenum-graecum*) seed extract supplemented with furostanolic saponins was tested on 50 premenopausal women (ages 18 to 45, BMI of 23.88 4.72 kg/m2, PCOS diagnosis) in an open label, one arm, non-randomized, post-marketing monitoring analysis (Furocyst). The research was done over the period of three months. The volume of the ovaries, the number of ovarian cysts, and other significant considerations were analyzed medically to see if they had transformed. In order to cure chronic illnesses, more individuals across the world are turning to

complementary and alternative medicines (CAMs), a distinct kind of treatment that utilizes herbs. Fenugreek, also known as Trigonella foenum-graecum, is a wild plant that has been used to treat hypercholesterolemia and diabetes in both human and animal studies. The fenugreek herb's active components, which include flavonoids, saponins, alkaloids, steroids, and fibers, cause the herb's hypoglycemic effects. The purpose of this study was to evaluate how fenugreek seeds affected the levels of androgen, lipids, insulin resistance, and reproductive biomarkers in PCOS caused by letrozole. Letrozole increases ovarian androgens and inhibits the aromatase enzyme by competitive binding, leading to hyperandrogenism, the disruption of the estrous cycle, polycystic ovaries, and hirsutism [24].

Flaxseed, usually known as *Linum usitatissiumum*, is rich in linolenic acid, phytosterogenic lignans (secoisolariciresinol diglycoside-SDG), and carbohydrates. Numerous studies have reported that consuming foods high in lignin boosted testosterone exertion by connecting it to enterohepatic circulation. The levels of SHBG may also rise due to lignans, which would reduce the bioavailability of free testosterone. The impact of flaxseed powder supplementation on individuals with PCOS's lipid profiles, insulin sensitivity, inflammatory markers, leptin, adiponectin levels, and monthly irregularities are not well known as far as we are aware. Flaxseed and soy are rich in lignans and isoflavones [25]. Protein makes approximately 20% to 30% of flaxseed, with globulins (linin and conlinin) responsible for almost 80% of the protein and glutelin for the remaining 20%. The nutritional profiles and amino acid composition of flaxseed and soya are comparable. Flaxseed proteins are composed mostly of arginine, aspartic acid, glutamic acid, and a little amount of lysine. The risk of cancer is decreased because flaxseed contains large amounts of antioxidants like cysteine and methionine. Peptides with bioactivities also lower the chance of developing cardiovascular disease [26].

Linseed, widely known as flaxseed or linseed (*Linum usitatissiumum L*), is a perennial or annual plant that is part of the Linaceae family and is largely exploited as an edible herb globally. High-quality protein and soluble fibre are both abundant in flaxseed. The important fatty acids alpha-linolenic acid (ALA), an omega-3 biological precursor, linoleic acid, an omega-6 fatty acid, and omega-9 essential fatty

acids are properly balanced in flaxseed oil which is mostly what provides health benefits. It has around 6% moisture. Vitamins A, B, D, and E are also present along with potassium, lecithin, magnesium, 6% mucilage, and trace amounts of the cyanogenic glycoside linamarin [27]. Flaxseeds include Phyto active chemicals such phenolic compounds, terpenoids, pigments, and other naturally occurring antioxidants. In a research by Jelodar et al., it was shown that giving 31-year-old PCOS women flaxseed supplementation (30 g/day for 4 months) reduced testosterone and increased insulin levels [28]. A 29-kD heterodimeric glycoprotein that is a distinctive feature of LH provides the biological specificity for hormone receptor binding in target tissues. The genes for the LH component are found on chromosome 19q13.32. It is crucial for encouraging follicular growth, oocyte maturation, ovulation, and the female body's generation of oestrogen. The anterior pituitary is responsible for its production and release. LH binds to and activates the lutropin receptor (LHR). On chromosome 2p21 is where the LHR gene is found. The testis, uterine, interstitial, and granulosa cells express the LH subunits' genes, which are found on chromosome 19q13.32 [29]. The testis and uterus, along with theca lutea, interstitial, and granulosa cells, all express LHR. An important biochemical feature of PCOS is a high amount of circulating LH. Luteinizing hormone hypersecretion is brought on by pulsatile LH release that is more frequent and powerful. When a result, theca cells in the ovary are stimulated to create more androgen as LH levels rise.

The bioactivity of LH and LHR may change due to microheterogeneity and genetic differences resulting in polycystic ovary. Because most pharmaceutical remedies for metabolic disorders are general, treatment for PCOS is still unsuccessful [30]. Clinical care must take genetic variants into account when considering personalized symptomatology in order to reduce the exposure of patients to medications that are useless for them.

SNPs in PCOS-vulnerable genes can be used to analyze a person's PCOS risk profile and treatment response. Investigating the polymorphism link between LH, LHR, and PCOS could therefore aid in our understanding of the disorder [31]. The anterior pituitary produces the hormone LH, a gonadotropic glycoprotein

hormone. When LH levels increase in female's ovulation and corpus luteum development are stimulated. LH promotes testosterone production and spermatogenesis in males [32]. In order to create the glycoprotein known as LH, GPH and LH subunits that are not covalently bonded together combined. The GPH subunit is shared by chorionic gonadotropin, thyroid-stimulating hormone, and follicle-stimulating hormone. The LH subunit incorporates cysteine-knot motifs, which are crucial for the development and biological activity of the heterodimer. The N-linked oligosaccharide chain is vital for the intracellular folding, secretion, metabolic clearance, and biological properties of the hormone [33].The LH subunit gene is synthesized by the basophilic gonadotropes in the anterior pituitary. Despite they co-express in gonadotropes in tetrapods, FSH and LH are synthesized in distinct cells in teleosts [34].

Docking is an *Insilco* technique that identifies the proper structure of the ligand within the target binding site and uses a particular scoring function to evaluate the strength of the connection between a ligand and a target protein. The input for docking is the target proteins' and ligands' three-dimensional structures. In order to assist in the selection of leads for the target, it is acknowledged that this innovative tiny molecular compound exhibits crucial qualities, such as strong target binding to target proteins interaction and appropriate absorption, distribution, metabolism, and excretion (ADME). Molecular docking simulates the process of identifying a target protein and its ligands at the molecular level. Additionally, it emphasizes obtaining the minimal independent energy of the entire system, which includes properly aligned proteins and ligands [34]. Patch dock, Auto Dock, CB Dock, and ICM are among the most popular docking software programs [35].

1.1 Problem Statement

A prevalent condition that influences how a woman's ovaries function is PCOS. Even if there are many therapies for PCOS problems, new herbal medicines need to be developed in order to have fewer negative effects than synthetic drugs that may

treat the condition and have minimal side effects. In ethno-medical treatments that are less likely to have negative effects than synthetic ones, plant extracts have been employed.

1.2 Aim and Objectives

The aim of this study was the in-silico evaluation of ligands that can be amicably for drug development against PCOS. To achieve the goal, we have following objectives:

1. To determine various bioactive compound of 2 ligands as potential inhibitors of PCOS.
2. To check the interaction between Target protein and the bioactive compounds obtained from two different seeds.
3. To compare the standard drug Letrozole with the ligands of flax and fenugreek seeds against Polycystic Ovary Syndrome.

Chapter 2

Review of Literature

Polycystic ovarian syndrome a common health problem in women, is caused by ovarian malfunction (PCOS). Any two of the following symptoms including irregular periods brought on by intermittent or absent ovulation, high levels of testosterone and other androgenic hormones could be signs of PCOS. A polycystic ovary is larger than normal and has a lot of follicles around the eggs [36]. In the world 6%–20% of women of reproductive age have PCOS. PCOS is a complicated endocrine condition that affects metabolic, reproductive, and mental health [37]. This condition is defined by two of its characteristics: polycystic ovaries, hyperandrogenism, and oligo/anovulation. An increase in testosterone, a decrease in sex hormone-binding globulin (SHBG), abnormal gonadotropin production additional [LH] and [FSH], and persistent low-grade inflammation are among the biochemical findings of the disease. PCOS is frequently associated with infertility, cardiovascular ailments, IR, T2D, dyslipidemia, visceral obesity, and endothelial dysfunction. As a result, this syndrome is regarded as a metabolic disorder that decreases the quality of life of women, as well as a health concern related to fertility [38]. Although PCOS is the most common cause of anovulatory infertility in women, the causes of irregular ovulation are yet unknown. The development of antral follicles in women with PCOS and anovulatory cycles is evidently stopped before the mature preovulatory stage. It aims to review the abnormalities of hormone secretion and action in anovulatory women with PCOS and how they may be related

to the mechanism of failed follicle development [39]. In their initial definition of PCOS, Stein and Leventhal recognized that women of all ages had enlarged ovaries, obesity, hirsutism, and persistent anovulation. A diagnostic criterion was developed to include hyperandrogenism and abnormal gonadotropin secretion as a result of the ability to monitor hormone concentrations. Ovarian morphology became more prominent after ultrasonography was introduced. The elevated risk of carbohydrate metabolic abnormalities in these individuals and the advancement of tools to evaluate insulin sensitivity in vivo increasing recognition of the condition's pathological conditions. Insulin resistance/hyperinsulinemia was recognized to play a role in PCOS [40]. In vitro vascular endothelial growth factor (VEGF) was first isolated from bovine pituitary follicular stellate cells and is one of the most active angiogenic factors ever discovered. A significant role is played in the occurrence and progression of various diseases by maintaining differentiation of vascular endothelial cells, stimulating microvascular permeability, etc. VEGF is intimately linked to PCOS's etiology and studies show that people with PCOS are significantly more likely to have high serum VEGF levels. The polycystic ovarian stroma also upregulates the VEGF gene which highlights its key role in PCOS pathogenesis [41].

2.1 PCOS Symptoms

PCOS can cause a number of symptoms in women including irregular menstrual cycles, excessive hair growth, infertility, and problems during pregnancy. Additionally, there is evidence that PCOS is linked to psychological problems including low self-esteem, anxiety, sadness, and body image illusions [42].

2.1.1 Clinically Hyperandrogenic Females

Hirsutism, acne, and androgenic alopecia commonly known as male pattern baldness—are symptoms of hyperandrogenism. Furthermore, according to clinical

data, PCOS is not frequently associated with virilization which includes bodily masculinization, severe or widespread male-pattern baldness, clitoromegaly etc. The most common cause of significant virilization is severe insulin resistance which is caused by mutations in the insulin receptor gene, androgen-secreting tumors and androgenic drug treatment. Rotterdam 2003 criteria identified 72.1% of 950 patients with clinical hyperandrogenism as having PCOS. There were 147 ovulatory patients (15.5% of total number of patients) and 538 anovulatory patients (56.6% of total number of patients) who were considered to have typical PCOS [43].

2.1.2 Women with Acne

Women who only suffer from acne are slightly susceptible to have PCOS (excluding those with hirsutism). 36% of 29 individuals had PCOS with treatment-resistant acne who did not have alopecia, menstrual irregularities or hirsutism [44]. There is evidence that 20% to 40% of acne patients have PCOS. Although most studies have examined the characteristics of acne patients (levels of androgen, severity of monthly disruption, polycystic ovaries on ultrasound, and so forth), they have not examined PCOS incidence using current criteria in detail. Despite this high prevalence, large population studies of acne sufferers are still required especially in individuals who do not exhibit additional signs of hyperandrogenism (such as hirsutism) [45].

2.2 Women with Menstrual and Ovulatory Dysfunction

It is considered oligomenorrhea if there are fewer than ten bleeds every year or intervals of over 35 days between menstrual cycles, whereas polymenorrhea is defined as bleeds that occur more frequently than 25 times annually [46]. The prevalence of PCOS among women with menstrual disorders can be estimated by analyzing four studies using the NIH 1990 criteria that have assessed PCOS prevalence in

the general population. According to these studies, which investigated at a group of more than 1,000 randomly selected women, menstrual dysfunction is present in 18.0% (14.6%–22.8%) of these women, a rate very similar to the 22.9% rate reported by 101,073 women participating in the Nurses' Health Study II for cycles lasting 32 days [47]. PCOS was diagnosed in 27.1% (17.4%-46.4%) of the women presenting with menstrual problems. PCOS is estimated to affect between 25 and 30% of women suffering from oligo-amenorrhea or menstrual disorders. As women with PCOS experience an increase in general sympathetic nerve activity and a decrease in vagal activity, it is thought that altered ovarian sympathetic activity may be a contributing factor to the disease. It has been found that women with PCOS have higher densities of catecholaminergic nerve fibers in their ovaries in addition to higher levels of NGF in their follicle fluid.

Additionally, it has been demonstrated in polycystic ovaries, increased ovarian sympathetic output occurs before the development of stopped follicles. Researchers have previously shown that overexpression of ovarian NGF together with sympathetic hyperactivity contributes to the development of the syndrome as well as reproductive and metabolic problems [48]. In addition to endometriosis, PCOS and unexplained infertility, oxidative stress (OS) caused by the imbalance between oxidants and antioxidants, may also be contributing factor. The use of OS can be effective in boosting androgen production, preventing the growth of ovarian follicles, and damaging ovarian tissue in patients with PCOS. The mechanism of this pathway allows even environmental factors associated with OS, such as smoking, drinking, a poor diet, and obesity, to lower fertility [49]. Health and disease of the offspring are thought to be affected by the intrauterine environment through a variety of mechanisms but epigenetic modifications in response to external stimuli seem to be the most likely mechanism.

It is believed that early trauma and hardship may permanently alter the epigenotype of germ and somatic cells, resulting in long-term effects. Covalent modifications of DNA and histones may lead to chromatin compaction and as a result gene accessibility. The 5 cytosine sequences of DNA's CpG sequences can be formylated, carboxylated, methylated, and hydroxylated. Histones can also be altered

in many different ways at their N-termini, as well as to a lesser extent at their C-termini and globular domains. In addition to histone methyl transferases and histone acetyltransferases, DNA methyltransferases and kinases, epigenetic "writers" are responsible for adding methyl or acetyl groups to the lysine and arginine residues of histone tails, DNA CpG sites, and amino acid residues in histones. Histones are reorganized as a result of these modifications being interpreted by effector proteins known as "readers" such as MBDs that bind methyl-CpG and proteins that bind histone methylation domains or acetylation domains [50]. Despite the fact that the exact cause of PCOS is unknown genetic, psychological and lifestyle factors have been linked to this condition. Several studies have shown that dysbiosis or an imbalance in the gut microbiota is one of the main causes of PCOS. The gut microbiota of women suffering from PCOS differs in composition, structure, and diversity from that of healthy women according to a recent study [51]. Endometrial receptivity refers to the capacity of the endometrium to accept an embryo for implantation. The endometrium can be compared to soil while the zygote can be compared to seeds. When blastocysts enter the endometrium, it is referred to as implantation. As part of the implantation process, the three steps of location, adhesion, and invasion will take place.

2.3 Androgen Excess

Luteinizing hormone in the ovaries and ACTH (adrenocorticotrophic hormone) in the adrenal glands regulate sex hormone production [[52], [53], [54]]. In females with normal or low levels of follicle stimulating hormone (FSH) chronically elevated GnRH pulse frequency results in an increase in LH pulse frequency and amplitude which raises the Luteinizing Hormone/FSH ratio [55]. An ovary is healthy when LH is released into the granulosa cells where it synthesizes androgens which are converted to estrogen by CYP19A1 (or P450aromatase). CYP17 which converts progesterone into 17-hydroxyprogesterone and from 17-hydroxyprogesterone to androstenedione (A4) has excessive activity in PCOS and a decrease in CYP19A1 activity can lead to the generation of androgens [56]. Contrarily, the adrenal

gland also plays a role in PCOS-affected women's hyperandrogenism. In addition to DHEA, A4 to a lesser extent testosterone (T) and the adrenal cortex is responsible for producing these substances primarily via 5 steroidogenic pathways [56]. A DHEA sulfotransferase enzyme can sulfate DHEA to release DHEA sulfate (DHEAS) into the bloodstream [57]. These adrenal precursor androgens (DHT) which act as pre-hormones (T) result in the production of stronger androgens, T and dihydrotestosterone. Among other things in the liver A4 is converted into 50% of the available T, while in the adrenals, another 25%, and in the ovaries the remaining 25% are converted into T [58].

The liver transforms DHEA directly into A4 without first forming T and certain peripheral tissues convert it directly into DHT. A4 is generated in 50% of the ovaries and DHEA in 20-30% while DHEAS is produced in the adrenal cortex to a very large extent. There is a tendency for PCOS to be associated with compensatory hyperinsulinemia and insulin resistance [59]. CYP17 is activated by insulin which helps convert progestogen precursors into androgens so that the adrenals and ovaries can produce androgen [60]. It has been reported that insulin levels are twenty times higher during obesity-induced hyperandrogenic anovulation and that this phenomenon can be reversed in transgenic mice lacking insulin receptors in the capillaries. Due to the fact that insulin reduces the liver's ability to synthesize insulin like growth factor binding protein (IGFBP-1) and sex hormone binding globulins (SHBG) androgen bioavailability is also enhanced. In response the concentration of IGF in the ovary increases, increasing the synthesis of androgen by activating IGF receptors.

The anti-Mullerian hormone may also be a contributing factor to the excessive levels of androgens of ovarian origin (AMH). The granulosa cells of the primary, preantral, and tiny antral follicles produce AMH. Primal follicles are not capable of making AMH. For the purpose of selecting for the dominant follicle AMH inhibits the recruitment of primordial follicles from the resting pool. Further, it reduces the susceptibility of tiny antral follicles to FSH activity decreasing the generation of AMH. AMH is responsible for controlling the rate at which primordial follicles are depleted and the selection of mature follicles. Because PCOS leads to

increased antral follicle numbers and higher production per antral follicle, AMH levels are significantly higher in women with PCOS. In order to explain the lack of follicular development and increased follicular unresponsiveness to FSH, it is believed that AMH suppresses aromatase and estradiol synthesis as well as granulosa cell acquisition of the FSH and LH receptors [61]. In spite of the fact that obesity is not considered part of the diagnosis it worsens the PCOS phenotype. An increase in testosterone levels and a decrease in SHBG secretion have been linked to obesity and a preference for the storage of abdominal fat. CYP19A1, which is modulated by insulin, converts A4 to the biologically active androgen testosterone through the enzyme aldoketoreductase type 1C3 (AKR1C3) . It is abundantly expressed in both women with simple obesity and PCOS with the latter also exhibiting lower gene expression of CYP19A1. The results of an in vitro study showed that while androgen stimulation increased adipocyte de novo lipid production which was reversed by pharmacological inhibition of AKR1C3 insulin stimulation increased AKR1C3 expression in adipose tissue and its activity [62]. PCOS has been linked to the hormone adiponectin which is thought to be an insulin sensitizer. Adiponectin levels are lower in obese PCOS patients compared to weight-matched controls studies have also suggested that an excess of androgen impairs adiponectin secretion. PCOS women's waist circumference, circulating adiponectin, and adipocyte size have been shown to be good indicators of insulin resistance [63]. Further, when mice are exposed to DHT, overexpression of adiponectin prevents them from developing a metabolic phenotype similar to PCOS but not one related to reproduction [64].

2.4 Factors in PCOS Pathogenesis

2.4.1 Genetics in PCOS

The genetic development of polycystic ovarian syndrome is influenced by a variety of factors, including individual genes, gene-gene interactions, and environmental factors. For a better understanding of PCOS genetics it is essential to identify the

most important variant gene that alters gene expression and sequence and thus modifies protein function. A cell's phenotypic response to environmental changes is mediated by epigenetic modifications at the tissue level. Detailed proteome profiles of sick tissues provide information regarding cellular proteome changes. In addition to genetic factors, epigenetic factors also have an impact on PCOS biomarkers, which in turn affect PCOS molecular pathogenesis [64].

2.4.2 Role of Insulin Resistance in PCOS

A polycystic ovarian syndrome patient is likely to experience irregular menstrual cycles, acne, hirsutism, and alopecia in addition to biochemical changes resulting from elevated testosterone levels, increased levels of dehydroepiandrosterone (DHEA), androstenedione (ASD), decreased levels of SHBG and insulin-related growth factor binding protein (IGFBP). Resistance to insulin and hyperinsulinemia are responsible for these changes. In PCOS, endothelial dysfunction and insulin resistance have long been associated. The concentration of nitric oxide (NO) in endothelial artery cells that are IR-induced is lower and the concentration of endothelin-1 (ET-1) is higher [64].

2.4.3 Environmental Factors

Numerous reviews have recently investigated the impact of environmental factors on PCOS pathogenesis including exposure to environmental contaminants, diet and nutrition, socioeconomic status, and location [65].

2.4.4 Obesity Impact on PCOS

There is a high prevalence of obesity among women with PCOS, particularly in the upper body and central area of the body. There have been reports of obesity rates ranging from 30% to 80% in PCOS, depending on ethnicity, PCOS diagnostic criteria, and study population recruitment standards. Furthermore, it has

been established that PCOS and overweight or obesity are related among adolescents. According to recent investigations of young American patients who were not selected for obesity, the average body mass index read 33.9 kg/m2 at 16.1 years old, which corresponds to a median percentile of 98 [66]. A significant contributor to the progression of PCOS can be found in nutrition when obesity is taken into account. Obesity and chronic overeating both adversely affect PCOS reproductive function. Particularly, obese women with PCOS have higher insulin resistance and hyperandrogenism levels than thin women with PCOS [66]. PCOS women who have undergone weight loss surgery or diet-induced weight loss have demonstrated that obesity adversely impacts PCOS development. During this period of time the patients' endocrine, metabolic, and menstrual conditions have significantly improved as well as their weight loss has been prolonged. In addition to obesity per se, visceral adiposity is also an important characteristic of women with PCOS. An analysis of visceral adipose tissue from morbidly obese PCOS patients supports the significance of visceral adiposity as an etiological factor. It is interesting to note that visceral obesity appears to play a more significant role in metabolic dysfunction and insulin resistance in PCOS-affected women than any other component of the disease [67].

It is based on the theory of adipose tissue expandability that there are strong associations between obesity, hyperinsulinemia, and hyperandrogenemia in women that start in prepuberty and persist into adulthood. Obesity resulting from persistently positive energy intake/output imbalance can impair the body's ability to store fat. Consequently, Sc adipocytes overproduce fat and cannot handle the additional energy. PCOS has been associated with hyperinsulinemia, androgen excess, and inability to increase Sc adipose tissue physiologically safely. There are a number of metabolic complications associated with this disease, including abnormal levels of adipocytokines, dyslipidemia, visceral fat accumulation, and ectopic fat deposition in extra-adipose tissue). As a general rule, obesity should not be considered the main cause of PCOS. As a result of the excess of calories in obesity, PCOS is likely to be exacerbated by a background of abnormalities already present in the body. Excessive nutritional intake can alter both metabolic

and reproductive function throughout the lifecycle influence the rate at which reproduction develops during crucial developmental windows, and have a trans-generational impact [68].

2.5 Medicinal Plant

In women with polycystic ovarian syndrome (PCOS), a class of plant-derived chemicals known as phytoestrogens appears to have protective effects on metabolic and hormonal disorders. Herbal estrogens known as phytoestrogens are found in many herbal preparations. Phytoestrogens seem to have less impact on the endocrine system than steroidal estrogens but they also possess both physiological estrogenic and antiestrogenic properties. According to research, phytoestrogens attach more strongly to oestrogen receptors than oestrogen receptors. Phytoestrogens are however, usually less potent than endogenous estrogens such as 17-beta estradiol. With clomiphene citrate, phytoestrogens enhance endometrial thickness, counteract clomiphene's anti-estrogenic effects, lower miscarriage rates, and increase conception rates [68].

Polyphenolic compounds with estrogenic properties, phytoestrogens are structurally similar to endogenous human hormones. Plant secondary metabolites are found most abundantly in fruits, vegetables, cereals, and legumes (particularly soy). Phytoestrogens are consumed in the most prevalent amounts in the diet. Enterolignans (such as enterodiol and enterolactone) and plant lignans (such as pinanediol, secoisolariciresinol, metaraminol, and sesamin) are two types of lignin produced by gut bacteria. Prenylflavonoids and coumestans, both estrogenic polyphenols ingested at lesser levels than isoflavones and lignans, are both estrogenic polyphenols from beer and soy, respectively [69]. There is a higher affinity for phytoestrogens to bind to the oestrogen receptor. Despite their reduced effectiveness as compared to mammalian estrogens. This characteristic enables them to exert estrogen-like effects on cells that express the ER receptor. A variety of phytoestrogens including genistein, daidzein, and glycitein, can affect animals

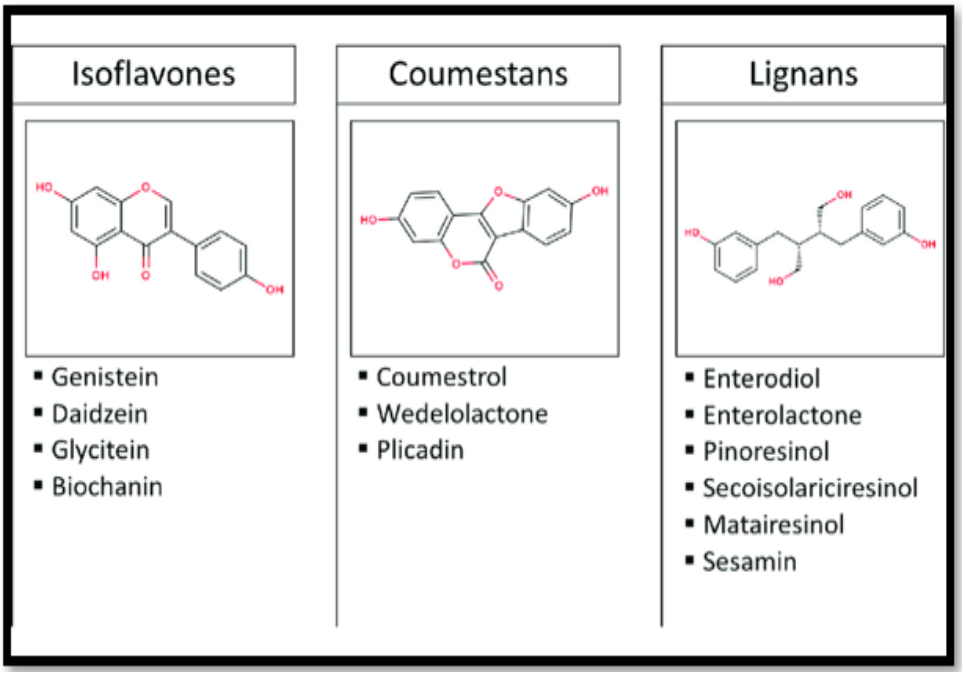


FIGURE 2.1: Classification and examples of the most common dietary phytoestrogens. Images are the chemical structures of genistein, coumestrol, and enterodiol [69].

as well as humans when ingested. Seeds vary by five times in their total amount of isoflavone as well as their amount of each type. There is an association between isoflavone content and quality and the biological activity of soy meals, dietary supplements, and nutraceuticals [70].

A variety of biochemical processes have been proposed to explain how dietary polyphenols, particularly phytoestrogens, may promote brain health. Nevertheless, there is little research supporting a connection between dietary phytoestrogen consumption and cognitive performance. Oestrogen receptor interaction regulates gene expression and alters processes at many different levels in the body, lowering the risk of osteoporosis, cancer, and cardiovascular diseases. Dietary phytoestrogen intake has been associated in the past with a number of chronic diseases and death. Phytoestrogens also appear to increase the production of neurotrophins, increase the bioavailability of nitric oxide and have anti-inflammatory and antioxidant properties. It is crucial to maintain cognitive function. Few studies have looked at the potential implications of phytoestrogen ingestion on human cognitive outcomes however the results are inconsistent although optimistic [71]. Plant

phytoestrogens are non-steroidal phenolic plant chemicals that occur naturally in plants. There is a structural similarity between these hormones and estrogen which is the primary female sex hormone. The phytoestrogens reduce the risk of cardiovascular disease, osteoporosis, and the effects of menopause. Additionally, they reduce the risk of brain disease. A phytoestrogen's and its derivatives' effects on cancer are primarily due to the suppression of oestrogen synthase and metabolism which results in antiangiogenic, antimetastatic, and epigenetic effects. Neuroprotective effects of phytoestrogens are associated with their antioxidant capacities as well as their interactions with oestrogen receptors. In general, no significant negative effects have been observed and these substances can be recommended as health-promoting dietary ingredients or supplements despite their possible effects on thyroid function [72]. The composition of foods varies and certain foods have a greater influence than others. A variety of soy beans can be processed differently resulting in a variation in the content, even within the same food group as in the case of soy beverages. Phytoestrogens are present in a variety of foods, including soybeans, tofu, tempeh, soy beverages, linseed (flax), sesame seeds, wheat, berries, oats, barley, dry beans, lentils, rice, alfalfa, mung beans, apples, carrots, wheat germ, rice bran, and soy linseed bread. A wide variety of medicinal plants, including red clover, black cohosh, alfalfa, hops, licorice, and turmeric, contain phytoestrogens of various types.

Estradiol, estrone, and estriol, collectively known as endogenous estrogens, are three different types of oestrogen produced by the human body and found in the ovaries, placenta and to a lesser extent the testes. As well as the oestrogen itself the blood contains a variety of oestrogen metabolites. According to their chemical composition all of the above are considered steroids. In spite of the fact that some plant seeds such as those from date palms and pomegranates contain trace amounts of estrone most phytoestrogens do not have steroidal properties.

Phytoestrogens are classified chemically into three categories: coumestans, isoflavones, and lignans. While there are several differences between them and steroids, they are similar enough to them to affect the oestrogen receptors and hormone metabolism in cells. Lignan should be distinguished from lignin which

is a stiff polymer found in wood that gives plants the ability to resist gravity and wind.

It is estimated that East and Southeast Asia consume the largest amount of phytoestrogen (between 20 and 50 mg per day). Phytoestrogen intake is normal for men and women in Europe where soy products are less commonly consumed. The phytoestrogens (isoflavones) have been linked to feminizing effects in men, including a reduction in testosterone levels and a rise in oestrogen levels. Recent research indicates that soy and isoflavone consumption has no significant effect on male reproductive hormones [73].

2.6 Oestrogenic Potencies of Phytoestrogens

Phytoestrogens are weak forms of oestrogen that require significantly higher concentrations than estradiol in order to have the same physiological effects. As potency levels might vary greatly between approaches, it can be difficult to determine the relative absolute estrogenic potency of phytoestrogens [74]. Moreover, isoflavones are found in a greater variety of foods than coumestans, believed to be the most potent phytoestrogens and commonly found in clover and alfalfa plants.

2.7 Fenugreek

The legume fenugreek plant (*Trigonella foenum-graecum*) is grown in North African and Indian nations. There are various names by which it is known in many languages including Fenugrec in French, Methi in Hindi, Bockshorklee in German, Fienogreco in Italian, Pazhitnik in Russian, Alholva in Spanish, Koroha in Japanese, Hulba in Arabic, Halba in Malaya, and K'U-Tou (China), which are all members of the Fabaceae family. While the leaves of the plant are consumed as green vegetables the seeds are used throughout the world as spices. Many years have been spent using the bitter-tasting fenugreek seed as a medicinal remedy.

There has been a use for fenugreek seeds for more than 2500 years. The majority of fenugreek produced and consumed worldwide is grown in India. Seasoning, flavoring, and larger amounts are used as a component of soups and pan cakes that contain fenugreek seeds.

According to the Indian traditional medical system it is effective against anorexia as a stomach stimulant [75]. The seeds and leaves of fenugreek which are native to Eastern Europe and parts of Asia are now farmed almost everywhere in the world for use as leafy vegetables and sauces. An annual fenugreek plant grows between 0.3 and 0.8 meters tall with trifoliate leaves. In addition to their white or yellow blooms, these plants produce long, thin, yellowish to brown pods. Fenugreek pods contain firm, brown seeds that are used in medicine as soon as they reach maturity. Although the green leaves are utilized in many cultures as vegetables, the dried leaves make an excellent addition to many meal preparations on the Indian subcontinent. Fenugreek seed has long been used as a stomachic, expectorant, laxative, and carminative remedy. It is known that the mature seed of fenugreek contains fibers, flavonoids, polysaccharides, fixed oils, and a variety of alkaloids, such as trigonelline and choline, in addition to amino acids, fatty acids, vitamins, saponins, such as diosgenin, gitogenin, neo gitogenin, homoorientin saponaretin, neogigogenin, and tigogenin [76].

In addition to treating diabetes, ulcers, bronchitis, fever, sore throats, wounds, swollen glands, and other conditions, the herb has also been used to treat other illnesses as well. The extensive use and beneficial qualities of fenugreek have led to many research studies being conducted in recent years to examine the possible use of fenugreek in health and other common diseases. From the early 1960s to the late 1990s, experimental research was conducted on fenugreek seed extracts and their effects on laboratory animals [75].

The pharmacological properties of fenugreek including its anticancer, antiulcerogenic, anti-inflammatory, antioxidative, hypocholesterolemia, antineoplastic, and anti-inflammatory properties are well established. Several active components of fenugreek seeds have been isolated and identified including polyphenolic flavonoids

which are commonly found to exhibit hypoglycemia, hypocholesterolemia, hypotriglyceridemic, and antiperoxidative properties, steroid saponins with anti-inflammatory and uterine- and lactation-stimulating properties, galactomannans with anti-diabetic effects, and the amino acid 4-hydroxyiso-leu [76].

2.7.1 Fenugreek Greens and Fenugreek Seeds

Fresh or dried fenugreek leaves and sensitive stems can be consumed. It is noteworthy that these leaves are a great source of a variety of minerals and vitamins but they are especially rich in choline. Biological value of leaf protein is 84%, with a true digestibility coefficient of 77%. There are many health benefits associated with fenugreek seeds which are aromatic, bitter, carminative, galactagogue, antimicrobial and can be consumed raw or cooked. Most of the seed is composed of carbohydrates that are inaccessible to the human body (50%) This bitterness is primarily due to the oil, steroidal saponins, and alkaloids in the seed. Insoluble fractions (30%) make up the fibre component, while the soluble fraction (20%) is primarily galactomannan [77].

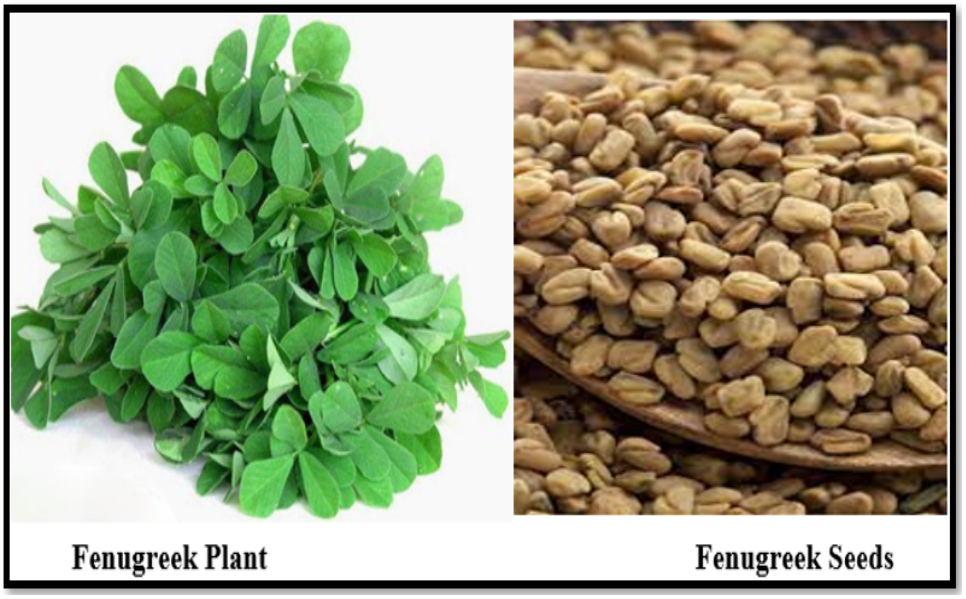


FIGURE 2.2: Fenugreek plant and seeds

7.5% of the seed's lipids are considered to be neutral lipids, with 6.3% of these being triglycerides and 450 mg/100 grammes being phospholipids. According to

the conclusions of an animal research adding fenugreek seeds to the diet in place of up to 10% of casein had no detrimental effects on the protein quality of casein as determined by the protein efficiency ratio, the protein digestibility, and the net protein intake. There is no proof that fenugreek's high-quality protein content would be harmed by cooking [78].

2.7.2 Fenugreek Oils

Oil that can be extracted from fenugreek contains a pungent smell, a bitter taste, and strong drying properties. With a specific gravity of 0.91, an acidity of 1-2, a saponification value of 178–183, an iodine value of 115, an unsaponifiable matter value of 3.9%, and fatty acid compositions of 35.1% oleic, 33.7% linolenic, 13.8% -linolenic, 9.6% palmitic, 4.9% stearic, and 9.6% arachidic, the soap has the following properties. Most of the volatile oil consists of anethole, which accounts for 0.02% (sp.gr. 0.87) of the total oil. There is a smell that may be reminiscent of roasted coffee or maple syrup [79].

2.7.3 Bioactive Constituents of Fenugreek

2.7.3.1 4-Hydroxyisoleucine

4-hydroxyisoleucine is one of the bioactive components in fenugreek that confers its anti-diabetic properties. 4-hydroxyisoleucine, a nonproteinogenic amino acid found in human and rat pancreatic islet cells significantly enhances lipid profiles and glucose-induced insulin release [77].

2.7.3.2 Diosgenin

Among the active steroid sapogenins found in fenugreek is diosgenin. Diosgenin may be beneficial for the treatment of several diseases including diabetes, hyperlipidemia, cancer, CD, OS and inflammation [78].

2.7.3.3 Galactomannan

There is evidence that fenugreek seed galactomannan shows prebiotic potential and can act as a nutrient source and carbon source for promoting the growth of the probiotic strain Bacillus cerevisiae [79].

2.7.3.4 Quercetin

A flavonoid important for health quercetin has antioxidant and anti-toxic properties. Chronically high GnRH pulse frequency causes an increase in LH pulse frequency and amplitude which raises the Luteinizing Hormone/FSH ratio in females with normal or low levels of follicle stimulating hormone (FSH). Quercetin chelates iron which is responsible for the production of reactive oxygen species [80].

2.7.3.5 Trigonelline

A plant hormone known as trigonelline regulates a number of processes including nodulation, oxidative stress, as well as assisting the plant in survival and growth. Fenugreek seeds and coffee beans contain abundant amounts of this compound [81].

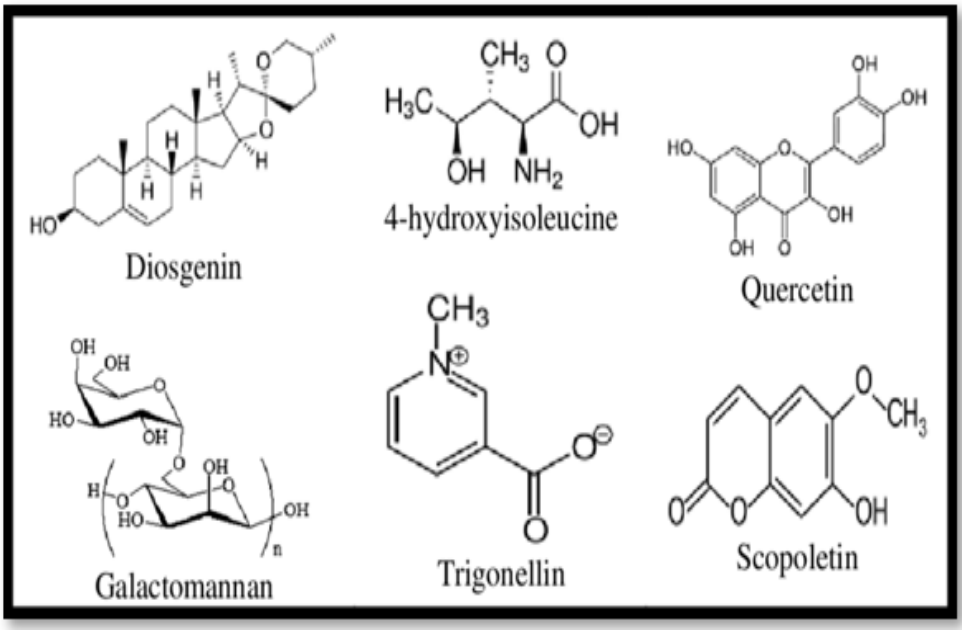


FIGURE 2.3: Bioactive chemical constituents of fenugreek [82].

2.7.3.6 Scopoletin

Scopoletin a ubiquitous constituent of the flavonoid's family is a 7-hydroxy-6-methoxy coumarin. There is a flavonoid skeleton core in the 1,2-benzopyrone structure and a carbon skeleton C6-C3. In addition to being methoxylated and hydroxylated, the benzene ring has also been modified. Plants that produce phenylpropane including Arabidopsis thaliana contain phenolic coumarins [82].

2.7.3.7 Dietary Fiber of Fenugreek

Fiber can be ingested as a plain powder blended in fruit juices or it can be added to dishes like soups, drinks, and sauces. Fenugreek dietary fibre which balances soluble and insoluble fibre can fortify refined flour. In bakeries, foods are made with flour fortified with 8–10% fenugreek fibre. As a result of fenugreek's slight maple syrup aroma these goods will gain flavour. In addition, fenugreek dietary fibre has been added to flour to make taco shells, chapatis, chips, and wafers. Fibre can be added to foods such as these in order to increase one's intake of dietary fibre on a daily basis. It is recommended that children consume 20 to 30 grams of dietary fiber each day. Consumption of fenugreek dietary fiber-rich diets is associated with a rapid transit of colonic contents. It enhances the faecal bulk reduces the risk of constipation, increases regularity, lowers colonic bioburden, has little to no flatulence and facilitates the process of defecation.

Plants that produce phenylpropane including Arabidopsis thaliana contain phenolic coumarins. As a consequence, hemorrhoids, anal fissures and diverticulosis are decreased. Health benefits from fibre in the diet include the reduction of calories, the lengthening of chewing time, the suppression of appetite, the reduction of overeating, and the prevention of weight gain. The oil has been claimed to have insect and pest repelling effects as a cosmetic and a pesticide. With 30 g of fenugreek dietary fibre per day and the appropriate level of physical activity, steady and considerable weight reduction can be readily accomplished without damaging protein-calorie malnutrition or other unfavorable dieting side effects [83].

2.8 Flaxseed

A flaxseed compound called lignan has been shown to suppress androgen levels in men with prostate cancer. Flaxseed is one of the best sources of dietary lignan having levels 800 times greater than most other foods. In addition to being a good source of omega-3 fatty acids flaxseed is also an excellent source of dietary lignan. A flax plant, also known as Linum usitatissiumum, has blue flowers and produces thin flat seeds that are either golden yellow or reddish brown in color. The most common forms of flaxseed are whole seeds, powdered seeds (powder or meal), and flaxseed oil. Whole and ground flaxseeds differ from flaxseed oil in that they contain no fibre or lignans. Flaxseed has a high nutritional value since it contains a lot of vitamins, minerals, and carbs in addition to having 41% fat, 28% dietary fibre, 21% protein and 28% carbohydrates. Flaxseed oil contains 9% saturated fatty acids, 18% monounsaturated fatty acids and 73% polyunsaturated fatty acids (PUFAs) [84].

Flaxseed oil is low in saturated fat. There is no known better source of omega 3 (n-3) fatty acids than flax seeds, which constitute around 55% of all fatty acids. Compared with walnuts and canola oil, flaxseed provides five times the amount of ALA as walnuts. Flaxseed is one of the greatest sources of dietary lignan with levels 800 times higher than those of most other foods in addition to having significant concentrations of omega-3 fatty acids. Plant phytoestrogens such as flax seeds have a taming effect on estrogen. This naturally leads to the production of healthy levels of oestrogen [84].

The whole flaxseed is flat, oval and has pointy tips. An embryo has two embryos and an embryo axis. There is a true hull also known as the seed coat or Testa, a thin endosperm, two embryos and a thin endosperm. Each component of the linseed plant is used economically regardless of whether it has been processed first. Oil from the seed contains omega-3 fatty acids, digestible proteins, and lignans, and the shell yields fibre with excellent mechanical properties and low density, which is useful for painting, varnishing, linoleum, oilcloth, printing ink, soaps, and a number of other products. Seed has a tendency to go rancid at

FIGURE 2.4: Flaxseed.

room temperature within a week whereas whole flaxseeds do not. Ground flax can be kept fresh for a longer period of time if it is refrigerated and stored in an airtight container. Milled flax can maintain its excellent oxidation resistance for nine months if packed immediately without exposure to air or light and for 20 months if packed under ambient conditions in the warehouse [84].

2.8.1 Nutrients Composition of Flaxseed and Health Benefits

Flaxseed contains soluble dietary fibre, polyunsaturated fatty acids (PUFAs), lignans, proteins and carbohydrates that have particular biological activity and function. In spite of this, it only contains trace amounts of harmful substances such as cyanogenic substances, protease inhibitors and cadmium. According to an analysis of brown Canadian flax, it consists primarily of fat 21% protein, 28% dietary fibers, 7.7% moisture and 3.4% ash, a mineral-rich residue left over after burning. The composition of flax can vary with genetics growing environment, seed processing and method of analysis [85].

2.8.2 Omega-3 Fatty Acids in Flaxseed and Health Benefits

In flaxseeds, approximately 30% of the fats are lipids composed primarily of -linolenic acid (ALA), 17% of which is linoleic acid (LA), 19% of which is oleic acid, 3% of which is stearic acid and 5% of which is palmitic acid. The great n-6 to n-3 fatty acid ratio of flaxseed is the result of this ratio. As marine foods contain the highest concentration of n-3 fatty acids the seed may be a viable option for providing people in areas where such foods are unavailable [86].

2.8.3 Proteins in Flaxseed and Health Benefits

Flaxseed proteins exhibit some technologically functional characteristics as do all plant proteins which influence their behavior in a food system by interacting with other nutrients. Water oil retention capacity and solubility are highly dependent on the mechanisms by which they are hydrated. The amino acid composition of soybean protein is the most similar to that of flax protein which is one of the most nutrient-dense plant proteins. Flaxseeds do not constitute a complete protein source because they are deficient in several amino acids [87].

Flaxseed paste and grain contain approximately 21% and 34% protein respectively, depending on hereditary and environmental factors. It is common for seeds to contain high oil content and low protein content at cool temperatures. Among the main components of linseed are two types of storage proteins: an albumin-like component (1.6–2S) and a salt-soluble fraction (11–12S; globulin; 18.6% nitrogen). There are three limiting amino acids in flaxseed which has a favorable amino acid ratio overall. These three amino acids are Lysine, Threonine, and Tyrosine. Additionally, it contains branched chain amino acids such as isoleucine, leucine, and valine as well as sulfur amino acids such as methionine and cysteine. As one of the richest sources of essential amino acids flaxseed plays a crucial role in the synthesis of proteins which enable cells, tissues and organs to maintain and repair themselves [88].

2.8.4 Micronutrients in Flaxseed and Health Benefits

Among the vitamins and minerals contained in flaxseed are calcium, magnesium, and phosphorus. A 30g serving of the seed provides 7–30% of the various minerals' Recommended Dietary Allowances (RDA). The most antioxidants (tocopherols -, -, and -) and niacin are found in flaxseed as a whole (vitamin B3). The quantity and placement of the methyl groups on the chromanol ring dictate the composition of alpha, beta, gamma and delta tocopherols. Despite the fact that gamma-tocopherol which has been mono-methylated is frequently the form of vitamin E present in oils, humans preferentially absorb and store alpha-tocopherol. The isomer -tocopherol of vitamin E, a fat-soluble vitamin is largely found in flaxseeds. As a potent antioxidant vitamin E guards against free radicals that if left unchecked can lead to the growth of cancer. Through its ability to stop the stomach from creating cancer-causing nitrosamines from nitrites in food vitamin E may help strengthen the immune system and prevent cancer. By boosting salt excretion in the urine vitamin E may also lower the risk of heart disease, a number of cancers, and Alzheimer's diseases [89].

2.8.5 Bioactive Constituents of Flaxseed

2.8.5.1 Alkaloids

The alkaloids in plants control their growth and guard them against predators. Alkaloids are particularly well known for their therapeutic uses as anesthetics, cardio protectants, and anti-inflammatory agents. There are several well-known alkaloids used in clinical settings including morphine, strychnine, quinine, ephedrine, and nicotine [89].

2.8.5.2 Cadmium

Cadmium can be absorbed by any plant including flaxseed and build up in the seed of the plant as a result. Plant items account for approximately two thirds of the

dietary cadmium. The sources of cadmium that are most abundant in this area are oil seeds and oilseed meals, particularly those made from sunflowers, peanuts and flaxseeds. The primary symptoms of cadmium toxicity are renal impairment and bone density loss. These impacts may require years of exposure as a result of their cumulative nature. A majority of the population is exposed to low levels of cadmium through food exposure through diet was predicted to be 2.3 g/kg b w/week in European countries (range: 1.9 to 3.0 g/kg b w/week), even though vegetarians consumed greater levels of cadmium [90].

2.8.5.3 Cyanogenic Glycosides

In addition to their biological effects, cyanogenic substances also have a significant environmental impact. Among the cyanogenic chemicals found in flaxseed are linamarin, linustatin, and neo linustatin. Each gram of flaxseed can release 7.8 M of cyanogenic chemicals. An amount of thirty grams of linseed can produce 240 milligrams of cyanide. A long-term intake of flaxseed was found not to have any adverse effects [91].

2.8.5.4 Lignans

In angiosperms as well as gymnosperms, lignans are abundant. Both biologically and structurally perform a multitude of functions. Several lignans have been shown to inhibit enzymes and to be anticancer, antimitotic and antiviral in nature. There are several lignans that are toxic to fungi, insects and vertebrates and they have a broad range of physiological effects [89].

2.8.5.5 Linatine

A substance called linatine may be responsible for the reduced quantity of flaxseed meal. In general, research suggests that vitamin B intake is associated with weight gain rather than direct health effects of linatine. In a diet of flaxseed and linatine,

glucose and linolenic acid did not affect the growth rate of swine given pyridoxine for instance [92].

2.8.5.6 Linoleic Acid

PUFAs are most prevalent in the human diet especially linoleic acid (18:2, cis, cis-9,12-octadecadienoic acid). When linoleic acid is absorbed, it produces four main effects. The potential energy source for this nutrient is the same as for all other fatty acids. By esterifying it, polar and neutral lipids can be formed, including cholesterol esters, triacylglycerols, and phospholipids. The linoleic acid in the epidermis serves as a structural element of membrane phospholipids and contributes to maintaining a specific level of membrane fluidity. In addition, by liberating it from membrane phospholipids, 13-hydroxy or 13-hydroperoxy octadecadienoic acid, 13-H, may be oxidised enzymatically to a number of compounds that are crucial for cell signaling [92].

2.9 Proteins Involve in PCOS

PCOS proteins and their structural characteristics are studied using computational techniques. Models of the relevant proteins are generated using Modeler 9v14 and Ab-initio. The relationship between the 43 genes encoding proteins was examined using Clustal Omega, a phylogenetic tool in order to identify the related proteins that are responsible for PCOS.

2.9.1 Genomics Biomarkers

The understanding of the main receptor and intracellular signaling pathways, underlying mechanisms and molecular participants in these processes has not kept pace with advancements in PCOS therapy, diagnosis and expression. Researchers have been able to find biomarkers and understand the molecular pathophysiology

of PCOS by researching genetic and epigenetic patterns and their influence on genetic profiles. Researchers have looked at a few good candidate genes to understand the genetic basis of PCOS and the symptoms that are related to it as well as to expose the basic molecular mechanisms that underlie it. This method enables scientists to comprehend the molecular causes of disease [93].

The main genetic indicators linked to PCOS are genes thought to have contributed to its development. The study also emphasized some relationships between genes, proteins and environment as well as the significance of genetic predisposition in the development of PCOS. It is thought that the relevant genes and other genetic variations linked to PCOS are important disease-causing factors. The most important PCOS candidate genes are those that encode for molecules involved in androgen synthesis, transportation and control insulin receptors their secretion and activity, signaling cascade proteins, and growth factors. Numerous susceptibility loci, such as those related to cell growth, insulin production and signaling, sexual hormone function, lipid metabolism, and apoptosis, have been identified in studies and may be enriched with potential candidates for the molecular mechanisms of PCOS. Genes involved in metabolism and biosynthetic pathways, reproductive function, signaling pathways, transportation and development, cellular senescence, and a number of other biological processes were the main focus of the numerous genomic investigations [94].

TABLE 2.1: List of several genes connected to genetic research on PCOS [94].

Sr. No	**Gene**	**Biochemical Processes**
1	GYS	Control of biological processes in the metabolic and endocrine systems
2	MTHFR Ovarian function	Ovarian function
3	DKK1	Increasing secretion of androgen
4	MAP3K15	Associated with adrenal and testicular steroidogenesis
5	GATA4	Impact PCOS sufferers androgen biosynthesis

2.9.2 Proteomics Biomarkers

Finding new biomarkers has benefited greatly from proteomics' links to genotype and phenotype. A high-throughput analysis of proteins and peptides known as proteomics considers each one's structure, function, degree of alteration and association with other proteins. Proteomics analysis has helped identify a number of unique proteins that may be involved in the pathophysiology of PCOS. It is essential to be knowledgeable about the systematic characterization and extensive investigation of dynamic changes in protein expression in order to comprehend complicated disorders. With broad alterations in protein expression and post-translational variation in protein function hold enormous promise for unravelling disease mechanisms and revealing novel insights into PCOS. Protein expression profiles from cells and tissues may be created using a protein array, 2D PAGE, mass spectrometry, and other techniques. To profile the protein expression from cells and tissues, the following techniques can be used: protein arrays, 2D PAGE, mass spectroscopy, and other protein separation techniques. This might result in the clarification and discovery of new proteins in PCOS illnesses that are crucial for the development of more precise diagnostic methods and novel treatment targets. Protein and enzyme indicators are extensively employed for predictive and therapeutic reasons to prevent the consequences of PCOS. Integrating a vast quantity of unique data from proteomics may help to clarify cellular alterations that affect a variety of metabolisms, androgen production or chronic inflammatory pathways, leading to PCOS illness and other conditions [95].

TABLE 2.2: List of some proteins related with proteomics based PCOS study

Sr. No	**Protein**	**Biological Functions**
1	LH	The increased androgen levels in PCOS are caused by high amounts of LH.
2	LH/choriogon-adotropin receptor	Linked to infertility due to anovulatory cycles

TABLE 2.2: List of some proteins related with proteomics based PCOS study

Sr. No	Protein	Biological Functions
3	FSH	follicle growth, oocyte maturation and steroidogenesis
4	AMH	Lower levels of fertilization and a higher proportion of immature oocytes are associated with folliculogenesis.
5	Homocysteine	Insulin resistance

2.10 Target Protein

2.10.1 Luteinizing hormone β-subunit

The polypeptide called LHB also known as lutropin subunit beta or LH, joins with an alpha subunit that is shared by all gonadotropin hormones in order to produce the reproductive signaling molecule called luteinizing hormone. Humans possess the LHB gene that encodes this protein. LH is a heterodimeric hormone belonging to the same family as thyroid-stimulating hormone (TSH), hCG, and FSH. All of these hormones have a common subunit and a unique subunit in their structure. This unique subunit confers the biological specificity of the hormone receptor in the target organ. LH is essential for the gonadal system's growth of follicle expansion, stimulation of steroidogenesis, and maturation of the oocyte. To explain the irregular menstrual cycles and infertility associated with PCOS, it is hypothesized that abnormal LH secretion which may be brought on by a mutant form of the LH gene, affects gonadal function and that microheterogeneity is connected to anovulation, luteal insufficiency and premature oocyte maturation. LH is a gonadotropic glycoprotein hormone secreted by the anterior pituitary. In females, an increased level of LH causes ovulation and increases the size of

the corpus luteum. Spermatogenesis and testosterone synthesis are promoted by LH in males. PCOS and the LH signaling system are still not fully understood. In PCOS, aberrant LH signaling is thought to contribute significantly to an increase in ovarian androgen production resulting in anovulation. LH and LHCGR gene mutations cause anovulatory disorders, amenorrhea and polycystic ovaries in women by modifying the structure or function of the LH and LH-CGR, either activating or inactivating their biological activity. In spite of the fact that the results of various populations and polymorphism loci may indicate discrepancies, several studies have demonstrated that LH and LHCGR polymorphisms are genetically related to PCOS [96]. PCOS is characterized by an increase in level of circulating LH, which is a biological characteristic. Hypersecretion of LH is caused by an increase in the amplitude and frequency of pulsatile LH release. Theca cells in the ovary are stimulated to create more androgen as a result of higher LH levels. The microheterogeneity of LH and LHR and genetic variations may contribute to polycystic ovarian syndrome. Generally, PCOS metabolic abnormalities are not treated effectively with pharmaceuticals due to their broad scope. The clinical therapy must take into account each patient's unique symptomatology as well as genetic variations in order to avoid subjecting patients to medications that are ineffective for them. Single nucleotide polymorphisms (SNPs) in genes that are vulnerable to PCOS can be used to determine a person's PCOS risk profile and treatment response. PCOS may therefore be better understood if we study its polymorphic relationship with LH and LHR. In addition to allowing for the selection of susceptible and non-susceptible patients [97].

2.11 Molecular Docking

Applications for molecular docking in drug discovery include structure-activity studies, lead optimization, finding potential leads through virtual screening, providing binding hypotheses to facilitate predictions for mutagenesis studies, assisting x-ray crystallography in the fitting of substrates and inhibitors to electron density, chemical mechanism studies, and combinatorial library design. Virtual

screening on the basis of molecular descriptors and physicochemical properties of (in) active ligands is a method that is frequently used for reducing and enriching the library of ligands for molecular docking; recent reports suggest that ligand shape-matching performs at least as well as docking. However, molecular docking when utilized as the last step in virtual screening helps to generate three-dimensional (3D) structural theories of how a ligand interacts with its target [96].

A tiny molecule and a target macromolecule engage in molecular docking most frequently. Although it is frequently referred to as ligand-protein docking, protein-protein docking is gaining popularity. As we concentrate on ligand-protein docking in this chapter we will refer to the larger protein, DNA, or RNA macromolecule that is being docked with a much smaller molecule (or ligand). Additionally, there are a number of small molecule databases including PubChem, Chem DB and Drug Bank that collectively include more than one million deposited structures of possible ligands. When it's required to quickly test a database of thousands (or perhaps millions) of compounds against one or more targets, the virtual screening through docking technique has become essential. Experimental replication of this search would not be achievable at such a low cost in terms of both money and time [97]. So, the docking process includes compounds which are discussed below.

- The docking process requires a 2sD structure of protein which is downloaded from protein data bank (PDB).
- Minimum size of molecules or compounds or virtual compounds that contain a database is required.
- A computational framework is also needed to perform the docking and find the scoring process.

Protein and ligand docking is one of the major subfields of molecular docking science, which is well regarded and appreciated for its use in the development of structure-based pharmaceuticals. The most widely recognized for molecular docking was CB Dock, Auto Dock, Patch Dock, and others. The most extensively used algorithms in molecular docking were Molecular dynamics, distance geometry

technique, and genetics algorithm, etc. There were 12 ligands including flaxseed (Alkaloids, Cadmium, lignans, linatine, linoleic acid and Cyanogenic glycosides) and fenugreek (4-Hydroxyisoleucine, diosgenin, galactomannan, quercetin, scopoletin, trigonelline) were selected for molecular docking [98].

Chapter 3

Research Methodology

3.1 Methodology Flow Chart

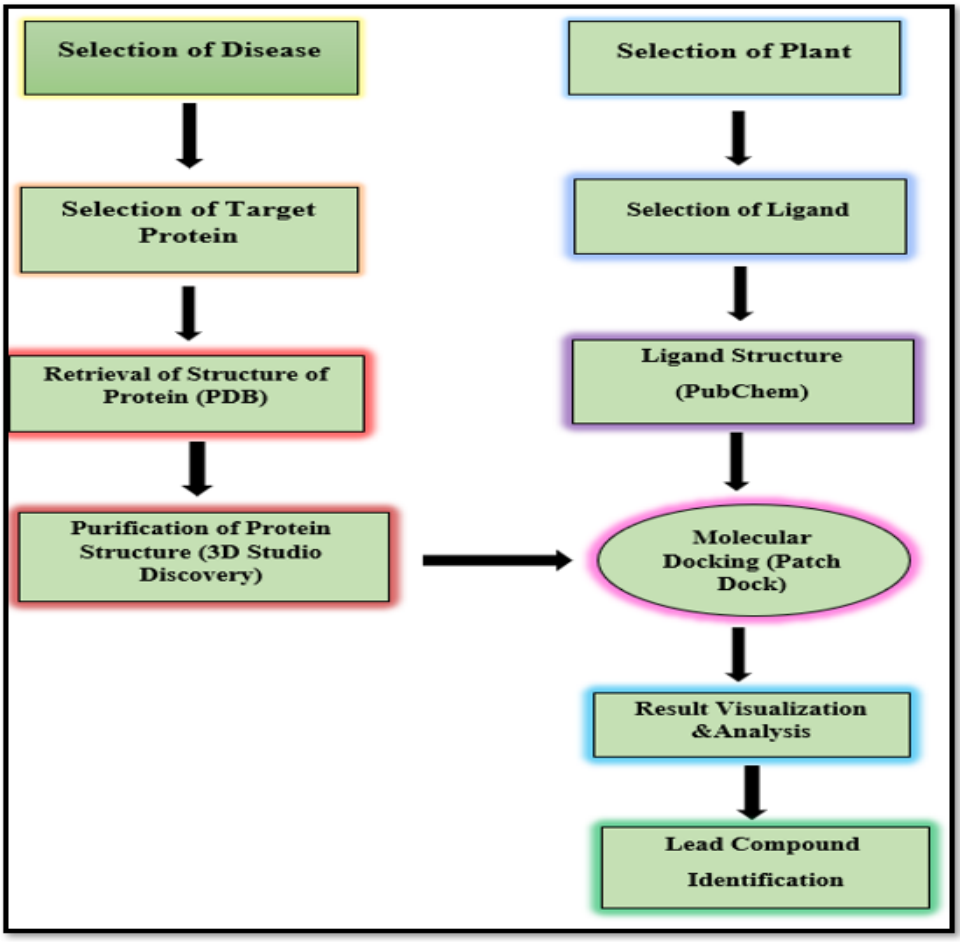


FIGURE 3.1: Flow chart of reserach methodology

3.2 Selection of Disease

The most prevalent endocrine condition is PCOS, which impacts 5–10% of women of reproductive age. Its distinguishing characteristics include polycystic ovarian morphology, biochemical and/or clinical hyperandrogenism, and chronic ovarian failure. PCOS has major clinical ramifications and can lead to health issues like obesity, IR, MS, and T2D that are linked to the buildup of adipose tissue [98].

3.3 Selection of Target Protein

The main contributing component luteinizing hormone subunit beta and other proteins can be controlled in PCOS illnesses. Therefore, these proteins are involved in PCOS and are essential in preventing it. Overweight, IR, MS and T2D are just a few of the illnesses that PCOS can contribute to becoming more prevalent [99]. Target proteins were identified from the protein databank which revealed that the 2D structure of the luteinizing hormone beta subunit is present in PDB.

3.4 Primary Sequence Retrieval

Primary sequence of target proteins Luteinizing hormone beta subunit was taken in FASTA format from protein sequence database Uniport `http://www.uniprot.org/` under accession number A0A0F7RQE6 [100].

3.5 Analysis of Physicochemical Properties of Protein

Protein function is significantly influenced by their physicochemical features. ProtParam available at `https://web.expasy.org/protparam` was employed to forecast these characteristics of the beta component of luteinizing hormone. Through

the use of ProtParam [101] the number of negatively charged residue (Asp+Glu) and positively charged residue (Arg+Lys), theoretical pI, molecular weight, Ext coefficient (Cys included), Ext coefficient (Cys not included), instability index and aliphatic index as well as the overall hydrophobicity were calculated [101].

3.6 Retrieval of Structure of Protein (PDB)

The multinational partnership that oversees the depositing, processing and dissemination of the PDB archive is known as the worldwide Protein Data Bank (www.PDB.com). More than 38 000 structures including proteins, nucleic acids and massive macromolecular complexes have had their coordinates and associated data stored in the online PDB database via means of X-ray crystallography, NMR and electron microscopy [102]. Protein Data Bank was used to extract the structure of the LHB protein which is primarily associated with PCOS for the current investigation (PDB) under the job id AF-AOAOF7RQE6-F1.

3.7 Purification of Protein Structure (3D Studio Discovery)

Software called 3D Discovery Studio was used to purify the protein. Through the use of 3D Studio Discovery the water molecule and other small molecules including ligands that are present in LHB protein were isolated. Purification is required to prevent unexpected docking results [103].

3.8 Ligand Structure (PubChem)

The greatest database of readily available chemical information is called PubChem. So, using the PubChem database, the chemical compounds that were chosen as ligands were chosen [104].

3.9 Analysis of Ligands and Toxicity Measurement

The ligands which are chemical substances were chosen from the PubChem database. The Lipinski rule of five is seen in some compounds and they are likely to be exploited as active pharmaceutical ingredients in humans [108].

The ADMET properties of a drug determine its likelihood of success. PkCSM is an online tool that aids in determining the compounds' ADMET characteristics. The rules are as follow:

1. The log P value of most "drug-like" molecules should be limited to 5.
2. Molecular weight should be under 500.
3. Maximum No. of H-bond acceptor should be 10.
4. Maximum No. of H-bond donor should be 5.

3.10 Molecular Docking (Patch Dock)

The goal of molecular docking is to identify the target proteins' and drugs' optimal conformational interaction. The target protein and the potential ligand are the two prerequisites for docking. The target protein is luteinizing hormone subunit beta and specific ligands from flaxseed

- (alkaloids, cadmium, lignans, linatine, linoleic acid and cyanogenic glycosides)
- and fenugreek are employed (4-Hydroxyisoleucine, diosgenin, galactomannan, quercetin, scopoletin, trigonelline)

An online docking service called Patch Dock and CB Dock is utilized to carry out docking and automatically detects binding points. By anticipating the target protein binding locations [105].

3.10.1 Process of Molecular docking

Since the early 1980s, the most popular computational approach for structure-based drug design (SBDD) has been molecular docking. When the protein target's three-dimensional (2D) structure is known, this is the instrument of choice. This is made possible by the exponential increase in computing power, the accessibility of protein and small molecule structures, and the rising popularity of molecular docking [106]. The first step in performing the docking process is to create a ligand and target protein files. First, the target protein file is compiled following a few steps. PDB file of target proteins (luteinizing hormone subunit beta) were given to Patch dock as input file.

3.10.2 Active Site Identification

The ligand shows maximum or highest interaction with the protein where the target protein has their active site. Amino acids are highly involved in the formation of complex of ligand to protein. Protein binding pockets were identified by CASTP.

3.11 Protein Ligand Interaction

For the purpose of interpreting docking data, the interaction between the active pockets of the ligand and the protein is computed.

3.12 Ligand ADME Properties

Lead needs to be more similar to drug in general for drug discovery to be more effective. The substances were subsequently examined for drug score, drug-likeness and toxicity. PkCSM was employed for ADMET experiments since it has a drug's ADMET features [108].

3.13 Result Visualization & Analysis

Software called Discovery Studio combines transcription of small compounds and macromolecules. Dassault Systems BIOVIA created it (Accelrys). Discovery Studio, a single integrated graphical user interface manages both research on protein modelling and advanced drug creation. This program offers a variety of plot viewers and data visualization viewers. This study helped people better grasp how to access, share and analyze protein and small molecule data using the Discovery Studio program. The DS program has applications in a variety of fields including as quantum physics, molecular dynamics and mechanism. Additionally, software is capable of doing hybrid QM/MM computations.

3.14 Lead Compound Identification

The most effective inhibitor was discovered using docking scores following a thorough examination of protein and ligand interactions. (Alkaloids, Cadmium, lignans, linatine, linoleic acid and cyanogenic glycosides) (4-Hydroxyisoleucine, diosgenin, galactomannan, quercetin, scopoletin, trigonelline) were among the twelve fenugreek and flaxseed ligands chosen for the current study project. Our lead compound was the one that was chosen.

3.15 Drug Selection

To pick the most potent medicine, the detected drugs must first be screened. This is accomplished by a detailed review of the medications that have been found, which identifies the most effective drug based on characteristics such as physiochemical qualities, effective ADMET properties, effective mechanisms of action and minimum side effects. From the databases PubChem and PKCSM was used for Physical and Chemical Properties, ADMET Properties, and mechanisms of action with drug side effects were gathered [110].

3.16 Drug Identification

The term "letrozole drug identification" relates to the labelling of medications used to treat PCOS. Drug bank databases were employed for drug identification since they facilitate in a detailed analysis of the disease including its route and available treatments [109].

3.17 Drug-Proposed Letrozole Agent Comparison

Comparing docking data, physiochemical parameters and ADMET properties allowed the non-steroidal letrozole drug and the suggested ligands to be compared [111].

Chapter 4

Results and Discussions

This chapter will explain the result that were obtained by following our methodological steps. The structure of protein and ligands was taken as input. After checking physiochemical properties of proteins were docked against the selected ligands. ADMET properties and lipin ski rule helped in prediction of drug-like features of compounds. Further the validation of selected compound was checked by comparing its properties with available antibiotic drug. All these steps are described under headings sequentially.

4.1 Structure Modeling

Structure modeling includes primary sequence retrieval, physiochemical properties prediction, 2D structures prediction and identification of proteins.

4.1.1 Primary Sequence Retrieval

Primary sequence of target proteins (Luteinizing hormone beta subunit polypeptide) was taken in FASTA format from Uniport database (http://www.uniprot.org) under accession number A0A0F7RQE6OS3565=Homo sapiens OX=9606 GN = LHB PE = 3 SV = 2

>trA0A0F7RQE6A0A0F7RQE6-HUMAN Luteinizing hormone beta polypeptide OS=Homo sapiens OX=9606 GN=LHB PE=3 SV=1

MEMLQGLLLLLLLSMGGAWASREPLRPWCHPINAILAVEKEGCPVCITVNT
TICAGYCPTMMRVLQAVLPPLPQVVCTYRDVRFESIRLPGCPRGVDPVVSFP
VALSCRCGPCRRSTSDCGGPKDHPLTCDHPQLSGLLFL

Primary sequence of target proteins (Luteinizing hormone beta subunit polypeptide) was taken in FASTA format from Uniport database (http://www.uniprot.org) under accession number A0A0F7RQE6.

4.1.2 Physiochemical Characterization of Luteinizing Hormone Beta Subunit

An online tool called ProtParam makes it possible to compute different physical and chemical properties for a given protein stored in Swiss-Port or TrEMBL as well as for user-inputted protein sequences. The numerous characteristics calculated by ProtParam include MW, theoretical PI, a.a composition (positive and negative charge), atomic compound, extinction coefficient, predicted half-life, instability index, aliphatic index, and grand average of hydropathicity (GRAVY). Greater than 7 signifies the protein's basic nature, whereas less than 7 indicates the protein's acidity. Extinction coefficient is a symbol for light absorption. Protein stability is indicated by an instability index below 40, whereas protein instability is indicated by an index over 40 [112]. The aliphatic composition of a protein is represented by the aliphatic index. The protein's high aliphatic index value implies that it is thermostable.

Both positively and negatively charged protein residues can be found in molecular weight. Tyr and Trp are present in high concentrations at 280 nm as shown by the extinction coefficient range of 73980, 67965, 20105, and 112270 [111]. Low GRAVY demonstrates improved interplay with water molecules. Better interactions with water molecules are seen at lower gravity. Table 4.1 displays the Physiochemical characteristics of luteinizing hormones beta subunit. Therefore, all of

the characteristics used for the current investigation were based on past research [112]. The physiochemical properties of target protein are shown in Table4.1.

TABLE 4.1: Physiochemical Properties of Luteinizing hormone beta subunit.

Target Proteins	**Ext.Co1**	**Ext.Co2**	**MW**	**PI**	**NR**	**PR**
LHB	9230	8480	13202.57	8.30	9	12

The below table show target proteins with the properties.

TABLE 4.2: Target proteins properties.

Instability Index	**Aliphatic Index**	**GRAVY**
53.49	87.69	0.128

MW stands for "Molecular weight," pI for "Theoretical pI," NR for "total negatively charged residues" (Asp + Glu), PR for "total positively charged residues" (Arg + Lys), ExtCo1 for "Ext. coefficient" (all Cys residues are reduced), ExtCo2 for "Ext. coefficient," II for "instability index," AI for "Aliphatic index," and GRAVY for "gravity (Grand average of hydropathicity).

4.1.3 Retrieval of Structure of Protein

In the fields of the life sciences, basic biology, biomedicine, biotechnology, bioengineering and energy, the PDB is widely considered as key and core data resource [113].

Large biological entities like proteins and nucleic acids have three-dimensional structural information stored in a database called the Protein Data Bank (PDB). The information is frequently gathered via X-ray crystallography, NMR spectroscopy or increasingly cryo-electron microscopy (PDB e, RCSB, and BMRB]) and is made available to the public on the Internet via the websites of its member organizations. The protein structures were prepared in 3D studio discovery by removing water molecules and ligands if existed. Saved the modified file in

PDB format. The refined structures are shown in figures below. The PDB id

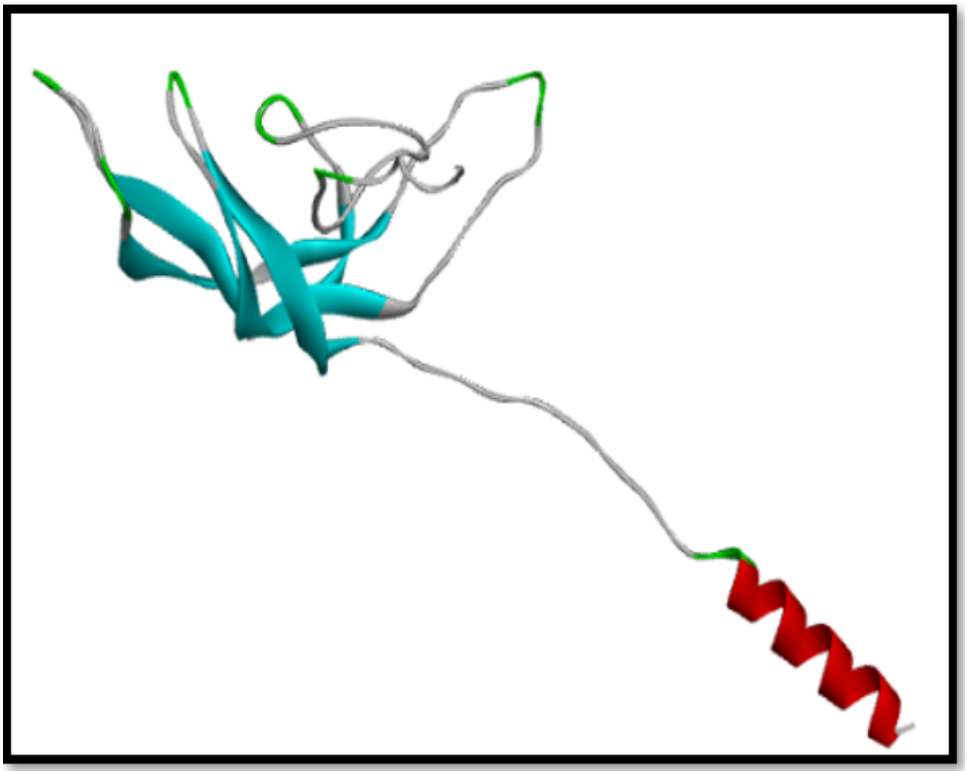


FIGURE 4.1: Structure of Luteinizing Hormone Beta protein

AF-A0A0F7RQE8-F1 was used to derive the luteinizing hormone beta protein structure for the current investigation. The Worldwide Protein Data Bank, or wwPDB, is the organization in charge of running the PDB. The Protein Data Bank (PDB) library of 3D structure data for big biological molecules (proteins, DNA, and RNA) is a vital resource for study and education in fundamental biology, health, energy, and biotechnology. RCSB PDB (www.RCSB.org) is the US data center for the PDB database.

4.1.4 Purification of Protein Structure

A strong graphical interface for accessing Discovery Studio Science is the Discovery Studio Visualizer Client. Research teams may install and manage DS Visualizer Clients on a budget while still receiving top-notch science. The DS Visualizer Client has unmatched capabilities for sharing data, processes, and computing resources when used in conjunction with a Pipeline Pilot Server. For the automation

and customization of typical modelling operations that operate on DS data models or mimic UI actions, a comprehensive collection of Perl-based scripting commands, as well as customized scripts, are also available. The extensive list of scriptable operations includes, as just a few examples, molecular overlay, chirality and valency checks, secondary structure prediction for DSC, access to electrostatics tools, management of constraints/restraints, and surface display [114].

3D Discovery Studio was used to purify the protein structure to remove water molecule and small molecules such as ligands present in the protein structure. The purification was done to prepared the structure of protein for docking purpose.

4.2 Retrieval of Structure of Ligand

The protein data library contains a large number of protein ligand complexes, notably for the protein target. The observed complex is the result of a procedure known as conformational selection, which occurs when a ligand binds to one of these conformers in particular, strengthening it and raising its population in comparison to the protein's overall population [115] .

PubChem, a freely available global collection of chemical knowledge, was used to search for ligands. PubChem can be accessed at https://pubchem.ncbi.nlm.nih.gov. They were obtained from PubChem in SDF-formatted 3D structures. Following ligand selection, energy minimization was carried out using the chem pro program (chem 3D v 12.0.2) [116].

The Lipinski rule concerns a number of parameters, including molecular weight, which should be less than or equal to 500, log P, which should be less than or equal to 5, hydrogen bond donors, which should be less than or equal to 5, and hydrogen bond acceptors, which should be less than or equal to 10.

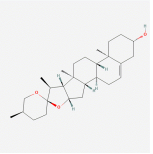
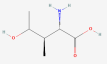
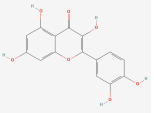
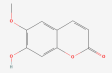
This was a necessary step in the ligand synthesis process since unstable ligands will produce unreliable docking data in terms of scores. Alkaloids, cadmium, lignans, linatine, linoleic acid, cyanogenic glycosides, 4-hydroxyisoleucine, diosgenin,

galactomannan, quercetin, scopoletin, and trigonelline were the chosen ligands for both fenugreek and flaxseed. The Lipinski rule of five is used to guide the selection of this ligand.

The Lipinski rule concerns a few parameters, including molecular weight, which should be less than or equal to 500, log P, which should be less than or equal to 5, hydrogen bond donors, which should be less than or equal to 5, and hydrogen bond acceptors, which should be less than or equal to 10 [117]. Table 4.3 and 4.4 lists a selection of ligands along with their chemical composition, molecular weight, and formula.

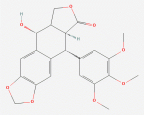
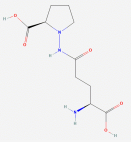
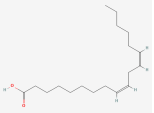
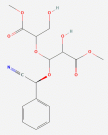
Fenugreek

TABLE 4.3: The following table represents structure of Fenugreek ligands.

Ligand Name	**Molecular Weight**	**Molecular Formula**	**Structure**
Diosgenin	414.6 g/Mol	$C_{27}H_{42}O_3$	
4-Hydroxy Isoleucine	147.17 g/Mol	$C_6H_{13}NO_3$	
Quercetin	302.23 g/Mol	$C_{15}H_{10}O_7$	
Trigonelline	137.14 g/Mol	$C_7H_7NO_2$	
Scopoletin	192.17 g/Mol	$C_{10}H_8O_4$	

Flaxseed

TABLE 4.4: The following table represents structure of Flaxseed ligands.

Ligand Name	**Molecular Weight**	**Molecular Formula**	**Structure**
Cadmium	112.41 g/mol	Cd	
Lignans	414.4 g/mol	$C_{22}H_{22}O_8$	
Linatine	259.26 g/mol	$C_{18}H_{32}O_2$	
Linolic acid	280.4 g/mol	$C_{18}H_{32}O_2$	
Cyanogenic Glycosides	353.32 g/mol	$C_{16}H_{19}NO_8$	

The Structure of ligands were retrieved through PubChem, the selected ligands such as Quercetin Trigonelline, Scopoletin, Cadmium, Linatine, Linolic Acids used for current research as shown in figure 4.1.

4.3 Molecular Docking

The goal of molecular docking approaches is to forecast the most effective way for a ligand to attach to a macromolecular partner (here just proteins are considered). It entails creating a variety of potential poses, or conformations and orientations, for the ligand inside the protein binding site. As a result, the presence of the molecular target's three-dimensional structure is a prerequisite; this structure may

have been determined experimentally (for example, by X-ray crystallography or NMR) or computationally (for example, through homology modelling) [118].

A few of the numerous uses and applications for molecular docking in drug discovery include structure-activity studies, lead optimization, virtual screening for potential leads, providing binding hypotheses to facilitate predictions for mutagenesis studies, assisting x-ray crystallography in the fitting of substrates and inhibitors to electron density, providing binding hypotheses to facilitate predictions for mutagenesis studies, and combinatorial library design [119].

It is possible to evaluate the amount of interaction between the ligand and the target proteins as well as the correct structure of the ligand within the target binding site by using a specific scoring function. Additionally, it makes it easier to recognize novel small molecule compounds by highlighting critical characteristics that help choose the best lead for the target, such as complexes formed with target proteins and suitable distribution, metabolism, and excretion.

Luteinizing hormone beta subunit proteins were used for the docking, along with fenugreek and flaxseed ligands (alkaloids, cadmium, lignans, linatine, linoleic acid, and cyanogenic glycosides) (4-Hydroxyisoleucine, diosgenin, galacto-mannan, quercetin, scopoletin, trigonelline). We utilized a user-friendly blind docking web server called Patch Dock to automatically anticipate binding modes without knowledge of binding sites. A geometry-based molecular docking method is Patch Dock. Its goal is to identify docking modifications that provide favorable complementarity of molecule shape. When these transformations are used, they result in both large interface regions and minor steric collisions. The attached molecules' local attributes that match and complement one another are included in a broad interface.

The Connolly dot surface representation of the molecules is divided into concave, convex, and flat patches using the Patch Dock method. Then, candidate transformations are created by matching complimentary patches. Patch Dock's quick transformational search, which is fueled by local feature matching rather than brute-force searching of the six-dimensional transformation space, is the primary

factor in its high efficiency [120]. The result of the patch dock were mentioned in table 4.5.

TABLE 4.5: Results of Patch Dock dock with ligands name, binding score and cavity size, grid Map, minimum Energy and maximum energy values

Ligands	**Binding Score**	**Cavity Size**	**Grid Map**	**Minimum Energy**	**Maximum Energy**
Quercetin	-6.9	276	21	0.00	1.6E.+00
Scopoletin	-5.6	276	18	0.00	1.6E.+00
Linatine	-5.3	276	21	0.00	1.6E.+00

4.4 Active Site Identification

Cast P program, which forecasts available pockets for binding and also discusses the surface area and volume of pockets [121], was used to find the active sites of proteins.

TABLE 4.6: Area and volume of binding pockets of LHB protein obtained by CASTp

Pocket Id	**Area (SA)**	**Volume (SA)**
1	600.249	809.900
2	56.575	22.671
3	10.055	2.504
4	6.629	1.447
5	6.093	1.243
6	6.461	0.602
7	2.413	0.350
8	1.203	0.097
9	0.389	0.048
10	0.308	0.005
11	0.207	0.003

Table 4.6 continued from previous page

12	0.233	0.003
13	0.041	0.000

The above table 4.6 shows the area and volume of the accessory gene regulator protein LHB's binding pocket IDs. It demonstrates that there are 13 accessible places for protein LHB. While the smallest binding pocket has a surface area of 0.041 and a volume of 0.000, the greatest binding pocket has a surface area of 600.249 and a volume of 809.900.

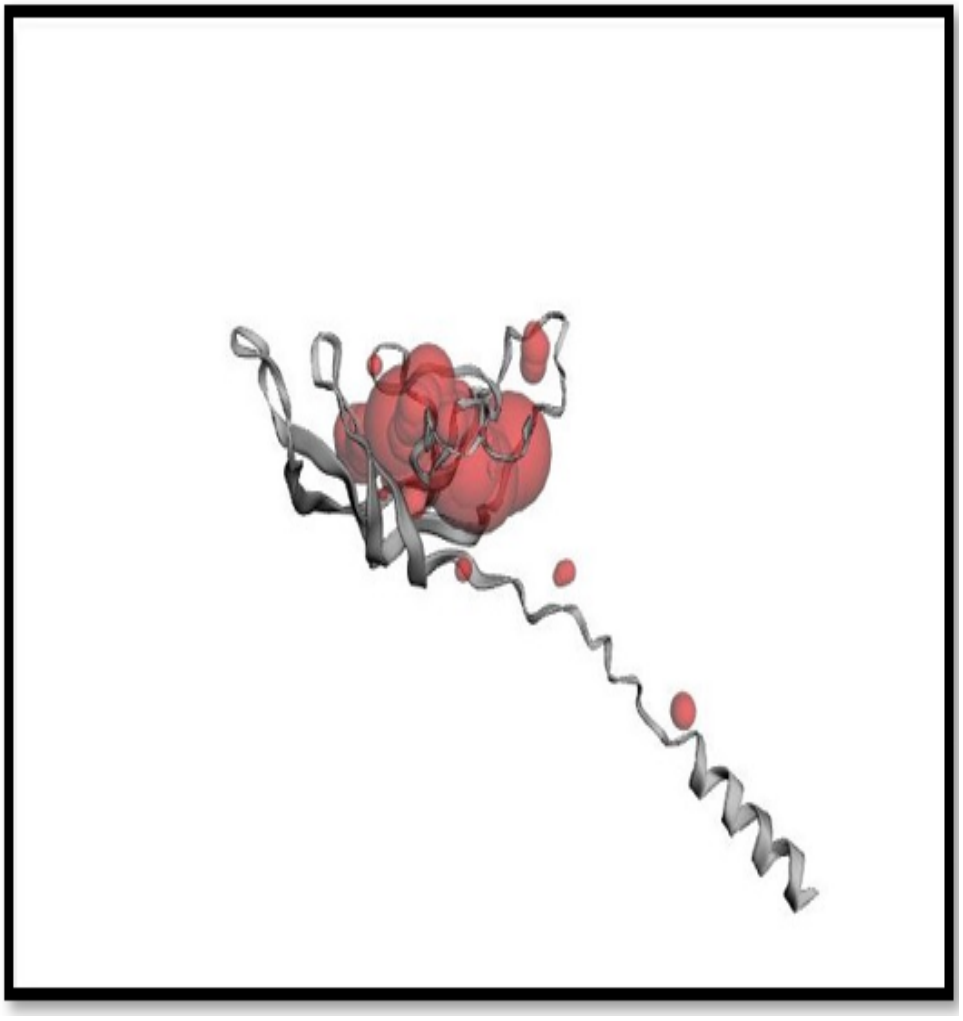


FIGURE 4.2: Structure of LHB protein showing available pockets for ligands.

Red color showing the available binding pocket for protein. Binding pocket is the region where ligand can bind. The number of pockets with size and volume is already shown in above table 4.6.

4.5 Interaction of Ligands and Target Proteins

Small molecules and macromolecules are both transcriptionally combined using a program called Discovery Studio. It was made by Dassault Systems BIOVIA (Accelrys). Discovery Studio, a single integrated graphical user interface, handles both advanced drug design and research on protein modeling. There are several plot viewers and data visualization viewers available in this application. This study improved people's understanding of how to use the Discovery Studio tool to access, distribute, and analyses data on proteins and small molecules. Numerous disciplines, such as quantum physics, molecular dynamics, and mechanism, can benefit from the DS program. Software also has the ability to do hybrid QM/MM calculations. Applications involving macro molecules and small molecules both employ it. Calculations and structural editing can be utilized to ascertain the molecular properties.

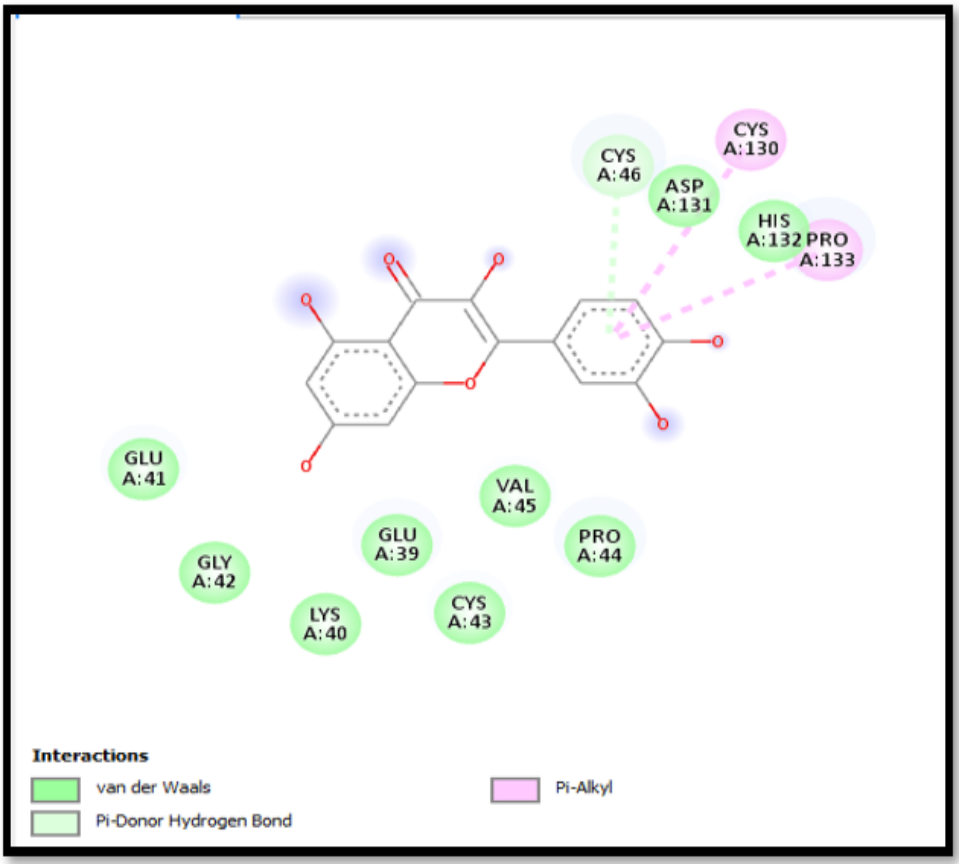


FIGURE 4.3: Quercetin interaction

The figure 4.3 interaction of Quercetin by 3D Studio Discovery.

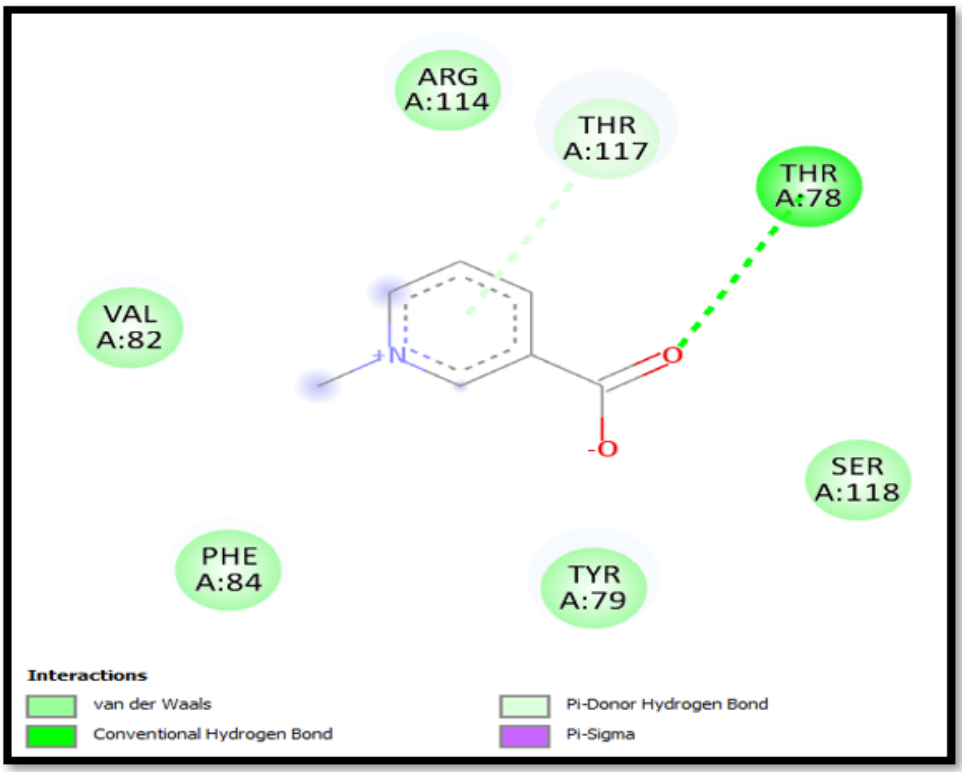


FIGURE 4.4: Trigonelline interaction

The figure 4.4 interaction of Trigonelline by 3D Studio Discovery.

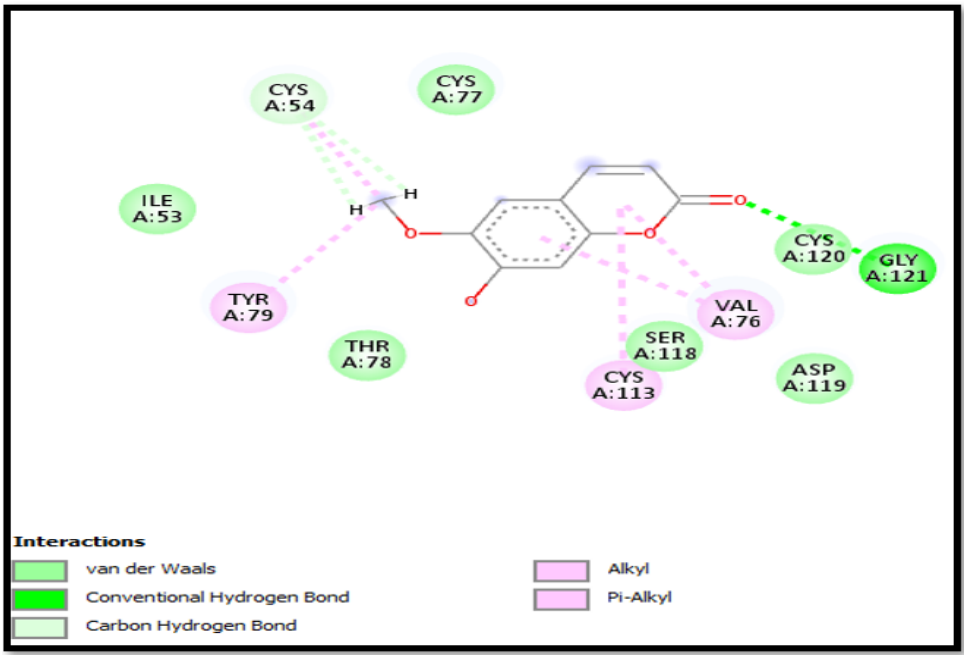


FIGURE 4.5: Scopoletin interaction

The figure 4.5 interaction of Scopoletin by 3D Studio Discovery.

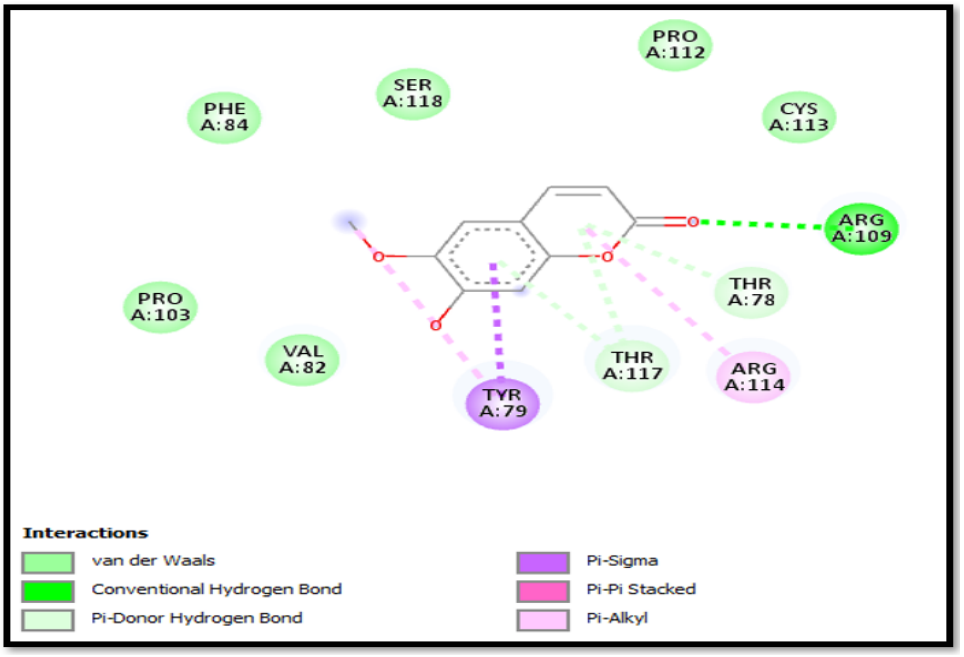


FIGURE 4.6: Scopoletin interaction

The figure 4.6 interaction of Scopoletin by 3D Studio Discovery.

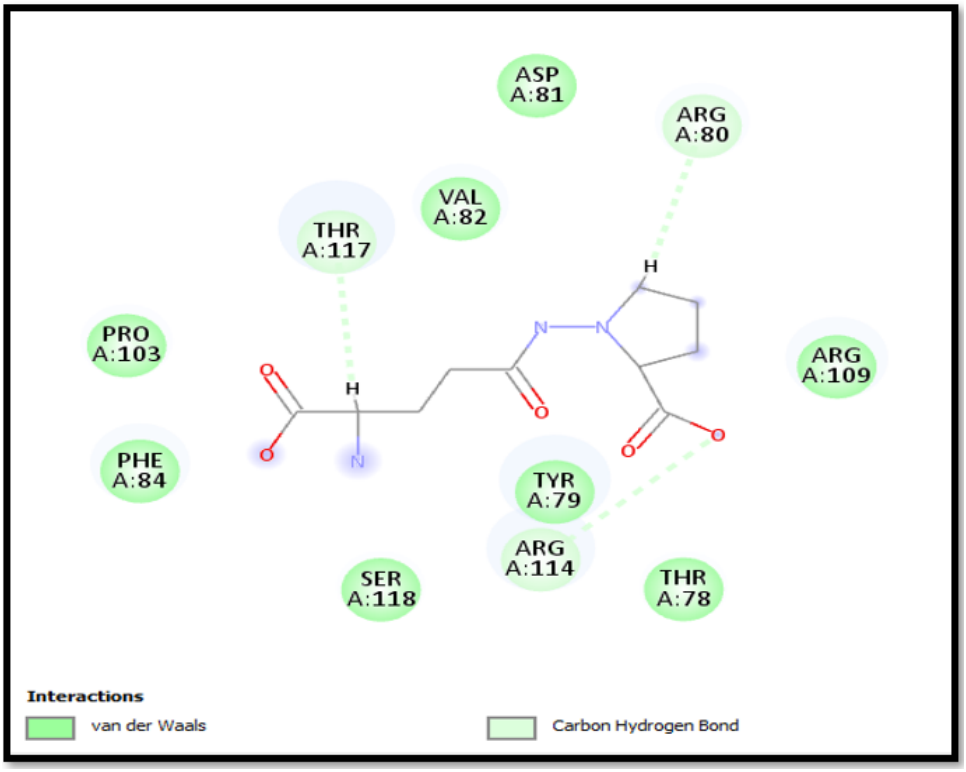


FIGURE 4.7: Linatine interaction

The figure 4.7 interaction of Linatine by 3D Studio Discovery.

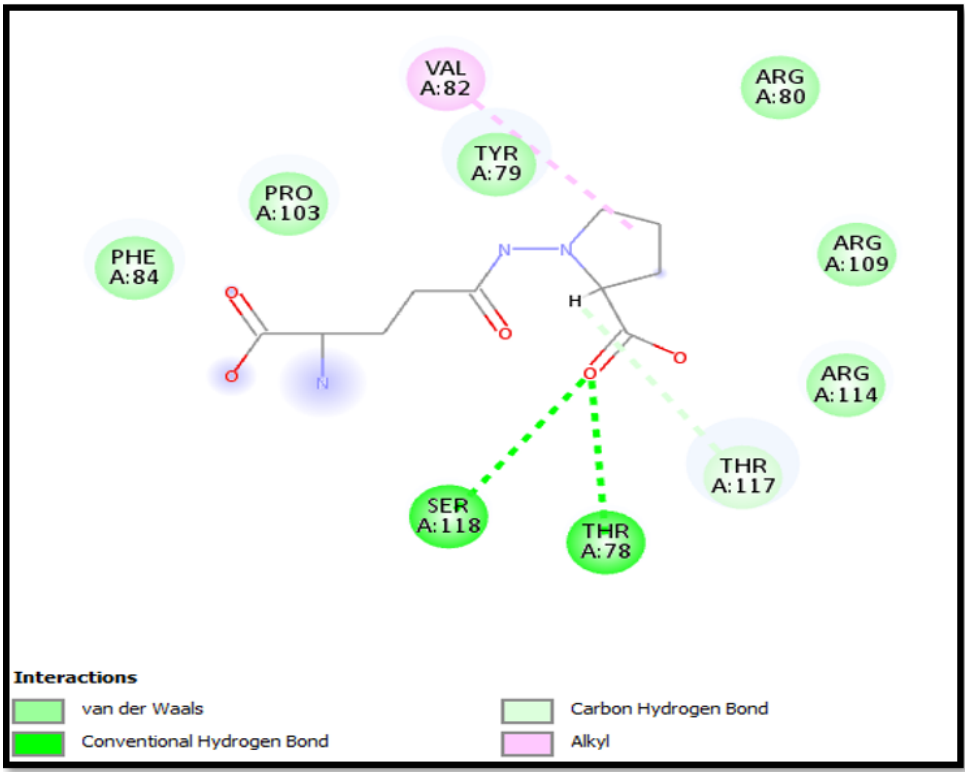


FIGURE 4.8: Linatine interaction

The figure 4.8 interaction of Linatine by 3D Studio Discovery.

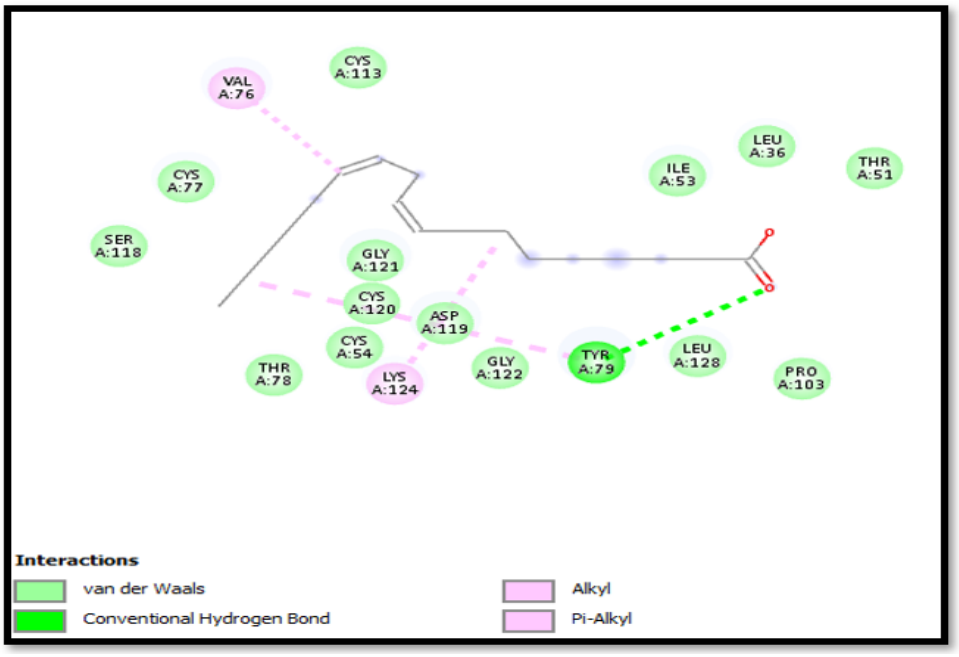


FIGURE 4.9: Linolic acid interaction

The figure 4.9 interaction of Linolic acid by 3D Studio Discovery.

4.5.1 Results of Protein and Ligand Interaction

Software called Discovery Studio combines transcription of small compounds and macromolecules. Dassault Systems BIOVIA created it (Accelrys). Discovery Studio, a single integrated graphical user interface, handles both advanced drug design and research on protein modelling. There are numerous plot viewers and data visualization viewers available in this application.

This study helped people better grasp how to access, share, and analyses protein and small molecule data using the Discovery Studio program. The DS program has applications in a variety of fields, including as quantum physics, molecular dynamics, and mechanism. Additionally, software is capable of doing hybrid QM/MM computations. It is used in applications involving both macromolecules and small molecules. Editing structures and doing calculations can be used to determine the molecular characteristics.

Table 4.7: Protein and Feenugreek ligand interaction

Name of Compound	**Vander wall force a.a**	**Conventional H2 bond**	**Pi sigma**	**Pi alkyl**	**Pi Donor H2 bond**
Quercetin	Glu(A:41) GLY(A:42) LYS(A:40) GLU(A:39) CYS(A:43) VAL(A:45) PRO(A:44) ASP(A:131)			CYS(A:130) PRO(A:133)	CYS(A:46)
Trigonelline	HIS(A:132) VAL(A:82) PHE(A:84)				THR(A:117)

Table 4.7: Protein and Feenugreek ligand interaction

Name of Compound	**Vander wall force a.a**	**Conventional H2 bond**	**Pi sigma**	**Pi alkyl**	**Pi Donor H2 bond**
Scopoletin	TYR(A:79) SER(A:118) ARG(A:117) VAL(A:82) PRO(A:103) PHE(A:84) SER(A:118) PRO(A:112) CYS(A:113)	ARG (A:109) THR (A:78)	TYR (A:79)	ARG (A:114)	THR(A:117) THR (A:78) THR (A:78)

Table 4.8: Protein and Flax seed ligated interaction

Name of Compound	**Vander wall a.a**	**Alkyl group**	**Conventional H2 bond**	**Pi Alkyl**	**Carbon H2 bond**
Cadmium	PRO (A:103) VAL (A:82) PHE (A:84) THR (A:78)	TYR (A:79)		TYR (A:79)	

Table 4.8: Protein and Flax seed ligated interaction

Name of Compound	**Vander wall a.a**	**Alkyl group**	**Conventional H2 bond**	**Pi Alkyl**	**Carbon H2 bond**
Linatine	SER (A:118) Arg (A:80) Arg (A:109) Arg (A:114) PRO (A:103) PHE (A:84) TYR (A:79)	VAL(A:82)	SER(A:118) THR(A:78)		THR (A:117)
Linolic Acids	ASP (A:119) LEU (A:128) LEU (A:36) THR (A:78) THR (A:51) ILE (A:53)	VAL(A:76)	TYR(A:79)	LYS (A:124)	

TABLE 4.8: Protein and Flax seed ligated interaction

Name of Compound	**Vander wall a.a**	**Alkyl group**	**Conventional H2 bond**	**Pi Alkyl**	**Carbon H2 bond**
	SER(A:118)				
	CYS (A:77)				
	CYS (A:113)				
	PRO (A:103)				
	GLY (A:122)				
	GLY (A:121)				
	CYS (A:54) CYS (A:120)				

The purified structure of protein and ligands were given as an input file to patch dock pdb form. The results of patch dock give 10 complex interactions of ligands and target protein. These interactions were visualized by 3D Discovery studio. Some ligands such as Quercetin Trigonelline, Scopoletin, Cadmium, Linatine,

Linolic Acids show good interactions with target protein. The interaction shows Vander wall a.a, Alkyl group, Conventional H2 bond, pi sigma, pi alkyl, Pi Donor H2 bond, Carbon H2 bond and pi sigma as shown in table 4.7 and in table 4.8.

4.6 Virtual Screening and Toxicity

Prediction PkCSM is a tool that used online to determine the ADMET properties (Absorption, distribution metabolism, execration, and the toxicity of drug [108]. The Lipinski rule has been applied as a filter the research project. Table demonstrated the Lipinski rules applicability to ligands. The Rule of 5 has the advantage of simplicity and a readily understood physicochemical basis. Followings rules are:

1. Molecular mass 500
2. Log P5
3. No. of H-bond donors 5
4. No of H-bond acceptors 10 [121].

So, rule was applied to our compound and analyses the different ligands of fenugreek and flaxseed is checked which are shown in table 4.9 and table 4.10

TABLE 4.9: Physiochemical properties of Fenugreek ligands

Name of Ligands	**M/W**	**Log p**	**H-Bond Donor**	**H-Bond Acceptor**
Quercetin	302.23 g/mol	1.22	5	7
Trigonelline	137.14 g/mol	-1.12	0	2
Scopoletin	192.17 g/mo	1.52	1	4

TABLE 4.10: Physiochemical properties of Flaxseed ligands

Name of Ligands	**M/W**	**Log p**	**H-Bond Donor**	**H-Bond Acceptor**
Cadmium	112.41 g/mol	0.21	0	0
Linatine	259.26 g/mol	-1.24	4	5
Linolic Acid	280.4 g/mo	5.88	1	1

Physicochemical and pharmacokinetics properties determine the final destiny of compounds as drug or non-drug compounds. Physicochemical properties or Lipinski rule of five works as primary filter and pharmacokinetics studies as secondary filter in screening of potential compounds. Trigonelline, cadmium, and linolic acid did not obey Lipinski rule of five so they knock out in primary screening. On the basis of binding score, ADMET properties, physicochemical properties and Lipinski rule of five, Scopoletin was selected as lead compound which could inhibit target proteins.

4.7 ADMET Properties of Ligands

In a second analysis, the online program PkCSM was used to determine the ADMET characteristics of ligands as an indicator of pharmacokinetics. Pharmacodynamics and pharmacokinetics are two basic words used in pharmacology. PkCSM online tool used to extracted the ADMET properties of ligands. Before creating a medicine, it is important to take into account toxicity since it sheds light on the characteristics of ligands. Prior to being used as a chemotherapy agent, a substance must undergo toxicity testing. PkCSM is utilized to determine the drug's

toxicity and ADME characteristics. SMILES can be entered into this server. These are the values from the dragged server.

4.7.1 Pharmacodynamics

Pharmacodynamics is a branch of pharmacology in which we study the effect of drugs on the body.

4.7.2 Pharmacokinetics

In pharmacokinetics we study the absorption of drugs, distribution of drugs, metabolism of the drug and excretion of the drugs.

4.8 Absorption

Absorption in pharmacology refers to the process by which a medication moves from the circulation into the tissues (more particularly, pharmacokinetics). The pace and extent of medication absorption are thus influenced by both the chemical makeup of a medicine and the environment in which it is administered. A medicine must pass through cellular barriers, such as epithelial or endothelial cells, in order for it to be absorbed. The majority of drugs cross cellular barriers through passive diffusion, which is when the drug simply diffuses through cell membranes from an area of higher concentration to an area of lower concentration.

TABLE 4.11: Absorption properties of ligands [111]

Model name	**Linatine**	**Linolic acid**	**Quercetin**	**Scopoletin**	**Trigonelline**
Water solubility	-2.891	-2.77	-2.925	-2.504	-1.931
$CaCO_2$ permeability	-0.354	1.541	-0.229	1.184	1.124

TABLE 4.11: Absorption properties of ligands [111]

Model name	**Linatine**	**Linolic acid**	**Quercetin**	**Scopoletin**	**Trigonelline**
Intestinal absorption	15.2	83.012	77.207	95.277	96.44
Skin permeability	-2.7	-2.556	-2.735	-2.944	-2.736
p-glycoprotein substrate	Yes	No	Yes	No	Yes
p-glycoprotein I inhibitor	No	No	No	No	No
p-glycoprotein II inhibitor	No	No	No	No	No

4.8.1 Distribution

Pharmacology's area of pharmacokinetics known as distribution studies how drugs migrate from one part of the body to another. A medicine must be transported into interstitial and intracellular fluids before it can be absorbed into the body or administered directly.

TABLE 4.12: Distribution properties of ligands [112]

Identified models	**Linatine**	**Linoleic acid**	**Quercetin**	**Scopoletin**	**Trigonelline**
VDSS	-0.48	-0.729	1.559	0.034	-0.758
Fraction unbound	0.45	0.054	0.206	0.363	0.857
BBB permeability	-0.92	-0.367	-1.098	-0.299	-0.234
CNS	-3.71	-3.186	-3.065	-2.32	-2.739

4.8.2 Metabolism

With the aid of enzymes, metabolism is the process of changing one chemical into another. Most metabolism takes place in the liver, lungs, gut, and blood plasma. Typically, the drug's polarity will increase during the metabolic process, making it a more water-soluble molecule.

TABLE 4.13: Metabolic properties of ligands [113]

Identified models	**Linatine**	**Linoleic acid**	**Quercetin**	**Scopoletin**	**Trigonelline**
CYP3A4 Inhibitors	No	No	No	No	No
CYP3A4 Inhibitors	No	No	No	No	No
CYP1A2 Inhibitors	No	No	Yes	Yes	No
CYP2C19 Inhibitors	No	No	No	No	No
CYP2C9 Inhibitors	No	No	No	No	No
CYP2D6 Inhibitors	No	No	No	No	No
CYP3A4 Inhibitors	No	No	No	No	No

4.8.3 Excretion

The kidneys, which play a significant part in excretion (renal excretion), and the liver are the organs involved in drug excretion (biliary excretion). The excretion process may also include other organs, such as the lungs in the case of volatile or gaseous substances.

Drug excretion can also occur in saliva, tears, and perspiration.

TABLE 4.14: Excretion properties of ligands [114]

Identified models	**Linatine**	**Linoleic acid**	**Quercetin**	**Scopoletin**	**Trigonelline**
Total clearance	0.367	1.869	0.407	0.73	0.378
Renal OCT_2 Substrate	No	No	No	No	No

4.8.4 Toxicity Prediction

PkCSM is an online tool that provides an integrated platform for swiftly evaluating the pharmacokinetic and toxicological properties of a medicine. This method is used to assess the PCOS-harmful potential of ligands.

TABLE 4.15: Toxicity properties of ligands [115]

Identified models	**Linatine**	**Linoleic acid**	**Quercetin**	**Scopoletin**	**Trigonelline**
Max. tolerated dose	1.306	0.651	0.499	0.614	0.743
hERG I inhibitor	No	No	No	No	No
hERG II inhibitor	No	No	No	No	No
Oral rat acute toxicity	2.386	2.482	2.471	1.95	1.878
Oral rat chronic toxicity	2.194	1.117	2.612	1.378	0.454

TABLE 4.15: Toxicity properties of ligands [115]

Identified models	**Linatine**	**Linoleic acid**	**Quercetin**	**Scopoletin**	**Trigonelline**
Hepatoxicity	No	NO	No	No	No
Skin sensitization	No	NO	No	No	No
T. pyriformis toxicity	0.285	0.478	0.288	0.516	-0.323
Minnow toxicity	3.881	4.299	3.721	1.614	2.536
LD50 (mg/kg)	1000	10000	159	1190	3720
Toxicity class	4	6	3	4	5

According to reports, the hERG I and II inhibitors model blocks the potassium channels that the hERG triggers, resulting in fatal ventricular arrhythmia and prolonged QT syndrome (human ether-a-go-go gene). Numerous medicinal drugs have been taken off the market because hERG channels are blocked. The LD50 is the medication concentration at which 50% of test animals, like mice, succumb. The LD50 (mol/kg) predicts toxicity while the LOAEL aims to identify the lowest dose of a chemical having a significant adverse effect. In medicine, prolonged exposure to low to moderate chemical doses expressed as a log (mg/kg - bw/day) is particularly important. Hepatotoxicity, which manifests as medication-induced liver injury, is a significant safety issue for drug development. Skin sensitivity is a possible side effect of skincare and applied products. It is common practise to use the protozoan bacteria textitT. pyriformis as a toxic endpoint since its toxin slows growth by 50%. (IGC50). It is regarded as hazardous when the anticipated value of p IGC50 (negative concentration logarithm necessary to halt 50% growth) is more than -0.5 log ug/L. Lethal concentrations (LC50) are defined as the number of molecules needed to kill 50% of Flathead Minnows (small bait fishes). According

to Minnow toxicity, high acute toxicity is indicated by LC50 values lower than 0.5 mm (log LC 50 less than -0.3) [122].

4.9 Identification of Lead Compound

The ultimate fate of substances as drugs or non-drugs is determined by their physicochemical and pharmacokinetic features. Pharmacokinetics studies serve as a secondary filter after physical-chemical properties, often known as the Lipinski rule of five, in the screening of prospective compounds. Trigonelline, Cadmium, and linolic acid failed to abide by the Lipinski rule of five and were eliminated from further consideration. Quercetin was chosen as the lead molecule that might inhibit target proteins based on binding score, ADMET qualities, physicochemical properties, and Lipinski rule of five.

4.10 Identification of Drug

The selection of most efficient anti-bacterial drug is based on the physiochemical, ADMET properties along mechanism of action with side effects. For physiochemical properties PubChem online database is used and for ADMET properties of drugs PkCSM online tool is used. Mechanism of action is identified through DB.

4.11 Selection of Drug

The most effective medication is chosen based on its physiochemical and ADMET qualities, as well as its mode of action and adverse effects.

Physiochemical characteristics of pharmaceuticals are determined using the PubChem online database, while ADMET properties are determined using the PkCSM online program. Drug Bank is used to identify the mechanism of action.

TABLE 4.16: Given table show the drug and their properties [116]

Sr.no	**Properties**	**Letrozole**
1	Chemical formula	$C_{17}H_{11}N_5$
2	Absorption	Letrozole is 99.9% orally bioavailable. A 2.5mg oral dose reaches a Cmax of 104nmol/L with a Tmax of 8.10h, and an AUC of 7387nmol*h/L
3	Water solubility	0.0799 mg/mL
4	H- bond acceptor	4
5	H-bond donor	0
6	Polarizability	29.59 Å3
7	Side effect	hot flashes, warmth or redness in your face or chest
8	Log P	2.4
9	Bioavailability	1
10	H-bond donor	0
11	H-bond Acceptor	4
12	Molecular weight	285.30g/mol

4.11.1 Letrozole

Nonsteroidal, third-generation aromatase inhibitor letrozole, which is taken orally once daily, has shown effective in treating postmenopausal women with hormone-sensitive breast cancer that is in the early or advanced stages.

Letrozole is a strong inhibitor of oestrogen production and a highly selective non-steroidal aromatase inhibitor. Letrozole dramatically decreased the plasma levels of estrone, estradiol, and ovariectomized in postmenopausal breast cancer patients. Letrozole lowered cellular indicators of proliferation more than tamoxifen in estrogen-dependent tumors that overexpressed human epidermal growth factor receptor (HER)1 and/or HER2 and exhibited anti-tumor effects in ovariectomized animal models of postmenopausal estrogen-dependent breast cancer. In healthy postmenopausal women and those with a history of breast illness, letrozole raised the levels of bone resorption indicators [122].

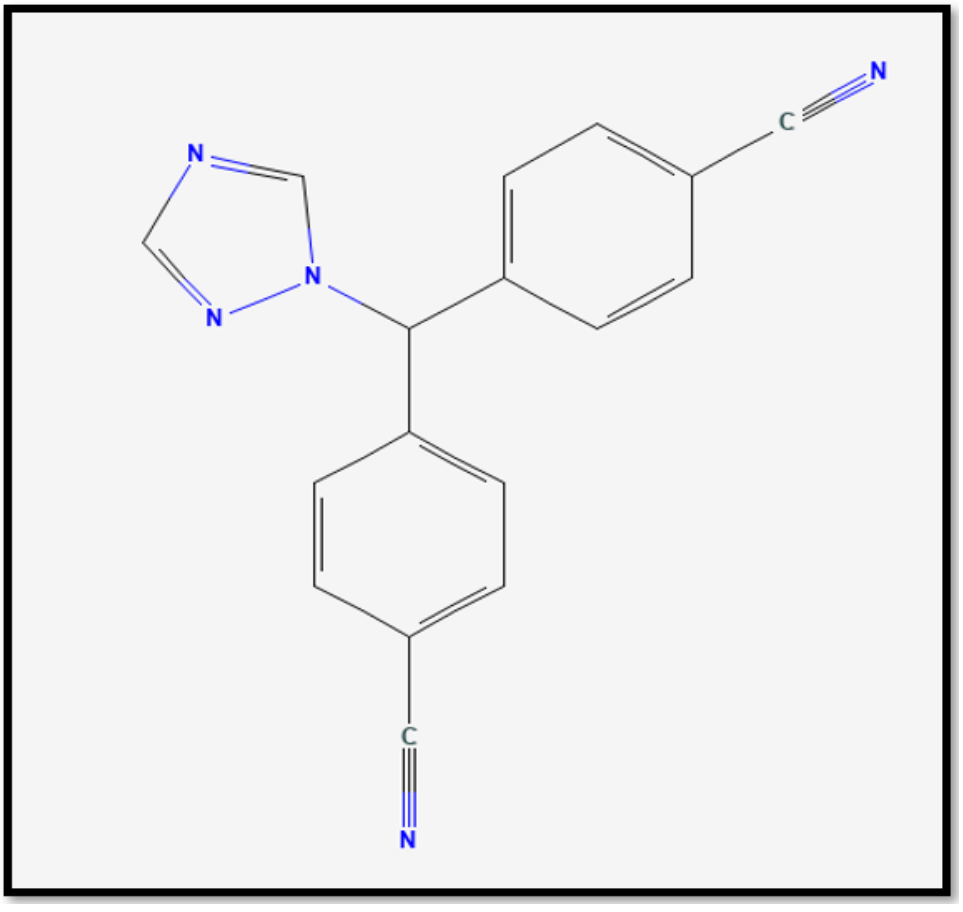


FIGURE 4.10: 2D Structure of Letrozole from the PubChem Database

4.11.2 Letrozole Mechanism of Action

Letrozole is a non-steroidal type II aromatase inhibitor. It blocks the active site, and therefore the electron transfer chain of CYP19A1. This competitive inhibition prevents the conversion of androgens to estrogen. This action leads to a reduction in uterine weight and elevated luteinizing hormone. In postmenopausal women, the action of aromatase is responsible for the majority of estrogen production. With reduced availability of estrogen, estrogen-dependent tumors regress. Third generation aromatase inhibitors do not significantly affect cortisol, aldosterone, and thyroxine level.

4.11.3 DRUG ADMET Properties

The ADMET properties of the selected drug letrozole were identified through PkCSM online prediction tool.

4.11.3.1 Toxicity Prediction of Selected Drug

PkCSM is an online tool that provides an integrated platform for swiftly evaluating the pharmacokinetic and toxicological properties of a medicine. This method is used to assess the PCOS-harmful potential of ligands.

TABLE 4.17: The Toxicity value of selected drug letrozole [116]

Identified Models	**Predicted Values**
Max.tolerated dose(human)	-0.592
hERG I inhibitor No	No
hERG II inhibitor No	No
Oral rat acute toxicity	2.085
Oral rat chronic toxicity	1.238
Hepatoxicity	No
Skin sensitization	No
T. pyriformis toxicity	0.851

TABLE 4.17: The Toxicity value of selected drug letrozole [116]

Identified Models	**Predicted Values**
Minnow toxicity	1.854
Toxicity class	4
LD50	1463mg/kg

4.11.3.2 Absorption Properties

Absorption in pharmacology refers to the process by which a medication moves from the circulation into the tissues (more particularly, pharmacokinetics).

TABLE 4.18: The Absorption value of selected drug letrozole [117]

Identified Models	**Predicted Values**
Water solubility	-3.793
$CaCO_2$ permeability	0.883
Intestinal absorption	99.83
Skin permeability	-2.492
p-glycoprotein substrate	No
p-glycoprotein I inhibitor	No
p-glycoprotein II inhibitor	No

4.11.3.3 Distribution Properties

Pharmacology's area of pharmacokinetics known as distribution studies how drugs migrate from one part of the body to another. A medicine must be transported into interstitial and intracellular fluids before it can be absorbed into the body or administered directly.

TABLE 4.19: The Distribution value of selected drug letrozole [118]

Identified Models	**Predicted Values**
VDSS	-0.031

TABLE 4.19: The Distribution value of selected drug letrozole [118]

Identified Models	**Predicted Values**
Fraction unbound	0.164
BBB permeability	-0.386
CNS	-2.05

4.11.3.4 Metabolic Properties

With the aid of enzymes, metabolism is the process of changing one chemical into another. Most metabolism takes place in the liver, lungs, gut, and blood plasma. Typically, the drug's polarity will increase during the metabolic process, making it a more water-soluble molecule.

TABLE 4.20: The Metabolic value of selected drug letrozole [119]

Identified Models	**Predicted Values**
CYP3A4 Inhibitors	No
CYP3A4 Inhibitors	Yes
CYP1A2 Inhibitors	Yes
CYP2C19 Inhibitors	Yes
CYP2C9 Inhibitors	No
CYP2D6 Inhibitors	No
CYP3A4 Inhibitors	Yes

4.11.3.5 Excretion Properties

The kidneys, which play a significant part in excretion (renal excretion), and the liver are the organs involved in drug excretion (biliary excretion). The excretion process may also include other organs, such as the lungs in the case of volatile or gaseous substances. Drug excretion can also occur in saliva, tears, and perspiration.

TABLE 4.21: The Excretion value of selected drug letrozole [120]

Total clearance	0.77
Renal OCT_2 Substrate	Yes

4.11.4 Letrozole Docking

For the docking purpose Patch Dock online docking tool was used. It gives 5 best confirmation results and finest is selected.

TABLE 4.22: Results of patch dock of Letrozole

Drug Name	**Binding Score**	**Cavity Size**	**Grid Map**	**Minimum energy (Kcal/mol)**	**Maximum energy (Kcal/mol)**
Letrozole	-6.1	276	20	0.00	160E+ 00

4.12 Letrozole and Lead Compound Comparison

When choosing chemical as therapeutic candidates ,pharmacokinetics qualities are a key consideration. To compare the ADMET characteristics, absorption, distributation,metabolism, exceretion and toxicity are examined. Tables contain a list of these attributes.

4.12.1 Absorption Properties Comparison

Absorption in pharmacology refers to the process by which a medication moves from the circulation into the tissues (more particularly, pharmacokinetics).

TABLE 4.23: Absorption properties comparison [121]

Properties	**Letrozole**	**Quercetin**
Water solubility	-3.793	-2.925

TABLE 4.23: Absorption properties comparison [121]

Properties	**Letrozole**	**Quercetin**
$CaCO_2$ permeability	0.883	-0.229
Intestinal absorption on Human	99.83	77.207
Skin permeability	-2.492	-2.735
P-glycoprotein substrate	No	Yes
P-glycoprotein in I inhibitor	No	No
P-glycoprotein in II inhibitor	No	No

Table 4.23 shows that Quercetin is easily soluble in water as compared to letrozole and it is permeable to skin. The intestine absorption of Quercetin is lower as compared to letrozole. But other properties follow all the rules Lipinski rule of 5 and ADMET properties.

4.12.2 Distribution Properties Comparison

Table 4.24 shows the comparison of distribution properties. Ligand and lead compounds have reasonable value for VDss, as if it exceeded 2.8 L/kg then the drug is more distributed in the tissue rather than blood plasma.

TABLE 4.24: Distribution properties comparison [122]

Properties	**Letrozole**	**Quercetin**
VDss Human (L/kg)	-0.031	1.559
Fraction unbound Human (Fu)	0.164	0.206
BBB Permeability (logBB)	-0.386	-1.098
CNS Permeability (log PS)	-2.05	-3.065

Table 4.24 shows that distribution properties of bioactive compound Quercetin lies better than drug Letrozole. Ligand and lead compounds have reasonable value.

4.12.3 Metabolic Properties Comparison

Table 4.25 elaborate on the compared metabolic properties of ligands and lead. The cytochrome P450 isoforms CYP2D6, CYP3A4, CYP1A2, CYP2C19, and CYP2C9 are used to predict the metabolic characteristics of substances.

TABLE 4.25: Metabolic properties comparison [122]

Properties	**Letrozole**	**Quercetin**
CYP2D6 Substrate	No	No
CYP3A4 Substrate	Yes	No
CYP1A2 Inhibitor	Yes	Yes
CYP2C19	Yes	No
CYP2C9	No	No
CYP2D6	No	No
CYP3A4	Yes	No

Table 4.25 shows that metabolism properties of bioactive compound Quercetin lies better than drug Letrozole.

4.12.4 Excretion Properties Comparison

Excretion attribute are represented in table 4.26 as two models with projected values. All substances fall under the "No" category for the "Renal OCT_2 Substrate Model," which means they do not disrupt the normal operation of the Organic Cation Transporter 2 (OCT_2), which is involved in the renal clearance of medicines.

TABLE 4.26: Excretion properties comparison [122]

Properties	**Letrozole**	**Quercetin**
Total clearance	0.77	0.407
Renal OCT_2 Substrate	Yes	No

Table 4.26 shows that Excretion properties of bioactive compound Quercetin lies better than drug Letrozole.

4.12.5 Toxicity Properties Comparison

Of all the pharmacokinetic (ADMET) qualities, toxicity is the most crucial one. Maximum tolerated dosage aids in determining the maximum suggested tolerated dose; if the value is equal to or less than 0.477 log mg/kg/day, it is regarded as low.

TABLE 4.27: Toxicity properties comparison [122]

Properties	**Letrozole**	**Quercetin**
Max. tolerated dose(human)	-0.592	0.499
hERG I inhibitor	No	No
hERG II inhibitor	No	No
Oral rat acute toxicity	2.085	2.471
Oral rat chronic toxicity	1.238	2.612
Hepatoxicity	No	No
Skin sensitization	No	No
T. pyriformis toxicity	0.851	0.288
Minnow toxicity	1.854	3.721
Toxicity class	4	159mg/kg
LD50	1463mg/kg	3

Table 4.27 shows ligand and the Lead Compound does not cause any allergic skin reaction. *T. pyriformis* toxicity value i.e., greater than - 0.5 is considered toxic according to which all compounds show toxicity against *T. pyriformis*. Toxicity levels for minnows are deemed toxic if they are less than 0.5mM.

4.13 Comparsion of Lipinski Rule of 5 between Drug and Ligand

An online tool (PkCSM) has been used to find the Lipinski rule of five. According to that rule, the logP value of the molecule should be limited to 5, molecular weight should be below 500, the maximum number of Hydrogen bond acceptors should be 10 and the maximum number of Hydrogen bond donors should be 5. The Lipinski Rule of Five has been applied to selected ligands and the durg are given in Table 4.28.

TABLE 4.28: Letrozole and Quercetin Lipinski rule of five [122]

Properties	**Letrozole**	**Quercetin**
Chemical formula	$C_{17}H_{11}N_5$	$C_{15}H_{10}O_7$
Molecular-weight	285.30g/mol	302.23g/mol
H. bond donor	0	5
H. bond acceptor	4	7
Log p value	2.4	1.22

Table 4.28 shows that Quercetin bioactive compound showed better result over Letrozole with respect to log P value and hydrogen bond donors and acceptors.

4.14 Docking Score Comparison

Both the standard and the lead compound were docked against the target proteins and the docking result gives us the best binding score.

TABLE 4.29: Docking results comparison [122]

Compounds Name	**Binding Score**	**Cavity Size**	**Grid Map**	**Minimum energy (Kcal/mol)**	**Maximum energy (Kcal/mol)**
Letrozole	-6.1	276	20	0.00	160E+ 00

TABLE 4.29: Docking results comparison [122]

Compounds Name	**Binding Score**	**Cavity Size**	**Grid Map**	**Minimum energy (Kcal/mol)**	**Maximum energy (Kcal/mol)**
Quercetin	-6.9	276	21	0.00	160E.00

Table 4.29 shows that the lead compound Quercetin has higher score than that of the standard drug which is Letrozole. So from this Quercetin has binding energy make that possible.

4.15 Discussion

In women polycystic ovarian syndrome (PCOS) is most chronic endocrinopathy, which affect 5-7% of women of reproductive age. Persistent anovulation and Hyperandrogenism are the characteristics of PCOS and its morbidities might such as high blood sugar, IR, T2D mellitus at an early stage, dyslipidemia, cardiovascular disease and infertility [1]. Ovulatory disfunction (that contains menstrual dysfunction), hyperandrogenism (i.e. Indication of excess male hormone or androgen effect for e.g. clinically such as hirsutism or biochemically such as hyperandrogenemia or excess levels of androgen) and polycystic ovarian morphology PCOMS an excessive number of preantral follicles in the ovaries) are all features of syndrome [2]. Another symptom of polycystic ovarian syndrome, which is usually related to obesity and IR and causes hyperinsulinemia conditions in 30% to 80% of lean women and 80% of obese women is a high LH/FSH ratio [3] progression of PCOS [4] to [6].

PCOS can cause wide range of problems in women because of harmful impact on the reproductive, endocrine, metabolic and psychological systems [7]. Pathophysiology of PCOS is not fully understood. In Recent studies the abnormal adrenocortical function elevated sympathetic nerve activity and abnormalities with the hypothalamus pituitary ovarian axis all contribute to the Hyperandrogenism is

associated with elevated blood levels of free testosterone, a key hormone influencing the pathophysiology of PCOS. These sophisticated disorders analyzed and improve elements are degraded. Genetics, neuroendocrine lifestyle/environment, obesity and some other risk factors all contribute to occurrence of polycystic syndrome [8]. Numerous studies has shown that PCOS may be influences by environmental factors, prenatal hormone imbalance, lifestyle, decisions, and genetic abnormalities [9]. The different medicine were used but the medical plants such as Fenugreek plant and Flaxseed were the new options for treating the PCOS [10].

The legume fenugreek plant (*Trigonella foenum-graecum*) is grown in North African and Indian nations. There are various names by which it is known in many languages including Fenugrec in French, Methi in Hindi, Bockshorklee in German, Fienogreco in Italian, Pazhitnik in Russian, Alholva in Spanish, Koroha in Japanese, Hulba in Arabic, Halba in Malaya, and K'U-Tou (China), which are all members of the Fabaceae family. While the leaves of the plant are consumed as green vegetables the seeds are used throughout the world as spices. Many years have been spent using the bitter-tasting fenugreek seed as a medicinal remedy. There has been a use for fenugreek seeds for more than 2500 years. The majority of fenugreek produced and consumed worldwide is grown in India. Seasoning, flavoring, and larger amounts are used as a component of soups and pan cakes that contain fenugreek seeds [11]. A flaxseed compound called lignan has been shown to suppress androgen levels in men with prostate cancer. Flaxseed is one of the best sources of dietary lignan having levels 800 times greater than most other foods. In addition to being a good source of omega-3 fatty acids flaxseed is also an excellent source of dietary lignan. A flax plant, also known as Linum usitatissiumum, has blue flowers and produces thin flat seeds that are either golden yellow or reddish brown in color. The most common forms of flaxseed are whole seeds, powdered seeds (powder or meal), and flaxseed oil [11], [12].

The Pituitary gland produce Luteinizing Hormone which stimulate the testes and ovaries to produce steroid hence promoting spermatogenesis and ovulation. On chromosome 19q13.3, the gene for Luteinizing Hormone and beta chains of chronic gonadotrophin are joined together. Infertility and pseudo hermaphroditism, two

symptoms of hypogonadism are linked to mutations in this gene. The Insilico evaluation of ligands of fenugreek and flaxseed as a therapeutic agent for PCOS. For this purpose, six Ligands of fenugreek and six ligands of flaxseed were identified from literature. The structure of ligands was visualized in PubChem and Patch dock was used by docking purpose. After that the protein ligand interaction of theses ligands were analyzed by using 3D discovery studio. After the comprehensive analysis of their physiochemical properties including molecular weight, log P value, H-bond Acceptors, H-bond donor are visualized as potent inhibitors. The above-mentioned properties from 5 selected ligands linatine, Linoleic acid, Quercetin, Scopoletin and Trigonelline shows the best activates but not all follow the Lipinski rule of 5 so they knock out. Among the five above mentioned ligands by two seeds Quercetin from fenugreek can be suggested as Potent lead compound because it follow all the properties as MW, Log P value, H-Bond donor and H-bond Acceptor which was discussed above in the results.

Chapter 5

Conclusions and Recommendations

Ovarian dysfunction leads to a health issue in women known as polycystic ovarian syndrome which is an endocrine disorder hitting women of middle age globally. After ruling out other possibly causes the illness is diagnosed when two or three symptoms oligo or anovulation, biochemical hyperandrogenism and polycystic ovaries on ultrasound are present. Insulin resistance, hyperlipidemia, obesity, high blood pressure and metabolic diseases are all severe risks for PCOS afflicted women. The main risk factor for PCOS is a history of early adrenarche, premenstrual weight gain and family history.

The aim of this study was to find a potential inhibitor that might be used as an effective drug development to treat PCOS problems utilizing computational methods. Twelve ligands from fenugreek and flax seeds were chosen for the current study endeavor from the literature. Luteinizing Hormone Beta Subunit Protein was the protein that underwent virtual screening. The docking trials were carried out using Patch Dock. By using 3D Discovery studio, the interactions between these ligands and proteins were explored.

The first objective was to identify the target protein which is involved in PCOS progression from PDB. The 3D structure of Luteinizing Hormone Beta subunit

protein was visualized in PubChem for amino acid residues. The Pituitary gland produce Luteinizing Hormone which stimulate the testes and ovaries to produce steroid hence promoting spermatogenesis and ovulation. On chromosome 19q13.3, the gene for Luteinizing Hormone and beta chains of chronic gonadotrophin are joined together. Infertility and pseudo hermaphroditism, two symptoms of hypogonadism are linked to mutations in this gene.

The second objective was Insilico evaluation of ligands of fenugreek and flaxseed as a therapeutic agent for PCOS. For this purpose, six Ligands of fenugreek and six ligands of flaxseed were identified from literature. The structure of ligands was visualized in PubChem and Patch dock was used by docking purpose. After that the protein ligand interaction of theses ligands were analyzed by using 3D discovery studio. After the comprehensive analysis of their physiochemical properties including molecular weight, log P value, H-bond Acceptors, H-bond donor are visualized as potent inhibitors. The above-mentioned properties from 5 selected ligands linatine, Linoleic acid, Quercetin, Scopoletin and Trigonelline shows the best activates but not all follow the Lipinski rule of 5 so they knock out. Among the five above mentioned ligands by two seeds Quercetin from fenugreek can be suggested as Potent lead compound because it follow all the properties as MW, Log P value, H-Bond donor and H-bond Acceptor.

Future Prospective

In-silico analysis must be evaluated on animal model for further clinical trials and the efficacy must also be evaluated as a potential therapeutic. As we know that letrozole is considered as best drug. In future the alternative treatment of PCOS with herbal drug. Then we select the Quercetin in fenugreek seed considered as best ligand.

Bibliography

[1] X. Jiang, J. A. Dias, and X. He, "Structural biology of glycoprotein hormones and their receptors: Insights to signaling," Mol. Cell. Endocrinol., vol. 382, no. 1, pp. 424–451, 2014, doi: 10.1016/j.mce.2013.08.021.

[2] R. Azziz et al., "Polycystic ovary syndrome," Nat. Rev. Dis. Prim., vol. 2, 2016, doi: 10.1038/nrdp.2016.57.

[3] P. A. Regidor, A. Mueller, M. Sailer, F. G. Santos, J. M. Rizo, and F. M. Egea, "Chronic inflammation in pcos: The potential benefits of specialized pro-resolving lipid mediators (spms) in the improvement of the resolutive response," Int. J. Mol. Sci., vol. 22, no. 1, pp. 1–14, 2021, doi: 10.3390/ijms22010384.

[4] Y. Li et al., "Effect of Acupuncture on Polycystic Ovary Syndrome in Animal Models: A Systematic Review," Evidence-based Complementary and Alternative Medicine, vol. 2021. Hindawi Limited, 2021. doi: 10.1155/2021/5595478.

[5] S. Franks, "Do animal models of polycystic ovary syndrome help to understand its pathogenesis and management? Yes, but their limitations should be recognized," Endocrinology, vol. 150, no. 9, pp. 3983–3985, 2009, doi: 10.1210/en.2009-0652.

[6] M. A. Sanchez-Garrido and M. Tena-Sempere, "Metabolic dysfunction in polycystic ovary syndrome: Pathogenic role of androgen excess and potential therapeutic strategies," Mol. Metab., vol. 35, no. February, p. 100937, 2020, doi: 10.1016/j.molmet.2020.01.001.

[7] I. E. Sucquart et al., "Neurokinin 3 Receptor Antagonism Ameliorates Key Metabolic Features in a Hyperandrogenic PCOS Mouse Model," Endocrinol. (United States), vol. 162, no. 5, pp. 1–15, 2021, doi: 10.1210/endocr/bqab020.

[8] J. Bulsara, P. Patel, A. Soni, and S. Acharya, "A review: Brief insight into Polycystic Ovarian syndrome," Endocr. Metab. Sci., vol. 3, no. November 2020, p. 100085, 2021, doi: 10.1016/j.endmts.2021.100085.

[9] W. Chu et al., "Continuous Light-Induced PCOS-Like Changes in Reproduction, Metabolism, and Gut Microbiota in Sprague-Dawley Rats," Front. Microbiol., vol. 10, no. January, pp. 1–13, 2020, doi: 10.3389/fmicb.2019.03145.

[10] R. S. Pawar, M. Dimri, A. Maithani, and K. Luv, "Asian Journal of Pharmaceutical Research and Development," Asian J. Pharm. Res. Dev., vol. 8, no. 6, pp. 77–80, 2020.

[11] A. Swaroop et al., "Efficacy of a novel fenugreek seed extract (Trigonella foenum-graecum, furocystTM) in polycystic ovary syndrome (PCOS)," Int. J. Med. Sci., vol. 12, no. 10, pp. 825–831, 2015, doi: 10.7150/ijms.13024.

[12] S. R. Emam et al., "Linum usitatissimum seeds oil down-regulates mrna expression for the steroidogenic acute regulatory protein and cyp11a1 genes, ameliorating letrozole-induced polycystic ovarian syndrome in a rat model," J. Physiol. Pharmacol., vol. 72, no. 1, 2021, doi: 10.26402/jpp.2021.1.06.

[13] M. Mehraban, G. Jelodar, and F. Rahmanifar, "A combination of spearmint and flaxseed extract improved endocrine and histomorphology of ovary in experimental PCOS," J. Ovarian Res., vol. 13, no. 1, pp. 1–8, 2020, doi: 10.1186/s13048-020-00633-8.

[14] J. Warren-Ulanch and S. Arslanian, "Treatment of PCOS in adolescence," Best Pract. Res. Clin. Endocrinol. Metab., vol. 20, no. 2, pp. 311–330, 2006, doi: 10.1016/j.beem.2006.02.002.

[15] F. Gharanjik, M. B. Shojaeifard, N. Karbalaei, and M. Nemati, "The Effect of Hydroalcoholic Calendula Officinalis Extract on Androgen-Induced Polycystic Ovary Syndrome Model in Female Rat," vol. 2022, 2022.

[16] F. Orio and S. Palomba, "Reproductive endocrinology: New guidelines for the diagnosis and treatment of PCOS," Nat. Rev. Endocrinol., vol. 10, no. 3, pp. 130–132, 2014, doi: 10.1038/nrendo.2013.248.

[17] G. G. C. Kuhnle, C. Dell'Aquila, S. M. Aspinall, S. A. Runswick, A. A. Mulligan, and S. A. Bingham, "Phytoestrogen content of beverages, nuts, seeds, and oils," J. Agric. Food Chem., vol. 56, no. 16, pp. 7311–7315, 2008, doi: 10.1021/jf801534g.

[18] B. Khani, F. Mehrabian, E. Khalesi, and A. Eshraghid, "Effect of soy phytoestrogen on metabolic and hormonal disturbance of women with polycystic ovary syndrome," J. Res. Med. Sci., vol. 16, no. 3, pp. 297–302, 2011.

[19] J. Waldschläger et al., "Flax-seed extracts with phytoestrogenic effects on a hormone receptor-positive tumour cell line," Anticancer Res., vol. 25, no. 3 A, pp. 1817–1822, 2005.

[20] T. Tamaya, "Phytoestrogens and reproductive biology," Reprod. Med. Biol., vol. 4, no. 4, pp. 225–229, 2005, doi: 10.1111/j.1447-0578.2005.00110.x.

[21] M. Jafari Khorchani, F. Zal, and A. Neisy, "The phytoestrogen, quercetin, in serum, uterus and ovary as a potential treatment for dehydroepiandrosterone-induced polycystic ovary syndrome in the rat," Reprod. Fertil. Dev., vol. 32, no. 3, pp. 313–321, 2019, doi: 10.1071/RD19072.

[22] S. Arentz, J. Abbott, and C. Smith, "Herbal medicine for the management of polycystic ovary syndrome (PCOS)," BMC Complim. Altern Med, vol. 14, no. 2015, pp. 511–530, 2014.

[23] K. Srinivasan, "Fenugreek (Trigonella foenum-graecum): A review of health beneficial physiological effects," Food Rev. Int., vol. 22, no. 2, pp. 203–224, 2006, doi: 10.1080/87559120600586315.

[24] B. manzoor Lodhi and Uzma Firdous, "The effect of fenugreek seeds (Trigonella foenum-Gracecum) supplementation on glycemic status, androgens, and lipid profile in letrozole induced polycystic ovarian syndrome (PCOS) model," BioSight, vol. 2, no. 2, pp. 13–21, 2021, doi: 10.46568/bios.v2i2.35.

[25] J. D. Brooks et al., "Supplementation with flaxseed alters estrogen metabolism in postmenopausal women to a greater extent than does supplementation with an equal amount of soy1-3," Am. J. Clin. Nutr., vol. 79, no. 2, pp. 318–325, 2004, doi: 10.1093/ajcn/79.2.318.

[26] B. Ebrahimi et al., "Biomedical features of flaxseed against different pathologic situations: A narrative review," Iran. J. Basic Med. Sci., vol. 24, no. 5, pp. 551–560, 2021, doi: 10.22038/IJBMS.2021.49821.11378.

[27] M. M. A. El-galil and S. F. Mohammed, the Possible Effect of Flaxseed Extract on Letrozole-Induced Polycystic Ovary Rat Model: Correlative Histological and Functional Study, vol. 50, no. 4. 2021. doi: 10.21608/amj.2021.196448.

[28] G. Kalidoss, S. Velraja, and P. Narayanan, "Development and formulation of phytoestrogen-rich supplement for women with polycystic ovary syndrome," Int. J. Infertil. Fetal Med., vol. 12, no. 2, pp. 31–36, 2021, doi: 10.5005/JP-JOURNALS-10016-1221.

[29] A. Badawy and A. Elnashar, "Treatment options for polycystic ovary syndrome," Int. J. Womens. Health, vol. 3, no. 1, pp. 25–35, 2011, doi: 10.2147/IJWH.S11304.

[30] E. Kit, Luteinizing Hormone (human), no. 500720. Elsevier Inc., 2016. doi: 10.1016/B978-0-12-801028-0.00531-6.

[31] A. Ulloa-Aguirre, A. Maldonado, P. Damián-Matsumura, and C. Timossi, "Endocrine regulation of gonadotropin glycosylation," Arch. Med. Res., vol. 32, no. 6, pp. 520–532, 2001, doi: 10.1016/S0188-4409(01)00319-8.

[32] L. G. Ferreira, R. N. Dos Santos, G. Oliva, and A. D. Andricopulo, Molecular docking and structure-based drug design strategies, vol. 20, no. 7. 2015. doi: 10.3390/molecules200713384.

[33] K. Shanmugaraj, S. Anandakumar, and M. Ilanchelian, "Probing the binding interaction of thionine with lysozyme: A spectroscopic and molecular docking investigation," Dye. Pigment., vol. 112, pp. 210–219, 2015, doi: 10.1016/j.dyepig.2014.07.003.

[34] R. A. Friesner et al., "Glide: A New Approach for Rapid, Accurate Docking and Scoring. 1. Method and Assessment of Docking Accuracy," J. Med. Chem., vol. 47, no. 7, pp. 1739–1749, 2004, doi: 10.1021/jm0306430.

[35] D. Rodríguez, Z. G. Gao, S. M. Moss, K. A. Jacobson, and J. Carlsson, "Molecular docking screening using agonist-bound GPCR structures: Probing the A2A adenosine receptor," J. Chem. Inf. Model., vol. 55, no. 3, pp. 550–563, 2015, doi: 10.1021/ci500639g.

[36] K. Walter, "What Is Polycystic Ovary Syndrome?," JAMA - J. Am. Med. Assoc., vol. 327, no. 3, p. 294, 2022, doi: 10.1001/jama.2021.19776.

[37] N. X. Jiang and X. L. Li, "The Disorders of Endometrial Receptivity in PCOS and Its Mechanisms," Reprod. Sci., 2021, doi: 10.1007/s43032-021-00629-9.

[38] G. Yurtdaş and Y. Akdevelioğlu, "A New Approach to Polycystic Ovary Syndrome: The Gut Microbiota," J. Am. Coll. Nutr., vol. 39, no. 4, pp. 371–382, 2020, doi: 10.1080/07315724.2019.1657515.

[39] S. Franks and K. Hardy, "What causes anovulation in polycystic ovary syndrome?," Curr. Opin. Endocr. Metab. Res., vol. 12, pp. 59–65, 2020, doi: 10.1016/j.coemr.2020.03.001.

[40] R. Azziz et al., The Androgen Excess and PCOS Society criteria for the polycystic ovary syndrome: the complete task force report, vol. 91, no. 2. 2009. doi: 10.1016/j.fertnstert.2008.06.035.

[41] J. Zhao, D. Li, H. Tang, and L. Tang, "Association of vascular endothelial growth factor polymorphisms with polycystic ovarian syndrome risk: A meta-analysis," Reprod. Biol. Endocrinol., vol. 18, no. 1, pp. 1–14, 2020, doi: 10.1186/s12958-020-00577-0.

[42] Y. V. Louwers and J. S. E. Laven, "Characteristics of polycystic ovary syndrome throughout life," Ther. Adv. Reprod. Heal., vol. 14, p. 263349412091103, 2020, doi: 10.1177/2633494120911038.

[43] E. Carmina, F. Rosato, A. Jannì, M. Rizzo, and R. A. Longo, "Relative prevalence of different androgen excess disorders in 950 women referred because of clinical hyperandrogenism,"

[44] J. Clin. Endocrinol. Metab., vol. 91, no. 1, pp. 2–6, 2006, doi: 10.1210/jc.2005-1457.27 thesis.pdf."

[45] F. Borgia, S. Cannavò, F. Guarneri, S. P. Cannavò, M. Vaccaro, and B. Guarneri, "Correlation between endocrinological parameters and acne severity in adult women," Acta Derm. Venereol., vol. 84, no. 3, pp. 201–204, 2004, doi: 10.1080/00015550410023248.

[46] L. Chiazze, F. T. Brayer, J. J. Macisco, M. P. Parker, and B. J. Duffy, "The Length and Variability of the Human Menstrual Cycle," JAMA J. Am. Med. Assoc., vol. 203, no. 6, pp. 377–380, 1968, doi: 10.1001/jama.1968.03140060001001.

[47] C. G. Solomon et al., "Long or highly irregular menstrual cycles as a marker for risk of type 2 diabetes mellitus," J. Am. Med. Assoc., vol. 286, no. 19, pp. 2421–2426, 2001, doi: 10.1001/jama.286.19.2421.

[48] M. Manti et al., "Excess of ovarian nerve growth factor impairs embryonic development and causes reproductive and metabolic dysfunction in adult female mice," FASEB J., vol. 34, no. 11, pp. 14440–14457, 2020, doi: 10.1096/fj.202001060R.

[49] P. Darabi, H. Khazali, and M. Mehrabani Natanzi, "Therapeutic potentials of the natural plant flavonoid apigenin in polycystic ovary syndrome in rat model: via modulation of pro-inflammatory cytokines and antioxidant activity," Gynecol. Endocrinol., vol. 36, no. 7, pp. 582–587, 2020, doi: 10.1080/09513590.2019.1706084.

[50] N. Sinha, S. Roy, B. Huang, J. Wang, V. Padmanabhan, and A. Sen, "Developmental programming: Prenatal testosterone-induced epigenetic modulation and its effect on gene expression in sheep ovary," Biol. Reprod., vol. 102, no. 5, pp. 1045–1054, 2020, doi: 10.1093/biolre/ioaa007.

[51] L. Corrie et al., "Combination therapy of curcumin and fecal microbiota transplant: Potential treatment of polycystic ovarian syndrome," Med. Hypotheses, vol. 154, no. July, p. 110644, 2021, doi: 10.1016/j.mehy.2021.110644.

[52] S. S. C. Yen, "Regulation of the hypothalamic-pituitary-ovarian axis in women," J. Reprod. Fertil., vol. 51, no. 1, pp. 181–191, 1977, doi: 10.1530/jrf.0.0510181.

[53] R. F. Arrais and S. A. Dib, "The hypothalamus-pituitary-ovary axis and type 1 diabetes mellitus: A mini review," Hum. Reprod., vol. 21, no. 2, pp. 327–337, 2006, doi: 10.1093/humrep/dei353.

[54] R. J. Handa and M. J. Weiser, "Gonadal steroid hormones and the hypothalamo-pituitary-adrenal axis," Front. Neuroendocrinol., vol. 35, no. 2, pp. 197–220, 2014, doi: 10.1016/j.yfrne.2013.11.001.

[55] F. Yang et al., "Follicular hyperandrogenism downregulates aromatase in luteinized granulosa cells in polycystic ovary syndrome women," Reproduction, vol. 150, no. 4, pp. 289–296, 2015, doi: 10.1530/REP-15-0044.

[56] L. Schiffer, W. Arlt, and K. H. Storbeck, "Intracrine androgen biosynthesis, metabolism and action revisited," Mol. Cell. Endocrinol., vol. 465, pp. 4–26, 2018, doi: 10.1016/j.mce.2017.08.016.

[57] M. O. Goodarzi, E. Carmina, and R. Azziz, "DHEA, DHEAS and PCOS," J. Steroid Biochem. Mol. Biol., vol. 145, pp. 213–225, 2015, doi: 10.1016/j.jsbmb.2014.06.003.

[58] G. Bachmann et al., "Female androgen insufficiency: The princeton consensus statement on definition, classification, and assessment," Fertil. Steril., vol. 77, no. 4, pp. 660–665, 2002, doi: 10.1016/S0015-0282(02)02969-2.

[59] L. Sèdes et al., "Anti-Müllerian hormone recruits BMPR-IA in immature granulosa cells," PLoS One, vol. 8, no. 11, 2013, doi: 10.1371/journal.pone.0081551.

[60] A. Kruszynska and J. Slowinska-Srzednicka, "Anti-Müllerian hormone (AMH) as a good predictor of time of menopause," Prz. Menopauzalny, vol. 16, no. 2, pp. 47–50, 2017, doi: 10.5114/pm.2017.68591.

[61] V. A. Kushnir, D. B. Seifer, D. H. Barad, A. Sen, and N. Gleicher, "Potential therapeutic applications of human anti-Müllerian hormone (AMH) analogues in reproductive medicine," J. Assist. Reprod. Genet., vol. 34, no. 9, pp. 1105–1113, 2017, doi: 10.1007/s10815-017-0977-4.

[62] M. W. O'Reilly et al., "AKR1C3-mediated adipose androgen generation drives lipotoxicity in women with polycystic ovary syndrome," J. Clin. Endocrinol. Metab., vol. 102, no. 9, pp. 3327–3339, 2017, doi: 10.1210/jc.2017-00947.

[63] H. Nautiyal, PCOS as a genetic issuse, pp. 1–26, 2022.

[64] H. Ding et al., "Resistance to the Insulin and Elevated Level of Androgen: A Major Cause of Polycystic Ovary Syndrome," Front. Endocrinol. (Lausanne)., vol. 12, no. October, pp. 1–14, 2021, doi: 10.3389/fendo.2021.741764.

[65] E. C. Costa, J. C. Ferezini De Sá, E. M. Mafaldo Soares, T. M. Araújo Moura Lemos, T. M. De Oliveira Maranhão, and G. Dantas Azevedo, "Anthropometric indices of central obesity how discriminators of metabolic syndrome

in Brazilian women with polycystic ovary syndrome," Gynecol. Endocrinol., vol. 28, no. 1, pp. 12–15, 2012, doi: 10.3109/09513590.2011.583956.

[66] R. Pasquali, A. Gambineri, and U. Pagotto, "The impact of obesity on reproduction in women with polycystic ovary syndrome," BJOG An Int. J. Obstet. Gynaecol., vol. 113, no. 10, pp. 1148–1159, 2006, doi: 10.1111/j.1471-0528.2006.00990.x.

[67] S. Virtue and A. Vidal-Puig, "It's not how fat you are, it's what you do with it that counts," PLoS Biol., vol. 6, no. 9, pp. 1819–1823, 2008, doi: 10.1371/journal.pbio.0060237.

[68] S. A. Pourhoseini, M. Mahmoudinia, M. Najaf Najafi, and F. Kamyabi, "The effect of phytoestrogens (Cimicifuga racemosa) in combination with clomiphene in ovulation induction in women with polycystic ovarian syndrome: A clinical trial study," Avicenna J. Phytomedicine, vol. 12, no. 1, pp. 8–15, 2022.

[69] D. El Khoury, J. Hansen, M. Tabakos, L. L. Spriet, and P. Brauer, Importance of flaxseed. vol.23, pp. 1–13, 2020.

[70] M. A. Kassem et al., "Definition of soybean genomic regions that control seed phytoestrogen amounts," J. Biomed. Biotechnol., vol. 2004, no. 1, pp. 52–60, 2004, doi: 10.1155/S1110724304304018.

[71] F. Giampieri et al., "Dietary Phytoestrogen Intake and Cognitive Status in Southern Italian Older Adults," pp. 1–12, 2022.

[72] J. Gorzkiewicz, G. Bartosz, and I. Sadowska-Bartosz, "The potential effects of phytoestrogens: The role in neuroprotection," Molecules, vol. 26, no. 10, pp. 1–12, 2021, doi: 10.3390/molecules26102954.

[73] K. E. Reed, J. Camargo, J. Hamilton-Reeves, M. Kurzer, and M. Messina, "Neither soy nor isoflavone intake affects male reproductive hormones: An expanded and updated meta-analysis of clinical studies,"

Reprod. Toxicol., vol. 100, no. December 2020, pp. 60–67, 2021, doi: 10.1016/j.reprotox.2020.12.019.

[74] H. F. Woods, "Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment," Comm. Toxic. Chem. Food, Consum. Prod. Environ., no. March, pp. 13–18, 1999, Available: http://cot.food.gov.uk/pdfs/opchap.pdf

[75] E. Basch, C. Ulbricht, G. Kuo, P. Szapary, and M. Smith, "Therapeutic Applications of TMS," Oxford Handb. Transcranial Stimul., vol. 8, no. 1, 2012.

[76] C. Broca et al., "Insulinotropic agent ID-1101 (4-hydroxyisoleucine) activates insulin signaling in rat," Am. J. Physiol. - Endocrinol. Metab., vol. 287, no. 3 50-3, pp. 463–471, 2004, doi 10.1152/ajpendo.00163.2003.

[77] K. Dev, E. Ramakrishna, and R. Maurya, "Glucose Transporter 4 Translocation Activators from Nature," Discov. Dev. Antidiabetic Agents from Nat. Prod. Nat. Prod. Drug Discov., pp. 113–145, 2017, doi 10.1016/B978-0-12-809450-1.00004-1.

[78] S. Fuller and J. M. Stephens, "Diosgenin, 4-hydroxyisoleucine, and fiber from fenugreek Mechanisms of actions and potential effects on metabolic syndrome," Adv. Nutr., vol. 6, no. 2, pp. 189–197, 2015, doi 10.3945/an.114.007807.

[79] M. Majeed et al., "Galactomannan from Trigonella foenum-graecum L. seed Prebiotic application and its fermentation by the probiotic Bacillus coagulans strain MTCC 5856," Food Sci. Nutr., vol. 6, no. 3, pp. 666–673, 2018, doi 10.1002/fsn3.606.

[80] M. Durga, S. Nathiya, and T. Devasena, "Protective role of fenugreek leaf extract and quercetin against petrol exhaust nanoparticle induced lipid peroxidation and oxidative stress in rat erythrocytes in vitro," Asian J. Pharm. Clin. Res., vol. 8, no. 1, pp. 237–241, 2015.

[81] A. Agent, C. Acid, N. Acid, H. Ashihara, and D. Prevention. Pant biochemistry, flaxseed,2018.

[82] N. Kumar, M. Kumar, M. K. Verma, S. Ramanarayanan, A. Ranjan, and R. Ranjan, "Bioactive effects and safety profiles of fenugreek (Trigonella foenum-graecum L.) for pharmaceutical and medicinal applications," vol. 10, no. 12, pp. 912–919, 2021, . Available https://www.researchgate.net/profile/Rakesh-Ranjan-7/publication/3

[83] S. K.B., "Effect of aqueous leaf extract of Andrographis paniculata in the reproductive organs and fertility of the male wild indian house rat (Rattus rattus)," Int. J. Curr. Pharm. Res., vol. 5, no. 4, pp. 71–76, 2013,Available: http://www.ijcpr.org/Issues/Vol5Issue4/746.pdf

[84] L. J. Malcolmson, R. Przybylski, and J. K. Daun, "Storage stability of milled flaxseed," JAOCS, J. Am. Oil Chem. Soc., vol. 77, no. 3, pp. 235–238, 2000, doi: 10.1007/s11746-000-0038-0.

[85] M. Rubilar, C. Gutiérrez, M. Verdugo, C. Shene, and J. Sineiro, "Flaxseed as a source of functional ingredients," J. Soil Sci. Plant Nutr., vol. 10, no. 3, pp. 373–377, 2010, doi: 10.4067/S0718-95162010000100010.

[86] E. A. Meagher, O. P. Barry, J. A. Lawson, J. Rokach, and G. A. FitzGerald, "Effects of vitamin E on lipid peroxidation in healthy persons," J. Am. Med. Assoc., vol. 285, no. 9, pp. 1178–1182, 2001, doi: 10.1001/jama.285.9.1178.

[87] M. Heinrich, J. Mah, and V. Amirkia, "Alkaloids used as medicines: Structural phytochemistry meets biodiversity—An update and forward look," Molecules, vol. 26, no. 7, pp. 1–18, 2021, doi: 10.3390/molecules26071836.

[88] Y. Y. Shim, B. Gui, P. G. Arnison, Y. Wang, and M. J. T. Reaney, "Flaxseed (Linum usitatissimum L.) bioactive compounds and peptide nomenclature: Areview," Trends Food Sci. Technol., vol. 38, no. 1, pp. 5–20, 2014, doi: 10.1016/j.tifs.2014.03.011.

[89] W. D. MacRae and G. H. N. Towers, "Biological activities of lignans," Phytochemistry, vol. 23, no. 6, pp. 1207–1220, 1984, doi: 10.1016/S0031-9422(00)80428-8.

[90] N. Information, "Linoleic Acid 1," pp. 311–312, 2013, doi: 10.3945/an.113.003772.311.

[91] S. Rani and P. Chandna, "Multiomics Analysis–Based Biomarkers in Diagnosis of Polycystic Ovary Syndrome," Reprod. Sci., vol. 2003, no. 0123456789, 2022, doi: 10.1007/s43032-022-00863-9.

[92] S. Dasgupta, P. V. S. Sirisha, K. Neelaveni, K. Anuradha, G. Sudhakar, and B. M. Reddy, "Role of luteinizing hormone β-subunit gene variants among South Indian women with polycystic ovary syndrome," Gene, vol. 494, no. 1, pp. 51–56, 2012, doi: 10.1016/j.gene.2011.11.054.

[93] U. Branavan, C. NV, W. WSS, and W. Chandrika N, "Polycystic Ovary Syndrome: Genetic Contributions from the Hypothalamic-Pituitary-Gonadal Axis," Int. Arch. Endocrinol. Clin. Res., vol. 4, no. 1, pp. 1–8, 2018, doi: 10.23937/2572-407x.1510013.

[94] R. Deswal, S. Nanda, and A. S. Dang, "Association of Luteinizing hormone and LH receptor gene polymorphism with susceptibility of Polycystic ovary syndrome," Syst. Biol. Reprod. Med., vol. 65, no. 5, pp. 400–408, 2019, doi: 10.1080/19396368.2019.1595217.

[95] S. B. Larson and A. McPherson, "The crystal structure of the β subunit of luteinizing hormone and a model for the intact hormone," Curr. Res. Struct. Biol., vol. 1, no. July, pp. 1–5, 2019, doi: 10.1016/j.crstbi.2019.07.001.

[96] N. S. Pagadala, K. Syed, and J. Tuszynski, "Software for molecular docking: a review," Biophys. Rev., vol. 9, no. 2, pp. 91–102, 2017, doi: 10.1007/s12551-016-0247-1.

[97] K. Gutberlet and R. Rudolph, "Angiosis carcinomatosa bei Mammatumoren der Hündin - Häufigkeit und Verbindung mit prognostisch wichtigen Faktoren," Kleintierpraxis, vol. 41, no. 7, pp. 473–482, 1996.

[98] E. Rudnicka et al., "Chronic low grade inflammation in pathogenesis of pcos," Int. J. Mol. Sci., vol. 22, no. 7, pp. 1–12, 2021, doi: 10.3390/ijms22073789.

[99] S. Karjula et al., "Psychological distress is more prevalent in fertile age and premenopausal women with pcos symptoms: 15-year follow-up," J. Clin. Endocrinol. Metab., vol. 102, no. 6, pp. 1861–1869, 2017, doi: 10.1210/jc.2016-3863.

[100] J. Cheng, M. J. Sweredoski, and P. Baldi, "Accurate prediction of protein disordered regions by mining protein structure data," Data Min. Knowl. Discov., vol. 11, no. 3, pp. 213–222, 2005, doi: 10.1007/s10618-005-0001-y.

[101] A. R. Oany, S. A. I. Ahmad, M. Siddikey, M. U. Hossain, and A. Ferdoushi, "Computational Structure Analysis and Function Prediction of an Uncharacterized Protein (I6U7D0) of Pyrococcus furiosus COM1," Austin J Comput Biol Bioinform, vol. 1, no. 2, pp. 0–5, 2014, . Available: https://s3.amazonaws.com/academia.edu.documents/36287515/ajcbb-v1

[102] H. Berman, K. Henrick, H. Nakamura, and J. L. Markley, "The worldwide Protein Data Bank (wwPDB): Ensuring a single, uniform archive of PDB data," Nucleic Acids Res., vol. 35, no. SUPPL. 1, pp. 2006–2008, 2007, doi: 10.1093/nar/gkl971.

[103] K. Sahu and A. Pradesh, "QSAR and Pharmacophore Modeling based Drug Designing for Spleen Tyrosine Kinase (Syk) Protein For Human using Accelrys Discovery Studio Software in Linux Server," Int. J. Pharm. Sci. Res., vol. 4, no. 11, pp. 4272–4280, 2013, doi: 10.13040/IJPSR.0975-8232.4(11).4272-80.

[104] T. Ginex, F. Spyrakis, and P. Cozzini, "FADB: A food additive molecular database for in silico screening in food toxicology," Food Addit. Contam. -

Part A Chem. Anal. Control. Expo. Risk Assess., vol. 31, no. 5, pp. 792–798, 2014, doi: 10.1080/19440049.2014.888784.

[105] V. S. S. Raj, M. Monisha, and G. Paramasivam, "In-silico Screening of Synthetic Inhibitors for Human Poly (Adp-ribose) Polymerase 2 Enzyme Using Patch Dock Software for Ovarian Cancer Therapy," Rev. Gestão Inovação e Tecnol., vol. 11, no. 1, pp. 5910–5923, 2021, doi: 10.47059/revistageintec.v11i1.1688.

[106] F. Stanzione, I. Giangreco, and J. C. Cole, Use of molecular docking computational tools in drug discovery, 1st ed., vol. 60. Elsevier B.V., 2021. doi: 10.1016/bs.pmch.2021.01.004.

[107] J. Dundas, Z. Ouyang, J. Tseng, A. Binkowski, Y. Turpaz, and J. Liang, "CASTp: Computed atlas of surface topography of proteins with structural and topographical mapping of functionally annotated residues," Nucleic Acids Res., vol. 34, no. WEB. SERV. ISS., pp. 116–118, 2006, doi: 10.1093/nar/gkl282.

[108] D. E. V. Pires, T. L. Blundell, and D. B. Ascher, "pkCSM: Predicting small-molecule pharmacokinetic and toxicity properties using graph-based signatures," J. Med. Chem., vol. 58, no. 9, pp. 4066–4072, 2015, doi: 10.1021/acs.jmedchem.5b00104.

[109] E. N. Chernyaeva et al., "Genome-wide Mycobacterium tuberculosis variation (GMTV) database: A new tool for integrating sequence variations and epidemiology," BMC Genomics, vol. 15, no. 1, pp. 1–8, 2014, doi: 10.1186/1471-2164-15-308.

[110] F. B. Mayr et al., "Infection rate and acute organ dysfunction risk as explanations for racial differences in severe sepsis," Jama, vol. 303, no. 24, pp. 2495–2503, 2010, doi: 10.1001/jama.2010.851.

[111] C. W. Park, K. W. Ma, S. W. Jang, M. Son, and M. J. Kang, "Comparison of Piroxicam Pharmacokinetics and Anti-Inflammatory Effect in Rats after

Intra-Articular and Intramuscular Administration," Biomol. Ther. (Seoul)., vol. 22, no. 3, pp. 260–266, 2014, doi: 10.4062/biomolther.2014.037.

[112] A. Jaiswal, A. Chhabra, U. Malhotra, S. Kohli, and V. R. Vibha Rani, "Comparative analysis of human matrix metalloproteinases: Emerging therapeutic targets in diseases," Bioinformation, vol. 6, no. 1, pp. 23–30, 2011, doi: 10.6026/97320630006023.

[113] M. L. Bucalo et al., "The anaemia control model: Does it help nephrologists in therapeutic decision-making in the management of anaemia?," Nefrologia, vol. 38, no. 5, pp. 491–502, 2018, doi: 10.1016/j.nefroe.2018.10.001.

[114] A. C. Headquarters, "Discovery Studio Life Science Modeling and Simulations,", pp. 1–8, 2008.

[115] A. Daina, O. Michielin, and V. Zoete, "SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules," Sci. Rep., vol. 7, no. March, pp. 1–13, 2017, doi: 10.1038/srep42717.

[116] A. Genoni, M. Pennati, G. Morra, N. Zaffaroni, and G. Colombo, "Ligand selection from the analysis of protein conformational substates: New leads targeting the N-terminal domain of Hsp90," RSC Adv., vol. 2, no. 10, pp. 4268–4282, 2012, doi: 10.1039/c2ra00911k.

[117] S. Kim et al., "PubChem substance and compound databases," Nucleic Acids Res., vol. 44, no. D1, pp. D1202–D1213, 2016, doi: 10.1093/nar/gkv951.

[118] V. Salmaso and S. Moro, "Bridging molecular docking to molecular dynamics in exploring ligand-protein recognition process: An overview," Front. Pharmacol., vol. 9, no. AUG, pp. 1–16, 2018, doi: 10.3389/fphar.2018.00923.

[119] K. Schleinkofer, T. Wang, and R. C. Wade, "Molecular Docking," Encycl. Ref. Genomics Proteomics Mol. Med., vol. 443, pp. 1149–1153, 2006, doi: 10.1007/3-540-29623-9-3820.

[120] D. Schneidman-Duhovny, Y. Inbar, R. Nussinov, and H. J. Wolfson, "PatchDock and SymmDock: Servers for rigid and symmetric docking," Nucleic Acids Res., vol. 33, no. SUPPL. 2, pp. 363–367, 2005, doi: 10.1093/nar/gki481.

[121] C. M. Tice, "Selecting the right compounds for screening: Does Lipinski's rule of 5 for pharmaceuticals apply to agrochemicals?," Pest Manag. Sci., vol. 57, no. 1, pp. 3–16, 2001, doi: 10.1002/1526-4998(200101)57:1¡3::AID-PS269¿3.0.CO;2-6.

[122] E. D. Deeks and L. J. Scott, "Exemestane: A review of its use in post-menopausal women with breast cancer," Drugs, vol. 69, no. 7, pp. 889–918, 2009, doi: 10.2165/00003495-200969070-00007.