CAPITAL UNIVERSITY OF SCIENCE AND TECHNOLOGY, ISLAMABAD

Zingiber officinale Metabolites Anti-Inflammatory and Inhibitory Effects on Prostaglandin Synthesis in Primary Dysmenorrhea

by

Asved Nawaz

A thesis submitted in partial fulfillment for the degree of Master of Science

in the

Faculty of Health and Life Sciences
Department of Bioinformatics and Biosciences

2024

I dedicate my work to my Baba, ammi, my siblings and my mentors.

Especial thanks to my supervisor, Dr. Arshia Amin and fellows who guided me well through it.

CERTIFICATE OF APPROVAL

Zingiber officinale Metabolites Anti-Inflammatory and Inhibitory Effects on Prostaglandin Synthesis in Primary Dysmenorrhea

by

Asved Nawaz

(Registration No: MBS221019)

THESIS EXAMINING COMMITTEE

S. No.	Examiner	Name	Organization
(a)	External Examiner	Dr. Nazia Bibi	COMSATS, Islamabad
(b)	Internal Examiner	Dr. Sahar Fazal	CUST, Islamabad
(c)	Supervisor	Dr. Arshia Amin Butt	CUST, Islamabad

Dr. Arshia Amin Butt
Thesis Supervisor
May, 2024

Dr. S. Maryiam Bakhtiar
Head
Dept. of Bioinfo. and Biosciences
May, 2024

Dr. Sahar Fazal
Dean
Faculty of Health and Life Sciences
May, 2024

Author's Declaration

I, **Asved Nawaz** hereby state that my MS thesis titled "***Zingiber officinale* Metabolites Anti-Inflammatory and Inhibitory Effects on Prostaglandin Synthesis in Primary Dysmenorrhea**" is my own work and has not been submitted previously by me for taking any degree from Capital University of Science and Technology, Islamabad or anywhere else in the country/abroad.

At any time if my statement is found to be incorrect even after my graduation, the University has the right to withdraw my MS Degree.

(Asved Nawaz)
Registration No: MBS221019

Plagiarism Undertaking

I solemnly declare that research work presented in this thesis titled "***Zingiber officinale* Metabolites Anti-Inflammatory and Inhibitory Effects on Prostaglandin Synthesis in Primary Dysmenorrhea**" is solely my research work with no significant contribution from any other person. Small contribution/help wherever taken has been duly acknowledged and that complete thesis has been written by me.

I understand the zero tolerance policy of the HEC and Capital University of Science and Technology towards plagiarism. Therefore, I as an author of the above titled thesis declare that no portion of my thesis has been plagiarized and any material used as reference is properly referred/cited.

I undertake that if I am found guilty of any formal plagiarism in the above titled thesis even after award of MS Degree, the University reserves the right to withdraw/revoke my MS degree and that HEC and the University have the right to publish my name on the HEC/University website on which names of students are placed who submitted plagiarized work.

(Asved Nawaz)
Registration No: MBS221019

Acknowledgement

I present the greatest gratitude to all those persons who provided me the possibility to complete this thesis. I am thankful to my thesis supervisor **Dr. Arshia Amin** for her unending support and encouragement during my thesis. Furthermore I would also like to acknowledge all of my friends who helped me in minutest ways for completion of this thesis. Their contribution and role was very crucial. Special thanks to all the teachers at Capital University of Science and Technology (CUST), who helped me to assemble the knowledge parts and their precious suggestion about my performance during my stay at CUST. Last but not the least, I am highly thankful to my parents who have invested their resources and efforts and believed in me in achieving this goal.

(Asved Nawaz)

Abstract

Primary Dysmenorrhea significantly affects 50-90% of women at their reproductive age by impacting their quality of life. It is characterized by lower abdomen, pelvic cramps, without any pathological condition; nausea, vomiting, headache, diarrhea, fatigue, irritability and depressive mood might be experienced; It could lead to even greater physical, psychological, social and behavioral distress. The biochemical marker of Dysmenorrhea is increase in uterine prostaglandins resulting in myometrium contractions, vasoconstriction, pain and ischemia. There is NSAIDs usage inclination for instant pain relief, with severe side effects like gastrointestinal ulcer, cardiovascular diseases, renal, hepatic insufficiency, hypercalcemia, interstitial nephritis, bronchoconstriction and acute renal dysfunction. NSAIDs non-specifically interfere with COX1 and COX2 pathways, inhibiting the constructive prostaglandin production as well. It is essential to have an herbal alternative to NSAIDs which will have less or no side effects. This study aims to ascertain and evaluate the potential of ginger derived bioactive metabolites which are effectual alternative to NSAIDs. Strong scientific evidence endorse diverse active metabolites in ginger for their anti-inflammatory and anti-oxidant properties. Six metabolites of ginger including 6-shogaol, 10-shogaol, 6-gingerol, 8-gingerol, 10-gingerol and zingerol considered as most suitable ligands were docked with "PGF2 receptor protein", present on uterine wall, which has crucial role in dysmenorrhea associated COX 2-Pathway. After considering multiple parameters including physicochemical properties, ADMET properties and LIPINSKI RO5, Dock score; the optimal potential ligand was selected followed by docked complexes interactions visualization using LigPlot. MD Simulations were performed to prove/disapprove the docking results. The ADMET Analysis, have proved the potential of ginger metabolites to be a good ligand for the receptor protein of COX2 Pathway of dysmenorrhea. The results of molecular docking and molecular dynamics simulations have specified the best ligand, turns out to be zingerol with docking score of -6.5, that could be an alternative to NSAIDs with a better efficiency with less side effects. The average RMSD value of zingerol with protein was 4.92 Å. These findings can be better alternative for NSAIDS as well might help in secondary dysmenorrhea treatment.

Contents

List of Figures

List of Tables

Chapter 1

Introduction

Dysmenorrhea is the Greek term for painful monthly bleeding or menstruation [1]. It is described as the pain or restlessness in the low abdominal and pelvic region of females before or during their menstruation [2]. Deligeoroglou described it as painful menstruation. Along with the abdominal pain, some women are reported to experience back pain, nausea, and vomiting as well [3]. Similarly, according to Nagy and Khan, dizziness, bloated feeling, and leg pain are also associated with dysmenorrhea [4]. There are two main types of dysmenorrhea [5]. One is primary dysmenorrhea which is not associated with any pathological condition and the other is secondary dysmenorrhea which is due to underlying conditions like endometriosis. It has been pointed out by Ibrahim et al., that although secondary dysmenorrhea is prevalent among 60.9% of the women under study, yet, primary dysmenorrhea still is the issue as it is due to biochemical alterations within the body. No matter which type of Dysmenorrhea it is, there is greater physical, psychological, social and behavioral distress [6]. Women usually do not go to the doctors for this pain as they consider as routine or a moment of embarrassment to tell the doctors about the details of their menstrual cycles.

Dysmenorrhea is one of the most common complaints among women globally, that is two- third of young women experience this condition [7]. According to Armour et al., the prevalence of dysmenorrhea is found among 72.70% of women of reproductive age [8]. Similarly, it has been reported by Berkley that Primary dysmenorrhea is more prevalent as compared to secondary and found to be among

90% of adolescents and 50% of women, and out of these, 10-20% of the women reported that their pain is quite extreme and significantly affects their lives [9]. The same is the situation in Pakistan and the study conducted by Ullah et al., showed that 91.5% of the sample population has been experiencing dysmenorrhea [10]. Although, dysmenorrhea does not lead to the death of women, yet it is still a matter of concern as it significantly affects the quality of women's lives. It has been pointed out by Dawood that the pain is the strongest during the initial 24-36 of the periods and these pains affect the personal as well as professional lives of women significantly [11]. It has been reported by Ju et al., that due to dysmenorrhea, the absenteeism of women from work may range from 16% to 92% [12]. Similarly, irritable moods are also due to dysmenorrhea, and it affects the social life of women. Mood alterations and pain during periods affect the relations of women with their families and friends [13]. Thus, it indicates that women are highly affected by this pain during menstruation.

The menstrual cycle is a series of hormonal secretions and interplays to produce the follicles, oocytes, and progesterone [14]. It is a complex phenomenon and may involve a range of phases that may last for 28 days on average [15]. The pathway of pain during menstruation (Dysmenorrhea), have complexity, as well as it has dependency upon several factors. It has been pointed out by Bulletti et al., that due to the complicated interplay of hormones, the contraction activity of the uterus is changed which may result in cramps [16]. However, Iacovides et al., raised the concern about the involvement of prostaglandin in the onset of dysmenorrhea [17]. Similarly, the study conducted by Barcikowska et al., also showed that females having dysmenorrhea have high levels of prostaglandins when measured through endometrial biopsies and examining their menstrual fluids in the late luteal phase as compared to the women who do not feel pain and restlessness during their periods [14]. Thus it can be the potential target for treating dysmenorrhea and a therapeutic agent should be looked at that may reduce its production.

There are different options available to treat dysmenorrhea. According to Schroll et al., dysmenorrhea can be treated with the help of non-steroidal anti-inflammatory drugs and oral contraceptive pills [18]. The Study conducted by Smith and Kaunitz showed that Non-steroidal anti-inflammatory drugs are quite effective in reducing

periods' pain, however, there are 60% chance of side effects related to gastrointestinal tract infections [19]. Also, Chen et al., mentioned that these drugs may also be ineffective for most of women. This study compared NSAID to oral ginger intake, proving the oral ginger to be most effective than NSAID [20]. Similarly, it has been reported by Smith and Kaunitz that oral contraception pills are also in use to treat dysmenorrhea and remain equally effective in reducing pain as NSAIDs. However, they cause some side effects like a reduction in bone density. These medications alter the hormonal levels significantly and thus it may lead to complications in conceiving [21]. Treating dysmenorrhea with drugs, have consequently led to some other disorders as side effects, like Stomach disorders, kidney malfunctions, dizziness, and blood and liver issues [22].

There are some other interventions like physical activities, but they require time as well as physical effort and most of the time women do not feel active enough to do these. To avoid the side effects of medications, most of the researchers recommend that the best option is to go for natural components like oils and plant extracts. It has been reported by Kashani et al., that natural and herbal treatments are quite effective in reducing problems related to the menstrual cycle [21]. Scientific researches reported that herbal medicines may play an important role in women's health and can treat different diseases, even depression. Niazi and Moradi reported that one natural element for treating dysmenorrhea is chamomile [22]. It has a mild sedative activity that relaxes the body and eases the pain, yet it may lead to the onset of drowsiness. Several other natural remedies are also reported by Ebrahimi Varzaneh et al., which include saffron, Thyme and Rosemary. Another promising agent to relieve pain is ginger extract [23].

Ginger is one of the most widely available and used plants around the world. Every household in Pakistan has ginger available in their homes and it can be used to cure certain diseases. Ginger extract has been reported to cure certain issues as it contains different compounds that are effective for particular ailments [24]. Kashani et al., reported that Ginger water may relieve pain and has the same effects as ibuprofen. Similarly, Rondanelli et al., reported that Ginger extract is considered a promising agent for relieving Dysmenorrhea. Ginger possesses the pain-reducing effect and it can do this by modulating pain through various

mechanisms: inhibition of prostaglandins via the COX and LOX pathways [25]. This is due to the chemical composition of Ginger extract as it contains different constituents that possess different functions.

It is reported by Rudrapal et al., that gingerol from the ginger extract is the effective component as it binds to the Ans 544 and Phe 177 amino acids of COX-2 and thus inhibits its functions [26]. It has also been reported by van Breemen et al., that gingerol is effective in binding with the COX-2 enzyme in COX-pathway thus it may reduce the production of prostaglandin [27].

Pain reduction with the help of ginger extract could be studied by a variety of methods. Different women could be approached and interviewed if they feel that the ginger extract has reduced their pain or not. However, it would be difficult and time-consuming as it has been reported that primary data collection is a time-consuming process [28].

Furthermore, the exact mechanism of pain inhibition and its molecular basis could not be understood with the help of collecting data from women regarding the efficacy of ginger extract in menstrual pain. Hence, the promising approach for this is detecting it through in silico methods, as it has been pointed out that molecular methods and the use of software is effective in understanding protein-protein interaction [29].

1.1 Problem Statement

Primary Dysmenorrhea is common condition in reproductive age of women which greatly impact the quality of life. To relieve this pain women generally intake the pain killer tablets or apply pain relieving creams.

There is widespread usage of NSAIDs, observed among the women suffering from Primary Dysmenorrhea. The use of such anti-inflammatory, non-steroidal drugs lead to a vast range of side effects including Gastrointestinal Ulcer, cardiovascular diseases, renal, hepatic insufficiency, hypercalemia, interstitial nephritis, bronchoconstriction and acute renal dysfunction.

1.2 Hypothesis

Primary Dysmenorrhea is one of the important public health concern as it affects the social and professional life of women significantly. Women are inclined to medicines to get instant relief from the pain so that they may continue their life chores. These medicines may have harmful effects on their overall health, thus, there is a need for an effective natural remedy to deal with this issue.

Ginger is one of the most used spice with great deal of metabolic implications. If Ginger metabolites could bind to PGF2a receptor, then it may stop PGF2α production, hence interfere with COX2 path of Primary Dysmenorrhea.

1.3 Aim and Objectives

The aim of this study is to analyze the effectiveness of Ginger metabolites in reducing prostaglandin levels by blocking the COX pathway. For this purpose, the following objectives are set:

- To determine the sequence of "PGF2α Receptor ", membrane protein involved in COX-2 Pathway of Dysmenorrhea.
- To analyze the conformational binding efficiency of 6 of metabolites of gin ger including 6-Gingerol, 6-Shaogoal and Zingerol as an inhibitor, of COX pathway.
- To score and visualize the "Ginger Metabolites- PGF2α Membrane Receptor" binding efficiency.

1.4 Gap Analysis

- Most of the studies are done to compare the different spices including curcumin, cinnamon, parsely and ginger, the general bioactivities of these all

are known but there is no clear comparison of potential metabolites of ginger which can best interfere with COX 2 pathway and reduced the Primary Dysmenorrhea and result in its herbal treatment.

- NSAIDs are frequently used to overcome Dysmenorrhea. "Targeted Herbal Treatment" is something we need to work on, so that more effective pain relieving effects with lesser side effects can be obtained.

1.5 Scope

This study is significant as it may increase awareness regarding the use of natural products to cure menstrual cramps as they are the routine of any woman's reproductive age. Women are now gradually moving towards natural and home remedies to treat their issues and menstrual problems. Similarly, it has also been pointed out by Gebeyehu et al., that two-thirds of the sample population use home remedies to treat their menstrual pain. This study will provide the scientific basis of ginger extract on pain relief from periods [30]. The dependency on NSAIDs and OCPs may be reduced if women believe that natural products like easily available ginger extracts can be used to relieve their pain without any side effects.

Chapter 2

Literature Review

2.1 Dysmenorrhea

Dysmenorrhea – a common gynecological disease which dramatically affects the quality of life for many women [31]. Understanding the different parts of dysmenorrhea is essential to understand how Ginger metabolites works to stop menstrual cramps. Dysmenorrhea, also known as menstrual cramping, a gynecological disorder, is commonly characterized by painful, recurrent contractions in uterus during menstruation. These contractions, which are frequently associated with the release of prostaglandin, contribute to discomfort and pain. The condition is broadly divided into two types: primary dysmenorrhea, which typically occurs in the absence of underlying reproductive disorders, and secondary dysmenorrhea, in which pain is a symptom of an underlying reproductive health condition [5]. Epidemiologically, dysmenorrhea impacts a substantial proportion of menstruating women, with a variety of risk factors contributing to its prevalence. The multidimensional nature of dysmenorrhea includes spasmodic and congestive dysmenorrhea. Congestive dysmenorrhea is characterized by pelvic obstruction and a feeling of fullness, whereas spasmodic dysmenorrhea is characterized by rhythmic uterine contractions. Pathophysiological, dysmenorrhea manifests as pelvic pain,lower abdominal pain, and sciatica [3]. Effective management requires an accurate diagnosis that incorporates a comprehensive comprehension of the type and severity of dysmenorrhea.

2.1.1 Epidemiology

Dysmenorrhea is a common problem with periods that affects a lot of women around the world. Studies have shown that many women have heavy periods with different pain levels during their reproductive years [8]. Among the two distinct forms of Dysmenorrhea, Primary Dysmenorrhea is most common, exceedingly. Approximately 50% Menstruating women and 90% of young, reproductive, adult girls reportedly suffer from Primary Dysmenorrhea. One-fourth of the reproductive aged women in the population face the primary dysmenorrhea.[9]

Different age groups and communities have different rates of dysmenorrhea. Several epidemiological studies have used incidence rates to show that many women, especially those in their late teens and early twenties, have dysmenorrhea [12]. People with this disease often miss work or school, which shows how it affects society.

Understanding the pervasive impact of dysmenorrhea has been greatly facilitated by epidemiological studies. A cross-sectional study conducted by Smith revealed a prevalence of 60% among 18- to 25-year-old women, highlighting the substantial burden of dysmenorrhea among young adults. Similarly, the findings of a seminal study by Molla et al., revealed a prevalence of 71% in the African population [32]. Moreover, the study conducted by Özder et al., also showed that dysmenorrhea was prevalent among 79.7% of the population [33].

2.1.2 Risk Factors

Looking into the risk factors helps us understand how complicated dysmenorrhea is. According to Chen, several things can cause or make dysmenorrhea worse. These include age, lifestyle choices, past sexual experience, and underlying health problems [34]. Age has a significant effect, with younger women usually having worse period pain. Stress, being inactive, and bad eating habits are lifestyle factors that can worsen symptoms. Also, reproductive factors like not having children or hitting puberty early may affect the chance and severity of dysmenorrhea [35]. People with long-term conditions like endometriosis or pelvic inflammatory disease

are more likely to develop secondary dysmenorrhea. Knowing these risk factors helps doctors make preventative and treatment plans that work best for each person.

2.1.3 Types of Dysmenorrhea

There are different kinds of dysmenorrhea, each with its symptoms and health effects on women. **Primary dysmenorrhea** is the most common type, and it usually happens when no other pelvic problem occurs [36]. It is often linked to the standard physical process of having your period. Women younger than thirty-five, especially teens and young adults, often have primary dysmenorrhea. The pain is usually crampy and starts right before or at the start of your period. It lasts for two to three days. **Secondary dysmenorrhea** is different from primary dysmenorrhea because it is linked to pelvic disease.

Some health problems, like endometriosis, adenomyosis, or pelvic inflammatory disease, can make secondary dysmenorrhea worse. When have secondary dysmenorrhea, the pain may be worse, and you may also have other symptoms that point to the main problem. Healthcare workers need to be able to tell the difference between primary and secondary dysmenorrhea to come up with the best ways to treat it. For primary dysmenorrhea, standard painkillers usually work well, but for secondary dysmenorrhea, the root problem may need to be treated specifically [5].

2.1.4 Pathophysiology (Symptoms)

According to Unuofin, Dysmenorrhea pathophysiology elucidates the complex mechanisms causing menstruation discomfort, laying the groundwork for specific treatments. Prostaglandins, which are released during menstruation, are thought to be the main cause of dysmenorrhea in the Cox pathway. Pain during your period, called dysmenorrhea, is closely connected to making more of these lipid molecules. Cyclooxygenase (Cox) breaks down arachidonic acid, which makes prostaglandins. This happens when the lining of the uterus sheds [37]. In this group, prostaglandin

F2α is the most important one. It causes uterine contractions to get stronger and creates an inflammatory environment that makes menstruation painful. Understanding the important part that prostaglandins play in the Cox pathway is the first step in understanding how dysmenorrheic pain works. Prostaglandins, which work similarly to hormones, are essential for the contractions of the uterus that are required for lining expulsion. Increased uterine contractions and pain might arise from an overabundance of prostaglandins [14]. Lower abdomen pain, known as cramps, is a classic sign of dysmenorrhea and usually begins either before or during menstruation. Lower back pain is a common complaint during menstruation, adding to a woman's general sense of unease.

In extreme cases of dysmenorrhea, the patient may also have nausea and vomiting, which significantly limits her ability to go about her daily routine. Some women also suffer headaches or migraines during period cramps [4]. Menstrual discomfort is a common cause of weariness and a general sense of malaise. Some people just have slight discomfort, while others have crippling agony that severely limits their ability to go about their everyday lives. Prostaglandins, especially prostaglandin F2, play a crucial role in mediating uterine contractions and menstrual discomfort. When prostaglandin levels are high, muscles contract excessively, resulting in ischemia and pain [17]. Prostaglandin inhibition arises as a viable treatment strategy.

2.2 Pathway of Pain

The synthesis and function of prostaglandins, and in particular the Cyclooxygenase (Cox) pathway, are closely related to the pain pathway in dysmenorrhea [14]. Initiation of menstrual discomfort and uterine contractions are both facilitated by prostaglandins, lipid chemicals that play a key role in these processes. Arachidonic acid is converted into prostaglandins via the Cox pathway, which consists of the two major isoforms Cox-1 and Cox-2. Cox-1 is involved in the upkeep of physiological systems and is expressed constitutively in a wide range of tissues. In contrast, Cox-2 is an inducible enzyme whose activity is frequently increased in response to cellular stress or inflammation [35]. Both isoforms are involved in dysmenorrhea's

increased prostaglandin production in the uterine tissues, which in turn causes more intense uterine contractions and pain.

2.2.1 Pathway of Dysmenorrhea

Kopustinskiene described that in the etiology of dysmenorrhea, the Cyclooxygenase enzymes (Mainly Cox-2) play a crucial role [14]. Extreme menstrual pain, known medically as dysmenorrhea, is connected to the production of prostaglandins, lipid chemicals implicated in a wide range of physiological activities. Increased uterine contractions and pain are common symptoms of dysmenorrhea, and the Cox pathway plays a crucial role in this process by mediating the conversion of arachidonic acid into prostaglandins. A constitutively produced isoform of COX, Cox-1 plays a role in the upkeep of other physiological processes in a wide variety of tissues, including the uterus. Cox-2 is an inducible enzyme that plays a critical role in the dysregulation of prostaglandin synthesis seen in dysmenorrhea because it is increased during inflammation and cellular stress. Elevated levels of prostaglandins, especially prostaglandin F2α, are linked to greater uterine contraction intensity and heightened pain perception, and higher expression of both Cox isoforms is responsible for this. Pain relief from dysmenorrhea can be achieved using nonsteroidal anti-inflammatory medicines (NSAIDs), which work by blocking the enzymes Cox-1 and Cox-2 [37].

Prostaglandins cause uterine contractions and discomfort, however, NSAIDs block these enzymes, reducing prostaglandin production. However, there are some restrictions on the use of NSAIDs because of the potential for gastrointestinal side effects and other issues with prolonged usage. This highlights the need for safer treatment alternatives that can modify the Cox pathway[38].

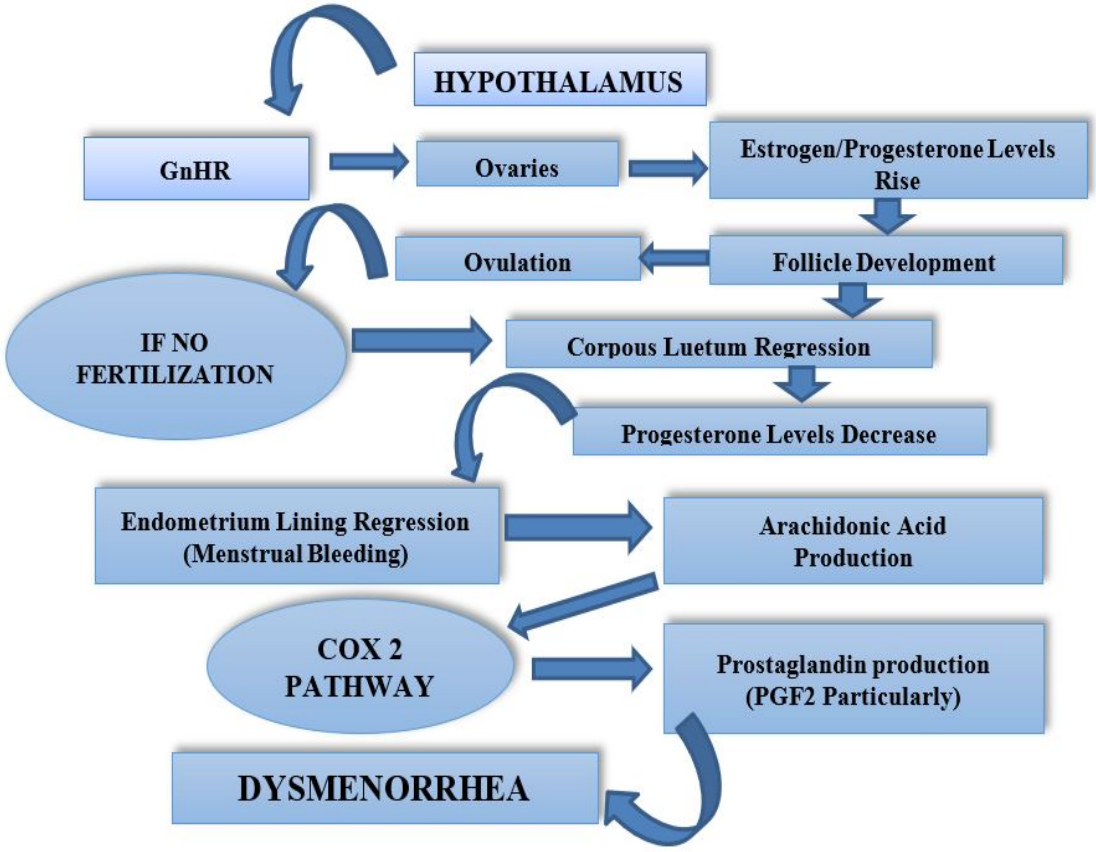


FIGURE 2.1: The pathway of Dysmenorrhea. Simplified form of [14]

2.2.2 Dysmenorrhea and Prostaglandins

The pathophysiology of dysmenorrhea revolves around prostaglandins because of their role as mediators in the Cox pathway. Prostaglandins are secreted during menstruation to facilitate the removal of the uterine lining. Increased uterine contractions and severe discomfort are the result of an overabundance of prostaglandins, specifically prostaglandin F2, in dysmenorrhea [14]. Due to ischemia, pain receptors in the uterus and its environs are activated by the increased levels of prostaglandins. This causes the cramping pain in the lower abdomen that is typical of dysmenorrhea. Oladosu et al., also asserted that prostaglandins can also increase the sensitivity of pain receptors, heightening the experience of pain [39]. Prostaglandins play more than just a role in uterine contractions when it comes to dysmenorrhea [14]. The pain response is amplified because prostaglandins cause inflammation and vascular permeability in the uterine tissues. The complex relationship between prostaglandins and dysmenorrhea can be

better understood, laying the groundwork for therapies that modify prostaglandin synthesis or action.

There is a range of drug-based treatments for dysmenorrhea that have been suggested by different researchers through their scientific writings, each has its own set of pros and cons. Nonsteroidal anti-inflammatory drugs (NSAIDs) and oral contraceptives have been common choices. They have helped, but they come with side effects like stomach pain, heart problems, and hormonal changes [4]. But in the world of drugs, there is a movement toward natural options that is gaining strength. As we look at the different treatments, it becomes clear that gingerol, a bioactive molecule found in ginger, has a lot of potential as one of its own kind way to change the production of prostaglandins in the Cox pathway. Researchers think that gingerol might work by stopping Cox enzymes from doing their job, which lowers the production of prostaglandins. Because of this possible mechanism, gingerol could be a natural and possibly well-tolerated option for the menstruating females who are having trouble with their periods. By looking into how gingerol works with the Cox pathway, scientists hope to find a scientific reason for its supposed anti-inflammatory effects in the treatment of dysmenorrhea [38]. The bioactive ingredient in ginger known as gingerol has shown itself as promising molecular tool for influencing prostaglandin production and the Cox pathway. It has been hypothesized from research that gingerol can inhibit Cox enzymes, leading to a decrease in prostaglandin formation and thus anti-inflammatory effects.

2.3 Ginger Metabolites

Ginger contains a lot of bioactive molecules, including terpenes, Phenolic Compounds, Polysaccharides, lipids, raw fibers and Organic acids. The phenolic compounds, among the ginger metabolites, are mainly gingerols, shogaols, and paradols, which mainly contribute to the various and notable bioactivities of ginger. Recently, there has been a lot of interest in gingerols and shoagoals because of its possible therapeutic properties, especially for dysmenorrhea. When looking into

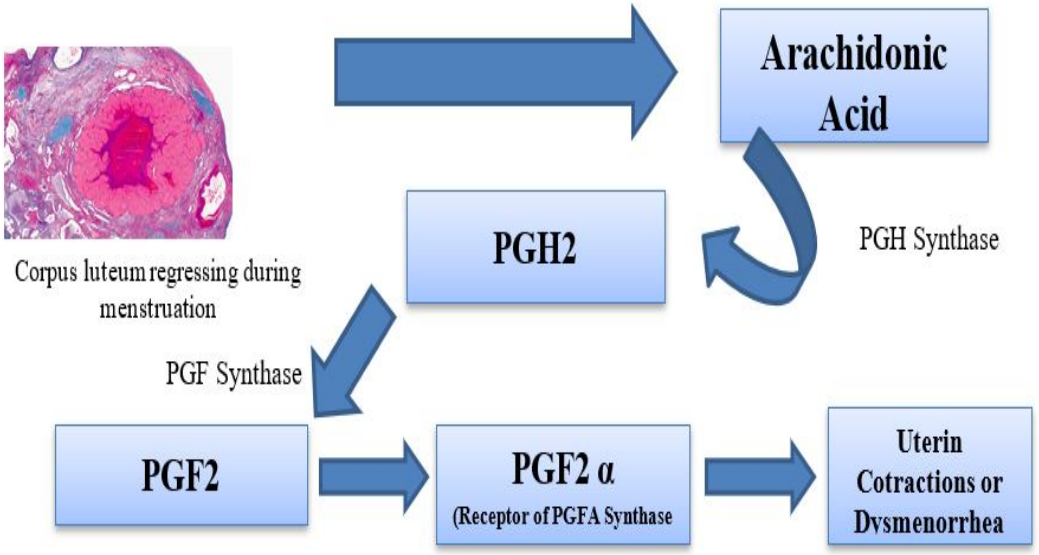


FIGURE 2.2: PGF Synthase and PGF2 α (Receptor of PGFA Synthase, can be used as Target proteins as they are key role players in Dysmenorrhea. [14]

the function of ginger metabolites especially, it is necessary to investigate its interactions as a pathway modulator as well as its influence on fundamental components including the Cox pathway and prostaglandins.

2.3.1 Ginger Metabolites as Anti-Inflammatory Agents

Ginger contains diverse bioactive compounds which possesses multiple bioactivities, such as antioxidant, anti-inflammatory, and antimicrobial properties, these bioactive compounds include gingerols, shogaols, and paradols. Ginger metabolites, mainly Phenolic Compounds of Ginger, possesses a variety of qualities, including those that reduce inflammation and pain, as well as those that act as antioxidants. Additionally, ginger has the potential to be the ingredient for functional foods or nutraceuticals. Persons who suffer from diseases associated with chronic inflammation needs alternative compounds for relieving symptoms, so that advantage of natural drug properties could be taken. An interplay of complex inflammatory cells and a large range of chemical mediator, especially natural derivatives, they are greatly associated with the initiation of the inflammatory response, recruiting, and activating other immune cells to the site and subsequently normalizing it [40]. Gingerol has shown the ability, in its capacity as a route regulator,

to modify signaling pathways that are implicated in inflammatory processes. According to certain studies, gingerol can reduce the amount of nuclear factor-kappa B (NF-kB) activity, which is a transcription factor that plays a role in the production of inflammatory genes. 6-shogaol showed protective effects against tumor necrosis factor α (TNF-α)-induced intestinal barrier dysfunction in human intestinal cell models. [41] Widespread studies have proved,both gingerols and shogaols for exhibiting key roles in biological activities, including anticancer, anti-oxidant, antimicrobial, anti-inflammatory and anti-allergic properties and various central nervous system activities. Shogaols are used as important biomarkers for the quality control of many ginger-containing products, as it has diverse biological activities.

As well as, Gingerols and Shoagoals have the ability to alleviate the inflammatory component of dysmenorrheic pain. They does this by inhibiting the activation of NF-B, which in turn may have anti-inflammatory properties. In addition, gingerol has been studied to see whether or not it can moderate the effects of oxidative stress [42]. Oxidative stress has been linked to several pathological disorders, including dysmenorrhea [43].

2.3.2 Ginger Metabolites and Cox Pathway

Dysmenorrhea management strategies should focus on the Cox pathway because of its prominent role in prostaglandin synthesis. The effects of ginger metabolites on the Cox pathway enzymes Cox-1 and Cox-2 have been studied. Studies have shown that gingerol can inhibit these enzymes, which in turn reduces prostaglandin synthesis. Ginger (Notably Gingerol)'s potential to modulate the Cox pathway and relieve dysmenorrheic pain without the negative side effects of conventional NSAIDs seems promising. Ginger had been proved to have a pain-reducing effect, through researches. Ginger Metabolites have pain relieving effects that can be modulated through several of the mentioned mechanisms: inhibition of prostaglandins via the COX pathway, antioxidant activity, inhibition of the transcription factor nf–kB, or acting as agonist vanilloid nociceptor. As Cox-2 is frequently elevated in inflammatory circumstances like those seen in dysmenorrhea, Ginger metabolites, mainly

Gingerols and shogoals, are involved in selective suppression of Cox-2, which may be proved be particularly useful, through this research. Due to ginger metabolites' ability to block the Cox route, gingerols, shogaols and zingerols could be promising natural option for treating menstruation pain. [26]

2.3.3 Ginger Metabolites and Prostaglandins

As a result of the complex interaction that exists between gingerol and prostaglandins [38], there is a potential for therapeutic intervention in the condition known as dysmenorrhea. Prostaglandins, and more specifically prostaglandin F2, play an essential role in the pathophysiology of dysmenorrhea, which contributes to increased uterine contractions as well as pain. Gingerol's inhibitory actions on the Cox pathway, leading to lower prostaglandin synthesis, may provide a method for reducing the symptoms of dysmenorrhea conditions. Several studies have investigated the effect that gingerol has on the different types of prostaglandins, including prostaglandin E2, which is involved in the inflammatory process [41], [44]. Gingerol has the potential to act as a regulator of the inflammatory processes that are linked with dysmenorrhea. This potential is reflected in gingerol's ability to modify prostaglandin levels.

2.4 Molecular Basis of Ginger Metabolites Action

Bioactive chemicals like gingerol have complex interactions with their targets, including enzymes in the Cox pathway, and molecular docking plays a critical role in understanding these interactions. The molecular docking of gingerol sheds light on its binding affinity, specificity, and potential inhibitory effects on important enzymes [29] and it may also work in the context of dysmenorrhea. In molecular docking, a ligand like gingerol is simulated on a computer to interact with a target protein like PGF2α Synthase. To fully comprehend the molecular nature of gingerol's interaction with these enzymes, it is necessary to forecast their

binding modes and energies. Researchers can determine the stability of ligand-protein complexes, predict the likelihood of inhibition, and locate possible binding sites through molecular docking experiments [28]. Results from molecular docking studies aid in the development of new treatments and the improvement of current ones.

2.5 Protein Simulation

Choo reported that the analysis of gingerol's effect on the Cox pathway is taken to a dynamic level using protein modeling - a potent computational method. Protein simulation allows researchers to monitor the dynamic behavior of the complex over time, in contrast to molecular docking, which only provides snapshots of ligand-protein interactions. To understand the complex interactions between gingerol and the Cox-2 enzymes in the setting of dysmenorrhea, protein simulation is a valuable tool [26]. The protein docking and molecular simulation process exposes the ligand-protein complex to dynamic settings that are analogous to the physiological environment. Considering environmental conditions, such as temperature, pressure, and solvation effects, a more accurate picture of the interactions is created as gingerol may decrease depending on the environmental conditions [27]. Gingerol's effects on the structural rigidity and pliability of Cox enzymes, can be studied through molecular dynamics simulations and other methods.

The conformational changes inside the Cox enzymes generated by gingerol binding can be better understood with the help of protein simulation [26]. To evaluate the possible long-term effects of gingerol on Cox-1 and Cox-2 enzymatic activity, an understanding of these dynamic variations is essential. Gingerol's time spent in the active site of Cox enzymes can be estimated with the help of protein simulations, providing insight into the length of inhibition. Protein modeling results provide useful data for the rational development of gingerol-based treatments for dysmenorrhea. Scientists can now determine the effects of gingerol on Cox enzyme kinetics, measure free energy changes associated with ligand binding, and identify stable conformations. This evolving viewpoint helps us better grasp the potential and longevity of gingerol as a medicinal agent.

In summary, Dysmenorrhea has been thoroughly investigated in this literature review, including its epidemiology, risk factors, kinds, pathophysiology, and diagnostic methods. Central to the understanding of dysmenorrheic pain is the Cox pathway, and more specifically the role of the Cox-1 and Cox-2 enzymes in prostaglandin synthesis. Increased uterine contractions and discomfort are both mediated by prostaglandins, most notably prostaglandin F2. Bioactive ginger component gingerol promises as a natural treatment for dysmenorrhea. It has anti-inflammatory and antioxidant actions, making it a candidate for use as a regulator of several pathways. The binding affinity and specificity of gingerol to Cox enzymes have been visulaized through molecular docking experiments, suggesting its ability to influence the Cox 2 pathway.

Research into the stability and long-term consequences of "gingerol- PGF2α Synthase enzyme complexes" benefits greatly in the dynamic perspective provided for protein simulation experiments. Analyzing the molecular mechanism by which gingerol inhibits prostaglandin production in the Cox pathway has potential character as a therapeutic intervention for dysmenorrhea. Due to gingerol's ability to lower prostaglandin synthesis, inhibit inflammatory processes, and preferentially targets Cox-2 without any side effects of conventional treatment of NSAIDs, hence gingerol is worthy of further study.

Chapter 3

Research Methodology

There is wide range of Prostaglandins, which play constructive role but there are specific prostaglandins which paly role in COX 2 pathway of Dysmenorrhea, hence this pathway should be inhibited at specific and suitable points. Selective Inhibition of membrane proteins will be done so that the constructive role of Prostaglandins is not hindered.

As an initial step, the sequence of the target protein is selected. The potential ligands sequences are obtained as well. Using the In- Silico tools, the ligand-Protein binding efficiency is checked. The process flow of the methodology will be as below

3.1 Selection of Problem

Primary Dysmenorrhea (menstrual pain) is very a common gynecological disease which dramatically affects the quality of life for many women. NSAIDs and oral contraceptive are used but they have adverse effects like GIT infections, bone density reduction, increased blood clot risk etc.

The problem here is the need of having such medication that have less side effects and with /more efficacy.[45]

3.2 Methodology Flowchart

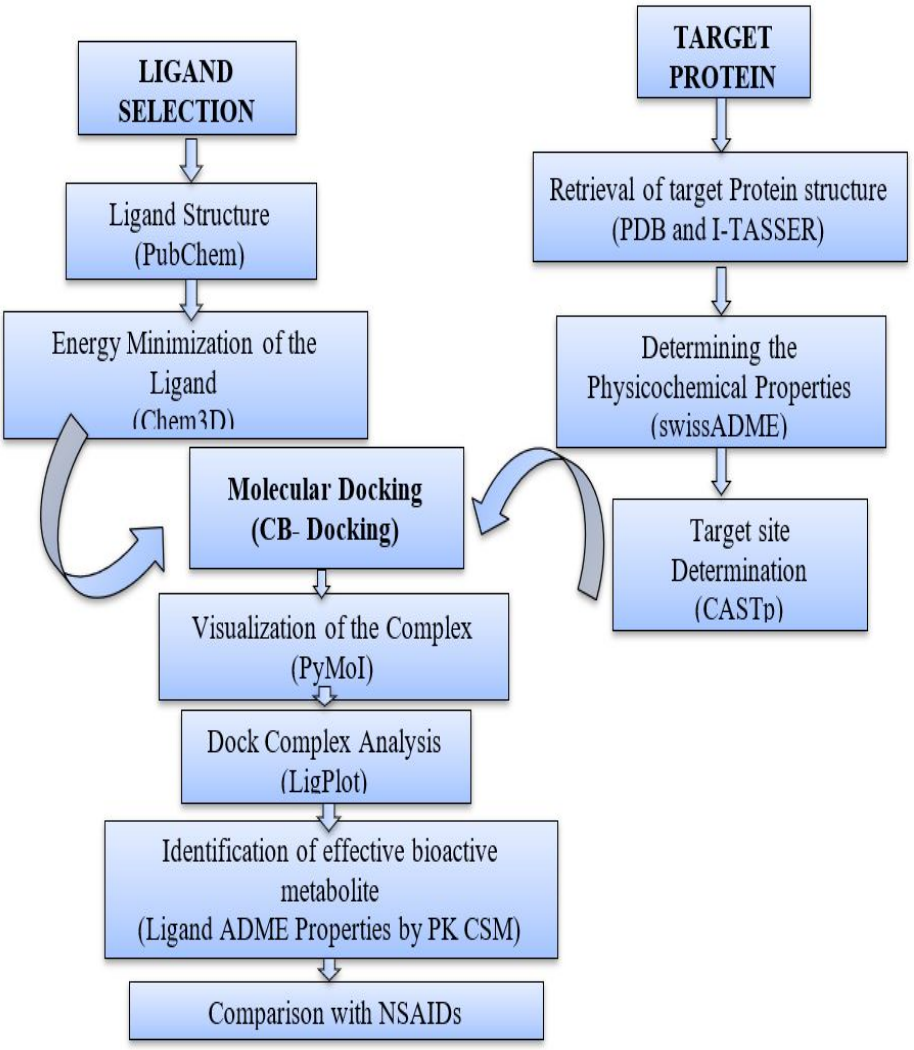


FIGURE 3.1: Methodology Flowchart

3.3 Target Protein Selection

The COX-2 pathway is involved in Dysmenorrhea and one of main receptor involved in production of prostaglandin which is in turn are involved menstrual cramps. PGF2α receptor is the membrane receptor which plays vital role in men strual cycle.

The targeted protein was selected on basis of purification technique used, nucleotide strength and resolution.

3.4 Primary Sequence Retrieval

Primary sequence of target proteins (PGF2) was taken in FASTA format from pro tein sequence database UniProt https://www.uniprot.org/ http://www.uniprot.org/ under accession number P43088 with residues length of 359.

3.5 Analysis of Physicochemical Properties of Protein

To determine the functional role of target protein, analyzing the physicochemical properties are the key role players. To predict the properties of target protein, PGF2α, ProtParam https://web.expasy.org/cgi-bin/protparam/protparam was used.

The total number of negatively charged residues (Asp + Glu), total number of positively charged residues (Arg + Lys), theoretical pI value, molecular weight, Ext coefficient (Cys included), Extinction coefficient (Cys reduced), instability index, aliphatic index and grand average of hydrophobicity were computed through ProtParam.

3.6 3D Structure Prediction of Protein

To obtain the 3D structure of a protein we need to have the protein sequence first which can be obtained through the online servers PDB https://www.rcsb.org/, alternatively I-TASSER https://zhanglab.ccmb.med.umich.edu/I-TASSER/.

I-TASSER is an online server that is used for the prediction of the structure and function of the protein in three dimensions.

Firstly, this online server identifies the structural model of the PDB, through various strategies inclusive of the atomic models of full length, that are built by using simulations of the different threading fragments.

The I-TASSER server can also predict the 3D structure of proteins so on the basis of C-score we can select the best 3D structure of the protein.

3.7 Structure Analysis of Target Protein by Py MOL

PyMOL https://pymol.org/ is a molecular graphics tool that has been used globally, widespread for the three-dimensional analysis and visualization of many proteins, small molecules including nucleic acids, densities of different electrons and varying surfaces and also the trajectories. It is also used for editing the molecules, tracing the ray and also to make animations and movies. This software tool is based on python and also contain many plugin tools in order to enhance its uses. The drug targeting and designing by the use of PyMOL software can be done.

After downloading the protein structure, the extra constituents attached to the protein need to be removed, like ligands and water molecules were removed, and polar hydrogens were added which was done by the use of an open-source system PyMol.

3.8 Functional Domain Identification of Targeted Proteins

Interpro http://www.interpro.com/is an online database which was used to identify the functional domains of targeted protein PGF2α Conserved domains are involved in sequence / structure / relationship.

3.9 Retrieval of Chemical Structure of Ligands

PubChem https://pubchem.ncbi.nlm.nih.gov/ **is the world's largest repository of easily accessible chemical information database.** So, the chemical

compounds that were used as ligands were selected from PubChem database. The refining of selected ligands was done through Chem3D ultra Pro Version 12.0.2. Those ligands were selected that had previously shown some anti-inflammatory properties. Those selected ligands include: 6- Shogaol, 6- Gingerol and zingerol. To refine the ligands, energy minimization was done using Chem3DPro.

3.10 Bioactivity Analysis of Ligands and Toxicity Measurement

Selected ligands Chemical compounds that were used as ligand were selected from PubChem database. If the compounds selected as ligands follow the "Lipinski rule of five" and those are likely to be used as an active drug. The potential success of a compound depends on its ADMET properties. PkCSM https://omictools.com/pkcsm-tool is an online tool that helps to find the ADMET properties of the compounds. The rules are described as under:

1. The logP value of most \drug-like" molecules should be limited to 5.
2. Maximum number of H-bond acceptor should be 10.
3. Maximum number of H-bond donor should be 5.
4. Rotatable bonds should be limited to five.
5. Molecular Weight should be less than 500.

3.11 Molecular Docking of Targeted Proteins

The purpose of molecular docking is to find the best conformational interaction between target proteins and compounds. The two essential requirements for docking are: the refined target protein and the refined candidate ligand. Active metabolites of Ginger,*Zingiber officinale* chosen after review of literature, structural retrieval

done with Pubchem and refined with Chem3DPro are the ligands; while Target Protein is surface protein (PGF2a).

CB dockhttp://clab.labshare.cn/cbdock/php/blinddock.php is an online docking server which automatically identifies binding sites and is used to perform docking. It can simplify docking procedures and improve accuracy by predicting target protein binding sites.

3.11.1 Process of Molecular Docking

The first step in Molecular Docking is creating potential ligands and target protein files. As an initial step the target protein is prepared for docking by refining it. For refining the target protein, ligands are removed, water molecules are removed and polar hydrogens are added. Then the refined target protein is saved in PDB file format. This refined target protein is undergoes the process of docking, with one by one ligands. In case of Dysmenorrhea COX Pathway is involved in inflammation and receptors of COX enzymes can be particularly docked with potential ligands. [46] For this process ligands also needs to be refined and these files need to be saved in SDF or PDB format. The ligand is refined through Chem3D Pro and in this process, Energy Minimization is done and then the files are stored. The refined files in PDB format will be an input to CB-DOCK. We picked particular ligands based on their ADME properties, refined them and saved in PDB. Refined Target Protein is one by one docked with the target protein.

3.11.2 Active Site Identification

Properties of protein structures including Geometric and topological for example surface pockets, interior cavities and cross channels, are vey important for proteins function. Computed Atlas of Surface Topography of proteins (CASTp) is a web server that provides online services for locating, and measuring these of protein structure properties. [47] 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 software http://sts.bioe.uic.edu/castp/

3.12 Protein Ligand Interaction

The interaction of the active pockets of the ligand and the protein are calculated for the interpretation of docking results. Two types of interactions are studied, hydrogen bonding and hydrophobic bonding. Using Ligplot plus (version v.1.4.5) the protein ligand interactions were studied. This software automatically generates schematic diagrams of the protein-ligand interaction of the given ligands in the PDB file.

3.13 Lead Compound Identification

After a detailed analysis of protein and ligand interactions, docking scores and toxicity studies, the most active inhibitor was identified. The selected compound was our lead compound.

3.14 Drug Identification and Selection

This step was performed for identification of drugs that were used for antibacterial diseases treatment purpose. KEGG and Drug Bank https://go.drugbank.com/ databases were used for drug identification because it helps to analyze the disease in details with its pathway and drugs.

3.15 Prediction of Different Parameters of Selected Drug

The identified drugs must be filtered in order to select the most effective drug. This is done through a detailed study of identified drugs and most effective drug

is identified setting parameters i.e. physiochemical properties, effective ADMET properties, effective mechanism of action and minimal side effects using PubChem, Drug Bank, pkCSM, and KEGG databases, respectively. The identified drug was then docked with target proteins to identify the inhibition efficiency. CB 2 dock is an online docking server which was used to perform docking. It can simplify docking procedures and improve accuracy.

3.16 Reference Drug and Lead Compound Comparison

The comparison between reference anti-bacterial drug and the proposed lead compound was done through comparing docking values, physiochemical properties and ADMET properties.

Chapter 4

Results and Discussion

This chapter will explain the results obtained after following systematic steps of methodological steps described in earlier sections. Initially, the 3D structure of protein and ligands were retrieved and given as input after refining them. The proteins Physiochemical properties and domain prediction of the protein were docked against selected ligands whose energy had already minimized. ADMET properties and lipinski 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, 3D structure prediction and functional domain identification of proteins.

4.1.1 Primary Sequence Retrieval

FASTA sequence of selected target proteins was retrieved through UniProt www.uniprot. The proteins was selected based upon its Biological significance in Dysmenorrhea Pathway; As well as the quality of the protein i.e NMR and X-ray Crystallography.

```
>sp|P43088|PF2R_HUMAN Prostaglandin F2-alpha receptor OS=Homo sapiens OX=9606 GN=PTGFR PE=1 SV=1
MSMNNSKQLVSPAAALLSNTTCQTENRLSVFFSVIFMTVGILSNSLAIAILMKAYQRFRQ
KSKASFLLLASGLVITDFFGHLINGAIAVFVYASDKEWIRFDQSNVLCSIFGICMVFSGL
CPLLLGSVMAIERCIGVTKPIFHSTKITSKHVKMMLSGVCLFAVFIALLPILGHRDYKIQ
ASRTWCFYNTEDIKDWEDRFYLLLFSFLGLLALGVSLLCNAITGITLLRVKFKSQQHRQG
RSHHLEMVIQLLAIMCVSCICWSPFLVTMANIGINGNHSLETCETTLFALRMATWNQILD
PWVYILLRKAVLKNLYKLASQCCGVHVISLHIWELSSIKNSLKVAAISESPVAEKSAST
```

FIGURE 4.1: Sequence Retrieval

4.1.2 Physiochemical Characterization of Protein

ProtParam is a tool of Expasy which is used online for the prediction of different parameters including both physical and chemical properties of selected proteins. These several parameters calculate and estimate the following: molecular weight, composition of amino acid, theoretical value of Protein index, atomic composition of protein, extinction coefficient, and estimated half-life of protein instability, aliphatic index and grand average of hydropathicity which was abbreviated as GRAVY. The calculated PI greater than 7 represents the basic nature of the protein while less than 7 shows acidic nature of protein. Extinction coefficient represents light absorption. Instability index if less than 40 show stability of the protein while greater than 40 indicates the instability of protein [48].

TABLE 4.1: Target Protein Properties

Prostaglandin F2 – Alpha Receptor (PGF2 α Receptor) Protein	
Number of Amino acids	359
Molecular Weight	40054.56
Theoretical PI	9.19
Extinction Coefficient (assuming all Cys residues form Cystines)	49805
Extinction Coefficient (assuming all Cys residues are reduced)	48930
Instability Index	33.7
Aliphatic Index	114.62
Grand Average of Hydropathacity (GRAVY)	0.545
Total number of negatively charged residues (Asp + Glu)	19
Total number of Positively charged residues (Arg + Lys)	32

The aliphatic index represents the aliphatic content of a protein. The high value of the aliphatic index indicates the thermo-stability of the protein. Molecular weight contains both positive and negative charged residues of protein.Low GRAVY shows better interaction with water molecules. All these parameters which were selected for this research work were taken according to previous research work [49].

MW stands for molecular weight, pl for theoretical isoelectric point at which protein is neutral, without any charge), NR for total number of negatively charged residues (Asp +ve Glu), PR for total number of positively charged residues (Arg+ve Lys), Ext.Co1 for extinction coefficients when assuming all pairs of Cys residues form cystines , Ext. Co2 for extinction coefficients when assuming all Cys residues are reduced and GRAVY for grand average of hydropathicity.

Prostaglandin F2α (PGF2α), is vital protein in cyclooxygenase (COX)-catalyzed prostaglandin, production Pathway that regulates a number of physiological functions like luteolysis, ovarian function, luteal maintenance of pregnancy, and parturition as a constitutive part of ongoing reproductive processes of the body. Through scientific studies, its role is emphasized in the regulation of prominent pathophysiological processes, including all kinds of inflammation (Actue and Chronic). With the discovery of a second isoform of COXs (COX2), it has been shown that PGF2α can be formed in vivo from arachidonic. [50]

4.1.3 3D Structure Prediction of Proteins

The 3D Structures of targeted proteins can easily be downloaded from RCSB PDB in the PDB format. Protein Data Bank is a database which is used to find three-dimensional structures of complex molecules like Proteins of living organism. I-TASSER (Iterative threading Assembly Renement) is a very special procedure used in order to predict the structure of the protein also structure and function of the target proteins. This is an online server, which initially identifies the templates of the given structure retrieved by the PDB obtained by applying different approaches including the multiple threading approach LOMETS, by involving atomic models of full length which are built by the simulations assembled by fragments that are template based; then this server has been widely used for

protein structure and performance predictions in biological and biomedical investigations. I-TASSER predicts regions of secondary protein structure which may include like alpha helix, beta sheet and coils obtained by the sequence of the organic compound [139]. I-TASSER server team mails complete results of job id with five models and on the base of C-score best 3D structural model often can be easily selected. Alphafold https://alphafold.com/ is also a protein structure database for 3D structure prediction of proteins. The 3D Structure of the Protein can be obtained from Alphafold by getting the Sequence of target protein under the PDB file ID:**8IUK**.

The protein structures were prepared by refining it with the help of PyMol. PyMol works by removing water molecules and ligands if exist. After the ligands been removed, the missing polar hydrogens were added. The energy of minimization for structure was performed to get the stable conformation by preventing overlap and the modified file in PDB format was saved. The refined structures is shown in figure below.

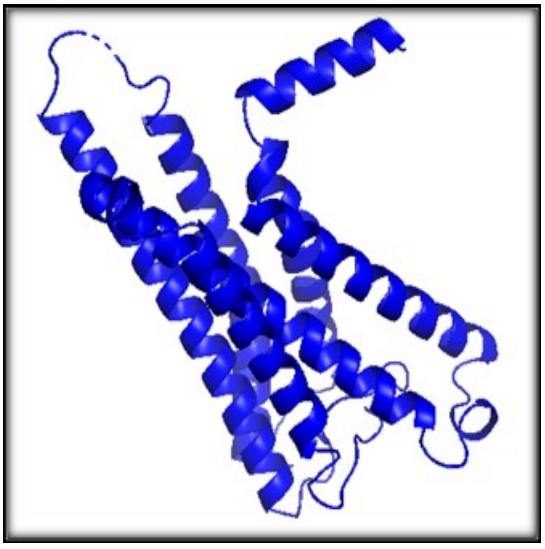


FIGURE 4.2: 3D Structure of PGF2 α Receptor Protein

PGF2 α Receptor Protein belongs to the G protein-coupled receptors (GPCRs) family. The GPCR superfamily is diverse, and sequencing of the human genome has revealed over 850 genes that encode these GPRCs. The function of proteins belonging to this family is that, they have mediation role in senses such as odor, taste, vision, and pain in mammals. In addition, to it, they act as receptors for ligands in metabolic pathways, like in production of Prostaglandins. They

are vital for cell recognition and communication processes often involve GPCRs. This diverse family of the GPCRs proteins match the variety of ligands that can activate them, including odorants, taste ligands, light, metals, biogenic amines, fatty acids, amino acids, peptides, proteins, nucleotides, lipids, and steroids. As there are many diseases which take place due to malfunction of GPCRs, this makes it important targets for drug development. An estimated more than 30% of all marketed therapeutics act on those GPCRs. Prostanoids (prostaglandins (PG) and thromboxanes (TX), both metabolites of arachidonic acid) have much important physiological roles in the cardiovascular and immune systems and in pain sensation in peripheral systems. We are interested in PGF2 α Receptor Protein which also belong to this family and involved in Pain path of menstruation (Primary Dysmenorrhea). [51]

4.1.4 Functional Domain Identification of Protein

Database Interpro was used to identify the domains and functional sites of selected proteins. Interpro is a resource for functional analysis of protein sequences. Conserved domains are involved in sequence/structure/relationship. Proteins can have more than one functional domain that perform different functions. Functional domain is the active part of a protein that is involved in interactions of proteins with other substances [52].

Prostaglandins F2 – alpha receptor is a protein made up of 359 amino acid. It belongs to G protein-coupled receptor, rhodopsin-like family. It has got three functional domains. One of these three domains is important in Dysmenorrhea, which is directly involved in PGF2 alpha synthase binding, resulting in Prostaglandin F production. The functional domains include: G protein-coupled receptor, rhodopsin-like (17-349 Residues), Prostanoids (43-304 Residues) and Prostaglandin F receptor (43-304 Residues)

G protein-coupled receptors (GPCRs) consist of a vast protein family that have a wide range of functions, inclusive of various autocrine, paracrine and endocrine processes. There is considerable diversity at the sequence level, on the basis of which they can be separated distinctly into groups.The rhodopsin-like GPCRs

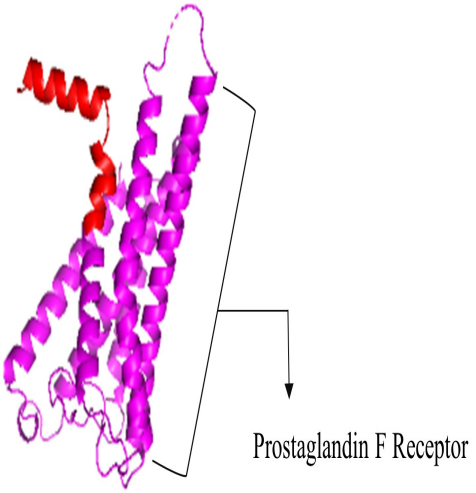


FIGURE 4.3: 3D Functional Domains of PGF2 α Receptor Protein (Residues 43-304)

(GPCRA) belong to a widespread protein family that includes the hormones, neurotransmitters and light receptors, all of these transduce extracellular signals by interacting with guanine nucleotide-binding (G) proteins. However, their activating ligands vary widely in their structure and features, but the amino acid sequences of the receptors are very similar and are believed to adopt a common structural framework comprising 7 transmembrane (TM) helices.

Prostanoids (prostaglandins (PG) and thromboxanes (TX)) play a wide variety of actions and play important physiological roles in the cardiovascular, immune systems, and in sensation of distinct in peripheral systems. PGI2 and TXA2 have opposing actions, involving regulation of the interaction of platelets with the vascular endothelium, while PGE2, PGI2 and PGD2 are powerful vasodilators that potentiate the action of various autocoids to induce plasma extravasation and pain sensation.

4.2 Active Site Identification

To identify active sites of protein, CASTp software was used which predicts available pockets for binding and also tells about surface area and volume of pockets. Table below illustrate the areas and volumes of PGF2a receptor Protein, binding Pockets:

TABLE 4.2: Illustrate The Areas and Volumes of PGF2a Receptor Protein, Binding Pockets:

Pock ID	Surface Area	Volume	Pock ID	Surface Area	Volume
1	156.591	139.073	**25**	3.895	0.177
2	220.602	91.377	**26**	1.974	0.104
3	163.96	66.613	**27**	1.476	0.077
4	141.845	41.897	**28**	0.808	0.036
5	40.863	14.729	**29**	0.728	0.033
6	27.522	8.544	**30**	0.527	0.013
7	32.32	8.282	**31**	0.627	0.012
8	23.772	8.159	**32**	0.447	0.008
9	25.471	5.771	**33**	0.206	0.007
10	13.352	5.691	**34**	0.36	0.007
11	25.47	4.793	**35**	0.292	0.007
12	22.936	3.654	**36**	0.304	0.006
13	18.749	2.947	**37**	0.237	0.004
14	10.998	2.74	**38**	0.171	0.004
15	12.453	2.678	**39**	0.177	0.003
16	26.827	2.295	**40**	0.105	0.001
17	6.679	2.15	**41**	0.014	0
18	15.003	2	**42**	0.046	0
19	8.638	1.615	**43**	0.001	0
20	7.649	1.288	**44**	0.037	0
21	8.821	1.015	**45**	0.005	0
22	10.18	0.858			
23	5.485	0.603			
24	7.159	0.521			

CASTp data depicts forty-five binding pockets for PGF2 α Receptor protein. Above table represents the binding pockets with area and volume of PGF2 α Receptor protein. The largest binding pocket has surface area 220.602 whereas its volume is 91.377. The smaller binding pocket has surface area 0.001 and volume 0.000. The below figure representing PGF2 α Receptor protein. Structure. Red color showing the available binding pockets 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.

4.3 Ligand Selection

In the Protein data bank have got a large amount of protein ligand complexes, with certain protein target. This facilitates the selection of ligands based on the

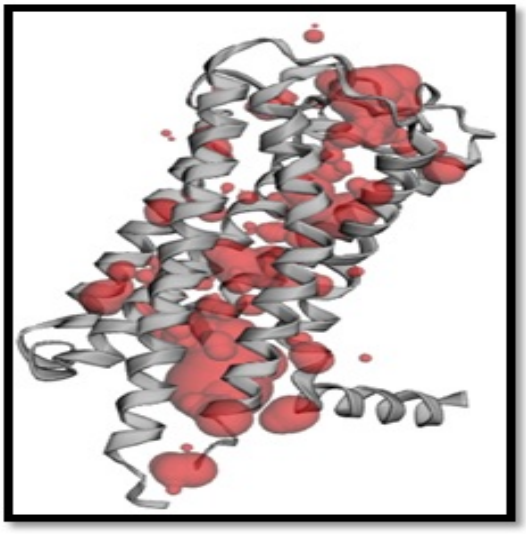


FIGURE 4.4: Binding pockets for PGF2 α Receptor Protein

best resolution of the structure, co-crystal ligand's chemical class that is bound to the protein structure and its best binding affinity. [53]

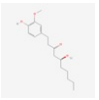
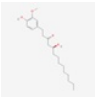
The chemical information database PubChem https://pubchem.ncbi.nlm.nih.gov/, is used to search out for Ligands. This database provides the information that can be accessed freely around the globe. The 3D structures of Ligands were downloaded from PubChem in SDF format. After selection of ligands, energy minimization was carried out by using a bioinformatics software chem3D pro software (chem 3D v 12.0.2)

While selecting the Ligands, the lipinski rule is considered as well, which deals with certain parameters like Molecular weight which should be is less than or equal to 500, log P is less than or equal to5, Hydrogen bond donors is less than or equal to 5, hydrogen bond acceptors is less than or equal to 10. This is a mandatory step in the preparation of ligands for docking because unstable ligands will show unreliable vina scores in docking results. The selected ligands include 6-Shogaol, 10-Shogaol, 6-Gingerol, 8-Gingerol, 10-Gingerol and Zingerol.

The ligand Selection was done on basis of lipinski rule of five. The lipinski rule deals with certain parameters like Molecular weight which should be is less than or equal to 500, log P is less than or equal to 5, Hydrogen bond donors is less than or equal to 5, hydrogen bond acceptors is less than or equal to 10 [55]. A compound is considered a drug when it follows 3 or more rules and if a compound

violates two or more rules it is considered poorly absorbed. All ligands which were considered after literature review, obeyed lipinski rule of five except Zingerone. Selected ligands with molecular formula, molecular weight and chemical structure are represented in table:

TABLE 4.3: Ligands Information

Sr. No	Ligands Name	Molecular Formula	Molecular Weight (g/mol)	Structure
1	6-Shogaol	C17H24O3	276.4	
2	10-Shogaol	C21H32O3	332.5	
3	6-Gingerol	C17H26O4	294.4	
4	8-Gingerol	C19H30O4	322.4	
5	10-Gingerol	C21H34O4	350.5	
6	Zingerol	C11H16O3	196.24	

4.4 Molecular Docking

Molecular Docking is technique which is used to estimate the strength of a bond between a ligand and a target protein. This is done through a special scoring function and to determine the correct structure of the ligand within the target binding site. As an input for docking, the 3D structure of the target proteins and the ligands is taken. It represents a frequently used approach in structure-based drug designing as it requires a 3D structure of a target protein. It determines the correct structure of the ligand that binds or fits into the target binding site, hence results in estimation of the strength of the binding between the ligand and the target proteins through a specific scoring function. Docking may also help in the recognition of new small molecular compounds revealing the essential properties such as high interaction between binding with target protein having reasonable

absorption, distribution, metabolism and excretion which help in the selection of lead compound for the target. [54]

To automatically predict binding modes without information about binding sites, a user-friendly blind docking web server called CB Dock was used. CB dock is involved in prediction and estimation of a binding site for a given protein, leading to calculation of centers and sizes with a novel rotational cavity detection method and perform docking with the popular docking program named Auto dock Vina. CB dock gives five best interacting confirmations for each ligand molecule. All of these five confirmations were arranged based on their binding affinities and then finest confirmation can be selected which has the highest affinity score of protein-ligand interaction. After docking process, the dock structures were selected for further analysis. On the basis of docking score, cavity size, Grid map, and binding energy, we can select the best docked structure.

The docking was performed using PGF2α receptor proteins and ligands 6-Shogaol, 10-Shogaol, 6-Gingerol, 8-Gingerol, 10-Gingerol and Zingerol. Ligands which showed the best binding score values with target proteins are represented in table:

TABLE 4.4: Binding Scores with target proteins

Sr. No	**Ligands Name**	**Binding Score/ Vina Score**	**Cavity Volume(A^3)**
1	6-Shogaol	-6.2	540
2	10-Shogaol	-6.4	540
3	6-Gingerol	-6.2	540
4	8-Gingerol	-6.4	540
5	10-Gingerol	-5.9	540
6	Zingerol	-6.5	540

4.5 Ligands Proteins – Interaction Analysis

The interaction of Target protein's active pockets and ligands were calculated to interpret of docking results. Two types of interactions were studied, hydrogen bonding and hydrophobic bonding interactions. Using Ligplot plus (version v.1.4.5) the protein ligand interactions were studied. [55] By using Ligplot plus the interaction of active confirmation of target protein and the ligands has been

identified. The saved conformations for ligand receptor complex of each molecule were analyzed in detail. The schematic diagrams of the protein-ligand interactions of the given ligands in the PDB file are automatically generated, by the Ligplot Software. The docked files were uploaded in PDB format to get hydrogen and hydrophobic bonding illustrations. The illustrations below shows the diagram of the ligand-receptor interactions.

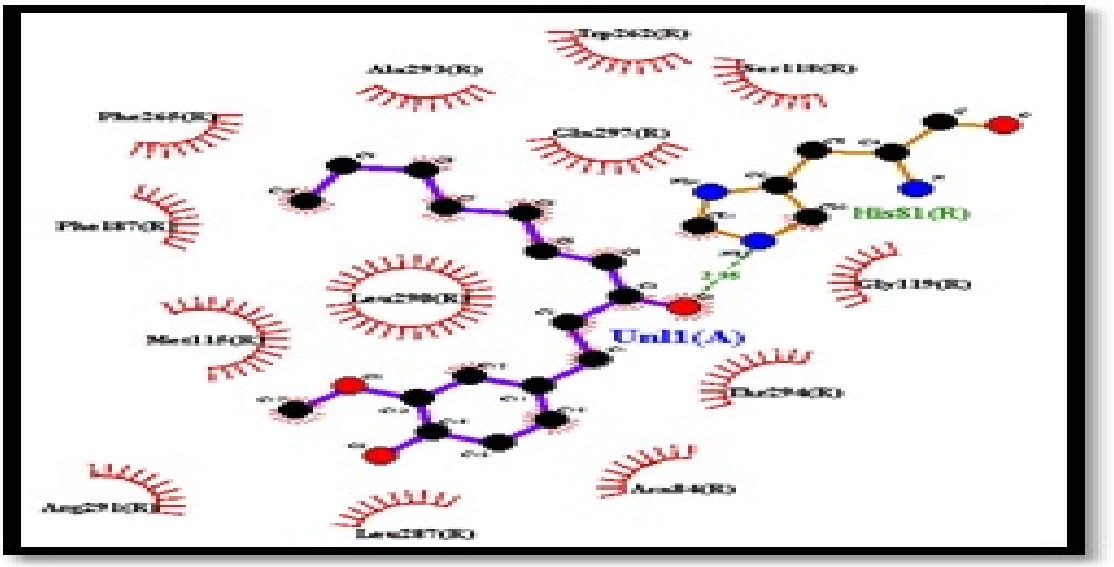


FIGURE 4.5: Interaction of PGF2a Receptor and 6 Shogoal by LigPlot

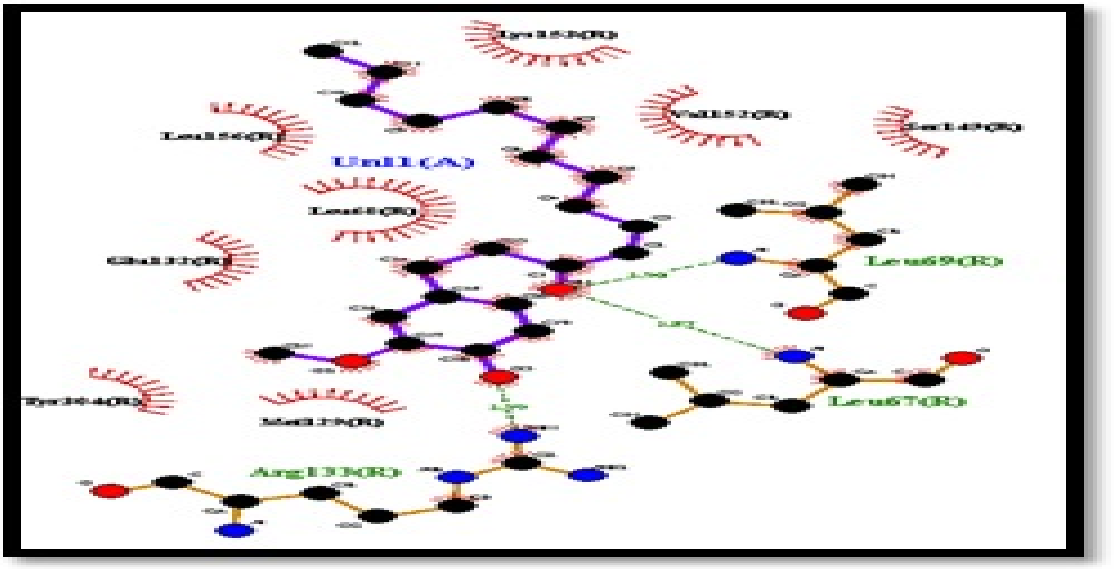


FIGURE 4.6: Interaction of PGF2a Receptor and 10 Shogoal by LigPlot

The in-silico method and Ligplot as a tool has enabled us to reveal that a how a multi-targeted small molecule can binds with any of its low affinity and respective targets, hence this binding affinity can be changed by the integration of integrating

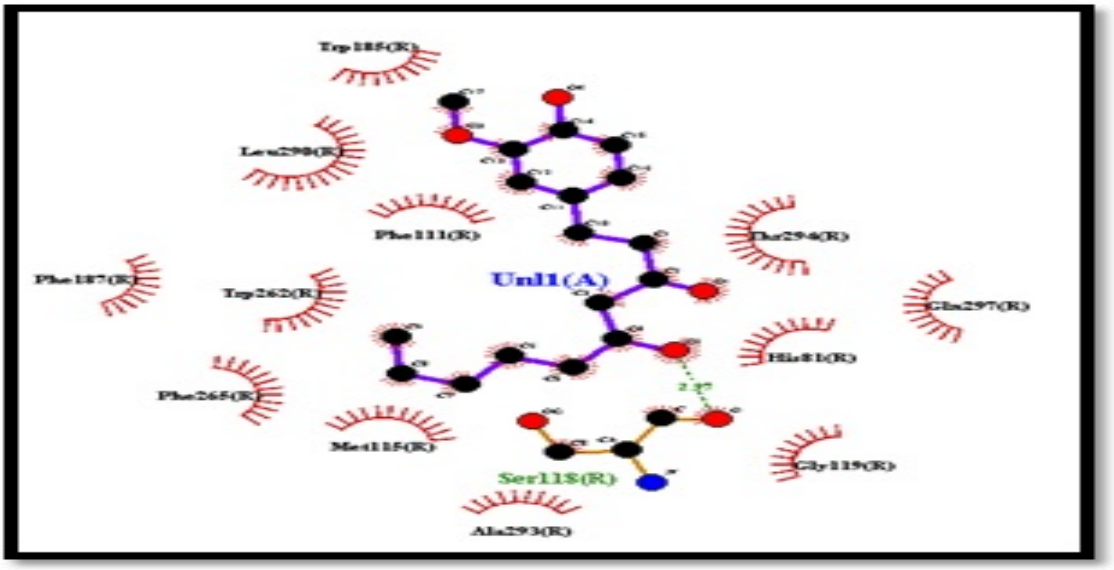


FIGURE 4.7: Interaction of PGF2a Receptor and 6 Gingerol by LigPlot

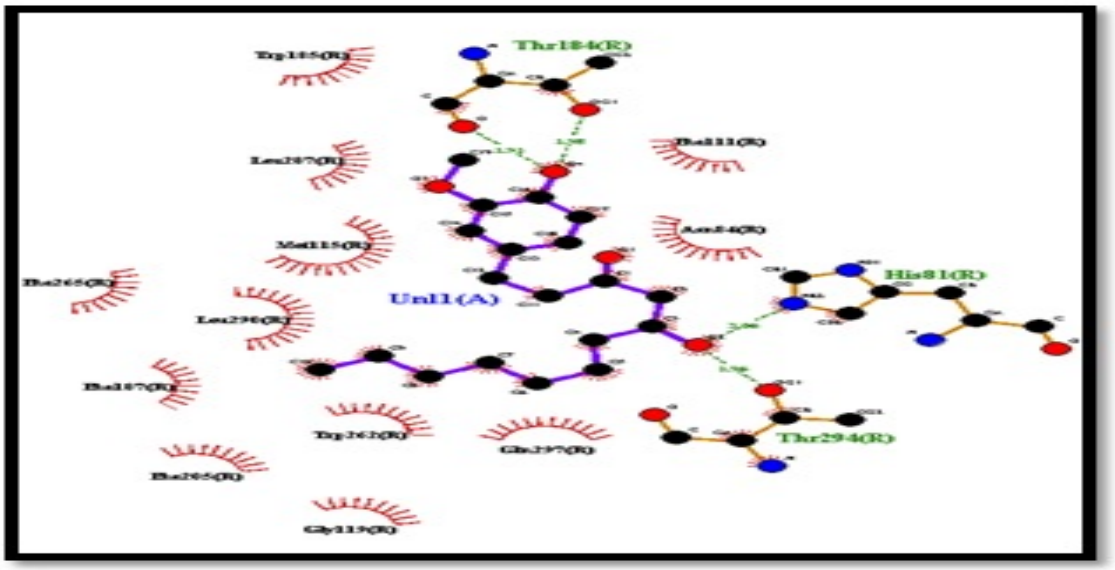


FIGURE 4.8: Interaction of PGF2a Receptor and 8 Gingerol by LigPlot

the conformational changes due to favored functional groups at the active site of the ligand-target interfacing one another. 3D structural folding at the protein-ligand grooves is hallmark for its molecular recognition and helps in predicting their biological activity. [56] The results present here that hydrogen bonding and optimized hydrophobic interactions both stabilize the ligands at the target site, and help to alter their binding affinity and increase their potential to be a drug.

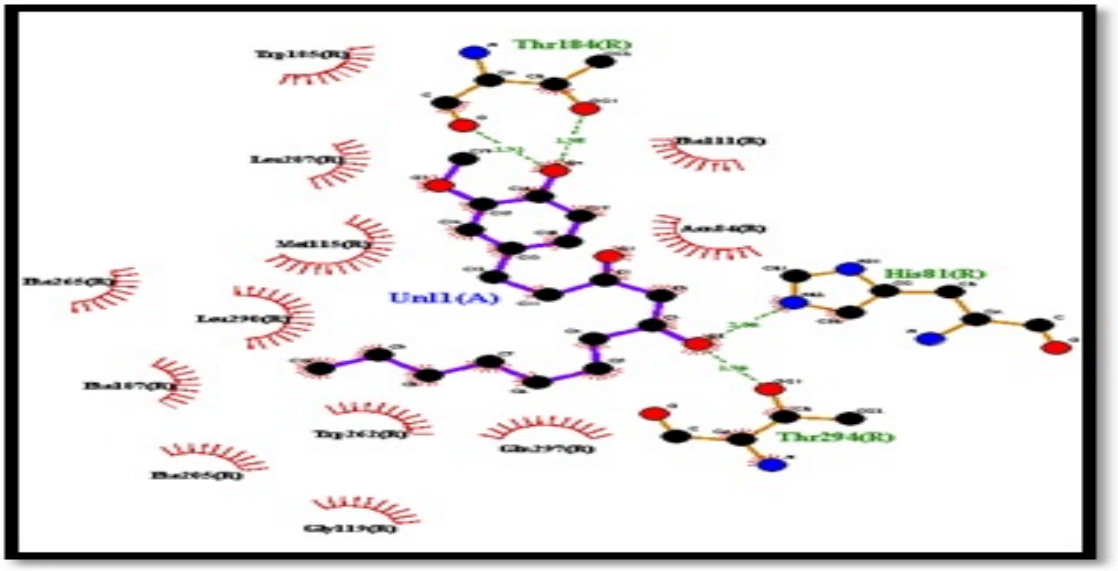


FIGURE 4.9: Interaction of PGF2a Receptor and 10 Gingerol by LigPlot

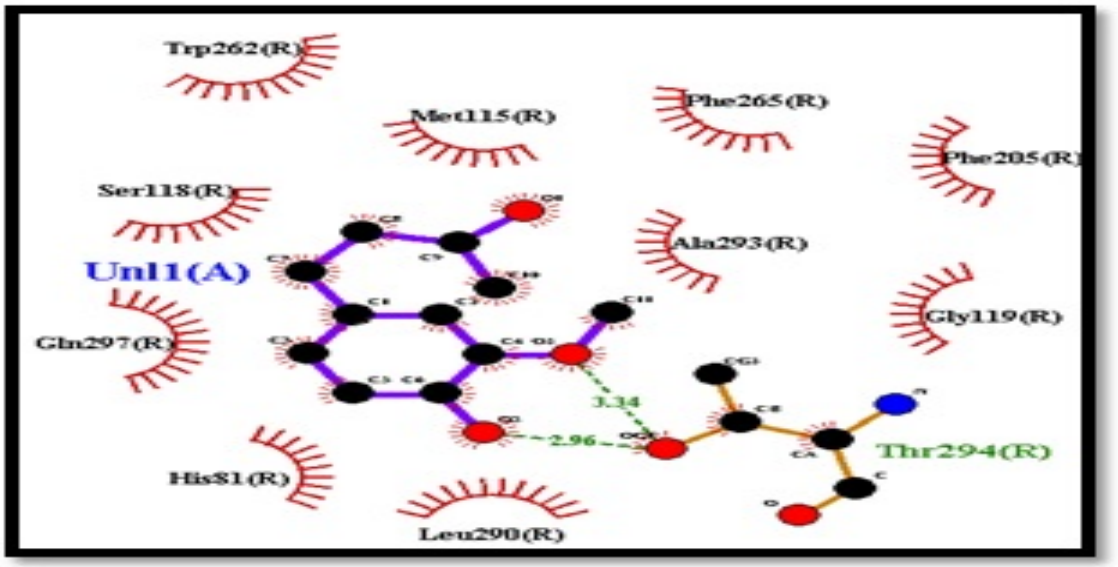


FIGURE 4.10: Interaction of PGF2a Receptor and Zingerol by LigPlot

Table 4.5: Hydrogen Bonding and Optimized Hydrophobic

Sr. No	Ligands Name	Binding Energy	No. Hydrogen Bonds	Hydrogen Bonding Distance	Amino Acids	Hydrophobic Interactions
						Trp 262
						Serl18
						Ala293
						Gln297
1	6-Shogaol	-6.2	1	2.95	His81	Phe265
						Phe187
						Met115
						Leu290
						Arg291
						Leu287
						Asn84
						Thr294
						Gly119
						Ser149
						Val152

						Lys153
				2.9	Leu69	Leu156
2	10-Shogoal	-6.8	3	2.57	Leu6	Leu168
				2.99	Arg133	Glu132
						Tyr304
						Met129
						Trp185
						Leu290
						Phe111
						Phe187
3	6-Gingerol	-6.2	1	2.97	Serl18	Trp262
						Phe262
						Met115
						Ala293
						Gly119
						His81
						Gln297
						Thr284

						Ehe111
						Asn84
				3.06	His81	Trp185
4	8- Gingerol	-6.2	4	2.96	Thr294	Leu287
				2.93	Thr184	Met115
				2.93		Ehe265
						Leu290
						Ehe187
						Trp262
						Gln297
						Ehe205
						Gly119
						Gln297
						Asn84
				3.21	Thr294	Arg291
5	10- Gingerol	-5.9	3	2.83	His81	Thr184
				2.94		Trp185
						Leu290

						Met115
						Phe265
						Ph187
						Trp262
						Gly119
						Ser118
						Gly119
						Phe205
				3.34	Thr294	Ala293
6	Zingerol	-6.5	2	2.96		Phe265
						Met115
						Trp262
						Ser118
						Gln297
						His81
						Leu290

4.6 ADMET Properties of Ligands

As a first step for assessing verbal bioavailability and artificial availability, Lipinski's five-drug law is used. As second assessment ADMET properties of ligands were calculated, as a measure of pharmacokinetics using the online tool pkCSM. In pharmacology there two broad terms the one is pharmacodynamics and pharmacokinetics.

4.6.1 Pharmacokinetics

In pharmacokinetics, the absorption of drugs, distribution of drugs, metabolism of the drug and excretion of the drugs, all of these factors are studied.

4.6.2 Absorption Properties of Ligands

In pharmacology specifically pharmacokinetics, we explain the transfer of a drug from the bloodstream into the tissues as absorption, hence the chemical composition of a drug, as well as the environment into which a drug is placed, all work together to determine the rate of absorption and extent to which drug absorption. Cellular barriers, such as epithelial or endothelial cells must be crossed by the medicine, in order to be absorbed. Only a few medications have ability to pass these cellular barriers in an active manner that demands the use of energy and then transports the drug from a low concentration to a higher concentration.

Most of the medications on the other hand, use the process of passive diffusion to pass past cellular barriers in which they travel from a high-concentration area to a low-concentration area by diffusing through cell membranes.
This sort of drug movement does not involve any energy expenditure but it is controlled by the drug size and solubility.
Water solubility and skin absorption for all ligands is low while CaCO2 permeability is normal.

absorption properties of ligands were shown in following tables:

Table 4.6: Absorption Properties of Ligands

Sr. No	Ligands Name	Water Solubility(mol/L)	CaCO2 Permeability(cm/S)	Intestinal Absorption(Human)%	Skin Permeability(Log/Kp)
1	6 Shogoal	-6.209	1.362	93.608	-2.572
2	10 Shogoal	-5.424	1.462	91.312	-2.681
3	6 Gingerol	-3.164	0.94	92.416	-2.817
4	8 Gingerol	-3.795	0.703	91.716	-2.781
5	10 Gingerol	-4.371	0.626	91.029	-2.759
6	Zingerol	-1.635	1.19	92.998	-2.633

Table 4.7: Absorption Properties of Ligands

Sr. No	**Ligand Name**	**P-Glycoprotein Substrate**	**P-Glycoprotein I Inhibitor**	**P-Glycoprotein II Inhibitor**
1	6 Shogoal	No	Yes	Yes
2	10 Shogoal	No	No	No
3	6 Gingerol	Yes	No	No
4	8 Gingerol	Yes	Yes	No
5	10 Gingerol	Yes	Yes	No
6	Zingerol	No	No	No

All ligands have average to Skin permeability for all ligands is low. All ligands show negative value for p-glucoprotein substrate, thid featur is favourable as If a compound is positive for Pgp substrate then its means that it can be casily pumped out of the cells to reduce its absorption.

4.6.2.1 Distribution Properties of Ligands

Central nervous system Permeability is expressed as log PS, it shows the total amount of drug that will be needed to be evenly distributed so that it provides the same concentration as in blood plasma. VDss less than 0.71 L/kg is considered low and it is considered higher if it is above 2.81L/kg.

The high VDss, means that more of the drug is still distributed to the tissues than to plasma. If a compound shows more Fu value it means it is more effective. BBB permeability is an important parameter as BBB protects the brain from exogenous compounds.

If predicted value of log BB is greater than 0.3 then it means given substance can cross BBB and if its value is less than -1 then there could be no harm to brain. Log PS is the product of blood brain permeability and surface area and its value greater than 2 considered to penetrate the Central Nervous System and less than -3 considered as safe. VDSS of all ligands is low, Fu values of all ligands are positive. BBB permeability of all ligands is in range of -0. Log PS value of 6- Shogoal, 10-Shogoal is -1.777 and -1.559 respectively, while for other ligands it is in range of more than -3. The distribution properties of ligands are shown in table

Table 4.8: Distribution Properties Ligands

Sr. No	LN	(Log L/Kg)	(Fu)	(Log BB)	(Log Ps)
01	6 Shogoal	0.501	0.147	-0.197	-1.777
02	10 Shogoal	0.572	0.037	-0.364	-1.559
03	6 Gingerol	0.524	0.258	-0.727	-2.788
04	8 Gingerol	0.588	0.183	-0.794	-2.799
05	10 Gingerol	0.605	0.123	-0.877	-2.815
06	Zingerol	0.384	0.47	-0.017	-2.502

4.6.2.2 Metabolic Property of Ligands

CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4 are the models of the various isoforms of Cytochrome P450. This, Cytochrome P450 is an important cleansing enzyme found in the liver. [149] The Metabolic Properties of ligands are presented as below:

TABLE 4.9: Metabolic Properties of Ligands

Sr. No	LN	CYP2D6 sub	CYP3A4 subs	CYP1A2 inh	CYP2C19 inh	CYP2C9 inh	CYP2D6 inh	CYP3A4 inh
01	6 Shogoal	No	Yes	Yes	Yes	Yes	No	No
02	10 Shogoal	No	Yes	No	Yes	No	No	No
03	6 Gingerol	No	No	Yes	Yes	Yes	No	No
04	8 Gingerol	No	Yes	Yes	Yes	Yes	No	No
05	10 Gingerol	No	Yes	No	Yes	Yes	No	No
06	Zingerol	No	No	Yes	No	No	No	No

4.6.2.3 Excretion Property of Ligands

There are two organs involved in drug excretion: the kidneys, involved in renal excretion and the liver which plays role in biliary excretion. Other organs may also be involved in excretion, such as the lungs for volatile or gaseous agents. Drugs may also be excreted in sweat, saliva and tears. Models of Excretion property can be evaluated through: Total Clearance expressed as log (CL tot) in ml/min/kg and Renal OCT2 substrate which predicts results as Yes /No.

TABLE 4.10: Pharmacokinetic Properties of Ligands

Sr. **No**	Names of **Ligands**	Total Clearance **(log ml/min/kg)**	Renal OCT2 **substrate (Yes/No)**
01	6 Shogoal	1.44	No
02	10 Shogoal	1.56	No
03	6 Gingerol	1.339	No
04	8 Gingerol	1.4	No
05	10 Gingerol	1.462	No
06	Zingerol	0.23	No

4.6.2.4 Ligand Toxicity

The MRTD (maximum tolerated dose) defines the toxicity of a hazardous substance in an individual. Ligand Toxicity information aids in directing a treatment's

initial indicated dosage in phase 1 clinical trials. The MRTD value is represented logarithmically (log mg/kg/day). A chemical has a low MRTD if its value is less than or equal to 0.477 log (mg/kg/day) and a high MRTD if its value is greater than 0.477 log (mg/kg/day). The 6 Gingerol showed no hERGI or hERGII inhibition. Hepatotoxicity was shown by none of the ligands. 6 Shogoal and 10 Shogoal showed skin sensitivity. Zingerol showed T. pyriformis activity less than -0.5 log µg/L. The minnow toxicity values of all ligands(except 10 Shogaol and 10 Gingerol) were greater than 0.5 mM, which is considered safe.

Ligand toxicity analysis is an essential component of modern computer-aided drug design strategies. Drug-induced toxicities can identify structural features and properties associated with toxicity. This allows medicinal chemists to optimize lead compounds to improve safety while maintaining potency. Integrating ligand-based toxicity predictions with structure-based design techniques like molecular docking provides a powerful approach to rationally design safer and more effective drug designing.

Table 4.11: Ligand Toxicity

Toxicity Property	**6 Shogoal**	**10 Shogoal**	**6 Gingerol**	**8 Gingerol**	**10 Gingerol**	**Zingerol**
AMES toxicity	No	No	No	No	No	No
Max. tolerated dose (human)	0.759	0.746	0.635	0.694	0.705	0.47
hERG I inhibitor	No	No	No	No	No	No
hERG II inhibitor	Yes	Yes	No	Yes	Yes	No
Oral Rat Acute Toxicity (LD50)	2.081	2.403	1.958	1.957	1.948	2.086
Oral Rat Chronic Toxicity (LOAEL)	2.159	2.321	1.631	1.702	1.783	1.866
Hepatotoxicity	No	No	No	No	No	No
Skin Sensitisation	Yes	Yes	No	No	No	No
T.Pyriformis toxicity	2.475	1.807	1.487	1.535	1.299	0.622
Minnow toxicity	0.15	-0.809	0.966	0.493	0.001	1.08

4.7 Lipinski Rule of Five Analysis of Ligands

If selected ligands, 6-Shogaol, 10-Shogaol, 6-Gingerol, 8-Gingerol, 10-Gingerol and Zingerol, follow the "Lipinski rule of five" then these have potential to be used as an active drug. The most considered and important rules are mentioned below:

1. The logP value of most \drug-like" molecules should be limited to 5.
2. Maximum number of H-bond acceptor should be 10.
3. Maximum number of H-bond donor should be 5
4. Rotatable bonds should be limited to five.
5. Molecular Weight should be less than 500.

The tables below show the properties of ligands according to Lipinski rule of five.

Table 4.12: Lipinski Properties of Ligands

Sr No	**Ligands Name**	**LogP value**	**Rotatable bonds**	**Molecular WT(g/mol)**	**H-Bond Acceptor**	**H-Bond Donor**
01	6-Shogaol	3.76	5	276.37	3	1
02	10-Shogaol	5.24	8	332.48	3	1
03	6-Gingerol	3.13	5	294.39	4	2
04	8 Gingerol	3.87	7	322.44	4	2
05	10 Gingerol	4.62	7	350.49	4	2
06	Zingerol	1.86	4	196.24	3	2

4.8 Reference Drug for Dysmenorrhea

There are some treatment options for primary dysmenorrhea, including hormonal and Non- hormonal. These both methods of treatment aim to provide the interference with the Prostaglandins production, hence reducing uterine contractions, or inhibiting the pain perception through direct analgesic effect. Among hormonal and non- hormonal treatments, the most widespread used medication and first line treatment is wide category of drugs: Non-Steroidal Anti-inflammatory Drugs (NSAIDs).

4.8.1 Ibuprofen (NSAIDs for Dysmenorrhea)

Nonsteroidal anti-inflammatory drugs (NSAIDs) are used as the first line treatment for primary dysmenorrhea and their choice should be based on their effectiveness and individual's tolerability, because as per scientific studies, there is no comparatively better effectiveness of any NSAID has been proven over any other. Ibuprofen, naproxen, mefenamic acid, and aspirin are the some of the NSAIDs used for Dysmenorrhea.

Ibuprofen, acts as a prostaglandin synthetase inhibitor and is one of the most frequent used NSAIDs by women for Dysmenorrhea. NSAIDs are such Anti-prostaglandins which can relieve dysmenorrheal pain.

Mefenamic acid from fenamate groups and ibuprofen from propionic acids act as inhibitors of PGs synthesis. The studies showed that ginger has similar effects as NSAIDs. In a search of this effectiveness the comparison of ginger metabolites need to be done with one of the most widespread NSAIDs ,i.e ibuprofen. [57]

4.8.2 Ibuprofen Mechanism of Action

As ibuprofen is a nonsteroidal anti-inflammatory agent, it has analgesic properties, it significantly reduces prostaglandins levels of menstrual fluid to normal levels, without getting any reduction in menstrual fluid volume.

Although there is evidence of some individual patients have reported some reduction in menstrual blood loss along with some great set of side effects.

Generally, the mode of action of Ibuprofen is by exerting its anti-inflammatory and analgesic effects through inhibiting both COX isoforms.

As Arachidonic acid is released from the cell membrane phospholipids by phospholipase A2 (PLA2), it is then converted to its unstable intermediate prostaglandin (PG) H2 by an enzyme cytosolic prostaglandin G/H synthases, termed cyclooxygenases (COX), that exist in two forms, COX-1 and COX-2, both of them are are encoded by genes PTGS1 and PTGS2, respectively.

PGH2, is then converted by tissue-specific synthases to various prostanoids – that is, PGE2, PGD2, PGF2α, PGI2, and TxA2. These bioactive lipids act through their corresponding receptors to trigger a series of biological effects.

In case of Dysmenorhea, PGF2α, is the major prostonoid that needs to be inhibited to proceed it COX Pathway. Ibuprofen does this effectively. [58]

4.9 Ibuprofen and Lead Ligand Comparison

To identify the better treatment for Dysmenorrhea and best ginger bioactive metabolite for inhibiting Prostaglandins and COX pathway, a comparison between ibuprufen and 6-Shogoal was done.

Comparison was being performed through parameters like ADMET properties and physiochemical properties of both compounds.

4.9.1 Absorption Properties

The absorption properties of selected drug and lead ligand is done and the results are laid below in form of table 4.13.

4.9.2 Distribution Properties Comparison

The distribution properties of Ibuprofen and Zingerol are given in Table 4.14.

4.9.3 Metabolic Properties Comparison

4.9.4 Excretion Properties Comparison

Total clearance of 6-Shogoal and Ibuprofen are close enough which helps in the excretion of drug from the body. The excretion properties of Ibuprofen and 6-Shogoal are given in Table 4.15.

4.9.5 Toxicity Properties Comparison

The table below shows the comparison between the toxicity of Ibuprofen and 6-Shogoal. The max tolerated dose for Ibuprofen is 0.263 and for 6- Shogoal is 1.44 and oral acute toxicity rat of Ibuprofen is greater.

Ibuprofen also have Hepatotoxicity and Skin Sensitization while 6- Shogoal do not have.

4.9.6 Lipinski Rule of Five

The values of Ibuprofen and 6 Gingerol, Lipinski rule of five are given in Table, Most values lie in the same range Zingerol shows best Lipinski Rule Properties as its log value is lesser leading to better absorption and less toxicity. Low molecular weight also makes it to be an effective ligand.

Lipinski Ro5 and makes better understanding of potential medicinal drugs by making chemists more aware of the any possible relationships between physical properties and ADMET leading to drug designing. [61] As in the case of this study, ginger metabolite (Zingerol) could be a better alternative to NSAIDs.

4.10 Molecular Dynamic Simulation of Docked Complexes

For better optimization and a better understanding of how the atoms interact, molecular dynamic simulations were run on the best docked poses using AMBER20 [62]The AMBER suit's ff14SB force field was used to build PGF2a topologies, whereas the GAFF force field was used to generate ligands topologies. A cubic TIP3P water box was utilized to solvate the systems after they were neutralized with Na+ ions. After that, to limit the chance of steric clashes, each atom in the complexes was subject to a 0.1kcal/mol constraint, and energy minimization was performed with a total of 5,000 steps using the steepest descent technique and the conjugate gradient. To achieve equilibrium, all atoms that were

covalently linked to hydrogen were run through the SHAKE algorithm, which allowed for an integration time step of 2 fs. An MD run that lasted for one hundred nanoseconds was initiated after each system had attained a state of equilibrium. During this time, the complexes' root mean square deviation (RMSD), root mean square fluctuation (RMSF), and radius of gyration (Rg) were investigated. To plot the graphs showing the parameters that were specified, the QtGrace software was utilized https://sourceforge.net/projects/QtGrace

4.11 Results – Molecular Dynamic Simulation (MD Simulation)

The optimum conformation of the studied ligands, which was identified through molecular docking at the PGF2a protein, was further analyzed using molecular dynamics simulation for a period of 100 nanoseconds. To determine the RMSD values, the protein's structure was superimposed along the trajectory with its original structure (at 0 ps). The average RMSD value of the 6-Shogaol complex with PGF2a protein was 3.73 Å, the average RMSD value of 6-Gingerol with protein was 4.24 Å, while and the average RMSD value of zingerol with protein was 4.92 Å (Figure a). It was noticed that 6-Gingerol and Zingerol displayed larger fluctuations in comparison to 6-Shogaol, even though all three systems reached equilibrium at approximately 18 nanoseconds. It became abundantly evident, upon examination of snapshots retrieved at various intervals that the changes in rmsd were caused by the alteration in the conformation of the protein, while all of the compounds occupied the binding pocket and remained stable throughout the simulation run with an average RMSD value less than 2 Å (Figure b). The estimated average RMSD values for 6-Shogoal, 6-Gingerol, and zingerol were 1.38, 1.62, and 0.97 Å, respectively.

In addition, the RMSF-graph, which illustrates the flexibility of amino acid residues across the simulation periods, was analyzed (Figure c).

In addition, the RMSF-graph, which illustrates the flexibility of amino acid residues across the simulation periods, was analyzed (Figure c).

The average RMSF value of the complex including 6-Shogoal, 6-Gingerol, and zingerol with PGF2a protein was 7.95 Å, 14.73 Å, and 24.45 Å, respectively. The RMSF values indicated that 6-Shogoal showed fewer fluctuations as compared to 6-Gingerol and zingerol from their initial conformation. This might mean that the amino acids aren't moving, which could be because the compound is stable in the protein's binding pocket.

Moreover, the values of the radius of gyration (Rg) of the protein and ligand complexes were computed (Figure d) to carry out an additional evaluation of the compactness and stability of the ligands. The average Rg values of 6-Shogoal, 6-Gingerol and zingerol with PGF2a were 42.11, 43.24, and 42.29 Å, respectively. While the average Rg values 6-Shogoal, 6-Gingerol and zingerol were 21.24, 21.60, and 21.02 Å, respectively, affirming the compactness and stability of studied complexes.

Overall, simulation plots analysis showed a stable behavior, suggesting that Ginger metabolites are better inhibitor of COX2 Pathway. They can inhibit the Prostaglandins Production by binding to PGF2a Receptor Proteins, hence blocking the pain pathway of Primary Dysmenorrhea.

Table 4.13: Properties of Ligands

Sr. No	LN	WS (mol/L)	CaCO2 P (cm/S)	IA (Human)%	SP (Log/Kp)	P-gly sub	P-gly I	P-glyco II I
01	Ibuprofen	-3.696	1.792	94.06	-2.685	No	No	No
02	6-Shogoal	-6.209	1.362	93.60	-2.572	No	Yes	Yes

Table 4.14: Distribution Properties Comparison

Sr. No	Ligands Name	VDss (Log L/Kg)	Fraction unbound (Fu)	BBB Permeability (Log BB)	CNS Permeability (Log Ps)
01	Ibuprofen	-0.803	0.239	0.31	-1.695
02	6-Shogoal	0.501	0.147	-0.197	-1.777

Table 4.15: Comparative Table for Metabollic Properties

Sr. No	LN	CYP2D6 sub	CYP3A4 sub	CYP1A2 inh	CYP2C19 inh	CYP2C9 inh	CYP2D6 inh	CYP3A4 inh
01	Ibuprofen	No	No	No	No	No	No	No
02	6-Shogoal	No	Yes	Yes	Yes	Yes	No	No

Table 4.16: Toxicity Properties Comparison

Toxicity Property	Ibuprofen	6- Shogoal
AMES toxicity	No	No
Max. tolerated dose (human)	1.015	0.759
hERG I inhibitor	No	No
hERG II inhibitor	No	Yes
Oral Rat Acute Toxicity (LD50)	2.303	2.081
Oral Rat Chronic Toxicity (LOAEL)	2.438	2.159
Hepatotoxicity	Yes	No
Skin Sensitisation	Yes	Yes
T.Pyriformis toxicity	0.528	2.475
Minnow toxicity	0.619	0.15

Table 4.17: Chemical Properties of Ligands

Sr. No	Ligands	LogP Value	No. of Rotatable bonds	Molecular Weight (g/mol)	H-Bond Acceptor	H-Bond Donor
01	6- Shogoal	3.76	6	276.4	3	1
02	Ibuprofen	3.00	4	206.28	2	1

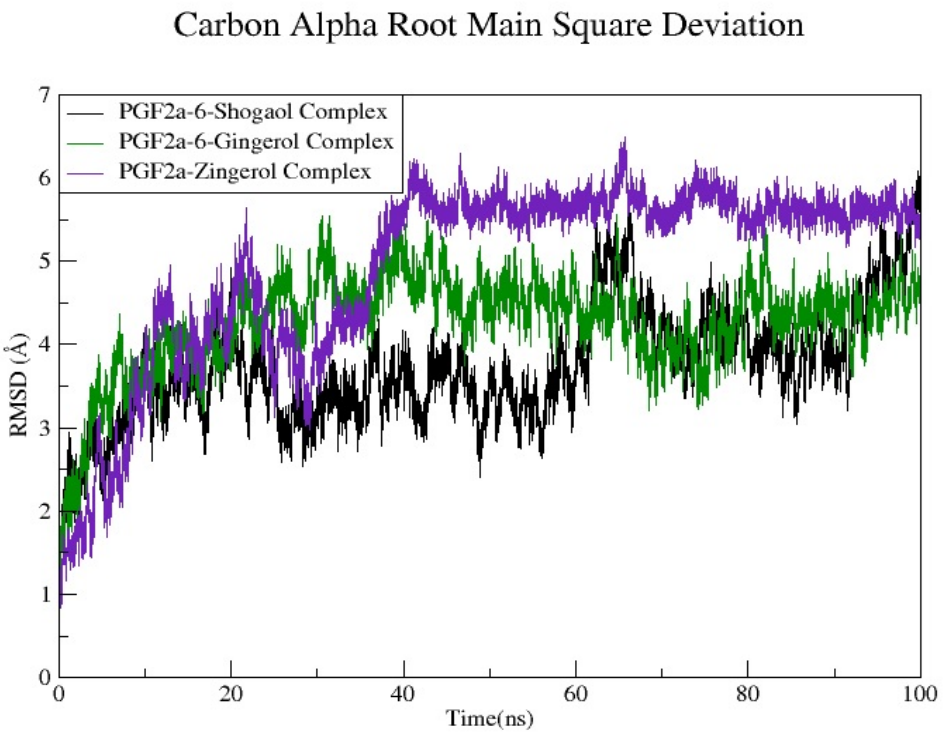


FIGURE 4.11: The average RMSD values of 6-Shogaol, 6-Gingerol and zingerol with protein PGF2a Protein

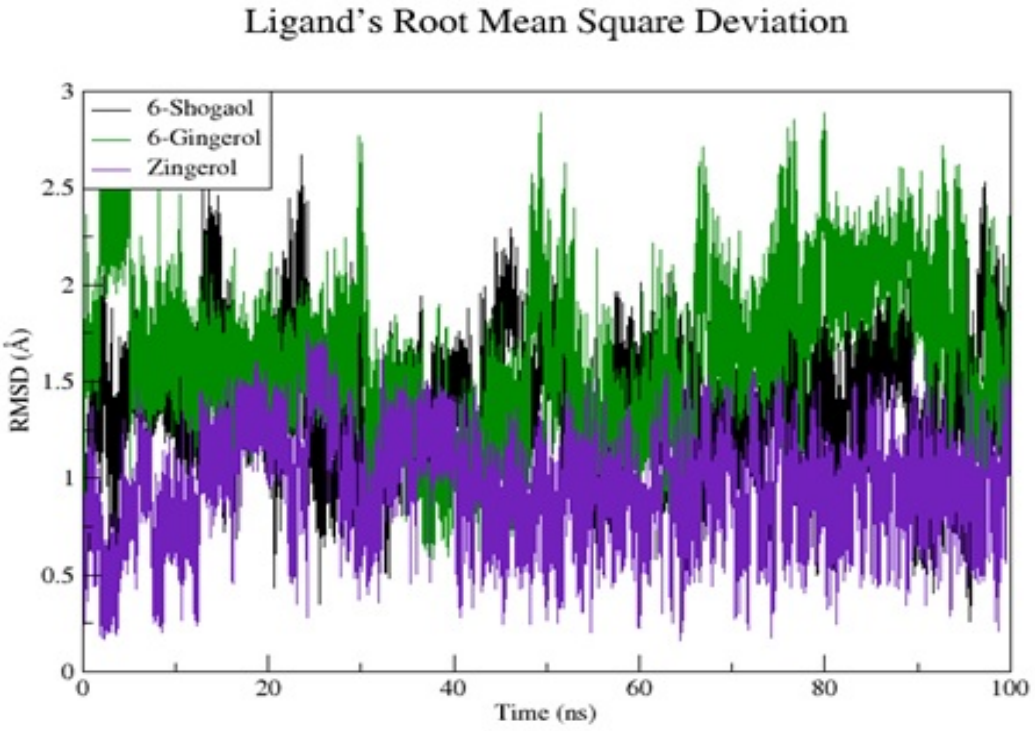


FIGURE 4.12: Ligand's root mean square Deviation

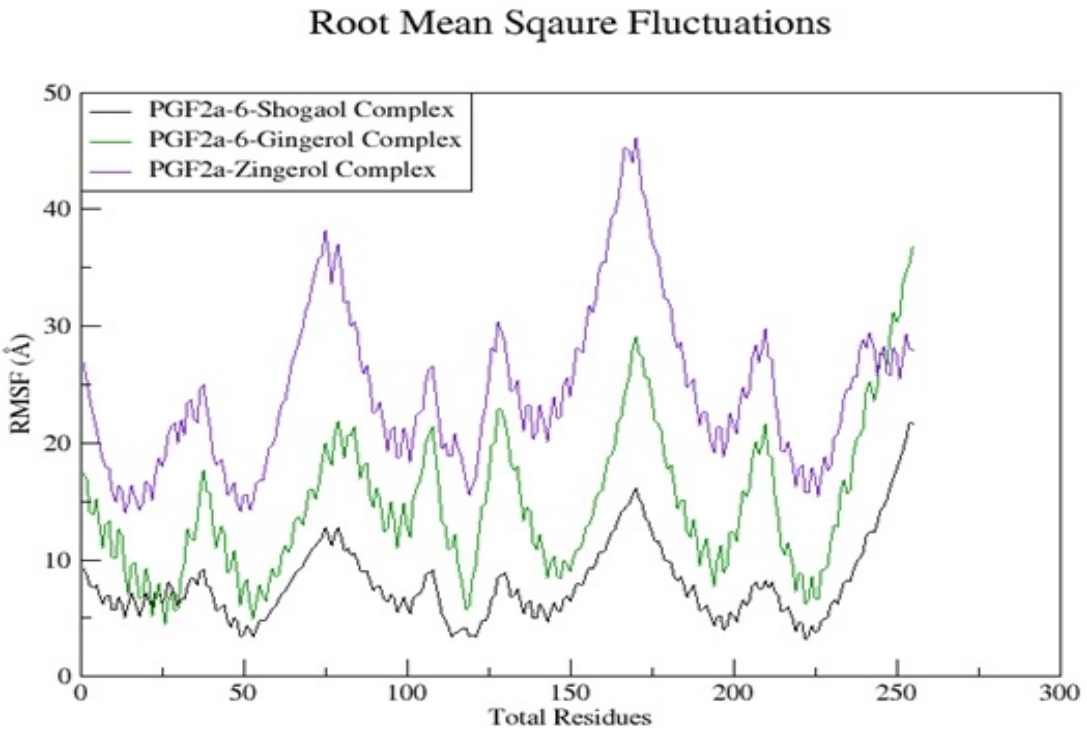


FIGURE 4.13: The average RMSD values of 6-Shogaol, 6-Gingerol and zingerol with protein PGF2a Protein

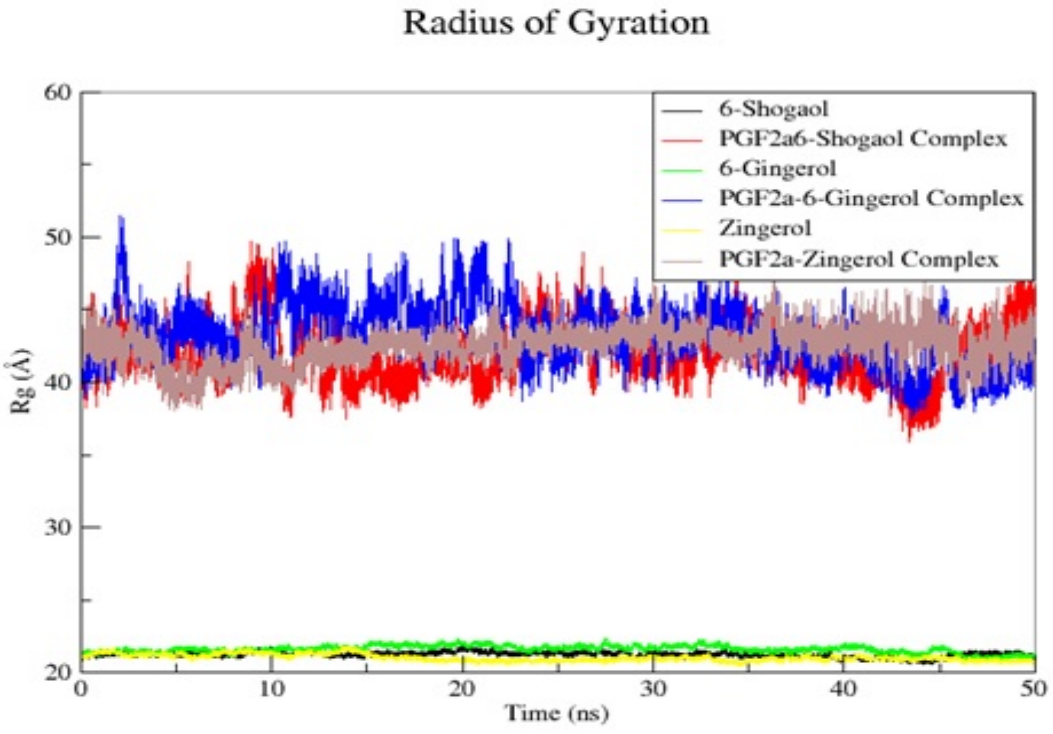


FIGURE 4.14: LThe average radius of Gyration of 6-Shogaol, 6-Gingerol and zingerol with protein PGF2a Protein

Chapter 5

Conclusion and Recommendation

5.1 Conclusion

This study aimed at identifying the best herbal Treatment for Dysmenorrhea, among the prominent Ginger Metabolites. This will lead to getting a better and efficient drug in near future that can be better alternative to NSAIDs. Data mining was done, through literature databases, to get some of the most suitable ligands which can have potential to be the best treatment of Dysmenorrhea. Through an in depth study of COX pathway of the Dysmenorrhea, the target protein most suited was found to be "PGF2α Receptor Protein" located on the cells of uterine wall. Ginger Metabolites were studied in detail through literature review as well. A great deal of Scientific evidences supported the role of Ginger and its metabolites in pain, inflammation and many other pathological, physiological or anatomical imbalances or disorders. Some of the most literature supported, biochemical of ginger were considered as potential ligands to bind with PGF2α Receptor Protein, so that they can play role in interrupting the COX pathway hence inhibiting Prostaglandin production ultimately reducing menstrual pain or Primary Dysmenorrhea.

A detailed analysis of both potential ligands and potential target protein was done.

Target protein was purified through PyMol and its active sites were found by CASTp; as well as the ligands were obtained from the PubChem, after analyzing their physicochemical properties, ADMET properties, then the step of energy

minimization was done with Chem3D. Molecular Docking was done for refined ligand and target protein using CB Dock automated version of Auto Dock vina. Further these docked complexes interactions were visualized and analyzed using LigPlot. After docking, the detailed analysis of binding score, physiochemical properties and ADMET properties, the best scoring ligands were identified as hitcompounds. Physicochemical and pharmacokinetics properties determined the final destiny of compounds as drug or non-drug.

From the above mentioned physiochemical and ADMET values it is concluded that the 6-Shogoal showed best binding ability with PGF2α Receptor Protein and its activity is also better in comparison to NSAIDs. All the software and tools used in research are very reliable and authentic. The finding suggests that 6-Shogoal is a bioactive molecule found in the Ginger could be a promising choice for treating Primary Dysmenorrhea. The exact mechanisms of action involves its inhibitory role which binds to the PGF2α Receptor Protein. Ginger is one of the most commonly and widely used spices.

Ginger root is used for attenuating and treating several common diseases, such as headaches, colds, nausea, and emesis. There is great potential possessed by Ginger to prevent and manage several diseases, such as neurodegenerative diseases, cardiovascular diseases, obesity, diabetes mellitus, chemotherapy-induced nausea and emesis, and respiratory disorders. There is readily availability of ginger because of its low cost so it can be widely used in the treatment of any inflammatory condition such as premenstrual syndrome. The usefulness of Ginger increases as there is an increased tendency of using traditional medicine and herbal medicine among people, as they have desire to avoid chemical drugs, which have more side effects. Mostly the previous studies on *Zingiber officinale* Roscoe proved it to be widely used as medicinal plant. Ginger tea and Ginger Candies are orally administered to treat for anti-inflammation, as an antioxidant.

The ADME Analysis, LIPINSKI RO5 have proved the potential of ginger metabolites to be a good ligand for the Receptor Protein of COX2 Pathway of Dysmenorrhea. Adding glory to it the results of Molecular Docking and Molecular Dynamics Simulations (MD Simulations) have specified the best ligand (6-Shogoal) among

potential ligands to be an alternative to NSAIDs with a better efficiency with less side effects.

5.2 Recommendation

This study will be effective for considering Primary Dysmenorrhea and also paves way towards an idea to specifically consider, whether these potential and lead compounds of Ginger could be a considerable option to treat the Secondary Dysmenorrhea or not.

Bibliography

[1] E. Vlachou, D. A. Owens, M. Lavdaniti, J. Kalemikerakis, E. Evagelou, N. Margari, G. Fasoi, E. Evangelidou, O. Govina, and A. N. Tsartsalis, "Prevalence, wellbeing, and symptoms of dysmenorrhea among university nursing students in greece," *Diseases*, vol. 7, no. 1, p. 5, 2019.

[2] D. Alateeq, L. Binsuwaidan, L. Alazwari, M. Algarni, M. Al Hussain, R. Alzahrani, and R. Aljohani, "Dysmenorrhea and depressive symptoms among female university students: a descriptive study from saudi arabia," *The Egyptian Journal of Neurology, Psychiatry and Neurosurgery*, vol. 58, no. 1, p. 106, 2022.

[3] E. Deligeoroglou, "Dysmenorrhea," *Annals of the New York Academy of Sciences*, vol. 900, no. 1, pp. 237–244, 2000.

[4] H. Nagy and M. A. Khan, "Dysmenorrhea," in *StatPearls [Internet]*, StatPearls Publishing, 2022.

[5] A. Abreu-Sánchez, M. L. Parra-Fernández, M. D. Onieva-Zafra, J. D. Ramos-Pichardo, and E. Fernández-Martínez, "Type of dysmenorrhea, menstrual characteristics and symptoms in nursing students in southern spain," in *Healthcare*, vol. 8, p. 302, MDPI, 2020.

[6] N. K. Ibrahim, M. S. AlGhamdi, A. N. Al-Shaibani, F. A. AlAmri, H. A. Alharbi, A. K. Al-Jadani, and R. A. Alfaidi, "Dysmenorrhea among female medical students in king abdulaziz university: prevalence, predictors and outcome," *Pakistan journal of medical sciences*, vol. 31, no. 6, p. 1312, 2015.

[7] N. Najafi, H. Khalkhali, F. Moghaddam Tabrizi, and R. Zarrin, "Major dietary patterns in relation to menstrual pain: a nested case control study," *BMC women's health*, vol. 18, pp. 1–7, 2018.

[8] M. Armour, K. Parry, N. Manohar, K. Holmes, T. Ferfolja, C. Curry, F. MacMillan, and C. A. Smith, "The prevalence and academic impact of dysmenorrhea in 21,573 young women: a systematic review and meta-analysis," *Journal of women's health*, vol. 28, no. 8, pp. 1161–1171, 2019.

[9] K. J. Berkley, "Primary dysmenorrhea: an urgent mandate," *Pain*, vol. 1, no. 1, p. 8, 2013.

[10] A. Ullah, K. Fayyaz, U. Javed, M. Usman, R. Malik, N. Arif, and A. Kaleem, "Prevalence of dysmenorrhea and determinants of pain intensity among university-age women," *Pain Medicine*, vol. 22, no. 12, pp. 2851–2862, 2021.

[11] A. Ullah, K. Fayyaz, U. Javed, M. Usman, R. Malik, N. Arif, and A. Kaleem, "Prevalence of dysmenorrhea and determinants of pain intensity among university-age women," *Pain Medicine*, vol. 22, no. 12, pp. 2851–2862, 2021.

[12] H. Ju, M. Jones, and G. Mishra, "The prevalence and risk factors of dysmenorrhea," *Epidemiologic reviews*, vol. 36, no. 1, pp. 104–113, 2014.

[13] G. Eryilmaz, F. Ozdemir, and T. Pasinlioglu, "Dysmenorrhea prevalence among adolescents in eastern turkey: its effects on school performance and relationships with family and friends," *Journal of pediatric and adolescent gynecology*, vol. 23, no. 5, pp. 267–272, 2010.

[14] Z. Barcikowska, E. Rajkowska-Labon, M. E. Grzybowska, R. Hansdorfer-Korzon, and K. Zorena, "Inflammatory markers in dysmenorrhea and therapeutic options," *International journal of environmental research and public health*, vol. 17, no. 4, p. 1191, 2020.

[15] S. A. Ryan, "The treatment of dysmenorrhea," *Pediatric Clinics*, vol. 64, no. 2, pp. 331–342, 2017.

[16] C. Bulletti, D. de Ziegler, V. Polli, L. Diotallevi, E. D. Ferro, and C. Flamigni, "Uterine contractility during the menstrual cycle," *Human reproduction*, vol. 15, no. suppl_1, pp. 81–89, 2000.

[17] S. Iacovides, I. Avidon, and F. C. Baker, "What we know about primary dysmenorrhea today: a critical review," *Human reproduction update*, vol. 21, no. 6, pp. 762–778, 2015.

[18] J. B. Schroll, A. Y. Black, C. Farquhar, and I. Chen, "Combined oral contraceptive pill for primary dysmenorrhoea," *Cochrane Database of Systematic Reviews*, no. 7, 2023.

[19] R. P. Smith and A. M. Kaunitz, "Dysmenorrhea in adult females: Treatment," 2022.

[20] C. X. Chen, B. Barrett, K. L. Kwekkeboom, *et al.*, "Efficacy of oral ginger (zingiber officinale) for dysmenorrhea: a systematic review and meta-analysis," *Evidence-Based Complementary and Alternative Medicine*, vol. 2016, 2016.

[21] L. Kashani, M. Mohammadi, M. Heidari, and S. Akhondzadeh, "Herbal medicine in the treatment of primary dysmenorrhea," 2015.

[22] A. Niazi and M. Moradi, "The effect of chamomile on pain and menstrual bleeding in primary dysmenorrhea: A systematic review," *International journal of community based nursing and midwifery*, vol. 9, no. 3, p. 174, 2021.

[23] F. Ebrahimi Varzaneh, F. Nahidi, F. Mojab, M. A. Pourhoseingholi, and Z. Panahi, "The effect of hydro alcoholic extract of achilleamillefolium capsule on duration and severity of primary dysmenorrhea pain," *The Iranian Journal of Obstetrics, Gynecology and Infertility*, vol. 20, no. 3, pp. 48–86, 2017.

[24] V. Menon, M. Elgharib, R. El-awady, and E. Saleh, "Ginger: From serving table to salient therapy," *Food Bioscience*, vol. 41, p. 100934, 2021.

[25] M. Rondanelli, F. Fossari, V. Vecchio, C. Gasparri, G. Peroni, D. Spadaccini, A. Riva, G. Petrangolini, G. Iannello, M. Nichetti, *et al.*, "Clinical trials on pain lowering effect of ginger: A narrative review," *Phytotherapy Research*, vol. 34, no. 11, pp. 2843–2856, 2020.

[26] M. Rudrapal, W. A. Eltayeb, G. Rakshit, A. A. El-Arabey, J. Khan, S. M. Aldosari, B. Alshehri, and M. Abdalla, "Dual synergistic inhibition of cox

and lox by potential chemicals from indian daily spices investigated through detailed computational studies," *Scientific Reports*, vol. 13, no. 1, p. 8656, 2023.

[27] R. B. van Breemen, Y. Tao, and W. Li, "Cyclooxygenase-2 inhibitors in ginger (zingiber officinale)," *Fitoterapia*, vol. 82, no. 1, pp. 38–43, 2011.

[28] J. J. Hox and H. R. Boeije, "Data collection, primary versus secondary," 2005.

[29] L.-P. Li, Y.-B. Wang, Z.-H. You, Y. Li, and J.-Y. An, "Pclpred: a bioinformatics method for predicting protein–protein interactions by combining relevance vector machine model with low-rank matrix approximation," *International journal of molecular sciences*, vol. 19, no. 4, p. 1029, 2018.

[30] M. B. Gebeyehu, A. B. Mekuria, Y. G. Tefera, D. A. Andarge, Y. B. Debay, G. S. Bejiga, B. M. Gebresillassie, *et al.*, "Prevalence, impact, and management practice of dysmenorrhea among university of gondar students, northwestern ethiopia: a cross-sectional study," *International journal of reproductive medicine*, vol. 2017, 2017.

[31] R. Mizuta, N. Maeda, T. Tashiro, Y. Suzuki, S. Oda, M. Komiya, and Y. Urabe, "Quality of life by dysmenorrhea severity in young and adult japanese females: A web-based cross-sectional study," *Plos one*, vol. 18, no. 3, p. e0283130, 2023.

[32] A. Molla, B. Duko, B. Girma, D. Madoro, J. Nigussie, Z. Belayneh, N. Mengistu, and B. Mekuriaw, "Prevalence of dysmenorrhea and associated factors among students in ethiopia: A systematic review and meta-analysis," *Women's Health*, vol. 18, p. 17455057221079443, 2022.

[33] S. M. Wikander, *PREVALENCE, IMPACT AND MANAGEMENT OF DYSMENORRHEA AMONG THE FEMALE STUDENTS AT THE UNIVERSITY OF SPLIT SCHOOL OF MEDICINE*. PhD thesis, University of Split. School of Medicine. Hystology and embryology, 2020.

[34] D. A. Bavil, M. Dolatian, Z. Mahmoodi, and A. A. Baghban, "Comparison of lifestyles of young women with and without primary dysmenorrhea," *Electronic physician*, vol. 8, no. 3, p. 2107, 2016.

[35] E. M. Cherenack, J. Rubli, A. Melara, N. Ezaldein, A. King, M. L. Alcaide, P. Raccamarich, L. A. Fein, and K. J. Sikkema, "Adolescent girls' descriptions of dysmenorrhea and barriers to dysmenorrhea management in moshi, tanzania: A qualitative study," *PLOS global public health*, vol. 3, no. 7, p. e0001544, 2023.

[36] S. F. Rahman, G. Wisnu Hardi, M. Artha Jabatsudewa Maras, and Y. Rachmia Riva, "Influence of curcumin and ginger in primary dysmenorrhea: a review," *International Journal of Applied Engineering Research*, vol. 15, no. 7, pp. 634–638, 2020.

[37] K. J. Sales and H. N. Jabbour, "Cyclooxygenase enzymes and prostaglandins in pathology of the endometrium," *Reproduction (Cambridge, England)*, vol. 126, no. 5, p. 559, 2003.

[38] Y.-H. Hwang, T. Kim, R. Kim, and H. Ha, "The natural product 6-gingerol inhibits inflammation-associated osteoclast differentiation via reduction of prostaglandin e2 levels," *International Journal of Molecular Sciences*, vol. 19, no. 7, p. 2068, 2018.

[39] F. A. Oladosu, F. F. Tu, and K. M. Hellman, "Nonsteroidal antiinflammatory drug resistance in dysmenorrhea: epidemiology, causes, and treatment," *American journal of obstetrics and gynecology*, vol. 218, no. 4, pp. 390–400, 2018.

[40] S. Wang, C. Zhang, G. Yang, and Y. Yang, "Biological properties of 6-gingerol: a brief review," *Natural product communications*, vol. 9, no. 7, p. 1934578X1400900736, 2014.

[41] P. Ballester, B. Cerdá, R. Arcusa, J. Marhuenda, K. Yamedjeu, and P. Zafrilla, "Effect of ginger on inflammatory diseases," *Molecules*, vol. 27, no. 21, p. 7223, 2022.

[42] R. Arcusa, D. Villaño, J. Marhuenda, M. Cano, B. Cerdà, and P. Zafrilla, "Potential role of ginger (zingiber officinale roscoe) in the prevention of neurodegenerative diseases," *Frontiers in nutrition*, vol. 9, p. 809621, 2022.

[43] M. K. Szmidt, D. Granda, E. Sicinska, and J. Kaluza, "Primary dysmenorrhea in relation to oxidative stress and antioxidant status: a systematic review of case-control studies," *Antioxidants*, vol. 9, no. 10, p. 994, 2020.

[44] T. A. Jiang, "Health benefits of culinary herbs and spices," *Journal of AOAC International*, vol. 102, no. 2, pp. 395–411, 2019.

[45] S. Bindu, S. Mazumder, and U. Bandyopadhyay, "Non-steroidal anti-inflammatory drugs (nsaids) and organ damage: A current perspective," *Biochemical pharmacology*, vol. 180, p. 114147, 2020.

[46] R. K. Vishwakarma, A. Negi, and D. S. Negi, "Development of potential cox inhibitor as anti-inflammatory agents from leaves of lantana camara by in-vitro analysis, molecular docking and admet prediction," *Journal of the Indian Chemical Society*, vol. 99, no. 10, p. 100694, 2022.

[47] W. Tian, C. Chen, X. Lei, J. Zhao, and J. Liang, "Castp 3.0: computed atlas of surface topography of proteins," *Nucleic acids research*, vol. 46, no. W1, pp. W363–W367, 2018.

[48] J. Yang and Y. Zhang, "I-tasser server: new development for protein structure and function predictions," *Nucleic acids research*, vol. 43, no. W1, pp. W174–W181, 2015.

[49] F. Huang, C. J. Oldfield, B. Xue, W.-L. Hsu, J. Meng, X. Liu, L. Shen, P. Romero, V. N. Uversky, and A. K. Dunker, "Improving protein order-disorder classification using charge-hydropathy plots," *BMC bioinformatics*, vol. 15, no. 17, pp. 1–13, 2014.

[50] S. Basu, "Novel cyclooxygenase-catalyzed bioactive prostaglandin f2α from physiology to new principles in inflammation," *Medicinal research reviews*, vol. 27, no. 4, pp. 435–468, 2007.

[51] Y. Li and W. A. GODDARD III, "Prediction of structure of g-protein coupled receptors and of bound ligands, with applications for drug design," in *Biocomputing 2008*, pp. 344–353, World Scientific, 2008.

[52] R. Apweiler, T. K. Attwood, A. Bairoch, A. Bateman, E. Birney, M. Biswas, P. Bucher, L. Cerutti, F. Corpet, M. D. Croning, *et al.*, "The interpro database, an integrated documentation resource for protein families, domains and functional sites," *Nucleic acids research*, vol. 29, no. 1, pp. 37–40, 2001.

[53] A. Daina, O. Michielin, and V. Zoete, "Swissadme: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules," *Scientific reports*, vol. 7, no. 1, p. 42717, 2017.

[54] D. E. Pires, T. L. Blundell, and D. B. Ascher, "pkcsm: predicting small-molecule pharmacokinetic and toxicity properties using graph-based signatures," *Journal of medicinal chemistry*, vol. 58, no. 9, pp. 4066–4072, 2015.

[55] R. A. Laskowski and M. B. Swindells, "Ligplot+: multiple ligand–protein interaction diagrams for drug discovery," 2011.

[56] X. Du, Y. Li, Y.-L. Xia, S.-M. Ai, J. Liang, P. Sang, X.-L. Ji, and S.-Q. Liu, "Insights into protein–ligand interactions: mechanisms, models, and methods," *International journal of molecular sciences*, vol. 17, no. 2, p. 144, 2016.

[57] G. Ozgoli, M. Goli, and F. Moattar, "Comparison of effects of ginger, mefenamic acid, and ibuprofen on pain in women with primary dysmenorrhea," *The journal of alternative and complementary medicine*, vol. 15, no. 2, pp. 129–132, 2009.

[58] L. L. Mazaleuskaya, K. N. Theken, L. Gong, C. F. Thorn, G. A. FitzGerald, R. B. Altman, and T. E. Klein, "Pharmgkb summary: ibuprofen pathways," *Pharmacogenetics and genomics*, vol. 25, no. 2, p. 96, 2015.