ANTI-ARTHRITIC AND ANTI-OBESITY STUDIES OF SECONDARY METABOLITES ISOLATED FROM PSORALEA CORYLIFOLIA

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Biotechnology

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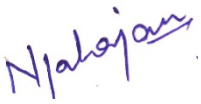
2023

DECLARATION

I, hereby declared that the presented work in the thesis entitled "**Anti-arthritic and anti-obesity studies of secondary metabolites isolated from *Psoralea corylifolia***" in fulfilment of degree of **Doctor of Philosophy (Ph.D.)** is outcome of research work carried out by me under the supervision of Dr. Joginder Singh, working as Professor, in the School of Bioengineering & Biosciences of Lovely Professional University, Punjab, India. In keeping with general practice of reporting scientific observations, due acknowledgements have been made whenever work described here has been based on findings of other investigator. This work has not been submitted in part or full to any other University or Institute for the award of any degree.



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CERTIFICATE

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Abstract

Obesity is now far more common than it was in the past and leads to many serious public health concerns worldwide. According to a WHO report, obesity prevalence is increasing not only among adults, but also among children and teenagers. It is found that obese people have higher relative risks from diabetes (type 2), gallbladder disease, hyper tension, heart disease, inflammatory bowel condition, neurological diseases, cancer and asthma than do people with a normal weight. The autoimmune disease rheumatoid arthritis (RA) is recognized by symmetrical, persistent synovial tissue inflammation that eventually results in joint degeneration, pannus development, tendon ruptures, and distinctive abnormalities. It has a 1% prevalence and is linked to severe morbidity and elevated death rate.

One of many frequently used medicinal plants is *Psoralea corylifolia* L. (family: Fabaceae), whose extracts or compounds have been shown to have oestrogenic, anticarcinogenic, antioxidant, antibacterial, counteracts depression, inflammation reduction, weight loss as well as osteoblastic effects, among others. Some major compounds of this species have received less research for their antihyperlipidemic, anti-inflammatory and anti-arthritic activity.

The main endeavor of the present experimentation work is to analyze the anti-obesity potential for dichloromethane seed extract of *P. corylifolia* or the major secondary metabolites present in the same extract by anti-adipogenesis (using 3T3L1 cell line), pancreatic lipase inhibitory and in silico study as well as to assess the inflammation or arthritis reduction effect of compound/s of *P. corylifolia* using the LPS mediated RAW 264.7 cell lines *in vitro* and carrageenan mediated paw edema and CFA-induced arthritis model *in vivo*. Three compounds bakuchiol, isopsoralen and psoralen were extracted from the DCME of *P. corylifolia* seeds. RAW 264.7 cell lines were utilized for the assessment of the effect of isolated compounds on the NO generation, reactive oxygen species and cytokines such as TNF -α. MTT assay was also conducted for the cell lines to know about the cell viability in presence of compounds.

Both the DCME and the compounds extracted from the DCME exhibited anti-lipase and anti-adipogenesis activities.

A dose dependent reduction in pancreatic lipase activity was observed. The highest anti-lipase activity was found in DCME (26.02 ±.041%) at 100 μg/ml conc., whereas bakuchiol out performed other isolates in terms of activity (24.2± .037%) at the same concentration. By conducting MTT assay, it was found that at 100 µg/ml of DCME the percentage cell viability of 3T3L1 was 97.02% whereas at 25 µM concentration of PC1, PC2 and PC3 the percentage cell viability was 93.97%, 91.90% and 94.11% respectively. These doses of DCME (100 μg/ml) as well as of isolated compounds (25 µM) were used for further studies, as at higher doses, a reduction in cell viability was observed.

At 100 µg/ml, DCME was found to have anti-adipogenesis properties, resulting in 75± 0.003% lipid accumulation than the control. Moreover, in 3T3-L1 preadipocytes, bakuchiol, isopsoralen, and psoralen also prevented lipid buildup. When compared with control at 25 μM dose, the mean values of lipid accumulation shown by PC1, PC2 and PC3 were 78.06 ± 0.002%, 80.91 ± 0.004%, and 80.91 ± 0.001%, respectively. During the insilico studies using SWISSADME tool, the three isolated compounds were predicted to have biocompatible physiochemical properties and are appropriate drug-like compounds. All the three ligands were found to be nontoxic and to have good absorption and solubility properties. Moreover, bakuchiol, isopsoralenand psoralen, were predicted to have higher negative binding energy: -7.8 kcal/mol, -7.6 kcal/mol.and -7.7 kcal/mol respectively, with PPAR-γ regulator.

Moreover, anti-inflammatory and anti-arthritic activity was also displayed by the compound/s of *P. corylifolia*. MTT assay revealed no significant reduction in cell viability of RAW cell line after treatment with the isolates PC1, PC2, PC3 at concentrations of 5µM, 2.5 µM and 1.25 µM. Nitric oxide (NO), reactive oxygen species and TNF- α levels which got elevated due to LPS stimulation were significantly reduced on treatment with bakuchiol, isopsoralen and psoralen. At 5µM concentration bakuchiol,

isopsoralen and psoralen reduced NO level to 21.23%, 20.19% and 21.30 % respectively. Corrected total cell fluorescent (CTCF) of LPS treated RAW cells was 14.32 (a.u), while that of PC1 (bakuchiol), PC2 (isopsoralen) and PC3 (psoralen) treated RAW cells was 3.49, 3.34 and 3.41 (a.u) respectively at 5μM concentration. The release of TNF-α was reduced by up to 55.33% ±6.28, 39.74% ±7.1and 43.76%±1.5 respectively by bakuchiol, isopsoralen and psoralen at 5 μM treatment as compared to LPS stimulated control cells. Studies on carrageenan-mediated paw swelling and CFA - mediated model for arthritis verified that Bakuchiol possesses potentially useful anti-inflammatory and anti-arthritic activity. BAK at dose of 4 mg/kg exhibited a remarkable decrease in edema with percentage inhibition of 35.65% when compared to control animals. BAK studies on adrenalectomized (ADX) rats using carrageenan mediated paw edema model suggests that it can efficiently suppress carrageenan mediated inflammatory edema and this inflammatory action does not require adrenal activity. Bakuchiol was found to be effective in decreasing joint inflammation in CFA-mediated arthritis animal model and the percentage inhibition was found out to be 34.30% as compared to control. Additionally, bakuchiol significantly decreased TNF-α, IL-1β and PGE_2 levels in arthritic model

Observations made in the present work provide the scientific substantiation to support and rationalize the conventional use of *Psoralea corylifolia* and its isolates particularly bakuchiol, isopsoralen and psoralen for the management of obesity and inflammatory disorders like rheumatoid arthritis. So, based on our research on PCSE, we assert that major constituents of *P. corylifolia* possesses anti-obesity and anti-arthritic potential.

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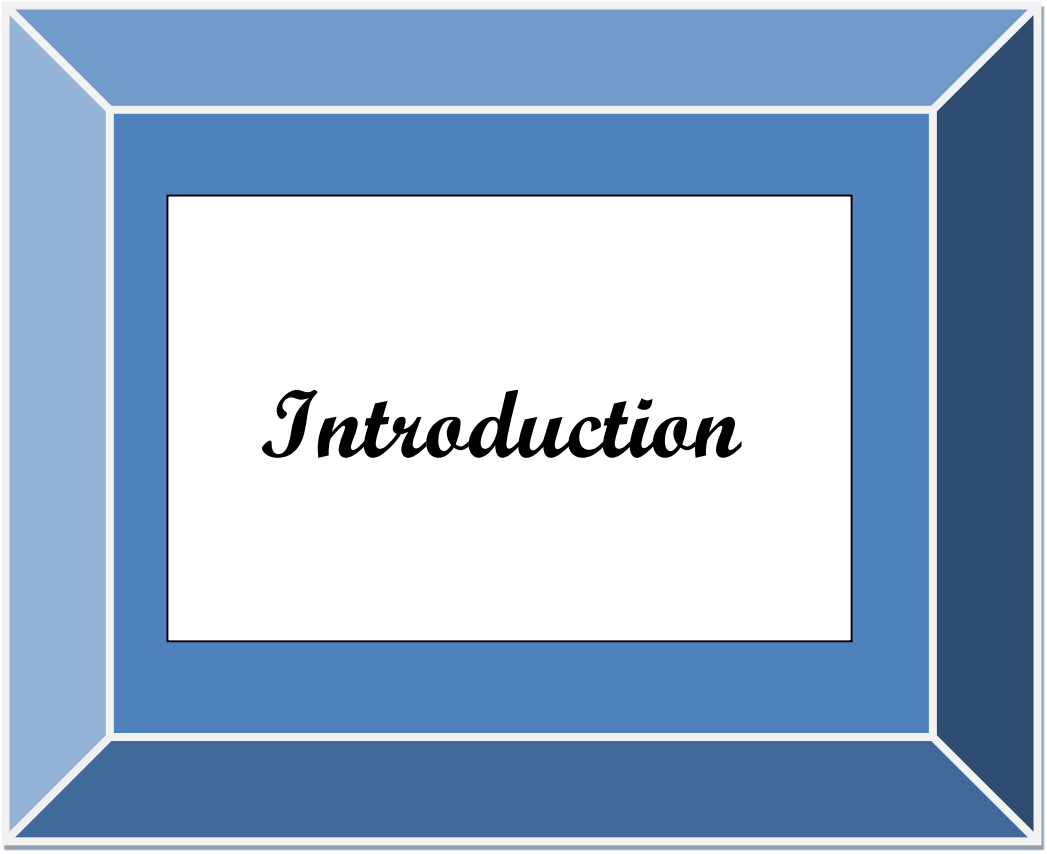
Chapter 1



1.0 Introduction:

Global security is becoming more and more dependent on global health. The appearance or reemergence of contagious diseases or other health issues somewhere around the world is frequently reported in the news each week. We are aware that Mother Nature has created incredibly complex compounds that are beyond the imagination of any synthetic chemist. Since the beginning of civilization, traditional medicines with botanical origins have been utilized to promote health and lessen disease related suffering in people. For thousands of years, medicinal plants have served as a source of healing in local communities around the globe. In the past, only unrefined or semi-refined extracts of herbs, animals, microbes and minerals were used to treat illnesses in people and domesticated animals. Later on, researchers came to the conclusion that specific chemical compounds found in extracts are responsible for the biological activity of herbal medicine. This resulted in the traditional therapies for diseases switching from extracts to pure, isolated molecules, resulting in an entirely new era in pharmacology (Lahlou, 2013). Traditional Indian Medicine, often known as ayurveda, is the world's oldest healthcare system. Ayurveda, which refers to "science of life," is made up of the words Ayur (the word for "life") and Veda (the word for "knowledge" or "science"). Body humours (dosas), as well as the internal life energy (prana), which is thought to support digestion and mental function, are the foundations of Ayurveda (Van and Wink, 2018). The Rigveda, a storehouse of wisdom and information that discusses the importance of 67 medicinal plants, contains the first reference to medicine. 81 medicinal plants are mentioned in the Yajurveda. 289 medicinal plants are mentioned in Atharvaveda. Chapters based on the therapeutic utilization of about 500 herbal plants can be found in Sushruta Samhita and Charaka Samhita (Ministry of AYUSH). The plant is said to have medicinal benefits that are described in Greek, Roman, and Unani approaches to medicine in addition to Ayurveda and Siddha. Because of the unrivalled profusion of chemical diversity, natural products - whether pure molecules or standardized extracts present a limitless possibility for new therapeutic discoveries. Traditional medicines continue to be widely regarded as a valuable therapeutic option to contemporary medications in many regions of the world (Tao et al., 2014). Despite magnificent improvements in synthetic pharmaceuticals, several plant-based medications have still retained their usefulness (Chatterjee, 1987). According to estimates,

traditional medicine serves 80% of the global population's healthcare demands (Akerele, 1993). There is an international "herbal renaissance" as botanicals are making a comeback. 21,000 plants are listed by the WHO as having a significant medical value worldwide. In India, more than 2,500 species have been identified, and pharmaceutical companies use 150 of them extensively and commercially as mainstream medicine (Yuan et al., 2016). The active chemicals found in a plant's diverse parts (leaves, roots, bark, fruit, or seeds) can vary greatly, making it possible for one part of the plant to be lethal while another is completely innocuous. In general, the effective dose of herbal medications differs significantly from the toxic dose (Van and Wink, 2018).

New and emerging diseases are posing a risk every day to people worldwide in the modern era. Pollution, poor lifestyles, and environmental toxins all raise the risk of disease in 21st century. Allopathic medication side effects and overuse/misuse are also important concerns. As evidenced by many research studies, in most illnesses, synthetic medications are recognised to relieve symptoms only. Herbal drugs, on the other hand, improve the body's internal healing mechanisms. Herbal remedies are mild in action, attempts to restore damaged body systems and processes in order to eliminate the abnormality in the system (Srivastava et al., 2019). As a result, traditional medical systems that are affordable, accessible, and compatible with the human body are in demand. As claimed by many more than 60% of the anti-cancer medications available and used today are derived from natural ingredients. Currently, around eighty per cent of cardiovascular, antibiotic, and anticancer medications are derivatives of plant products. Morphine, introduced by Merck in 1827, was the first natural plant alkaloid extracted for medicinal use. In 1899, Bayer developed Aspirin, the very first semi-synthetic pure medication based on a natural material called Salicin, which was obtained from the *Salix alba* plant (Rungsung et al., 2015). Vinblastin and vincristine, both derived from the important ethnomedicinal plant *Catharanthus roseus*, are two exceptional pharmaceuticals that are employed in the treatment of acute lymphoma and leukaemia. Among the 177 approved anti-cancer drugs, more than 70% of the entities are based on natural products (Sen and Chakraborty, 2017). Over 400 conventional plants or items derived from plants have been utilized to treat type 2 diabetes worldwide. An interesting illustration of such a discovery is the chemical galegrin,

which is obtained from herb *Galega officinalis*. Galegine investigations, both *in vitro* and *in vivo*, gave the pharmacological and molecular foundations in the discovery of metformin, the mainstay treatment for type 2 diabetes (Akinyemi et al., 2018). Natural medicines have so far been the preferred medications and have played a significant role in augmenting human health. A variety of secondary metabolites derived from plants can be used as molecular models or templates for the development, synthesis, and semi-synthesis of new novel medications in addition to those plant secondary metabolites that have found direct medical application as pharmacological entities.

Modern drug development has a tremendous option to utilize active compounds and their potential molecular targets from traditional herbal therapy. Finding strong and feasible lead candidates for drugs is turning out to be a difficult scientific job that demands expertise and experience. It all starts with screening natural products for potential drugs. For discovering new herbal medications, it is necessary to investigate the various bioactive fractions as well as perform phytoanalysis and phytopharmacological evaluation. However, novel pharmaceutical compounds have been created from natural ingredients, and they will continue to do so. (Koparde et al., 2019). Traditional Knowledge Digital Library (TKDL) is an exceptional proprietary database that is fully protected by national and international intellectual property laws and is managed by the government. The TKDL contains information such as medicinal plant names, disease descriptions under current names, and therapeutic formulations (Heinrich et al., 2017).

Man has always had an obligatory relationship with plants and has been dependent on them since prehistoric times for food, shelter, cure for diseases and oxygen to breathe. Life wouldn’t have been possible without plants. All forms of life on earth are supported by them directly or indirectly. There's even a long-held perception that every plant that exists on Mother Earth possesses some sort of physiological or therapeutic quality.

Humans have used plants as remedies at least since the Middle Paleolithic period, according to fossil evidence. Evidence of this early connection was discovered in a Neanderthal man's burial that was excavated 60 thousand years ago. Early humans learned about using plants

for medicinal purposes through years of careful observation, personal experience, and trial-and-error attempts (Karunamoorth et al., 2013). India and China are the two largest users of medicinal plants, and their ancient systems of medicine and literature in different cultures unveil the use of herbal preparation for human benefit. Indian and Chinese medicinal systems, as well as the ones used by African tribes, are rich stores of traditional knowledge. Indian Ayurvedic system uses some 7000 plant species, and traditional Chinese medicine uses over 5000. Worldwide, 5.1 billion individuals use natural plant-based medicines for both immediate and long-term health concerns, from treating the common cold to lowering cholesterol and blood pressure. About 70% of the population of India, Pakistan, China and Bangladesh depend on their herbal formulations based on their indigenous system of medicine, and with the help of modern scientific methods, they will be the basis of the development of new therapeutic agents in future. WHO estimates that almost 80% of people worldwide utilize plant medicines, either wholly or in part (Farnsworth et al., 1985). Many people do this out of necessity because they cannot pay the hefty prices of pharmaceutical medications. For a variety of reasons, including affordability and the desire for natural alternatives with fewer adverse effects, more and more Americans are turning to plant-based medications for their medical needs.

Over the last few decades, there has been a constant decline in drug development from natural products. After a brief setback of a few decades, the last 4-5 years have seen a revival in interest and appreciation of herbal drugs. Compared to synthetic pharmaceuticals, herbal remedies are far safer, kinder, and more beneficial for human health. This is true because, during the past several million years, humans and plants have co-evolved, as evidenced by studies comparing sea squirt *Ciona intestinalis* genome with those of humans (Dehal et al., 2002). We consume plants in a variety of ways, including eating them, drinking their juices, fermenting and distilling them into alcoholic beverages. Plant ingredients, ranging from carbohydrates, lipids and proteins to minerals and vitamins, contribute to our body's makeup and chemistry. Some substances serve the same purposes in plants and the human body. For instance, naturally occurring antioxidant phenolic compounds in plants shield plant cells against oxidation and frequently serve a similar purpose in the human body. Our bodies have sophisticated processes for metabolizing plant components, and they are able to detect the chemicals that are present in

plants. Synthetic medications, however, cannot be considered to be the same. These substances frequently are foreign to the human body's chemistry. Synthetic medications frequently function inside the body as irritants and toxins, disturbing the homeostasis of various systems and leading to potentially fatal side effects. The terrible effects of the synthetic drug surge have led to drugs becoming the sixth leading cause of mortality for American adults today. This is more than the sum of deaths from traffic accidents, pistols, and narcotics. The numbers are much worse when we include in the record number of child and adult deaths linked to over-the-counter and prescription medications administered outside of hospitals. On the other hand, a wise approach to one's own health is to regularly and wisely employ herbs as remedies to assist in the treatment of common maladies as well as to safeguard and promote health. The health of people can be affected by plants. Drinking oleander leaf tea or taking a bite of foxglove can both result in instant death. On the other hand, using any of the thousands of healthy herbs that have been used as folk remedies over the past several millennia, in dosage levels that have been established through millennia of trial and error, leads to therapeutic results without adverse effects (Lacassie et al., 2000).

1.1 Important Drugs from Plants:

Plants have traditionally been utilized as an essential source of medicine. The very first isolated drugs include morphine **1**, strychnine **2**, atropine **3**, and colchicines **4**. The first isolated natural substance that was commercially pure and utilized as an analgesic was morphine. It is still used to treat several types of pain, including cancer pain, post-surgery pain, pain from kidney stones, and pain from myocardial infractions (Murphy et al., 2022). One of the oldest medicinal herbs still used today is Saffron (*Colchicum autumnale*), which contains the important alkaloid colchicine as its main component. Its usage for treating acute gout and joint discomfort was first documented in 100 AD. Since ancient times, numerous substances based on alkaloids have been used to treat a wide range of diseases. Ephedrine **5**, an alkaloid with an adrenaline-like function, has long been utilized in the Chinese traditional medical system to treat bronchitis and asthma (Ford et al., 2001). Additionally, it is utilized when a patient has an overdose of ganglionic blockers, antiadrenergic drugs, or other blood pressure-lowering treatments (Bicopoulos, 2002). Piperine **6** is used as a flavoring agent in brandy and also

functions as a bio-enhancer (McNamara, 2005). Pomegranate alkaloids are found in the root bark of the pomegranate tree. Pelletierine (**7**), isopelletierine (**8**), methylisopelletierine (**9**) and pseudopelletierine are the main four alkaloids present in pomegranates. These alkaloids are what give pomegranates their distinctive vermicide activity (Lansky and Newman, 2007).

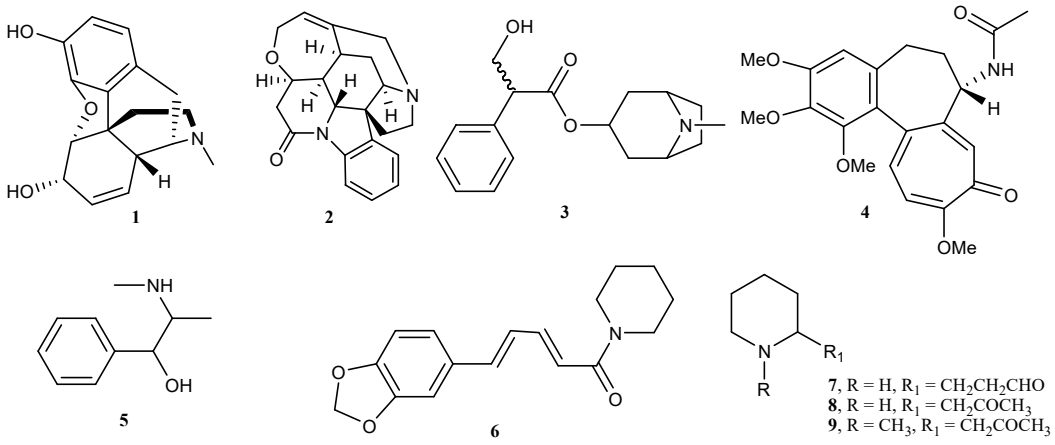


Figure 1.1 Structure of different drugs from plants

Drugs made from sources of nature have been utilized as analgesics, anti-infectives/antibiotics, inflammation reduction, anti-cancer, and more. Penicillin G **10**, the first breakthrough in antibiotic medicine, suddenly made it feasible to treat illnesses that were previously thought to be fatal. Later, other varieties of synthetic penicillin, including procaine penicillin, benzathin penicillin G, penicillin V were also produced (Lalchhandama, 2021). The discovery of streptomycin **11**, the antibiotic of practical importance, isolated from *Actinomycetacea*, marked another advancement in the medical field. A broad-spectrum antibiotic called chloromycetin **12** was discovered in a *Streptomyces* strain. Later erythromycin A **13** was discovered (Miller, 2000).

In the development of anti-malarial drugs, plants have played an exceptional role. For three centuries, an extract of Cinchona bark (quinine) was indeed the standard malaria treatment. Quinine **14** was the first anti-malarial drug, which paved the way for the development of other drugs like chloroquine **15**. The Chinese have used artemisinin to treat malaria for over 2000

years. Artemisinin, in particular, shows promise for treating malaria cases that have developed resistance to chloroquine treatment (Tse et al., 2019).

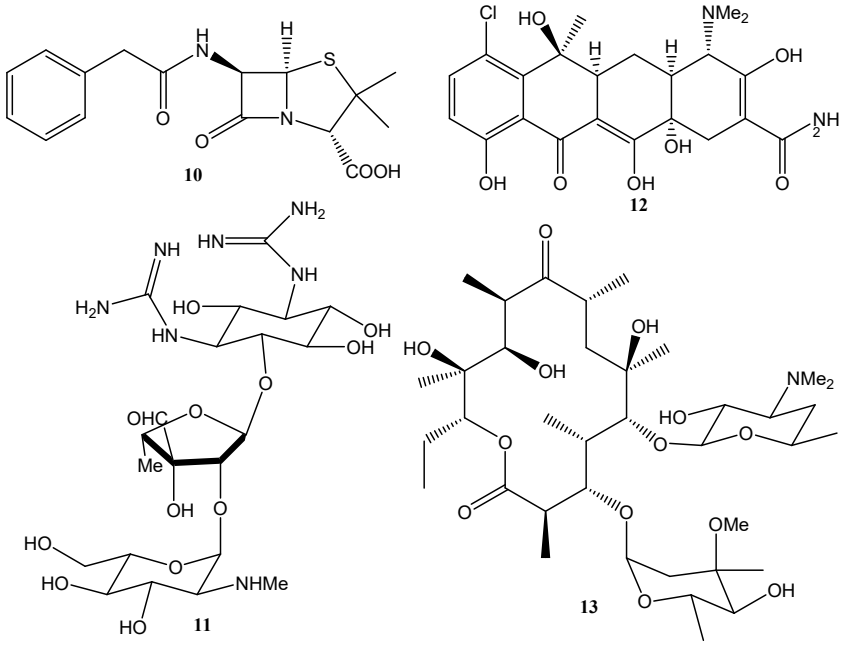


Figure 1.2 Structure of different anti-infective drugs

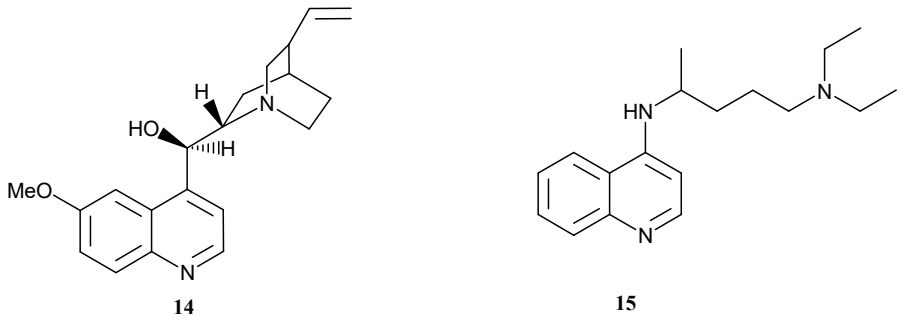


Figure 1.3 Structure of anti-malarial drugs

1.2 Development of Herbal Drugs and its challenges: Different countries have different definitions and classifications of herbal medicines. The same medicinal plant may well be

classified across many nations as food, a nutritional supplement, or a natural medication, depending on the laws governing foods and medicines. This confuses patients and customers while also complicating how herbal medicines are defined by national medication regulations (Ekor., 2014). The scientific validity of herbal remedies in research and development studies is still frequently questioned, despite the fact that herbal medicine practitioners are frequently confident in the application of herbal drugs and are also generally convinced by the results they observe with patients (Karbwang et al., 2019). There are a number of reasons why using herbal medicines can have negative side effects, including using the incorrect plant species, adulterating herbal products, using unregistered medications, contaminating herbal products, overdosing, misusing herbal medications by patients or healthcare professionals, and combining herbal medications with other medications (Bhardwaj et al., 2018). The active principles are frequently unknown, and quality control is a major bottleneck (Calixto., 2000). Bioavailability is an extremely important factor for any pharmacologically active molecule.

The practice of using plants traditionally is the consequence of centuries-old learning, being transmitted orally. This approach to information sharing runs the risk of distorting or losing local awareness of plant use (Karunamoorthi et al., 2009). Because of human activity and natural reasons, conventional medicinal plants are lost (Lulekal et al., 2008).

Though the market size of the Indian AYUSH sector as a whole increased by 17% between 2014 and 2020, with a current turnover of US $18.1 billion. But still, India has a large local market and extensive experience with herbal medicines but hasn't been able to capitalize on these factors by encouraging use in developed foreign markets. India needs to find products that could be useful for treating conditions that are common in the industrialized world but for which there are currently no treatments or only palliative care options available. One more problem with herbal drugs is ensuring that quality is constant and appropriate. Indian herbal therapies are not widely recognized because there are few herbal practitioners in foreign nations, especially for traditional systems of Indian medicine. Foreign companies believe that providing enough support for promoting Indian herbal medicines abroad through trade shows and exhibits will undoubtedly help improve the export potential (Sahoo and Manchikanti, 2013).

Globally, regulatory frameworks for herbal remedies need to be standardised and improved. In addition, when linked with a drug delivery system, a number of herbal bioactive compounds, including alkaloids, glycosides, terpenoids, flavonoids, etc., exhibited improved therapeutic or pharmacological action at an equal or lower dose as compared to standard herbal formulation (Rane et al., 2021).

Chapter 2

2.0 Review of literature:

2.1 Obesity:

Obesity is characterised by an abnormal rise in the proportion of adipocytes, primarily in the body's viscera and subcutaneous tissues. Obesity can be a) hyperplastic obesity, which is brought by an increase in the number of fat cells number or b) hypertrophic obesity, which involves an increase in the size of the fat cells (Moini et al., 2020). The difference between the number of calories you consume and spend is the primary factor in weight gain and obesity.

2.1.1 Obesity prevalence: Obesity prevalence has sharply increased in recent years, which has major implications for global public health. 93.3 million US adults, or 39.8% of the U.S. population, were obese in 2015–2016, and according to recent estimates, this number will rise to nearly 1 in 2 by 2030. According to a WHO report, obesity prevalence is increasing not only among adults but also among children and teenagers. Between the years 1975 and 2016, the frequency of being overweight or obese in children and teenagers aged 5 to 19 increased more than four times, from 4% to 18% globally. Previously believed to be trouble only in high-income nations, the prevalence of overweight and obesity is increasing, especially in metropolitan areas, in low and middle-income nations (WHO, 2021). By 2025, the International Obesity Task Force (IOTF) predicts that obesity prevalence will be between 45% and 50% in the United States, about 30-40% in Australia, England, and Mauritius, and about 20% in Brazil (Low et al., 2009).

BMI, which is heaviness in kilogrammes by tallness in metres squared (kg/m^2), is regularly employed as a measure of body fatness. Due to the fact that it evaluates excess weight rather than an excess of fat, it is a rough indicator of body fat (Weir and Jan, 2021). When evaluated using the standard weight status classification for individuals over the age of 20, the body mass index is the same for both men and women of all ages. In 2013, there were 36.9% of men and 38% of women worldwide had a BMI of more than 25 kg/m^2, up from 28.8% of men

and 29.8% of women in 1980. **Table 2.1** describes the weight status categories associated with BMI.

Table 2.1 Body mass index (BMI) related weight status classifications

S. No	Body Mass Index	Weight category	S. No	Body Mass Index	Weight category
1	<18.5	Underweight	4	30 -34.9	Obesity class 1
2	18.5-24.9	Healthy weight	5	35-39.9	Obesity class 2
3	25-29.9	Overweight/ preobesity	6	Above 40	Obesity class 3

2.1.2 Health risk of obese people: Obesity has become an increasing public health problem worldwide, and obese people have higher relative risks of developing diabetes (type 2), gallstone disease, hypertension, heart disease, inflammatory bowel conditions, neurological diseases, various cancer against people who are of normal weight. Obesity is a major reason for concern since it is allied to degenerative disorders such as diabetes, heart disease, hepatic disease, stroke, hypertension, high cholesterol, renal failure and osteoarthritis (Hasim et al., 2021), impaired immune function (Tanaka et al., 2021). According to a study of COVID-19 cases, an increase in BMI is associated with a greater likelihood of hospitalisation, ICU admission, ventilation, and fatality (Kompaniyets et al., 2021). Amendments in diet, social and demographic factors, and an inactive lifestyle may all be responsible for the disease's rising prevalence (Saad et al., 2017). Other factors contributing to the rise in obesity include inadequate sleep, endocrine disruptors, decreased smoking rates (since smoking lowers hunger), increased usage of drugs that might cause weight gains, such as common antipsychotics, and pregnancy at a later age (which could raise the risk of obesity) (Keith et al., 2006). Globally, there has been a considerable trend toward less physically demanding work, and at the very least, 60% of people do not get enough

exercise at present times (Abram and Apovian, 2006). In developing countries, ladies are more likely than males to be obese. Extra weight is a key risk factor causing medical issues, despite the fact that the general populace and some medical experts consider it more of a cosmetic issue than a health worry (Robinson and Thomas, 2006). According to findings from the National Centre for Health Statistics and the Framingham Heart Study, when compared to people with a normal BMI, natives with a BMI of 37.5 kg/m^2 had lifetime expenses for treating hypertension, high cholesterol, type 2 diabetes, cardiovascular disease, and stroke that were $ 10,000 more (Thompson et al., 1999).

2.1.3 Current Indian situation of Obesity: India, which is known for its malnourished population, is now seeing an increasing number of overweight and obese people who are at high risk of adverse consequences. In a study, it has been reported that by 2030, there would be 5.0% obese Indians and 27.8% overweight Indians (Kelly et al., 2008). Obesity in India differs from the rest of the world in that the proportion of body fatness, abdominal obesity, subcutaneous fat, intra-abdominal fat, and ectopic fat deposition is higher in the Indian overweight and obese population. According to a recent study, India is in 3rd position after the United States of America and China in terms of the number of obese people worldwide (Mehta and Vanan, 2006). In India, 40.3% of the population is obese. The greatest was in the south of India at 46.51 %, while the lowest was in the east at 32.96 per cent. Obesity was more prevalent in i) women than in males, ii) urban than rural areas, and iii) those > 40 years than those ≤ 40 years of age. Less physical activity (43.71 per cent inactive vs 32.56 per cent more active) and higher education (44.6 per cent of college graduates versus 38 per cent of non-graduates) were both linked to higher obesity (Venkatrao et al., 2020).

2.1.4 Current strategies for management of obesity: Diet, exercise, pharmaceutical treatment, and weight loss surgery are the current methods for managing obesity, either singly or in combination. Obesity prevention and treatment continue to be challenging. In general, efforts should target emotional and genetic aspects that lead to obesity, as well as guided therapies that incorporate lifestyle methods like food and exercise.

2.1.4.1 Role of Diet: An imbalance in energy between calories consumed and spent is the primary factor contributing to obesity and overweight. The epidemic of overweight and obesity is therefore being driven by societal changes and a global shift in nutrition. The primary causes of obesity are believed to be obese-promoting circumstances like a sedentary lifestyle and poor eating behaviours. In the emergence of obesity, the diet has a major role. Both proteins and carbohydrates provide 4 calories per gram, whereas fats have 9 calories per gram. Suggestions for weight management emphasize the need for good eating practices that should comprise a variety of nutrient-dense foods and minimize the intake of foods high in calories (Takada and Himmerich, 2021). There is ample evidence that some meal selections may aid with weight management. Even while each diet modification may only have a slight effect on weight control when combined, they could have a substantial long-term influence on society (Mozaffarian et al., 2011)

2.1.4.2 Role of Exercise: Exercise is crucial for prevention, preliminary weight decline, and weight loss maintenance, but requirements for physical activity vary depending on the category (Secor, 2020). Although abundant evidence suggests that increasing one's physical activity is an efficient way to avoid weight gain and regain weight, generating such changes remains a significant issue. The world we have created does not encourage physical exercise, and numerous technological improvements in recent decades have undoubtedly resulted in significant declines in physical activity. Although there is evidence that suggests 60 to 90 minutes of physical exercise are linked to better success in maintaining weight loss, it is unclear why this might be the case (Hill and Wyatt, 2005).

2.1.4.3 Yoga: Although symptoms associated with obesity and overweight, like low back pain, may be managed with yoga, it is yet unknown whether yoga aids in losing weight or maintenance in addition to what can be accomplished with diet and exercise (Bernstein et al., 2014). Dietary changes and exercise are two proven weight loss methods, but the majority of the weight lost is eventually gained back. Some studies have revealed yoga to be a promising method for helping adults manage and lose weight. According to some of the older research, yoga practice may help men and women who are overweight to lose weight when combined with

other weight loss methods (such as behaviour modification or dietary counselling) (Dai et al., 2021).

2.1.4.4 Surgery: Gastric banding and gastric bypass are two examples of bariatric surgical procedures that are successful at causing persistent weight loss. These procedures do, however, undoubtedly involve invasive procedures and typically have significant rates of complications. Surgery is often required for people with extreme obesity (BMI > 40 kg/m^2) and for people with complications related to obesity (Elangbam, 2009). Although surgery is the most successful therapy for treating obesity, it also is the most invasive, carrying perioperative risk in addition to the operation's associated harm. Studies have revealed that surgery is linked to some unfavourable symptoms that one would anticipate to be more common after surgery (e. g., bowel obstruction) (Albaugh and Abumrad, 2018).

2.1.5 Obesity medicines in the market: US Food and Drug Administration (FDA) has approved only a few numbers of medications in the last three decades for obesity. However, some of the anti-obesity drugs that were authorized and promoted have now been pulled from the market as a result of suspected side effects (Kang and Park, 2012).

2.1.5.1 Phentermine /topiramate (Qysmia®): The FDA authorized this drug combination in 2012, but the EMA rejected its application due to safety concerns. By increasing norepinephrine release and reducing its uptake in hypothalamic nuclei, phentermine is thought to aid in weight loss by causing a decrease in appetite. It has been observed that topiramate promotes weight loss by reducing calorie intake. Side effects from short-term use include paresthesia, dizziness, and sleeplessness. Phentermine can only be used for a brief time period and, on average, results in a 7.5 per cent weight loss. Even that much weight is regained once stopped (Tchang et al., 2005).

2.1.5.2 Sibutramine: FDA approved this medication in 1997. However, it was removed from the Italian market after there were 47 reports of adverse events (arrhythmias, mostly tachycardia and hypertension) and two cardiovascular disease deaths there (Bosello et al., 2002).

2.1.5.3 Orlistat: Orlistat, a powerful suppressor of lipase that was approved in 1998, is a hydrogenated derivative of lipstatin prepared from *Streptomyces toxytricini* that lessens the absorption of dietary fat. The gastrointestinal adverse effects of orlistat that are most frequently reported are dyspepsia, bloating, flatulence, faecal incontinence, diarrhoea, and oily spotting (Rucker et al., 2007). After receiving 32 reports of serious hepatic injury between the years 1999 and 2008, including 6 instances of liver problems in orlistat users, the U.S. FDA conducted a review of the therapeutic safety of orlistat (Morris et al., 2012).

2.1.5.4 Lorcaserin: In 2012, the FDA authorized the use of this medication. The most frequent negative effects of lorcaserin use include headache, nausea, hypoglycemia, back discomfort and dizziness (Chan et al., 2013). Because it was discovered that there might be an enhanced risk of cancer connected with lorcaserin, the FDA requested on February 13, 2020, that the drug manufacturer voluntarily remove lorcaserin from the US market (Tak et al., 2021).

2.1.5.5 Liraglutide: The FDA certified it as a glucagon-like peptide 1 (GLP-1) derivative in 2014. By working on the hypothalamus and cortex, GLP-1 reduces hunger and food consumption by causing postprandial satisfaction and fullness, slowing stomach emptying, and increasing satiety (Kulve et al., 2016). Liraglutide has a longer half-life (13 hours) and is more stable than human GLP-1. The usage of this medication might cause a number of side effects, including dyspepsia, diarrhoea, constipation, nausea, and vomiting.

2.1.5.6 Semaglutide: Wegovy (semaglutide) injection (2.4 mg once weekly) was approved by the FDA in 2021 for the treatment of acute weight gain in those who are obese or overweight and have a minimum of one problem associated with excess fat (such as high blood pressure, type 2 diabetes, or high cholesterol). The hormone glucagon-like peptide-1 (GLP-1) mimicked by this drug affects regions of the brain that regulate hunger and food intake. The most typical side effects are low blood sugar in people with type 2 diabetes, indigestion, dizziness, abdominal distension, nausea, diarrhoea, vomiting, constipation, abdominal (stomach) pain, headaches, fatigue, flatulence, intestinal infection and gastroesophageal reflux problem. A weight reduction

of 4% to 8% was normal in the majority of trials that were clinically conducted and assessed pharmacologic studies for longer than 12 months (Khera et al., 2016).

2.1.6 Challenges in treating obesity:

Long-term medication to attain body weight balance while maintaining tolerability and safety seemed an intractable challenge (Muller et al., 2018). There have been a variety of new anti-obesity medications flooding the marketplace recently. Even though these products are expensive, it is still not advisable to use them because they have associated negative side effects, including digestive and kidney issues (Calixto, 2000). However, the usage of natural weight loss solutions has surged. Botanical sources, according to scientists, appear more trustworthy, safer, and less expensive than existing approaches like synthetic medications or surgical treatments (Kazemipoor et al., 2012).

2.1.7 Natural anti obesity products and their classification based on mode of action:

Natural products potential for use in medication development has been thoroughly investigated and shown to be a trustworthy and productive source. So much interest has been drawn to the isolation and characterisation of new functional chemicals from living creatures. Numerous natural products have been investigated for their potential to prevent and treat obesity. These have been found to affect fat storage, weight loss and prevent diet-induced obesity. Because of this, such products have been widely used to treat belly obesity and overweight. The majority of studies used these products in *in vitro* methods (such as modulating 3T3-L1 cell development), *in vivo* methods (such as adipose tissue decreases in mice or rats), or straight enzymatic testing (anti lipase activity). Therefore, depending on their unique modes of action, anti-obesogenic treatments can be divided into a number of groups:

2.1.7.1 Pancreatic Lipase Inhibitory Effect:

Obesity causes an increase in circulating free fatty acid (FFA) levels, which leads to lipotoxicity and dyslipidemia. The goal of current therapeutic strategies in obesity is to treat or prevent lipotoxicity, which is brought on by excessive triglyceride (TG) buildup and lipolysis (Palatty and Saldanha, 2012). Enzymes implicated in lipid metabolic processes are being

discovered and studied more and more often. As a result, a wide range of new enzyme targets for obesity as well as other metabolic diseases, are emerging. The development of food digestion and absorption inhibitors, a method of reducing energy intake without disrupting any central systems, is one of the most crucial approaches in obesity treatment. Since dietary lipids are the main source of surplus calories, a unique strategy for lowering fat absorption involves precisely blocking triglyceride (TG) digestion (Birari and Bhutani, 2007). Lipase enzyme has a crucial role in the digestion of dietary fats and guards against its absorption in the small intestine. The primary cause of obesity is straightforwardly impacted by pancreatic lipase enzyme inhibitors. It is, therefore, a widely established assay for the therapy against overweight/obesity and its associated morbidities (Kumar and Chauhan, 2021).

Table 2.2 Plants with their compounds having pancreatic lipase inhibitory activity

Source	**Phytocompound**	**Mode of Action**	**Reference**
Eremochloa ophiuroides	Luteolin 6-*C*-β-D-boivinopyranoside Orientin Isoorientin Derhamnosylmaysin Isoorientin 2-*O*-α-L-rhamnoside	Pancreatic lipase inhibitory activity	Lee et al., 2010
Eisenia bicyclis	Fucofuroeckol A 7-phloroeckol		Eom et al., 2012
Oolong tea	Epigallocatechin-3,5-digallate		Nakai et al., 2005
Eugenia polyantha	Hydroxychavicol		Kato et al., 2013.
Annona crassiflora	Stephalagine		Pereira et al., 2017
Spartina anglica (Cord-Grass)	1,3-di-*O*-*trans*-feruloyl quinic acid and *p*-hydroxy benzaldehyde		Kim et al., 2021
Cornus mas	Pelargonidin 3-*O*-galactoside		Swierczewska et al.,

			2019

2.1.7.2 Appetite suppressive effect:

The primary line of treatment for obesity is regarded to be appetite suppression. The brain (centrally), liver, gastrointestinal tract, and adipose tissue (peripherally) create hunger and satiety signalling molecules. Plant-derived substances that reduce appetite have a direct impact on the pathways involved in appetite and satiety control. In order to increase satiety, appetite suppressants impede the reuptake of the brain chemicals serotonin and norepinephrine, which impact the hypothalamus, the area of the brain responsible for controlling hunger (Aronne et al., 2013). An appetite suppressant's primary function is to reduce hunger and increase the feeling of fullness in the stomach. It will cause one to eat less by decreasing cravings for food, enhancing sensations of fullness, or decreasing appetite.

Table 2.3 Plants with their compounds having appetite suppressive activity

Source	**Phytocompound**	**Mode of Action**	**Reference**
Cruciferous vegetables	Sulforaphanes	Appetite suppressant	Shawky and Segar (2018)
Caralluma fimbriata	Pregnane glycosides		Kuriyan et al., 2006
Garcinia cambogia	Hydroxycitric acid		Astell et al., 2016
Hoodia gordonii	14β-hydroxy pregnane glycosides		Geoffroy et al., 2011
Griffonia simplicifolia	5-hydroxytryptophan (5-HTP)		Rondanelli et al., 2012
Ilex paraguariensis (Mate tea)	Caffeoyl quinic acids and caffeine		Hussein et al., 2011
Catha edulis	D-norpseudoephedrine and cathinone		Kalyanasundar et al ., 2020, Murray et al ., 2008

2.1.7.3 Energy Expenditure Stimulatory Effect:

Obesity is linked to increased caloric intake, decreased energy expenditure, and adipose biology malfunction. Therefore, increasing energy expenditure can also help to lower the risk of obesity. There is currently no recognized treatment for stimulating energy expenditure, so using natural medications has become a popular substitute (Torres-Fuentes et al., 2015). Studies have shown that during fasting or under a calorie restriction, the level of circulating leptin declines while overfeeding or refeeding causes it to rise. Adipocytes produce leptin. Leptin lowers calorie intake, reduces body mass, and increases energy expenditure. Leptin levels rise proportionally due to an increase in fat cells, which then attach to their receptor, i.e. leptin receptors in the brain and release signals that reduce appetite and boost energy expenditure (Obradovic et al., 2021).

Table 2.4 Plants with their compounds having energy expenditure stimulatory effect

Source	Phytocompound	Mode of Action	Reference
Ophiopogon japonicas (Mondo grass)	MDG-1	Enhance energy expenditure, reduce the body weight and adipose tissue	Wang et al., 2014
Lactuca sativa (Purple lettuce)	Esculin, chlorogenic acid	Increasing energy consumption	Ju et al., 2017
Vitis vinifera (grape)	Resveratrol	Increase energy expenditure	Lagouge et al., 2006
Coffea	Caffeine	Increase energy expenditure	Yuliana et al., 2014
Ephedra sinica	Ephedrine	Increase in energy expenditure	Torres-Fuentes et al., 2013

2.1.7.4 Adipocyte Differentiation Inhibitory Effect:

Adipocytes, commonly referred to as fat cells and lipocytes, are crucial because they store triglycerides and release fatty acids according to varying body requirements. The size and mass of adipocytes are crucial indications of obesity (Seyedan et al., 2015).

Adiposity and adipocyte hyperplasia are encouraged if there is too much caloric intake without an increase in energy spending. Overconsumption of energy and prolonged increases in glucose uptake causes the stem cells to be recruited to the adipocyte lineage *in vivo*. These signaling factors induce MSC to transform into preadipocytes and then into adipocytes, causing an increase in adipocyte numbers (Tang and Lane., 2012). Direct or indirect targets of numerous signalling pathways include transcription factors that aid in initiating and promoting the differentiation process. It is said that the masters of adipogenesis are two transcription factors, peroxisome proliferator-activated receptor (PPAR) type and CCAAT/enhancer-binding protein (C/EBP) type. Adipose tissue has a high expression of PPARγ. Obese people are known to express PPARγ at higher levels, which leads to having more adipocytes and less bone density. Additionally, it was discovered that C/EBP is expressed at later stages of adipocyte development in culture cells and remains active in mature adipocytes (Boughanem et al., 2019).

Table 2.5 Plants with their compounds having adipogenesis differentiation inhibitory effect

Source	**Compound**	**Mode of Action**	**Reference**
Rosmarinus officinalis	Carnosic acid	Inhibits the preadipocytes differentiation	Gaya et al., 2013
Cudrania tricuspidata (Zhemu fruit)	6,8-Diprenylgenist-ein	Decrease fat accumulation and decrease PPAR-γ and C/EBPα	Jo et al., 2015
Barley	Ferulic acid and Coumaric acid	Inhibit body weight rise, reduce adipocyte differentiation, and alter lipid profiles	Seo et al., 2015
Sugar cane, bamboo, and cereals	Tricin	Block the transcription factors involved in	Lee and Imm, 2018

		adipocyte differentiation	
Curcuma longa (turmeric)	Curcumin	Inhibit adipogenesis	Ejaz et al., 2009
Ulmus davidiana	Catechin	Inhibit adipogenesis	Lee and Kang, 2021
Moringa oleifera	2(4-[α-L-rhamnosyloxy)benzyl] isothiocynate	Impede the accumulation of lipids in pre- adipocytes	Huang et al., 2020

2.1.7.5 Lipid Metabolism Regulatory Effect:

Adipocyte-stored triglycerides are hydrolyzed during the catabolic process of adipose tissue lipolysis, which releases glycerol and fatty acids into the plasma. Inducing this lipolysis mechanism may help fight obesity by reducing fat storage.

Table 2.6 Plants with their compounds having lipid metabolism regulatory effect

Source	**Phytocompound**	**Mode of Action**	**Reference**
Nelumbo nucifera	Catechin, isoquercitrin	Lipolytic activity	Ohkoshi et al., 2007
Magnolia officinalis	Honokiol	Effect lipolysis and adipogenesis	Weng et al., 2021
Aegle marmelos	Umbelliferone and esculetin	Lipolysis	Karmase et al., 2013

2.2 Inflammation:

The immune system's response to stimuli that could be detrimental, such as microorganisms, damaged cells, poisonous substances, or radiation, is inflammation. Inflammation is a common factor in the development of many chronic illnesses, including arthritis, diabetes, rheumatoid arthritis, cancer, and digestive and cardiovascular disorders (Chen et al., 2017). Any negative effect that compromises the integrity of cellular homeostasis is thought to trigger inflammation as an adaptive response. The host will experience more adverse

affects if this response lasts longer. So, the inflammatory response must be as short-lived as possible to prevent any of its unfavourable effects, despite its positive function as a protection for cellular physiology (Ahmed, 2011).

A class of clinical conditions known as "inflammatory diseases" is characterised by aberrant inflammatory reactions (i.e., chronic inflammation). Obesity can be a cause of inflammation, whereas chronic inflammation is associated with rheumatoid arthritis and obesity-associated diabetes. Literature studies revealed that numerous neurodegenerative conditions are associated with inflammation (Glass et al., 2010).

2.2.1 Arthritis:

Arthritis is a chronic inflammatory condition which mainly impinges the joints leading to joint erosions and cartilage erosions. According to the report published by the arthritis foundation, arthritis is the second most frequently reported chronic health condition and one of the nation's most common causes of disability (Arthritis Foundation, 2008). More than 100 different forms of arthritis have been identified. The most common types of arthritis include Osteoarthritis, Juvenile arthritis, Rheumatoid arthritis (autoimmune)**,** Psoriatic arthritis (autoimmune), and Gout (crystal deposition induced inflammation).

2.2.1.1 Rheumatoid arthritis:

The autoimmune disease rheumatoid arthritis (RA) is characterised by symmetrical, persistent synovial tissue inflammation that eventually results in joint degeneration, pannus development, tendon ruptures, and distinctive abnormalities. It has a 1% prevalence and is linked to severe morbidity and elevated mortality (Van den et al., 2017). It is categorised as an inflammatory poly arthropathy and a disease affecting the musculoskeletal system and connective tissue (Wolff, 2007). Additionally, systemic inflammation can damage a number of major organs, including the neurological system, kidneys, lung tissue, and cardiac and vascular systems (Nerurkar et al., 2019). This condition usually appears between the age of 35 and 60, with remission and exasperation. The term rheumatoid arthritis was first given by Garrod in 1858.

2.2.1.1.1 Etiology of RA:

The cause of rheumatoid arthritis (RA) is uncertain. However, variables, including genetic and epigenetic components, influence its incidence. Environment, tobacco smoke, dust exposure, and particularly the microbiota are other elements that significantly contribute (Scherer et al., 2020).

2.2.1.1.2 Genetic:

Studies have shown that between 30% and 60% of cases of rheumatoid arthritis are inherited. A significant factor in the genetic association with RA is HLA-DRB1. Autoantigens are presented by HLA-DR proteins. Seropositive individuals for rheumatoid factors (RFs) or anti-citrullinated protein antibodies (ACPAs) have an increased chance of developing RA (Viatte et al., 2013). Additionally, variation in PTPN22, a protein tyrosine phosphatase produced in lymphoid cells, has been connected to a higher risk of autoimmune diseases like RA. Studies conducted by Zhoo and co-workers disclosed that sometimes more than one of the genetic factors is responsible for RA (Zhao et al., 2021).

2.2.1.1.3 Gender:

Women have a two to three-fold higher risk of having RA vs men (National Library Medicine, 2019). Breastfeeding mothers tend to have a decreased risk of RA than non-breastfeeding mothers (Iqbal and Rattu, 2019).

2.2.1.1.4 Smoking:

Cigarette smoking causes oxidative stress in the body, which causes increased rheumatoid inflammation brought on by compromised antioxidant systems spurred on by free radicals (Kalpakcioglu and Senel, 2008). The expression of Matrix metalloproteinase (MMP)-12 (which can cause rheumatoid arthritis) has been found to increase in Cigarette smoking persons (Bracke et al., 2005)

2.2.1.1.5 Microbiota:

The development and advancement of RA are significantly influenced by the host microbiota, particularly the gut microbiome. Before birth, microbial colonisation begins, and it

changes and diversifies until it stabilises at about three years of age. A healthy microbiota interacts with intestinal epithelial cells to sustain immunological responses, which supports the maintenance of a tolerant state in the gastrointestinal tract. *Prevotella copri* was found to be more prevalent and *Bacteroides* to be less prevalent in RA patients in a study that evaluated the microbial makeup of healthy participants and untreated RA patients. This suggests that *P. copri* may be pathogenic. In another investigation, it was shown that *Prevotella histicola*, a new type of the *Prevotella* genus separated from the upper gut of humans, inhibited inflammation in HLA-DQ8 rats, which indicates that different *Prevotella* species have varied functional capabilities and have diverse effects on clinical outcome (Bodkhe et al., 2019).

2.2.1.1.6 Other factors:

Other factors that have reportedly been linked to an augmented risk of rheumatoid arthritis include a lesser consumption of vitamin D and antioxidants, as well as a diet having higher levels of sugar, sodium, proteins, red meats and iron (Nithyashree and Deveswaran, 2020). Rheumatoid arthritis risk factors in the environment include exposure to silica. It has also been determined that being exposed to textile dust is strongly linked to a higher likelihood of developing RA in Malaysian women (Smolen et al., 2018).

2.2.1.2 Diagnosis of Rheumatoid arthritis:

Joint pain and stiffness are frequent symptoms experienced by RA patients. The joints that are most usually affected include the wrists, metacarpophalangeal joints, and proximal interphalangeal joints. RF, anti-antibody against citrulline, and C-reactive protein concentration are all used to make the diagnosis of rheumatoid arthritis.

2.2.1.3 Rheumatoid arthritis pathogenesis:

An immune-mediated synovial illness called RA is brought on by the interaction between hereditary and environmental variables (Klareskog et al., 2009). The hallmark of RA is immune system dysfunction, which is characterised by an aberrant rise in immune cells, macrophages, cytokines and autoantibodies. In the synovial lining of diarthrodial joints, the initial inflammation takes place. Synovial lining cell proliferation, enhanced vascularization, and

inflammatory cell infiltration of the tissue are all seen. Subsequently, the adjacent articular cartilage is affected by the inflammatory tissue mass leading to the development of a pannus. At the junction of the inflammatory synovium and surrounding subchondral bone, there is localized activation of bone resorption leading to the loss of the mineralized bone matrix (Otero and Goldring, 2007). Despite the fact that the molecular mechanisms of cartilage and bone deterioration differ, current evidence from human and animal studies suggests that TNF-α, IL-1β, and other proinflammatory cytokines and mediators can drive both processes.

2.2.1.4 Synovial joint: Synovial joints allow neighbouring bones to move smoothly. The joint is covered by an articular capsule (joint capsule), which encloses a joint cavity having synovial fluid. A synovial joint has three primary components: i) an articular capsule, ii) articular cartilage, and iii) synovial fluid.

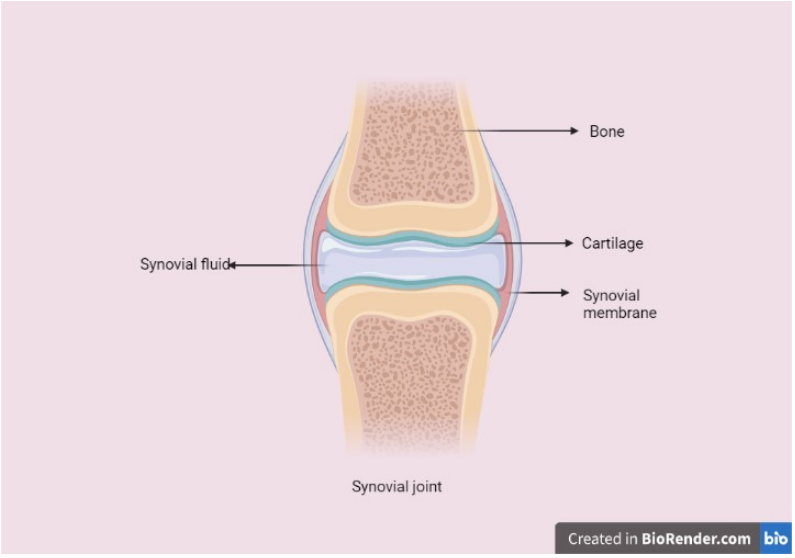


Figure 2.1 A typical synovial joint

Articular capsule: The articular capsule is a connective tissue that extends beyond the articulating surface of each involved bone. The smooth (hyaline) cartilage layer that covers the bone heads functions as a shock absorber.

Synovial membrane: The synovium, also known as the **synovial membrane** is a connective tissue that lines diarthrodial joints, surrounds tendons, and lines bursae and fat pads. The synovium has a significant role in preserving the volume and content of synovial fluid, mostly through the production of lubricin and hyaluronic acid (Scanzello and Goldring, 2012). Synovial membrane is made up of two layers: intimal and subintimal. In immune response in the synovium, MHC class II expressed by fibroblast-like cells, have an important role. The intimal layer's cellular population mainly performs two basic functions (i) phagocytosis- the process of removing undesirable particles from the joint (ii) production of components such as hyaluronan, fibronectin and collagen.

Synovial fluid: The synovial cells that line the joint capsule secrete synovial fluid. Normally, synovial fluid (SF) functions as both a biochemical pool for nutrients and regulatory cytokines and a biological lubricant for joints. Synovial fluid, which can be as much as 3- 4 ml in a knee, is very viscous, transparent, and can be an either colourless or pale straw in colour. White blood cells, primarily monocytes, lymphocytes, macrophages, and a few polymorphonuclear cells, are seen in SF. In illnesses such as rheumatoid arthritis, the leukocyte count can range from 2000 to 75,000/mm^3 (Faryna and Goldenberg, 1990). Clinically inflamed joints have low viscosity SF due to hyaluronic acid depolymerization by polymorphonuclear (PMN) leukocyte enzymes (Hogan and Pritzker, 1985). When macrophages in the SF are activated, they produce TNF-α and IL-1, which are involved in the pathophysiology of RA.

2.2.1.5 Role of Immune cells in RA

B cells: In the adaptive immune response, B cells have a major contribution. B-cells that are autoreactive are those that recognise host antigens and then obliterate the targeted cells or tissues. Autoreactive B-cells are generally removed by repairing processes that are governed by two checkpoints. Both checkpoints are generally faulty in RA patients, resulting in an overabundance of autoreactive mature naive B-cells. Rheumatoid factors (directed to the Fc region of IgG) and antibodies against cyclic citrullinated peptides (CCP) are important in RA (Yap et al., 2018).

B lymphocytes can produce proinflammatory cytokines and antibodies in addition to acting as antigen-presenting cells for T lymphocytes. Rheumatoid factor efficiently activates the

classic pathway of the complement system. The downstream products of complement activation are known to recruit infiltrating leukocytes. Leukocyte infiltration and the release of pro-inflammatory cytokines promote the maturation of pre-osteoclasts into osteoclasts, resulting in bone degradation (Gravallese et al., 2000). B cells in synovial fluid have been found to have elevated expression of the subunits necessary for functional IL-12 and IL-23 synthesis, as well as IL-1 and tumour necrosis factor (TNF) (Bugatti et al., 2014).

T cells: T cell development or maturation begins in the thymus, where developing thymocytes rearrange their TCR gene. Thymic maturation is essential for the selection of those T cells that recognise self-MHC molecules and for the rejection of T cells against autoantigens. A major portion of the thymocytes gets removed in the dual selection steps, and only left over T cells leave the thymus and become part of the peripheral T-cell repertoire. On stimulation of T cells, $CD4^+$ T-cells multiply and express rearranged antigen T cell receptors. When they recognise and connect with antigenic peptide fragments on the surface of antigen-presenting cells (APC) along with the HLA class II molecules and co-stimulatory molecules like CD80/CD86, activation takes place. Although the precise antigen(s) that autoreactive CD4+ T cells in RA recognise are unknown, when activated, Th1 cells produce proinflammatory cytokines such as IL-2, IFN-γ, and TNF-α (Skapenko et al., 2005). Furthermore, highly elevated quantities of TNF-α and IFN-γ produced by activated CD8+ T lymphocytes may lead to the destruction of target cells in autoimmune disorders.

The formation of inflammatory infiltrates in the synovial membrane, looking like tertiary lymphoid structures, is one of the most prominent symptoms of patients with moderate to severe RA. These structures are most likely important in the interaction between T cells, B cells, macrophages and fibroblasts. Synovial T cells stimulate macrophages to produce reactive oxygen species, IL-1, TNF-α, IL-6 and chemokines in a cell-contact dependent manner. (Cope et al., 2007).

T cell and RANKL pathway: Throughout adulthood, the body's skeleton is remodelled, with little bone packets resorbed and substituted for new bone. These are made up of a vascular area in which osteoclasts cause resorbtion of the bone at the structure's leading edge, and osteoblasts

follow, forming osteoid, which is then calcified. The quantity of bone resorbed and replenished is balanced by RANK and RANKL. The activation of the RANKL/RANK pathway causes NF-κB activation, which regulates the expression of the cytokines IL-1 and TNF, which are important mediators of inflammation in RA (Makarov, 2001). Their relative amount is susceptible to both local and systemic stressors, such as mechanical strain, cytokines including TNF- α, IL-1, and prostaglandin (PG) E2. The expression of RANKL in the lymphoid aggregates of the inflamed synovial tissues in patients with RA has been found to be enhanced, causing increased bone resorption (Horwood et al., 1999) (Gradaigh and Compston, 2004).

Macrophages: Macrophages are known to be a key player in any inflammatory disease and serve as a major connecting link between the development of the chronic disease from its acute form. It has also been reported that inflammatory cells like macrophages are crucial in arthritis pathogenesis. A huge influx of macrophages is observed in the synovial lining as well as junctions of cartilage during the arthritic condition (Mulherin D, 1996). These cells release various cytokines (Kinne et al., 2000), which further leads to bone destruction and recruitment of osteoclast precursors (Yingyu and Pope, 2005).

2.2.1.6 Role of cytokines in RA

With several cytokines exhibiting pleiotropic activities and a wide range of targets, the cytokine system in RA is indeed a complicated area. IL-1 and TNF are two essential pro-inflammatory cytokines in RA. It is crucially important to regulate these cytokines in the RA condition (Lubberts. and Van den, 2013).

TNF-α: TNF- **α** is unquestionably central to the pathogenesis of rheumatoid arthritis. TNF- α, which is largely synthesized by NK cells, T lymphocytes, and activated macrophages, is, in fact, a homotrimer protein. Numerous inflammatory molecules, including cytokines and chemokines, could be activated by it. When TNF-**α** interacts with its receptors, it sends out chemical signals that cause biological processes like cell death and inflammation. TNF- **α** exerts its effects by triggering a variety of secondary proteins that result in a range of cellular reactions, such as the

stimulation of gene transcription and/or the synthesis of reactive oxygen or nitrogen radicals. Nuclear factor κB (NF-κB) and mitogen-activated protein kinases (MAPKs) are activated as a result of the signalling pathways activated by TNF- **α**, and this might cause inflammation (Jang et al., 2021). TNF- **α** is thought to be the primary inflammatory factor in rheumatoid arthritis, and it is abundant in those who have the condition. Its improper release by macrophages contributes to the development and maintenance of synovitis (Parameswaran and Patial, 2010).

IL1: When it comes to the erosive changes in bone and cartilage that go along with chronic joint inflammation, TNF- α and IL-1 play a key role. TNF-α was hardly destructive on its own, but it could synergistically enhance the destructive behaviour of IL-1 (VandeLoo A A J, VandenBerg, 1990). The Ritchie Articular Index, the time period of morning stiffness, and the pain score have all been demonstrated to correlate with elevated plasma IL-1 concentrations in RA patients. (Kay and Calabrese, 2004). IL-1 beta and TNF-alpha, two inflammatory cytokines, promote the synthesis of matrix metalloproteinases (MMPs), which are the enzymes that have the ability to break down every part of the cartilage and the extracellular matrix. MMP gene expression is regulated by IL-1 β and TNF-α via signal transduction pathways such as those regulated by mitogen-activated protein kinases (MAPKs) (Burrage et al., 2006).

2.2.1.7 Arthritis medicines in market:

By focusing on inflammatory mediators and reducing inflammation and pain, the medication therapy for arthritis aims to stop the degradation of joints. **Table 2.7.** depicts some synthetic drugs available in the market for the management of arthritis along with their side effects and pausible mode of action.

Table 2.7. Synthetic drugs along with their mode of action for the treatment of arthritis

Type	Drug	Mode of Action	Side effects	Reference
Non biological Disease-modifying antirheumatic drugs (DMARDs)	Methotrexate, Hydroxychloroquine.	Inhibit inflammatory and other symptoms of rheumatoid arthritis	Alopecia, pneumonitis, photosensitivity, painful mouth ulcers, pulmonary fibrosis, hepatotoxicity, and nephrotoxicity.	Farzaei et al., 2016, Ruderman, 2012

Non-steroidal anti-inflammatory drugs (NSAIDs)	Naproxen, Ibuprofen, Piroxicam Paracetamol, diclofenac	Inhibit cyclooxygenase enzyme	Dyspepsia, peptic ulcer disease, bleeding, myocardial infarction, Increased blood pressure, heart failure.	Kesharwani et al., 2019
Biological agent	Adalimumab Infliximab Etanercept	TNF-α inhibitor	Infections, TB reactivation, malignancy, heart failure	Findeisen et al., 2021
	Tocilizumab Sarilumab	IL-6 inhibitor	GI perforation, hyperlipidaemia, infections	
Corticosteroids	Prednisone, prednisolone, triamcinolone and methyl prednisolone	anti-inflammatory, immunosuppressive	diabetes, osteoporosis, metabolic syndrome, cataract, and peptic ulcers among others	Mrid et al., 2022

2.2.1.8 Role of Plants in Anti-Arthritic Drug Development:

The current medication therapies can cause a reduction in the onset of disease and improve quality of life, but long-term use of synthetic pharmaceuticals has certain negative effects on the body. In light of the present medical scenario, which favours traditional herbal plants over allopathic medicines since they have fewer side effects and are more compatible with long-term use, people are becoming more interested in conventional therapy. Natural products have long served as a valuable source of potential therapeutic lead molecules. Rose, Capparis, Acacia, and Panax are among those linked to significant biological impacts and health-promoting properties (Grando and Fierro, 2017). **Table 2.8** depicts some phytocopmpounds along with their mode of action in the management of arthritis.

Table 2.8 Phyto compounds with their mode of action in management of arthritis

Source	Phytocompound	Mode of action	Reference
Rhodophiala bifida	Montanine	In the CIA model, montanine treatment decreased arthritis severity and joint destruction	Farinon et al., 2017

Berberis aristate	Jatrorrhizine hydrochloride	Reduce bone deterioration and inflammation, inhibit tumour necrosis factor-alpha, activation of NF-kappa-β, and MAPKs.	Qiu et al., 2018
Caesalpinia sappan	Brazilin	Decrease arthritis index score, acute inflammatory paw edema, and inflammatory cytokines in the CIA-mice model.	Jung et al., 2015
Cannabis sativum	Cannabidiol	Decrease the arthritic score and release of inflammatory mediators.	Malfait et al., 2000
Cinnamon	Cinnamaldehyde	Decrease the ROS and NO levels and increase in glutathione level in arthritic rats	Mateen et al., 2019
Nigella sativa	Thymoquinone	Decrease pro-inflammatory cytokines, such as TNF-α and IL-1β levels	Tekeoglu et al., 2007
Boswellia serrata	Acetyl Keto Boswellic acid	Reduce paw edema in the CFA arthritic model.	Banji et al., 2022
Curcuma longa	Curcumin	Inhibit matrix metalloproteinase (MMP-9), decrease TNF-α, reduce inflammation	McFarlin et al., 2016
Zingiber officinale	Shagoal	Reduce IL-1 β, IL-6, TNF-α, and INF-γ	Sabina et al., 2010

2.3 Secondary Metabolites

During the last few decades, there has been an amazing increase in interest among people living in both developing and developed countries for natural therapies. These herbal remedies are nowadays available in supermarkets also (Ekor, 2014). The term "natural product" refers to a broad range of secondary metabolites with definite physiological effects on the human body. Contrary to synthetic pharmaceuticals built on a single chemical, many phytomedicines work by combining or synergizing the effects of a number of chemical compounds acting at one or more target sites involved in a physiological process (Briskin et al., 2000). Secondary metabolites, which comprise significant subgroups like phenolics, terpenes, and nitrogen-containing chemicals, are frequently lineage-specific and support plant interaction with both biotic and abiotic environments. Earlier, there was a great misconception regarding the function of secondary metabolites, which were considered to be a waste product. But it is now understood that they work as pollinator-attracting chemicals, chemical responses to environmental challenges or chemical defences against microbes, insects, and larger predators. Pharmaceuticals, flavours, fragrances, and pesticides are examples of secondary metabolites that are industrially important biological compounds.

2.3.1 Terpenes:

Structurally terpenoids have a major disparity among the other secondary metabolites. Since the first compound of this family was extracted from turpentine oil, their names, terpene or terpenoid, were given for that reason. All terpenoids are derived from a repeated branched isopentane skeleton, which is known as an isoprene unit. They are referred to as monoterpenes or sesquiterpenes, depending on how many isoprene units they contain. Terpenoids are liquid, unsaturated hydrocarbons that are mostly found in resins and essential oils. Menthol, eugenol, and camphor are prominent monoterpenoids that are said to have excellent antioxidant properties, and resins and taxol are categories of diterpenoids that significantly inhibit cancer growth. The cytotoxic and sedative triterpenoids include cardiac glycosides, ursolic acid, and steroids (Velu et al., 2018). Essential oils are combinations of sesquiterpenes and volatile monoterpenes that give plants their distinctive odour. Terpenes are commonly employed in traditional herbal treatment, and curcumin is one such terpene. Paclitaxel is a popular terpene drug in the market today, and

it's used to treat malignancies of the breast, lung, ovary, pancreatic, cervix, and blood (Georgian et al., 2019).

2.3.2 Phenolic compounds:

When environmental challenges, including intense light, less warmth, pathogen infection, and nutritional deprivation, lead to an increase in free radicals or any of the oxidative species in plants, phenolics play a significant role as defensive chemicals (Lattanzio, 2013). Flavonoids are a massive class of phenolic natural products with over 4500 different representatives currently known. Flavonoids are found as monomers or dimers or higher oligomers in most the plant tissues, most notably in the vacuoles. Chalcones, aurones, flavanones, isoflavonoids, flavones, flavonols, catechins, and anthocyanins are some examples of flavonoids. Plants are estimated to convert about 2% of their total carbon into flavonoids or similarly related chemicals (Zuiter, 2014).

2.3.3 Nitrogen containing secondary metabolites:

A large percentage of molecules derived from plant sources contain nitrogen as one of their structural components. Alkaloids, glucosinolates, and nonprotein amino acids are the four categories into which secondary metabolites having nitrogen can be divided.

2.3.3.1 Alkaloids:

Alkaloids are composed of protein molecules that have an amino acid structural component that effectively contains an N-atom within it. This often happens when the H- atom of a peptide structure is swapped out for other radicals together with oxygen. While most alkaloids contain carbon, oxygen, hydrogen as well as nitrogen, certain alkaloids also contain additional elements, viz., phosphorus, chlorine, sulphur, and bromine (Nicolaou et al., 2011). Alkaloids' nitrogen atom functions as a defence mechanism, preventing bacterial, viral, or microbial infection of plant cells as well as damage caused by other factors such as herbivore attacks, ecological disturbances, and climatic changes. After the discovery of morphine, a well recognized alkaloid produced from the *Papaver somniferum*, or poppy, alkaloids like morphine, quinine, and codeine have been utilised extensively as medications in healthcare (Rolin, 2013). Vincristine and

vinblastine are alkaloids that are utilized as chemotherapeutic agents for the cure of a variety of cancers. Because of their strong pharmacological activity, these nitrogenous-based alkaloids can be employed successfully in current medicinal research to identify and find potent therapeutic moiety.

2.3.3.2 Cyanogenic Glycosides (CGs):

Plant secondary metabolites called cyanogenic glycosides to contain nitrile and are enzymatically broken down to create cyanide. At least 2600 plant species, including those in the Fabaceae, Rosaceae, Leguminosae, Linaceae, and Compositae families, have at least 25 identified CGs (Singh, 2018). Examples of cyanogenic glycosides include prunasin, which is found in wild cherry bark, and amygdalin, which can be found in bitter almonds and peach kernels (both of which are utilised in Chinese medicine). Its antitussive qualities are thought to be caused by the small amounts of cyanide produced by the bark (Bone and Mills, 2012).

2.3.3.3 Glucosinolates:

Glucosinolates are secondary metabolites of plants that are present in the agriculturally significant Brassicaceae family and contain both sulphur and nitrogen. When glucosinolates break down, they produce molecules that give crops like cabbage, broccoli and radish their flavour and aroma, as well as toxins and herbivore repellents. Plants have been found to contain over 130 glucosinolates. Brassica glucosinolates inhibit growth or deter feeding in a variety of herbivores, including birds, land slugs, and insects (Redovnikovic et al., 2008).

2.3.3.4 Nonprotein Amino Acids:

Certain plant families, such as legumes and grasses, have high levels of non-protein amino acids. Non-protein amino acids have anti herbivory, antibacterial, and allelochemical activity because they can mimic and interfere with insect neurological processes, misincorporate into proteins, impede primary metabolism, and have direct toxic effects. For example, canavanine is abundant in *Medicago sativa*, while homoarginine is abundant in *Lens culinaris*, a popular edible pulse (Huang et al., 2011).

Numerous plant genera have made significant contributions to both modern and traditional medicine. One of them is the genus *Psoralea* belonging to the legume family (Fabaceae), which Linnaeus discovered in 1742.

2.4 *Psoralea* Genus:

From the Greek term “psoraleos” comes the English word Psoralea, meaning "afflicted with an itch or leprosy" (Chopra et al., 2013). Morphologically *Psoralea* genus is extremely diverse. The genus *Psoralea* comprises 130 species which include herbs, shrubs or undershrubs and are primarily found in Australia, America and South part of Africa, with a small number also occurring in Asia and temperate Europe. It is widely dispersed throughout Asia, from China to Pakistan to India. They often grow in dry or semi-arid regions and are either annual or perennial. *Psoralea plicata* an indigenous to Pakistan and other Arab nations; *Psoralea pinnata* and *P. arborea* an indigenous to South Africa; *P. argophylla* is indigenous to Central America. *Psoralea esculenta* is found in America. Although an Asian native plant, *Psoralea corylifolia* L. is also grown on other continents (Aussie, North America, and African land). This plant is widely cultivated in flat, subtropical & tropical land regions (Koul et al., 2019). This genus is distinguished by the resin gland occurrence on almost every portion of *P. corylifolia*. Most of the species have beautifully scented flowers that are either blue, purple or very seldom yellow in colour. The pharmacological effects of the genus *Psoralea* are well-established and confirmed by research. In summary, *Psoralea* genus has been useful to humanity as a medicinal herb and is an unrivalled treatment for leprosy, leucoderma, psoriasis, and many other ailments (Khushboo et al., 2010).

P. corylifolia, an Indian native and one of the most well-known *Psoralea* plants in the world, deserves special notice for its historical role in medicine. Since ancient times, the powdered seeds of this plant have been employed in Ayurvedic medical system to cure skin depigmentation, commonly known as vitiligo or leukoderma. The Atharva Veda, a holy book, contains descriptions of the ancient Hindu practice known as eure that served as the foundation for this therapy as early as 2000 B.C. (Bourgaud et al., 1995). A secondary metabolite named psoralen, after the plant's name, is the cause of the pigmentation and is present in the seeds of *P. corylifolia*.

2.4.1 *Psoralea corylifolia*

Leguminosae member *P. corylifolia*, also known as *Cullen corylifolium* (L.) Medik, is a highly beneficial plant from the perspectives of ethnobotany, pharmacology, and phytochemistry. It has its mention in Ayurvedic, Chinese medicine and Chinese/British/American pharmacopoeias (Khushboo et al., 2010). Fruits and seeds of this plant are traditionally known as aphrodisiac, purgative, anti-helminthic, and diuretic. It is also known by the name "Kushtanashini" as its seeds are famous for chronic skin diseases. More than 150 pharmacologically active molecules have been identified in seeds/fruits of *P. corylifolia* (Zang et al., 2016), due to which these are utilized as a 'panacea to several maladies'. Each country and region has a common name for this plant. Some regional/ tropical names of *P. corylifolia* are summarized in **Table 2.9**.

Table 2.9 Regional/Topical name of the plant

Bengali	Bavachi, Hakuch, Barachi, Bakuchi	**Punjabi**	Babchi	**Telgu**	Bavanchalu, Bavanchi-vittulu, Bogi-vittulu
Hindi	Babachi, Babchi, Bavanchiyan, Bemchi	**Sanskrit**	Bakuchi, Chanderlekha, Chanderprabha, Bakuchi, Sasankarekha	**Tamil**	Karpokarishi, Karpuvanshi, Kaarboka-arisi, Karpogalarisi
Gujarati	Babchi, Bavacha, Babichi, Bawchi	**Urdu**	Babechi	**Chinese**	Ku Tzu, Bu Ku Zhi, Cot Chu

Marathi	Babachi, Bavachi, Bavanchi	**English**	Malay tea, scurf-pea, fountain bush, babchi seeds, psoralea seeds, and west indian satinwood	**Kashmiri**	Babchi

2.4.1.1 Taxonomic position

Kingdom	Plantae
Divison	Angiospermae
Class	Dicotyledoneae
Order	Rosales
Family	Leguminosae
Genus	*Psoralea*
Species	*corylifolia*

2.4.1.2 Botanical description

Psoralea corylifolia is an erect, dicot annual herb that grows to 0.6-1.2 m in height. This plant germinates epigeal and is cultivated through seeds. It grows well in sandy, loamy and well-drained soil. *P. corylifolia* is a hermaphrodite and is pollinated by insects. Shoot tip and auxiliary bud culture are used to propagate *P. corylifolia.* The petioles feature hairs and glands, whereas simple, broadly elliptic, mucronate leaves coated in white hairs on both sides are punctuated.

Flowers are axillary with 10–30 flowered racemes and are bluish-purple in colour. Fruit is a tiny, one-seeded pod that is indehiscent, 5 mm in length, subglobular with compression, pitted black and beaked without hairs. The seeds are kidney shape, brownish-black, have a fragrant, bitter flavor and are 2-4 mm long, 2-3 mm in width, and 1-15 mm thick.

2.4.1.3 Geographical distribution

Despite being an Asian native, *Psoralea corylifolia* is grown on other continents as well. Around the world, the plant can be found in tropical and subtropical climates, including Southern Africa,

China, India, Iraq, Jawa, Laos, Malaya, Myanmar, Oman, Pakistan, Somalia, Bangladesh, Sri Lanka, Vietnam, and Yemen (**Figure 2**). In India, we can see it in the Himalayas, Dehradun, Assam, Oudh, Rajasthan, Bundelkhand, Eastern Punjab, Bengal, Mumbai, Bihar, Deccan, and Karnataka.

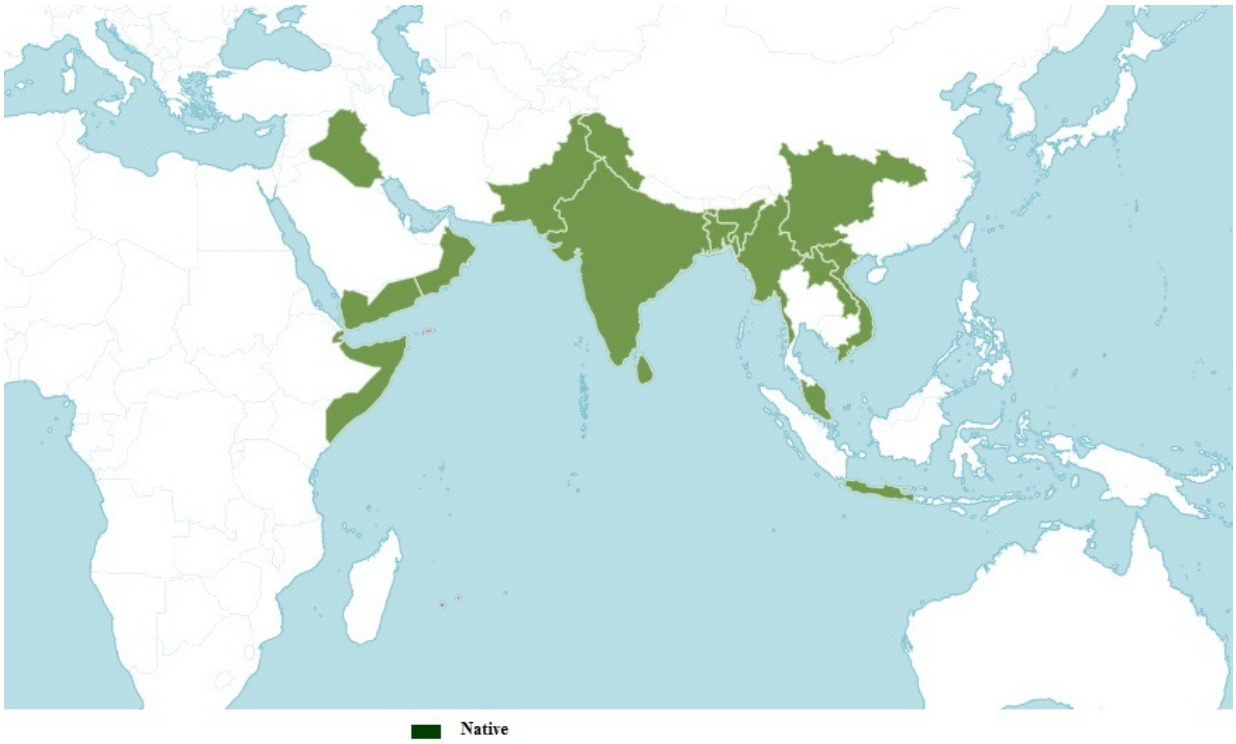


Figure 2.2 Geographical distribution of *Psoralea corylifolia*
(Source: http://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:489225-1)

2.4.1.4 Babchi seeds cultivation and drying

Babchi seeds are obtained from *P. corylifolia*. The plant grows well on red loamy soil with high organic content (pH range from 6.5-7.5). Seeds do not require any specific pre-treatment before sowing. Crops grown in November have the highest seed yield (1931 kg/ha) and consistency, followed by seeds sown in October (Sumathi and Srimathi, 2013). The oil content of the seeds obtained from plants that are sown in November is generally high (~ 6.7 %), and its value varies from 6.2% to 6.6% for other months. Thus, the time of sowing seeds had a great influence on the yield.

In winter, the common fungal disease observed is 'Powder mildew', which can be controlled by spraying 3% weekly wettable sulphur (sulfex) 3-4 times/week. Another threat is the 'Leaf roller caterpillar', controlled by spraying 0.2% Endosulfan, 2-3 times fortnightly.

In order to obtain a maximum yield of up to 2404 kg/ha, nitrogen, phosphorus, and potassium (NPK) should be applied at a ratio of 100:60:50 kg/ha, and spacing between the plants should be 60×30 cm (Sumathi et al., 2013). Pods turn purple 200 days after planting, signalling the crop's maturity. After harvesting, babchi seeds are transported for drying and purification. After shade drying, purification is done through a sieve using gravity and wind power. After sieving/screening, seeds are stored in gunny bags for marketing.

2.4.1.5 Traditional medicinal applications of *P. corylifolia*

One of the foremost critical highlights of *P. corylifolia* is that each and every part (roots, leaves, seeds etc.) of the plant possesses some or the other medicinal property. About thousands of years ago, an ayurvedic system of medicine recognized the relevance of seeds of *P. corylifolia* for repigmentation in vitiligo. Tribes such as Gond, Bharia and Korku in Madhya Pradesh use *P. corylifolia* seeds for leucoderma (Pandey et al., 2008). In Chinese medicine, *P. corylifolia* is considered to be warm in nature and is used to tonify the kidneys, particularly in kidney yang. Kidney Yang disease syndrome, which is linked to the loss of Mingmen (Fire of Life), is characterised by warm dysfunction and a metabolic problem of the body fluid, which leads to cold limbs, a cold back and waist, soreness and weakness in the knee and waist, tinnitus, exhaustion, hearing loss, and loose teeth. In Korean traditional medicine, seeds of this plant are used for skin diseases and to improve the male reproductive system (Yang et al., 2008). A few conventional applications of *P. corylifolia* are mentioned in table 2.

Table 2.10 Traditional medicinal applications of *P. corylifolia*

S.No.	Conditions	Plant part/Methods of Application	References
1	Anti-microbial	Seeds	(Kaul 1976)
2	Cytotoxic	*P. corylifolia* seed, also called	(Heo et al.,

		"Boh-Gol-Zhee" in Korea	1980)
3	Healing actions on kidney and spleen meridians	Seed	(Chopra et al., 2013)
4	Diuretic	Seed powder	(Chishty et al., 2016)
5	Anti-helminthic	Seed	(Ayurveda et al., 2016)
6	Aphrodisiac	Seed, fruits	(Chauhan et al., 2014)
7	Laxative	Seed powder	(Chandrasekar et al., 2016)
8	Febrile	Seed	(Suman et al., 2013)
9	Alopecia	As a good hair tonic, seeds are used to treat alopecia and hair loss. Over the damaged area, leaf paste is also used.	(Kaur et al., 2013)
10	Inflammation	Seed	(Choudhary et al., 2015; Gupta et al. 2014)
11	Leukoderma	Seeds are used as a paste or ointment for local application.	(Niranjan et al., 2017)
12	Leprosy	Seed. In leprosy and dermatoses, oil of the seeds is advised to be consumed orally along with the betel leaf, amalaki, and khandira. The lotion of seed with “Gau Mutra” is used	(Nabi et al., 2017) (Sharma et al.,2010); (Singh et al., 2011); (Ghosh, 2017)
13	Psoriasis	Seed as Powder/Paste	(Khare 2007; Hazra et al.,2013)
14	Eczema	Seed	(Rasheed et al., 2013)
15	Leukoderma	Seed. Oral ingestion and topical external use as paste or ointment	(Shadab et al., 2019)
16	Ringworm	Seed. Powered seeds of *Psoralea*	(Koul et al., 2019)

		corylifolia and Cassia tora with lime juice	
17	Scabies	Seed. External application of bakuchi seed powder containing buttermilk.	(Shuddhhi, 2020)
18	Dermatosis	Seed	(Jadhav et al., 2010)
19	Snakebite	Seed. Seeds are grinded and mixed with water and then put in nose. Given internally also	(Anand et al., 2006)
20	Scorpion sting	Seed	(Filipps et al. 2018)
21	Dental caries	Root	(Tiwari, 2012)
22	Laxative	Fruits, seeds	(Singh et al., 2018)
23	Anti- diarrheal	Leaves	(Khushali et al., 2020)
24	Vitiligo	Seed. The decoction of its seeds with gooseberry or “khair” is given to cure vitiligo	(Balkrishna et al.,2018)
25	Deobstruent	Seed	(Rajgopal et al.,2013)
26	Piles	Whole plant	(Sharma, 2016)
27	Tonic	Seed	(Krishna et al., 2009)
28	Wounds	Seed, Leaves. Seed oil applied on wounds.	(Tabassum et al., 2017)
29	Cough and Asthma	Seed	(Dabhadkar et al., 2013)
30	Deafness	Seed. Seed powder mixed with musali (*Curculigo orchioides*)	(Rajput et al., 2014)
31	Edema	Seed	(Nabi et al.,2017)
32	Post-natal care	Seed	(Anvar et al., 2015)
33	Gynecological bleeding	Seed	(Uikey et al., 2010)
34	Impotence	Seed	(Yang et al., 2008)

35	In Japan, used as Preservative in food or pickle	Seed	(Qiao et al., 2007)

Nowadays, with advancements in medical sciences, several formulations using different components of *P. corylifolia* are available in the market. A list of some commercialized medicinal formulations using *P. corylifolia* is depicted in **Table 2.11**.

Table 2.11 A non-exhaustive list of some commercialized medicinal formulations of *Psoralea corylifolia*.

Medicine name/Trade name	**Formulations**	**Uses**	**Company**
Avalgujadi Lepam	Cream /paste	Used to apply externally for leucoderma disease	Annapurna bioveda pvt ltd. Hyderabad India. Amrit ayurveda chikitsalya, Nabha, Punjab India.
Pancha Nimba Churna	Cream/powder	Used to cure Psoriasis and for non-healing wounds	Vyas pharmaceuticals, Indore, India.
Mahamanjistadi kashayam	Liquid	For purifying the blood as well as curing numerous skin conditions.	Shree baidyanath ayurved bhawan private ltd. Kolkata india. Dabur india ltd. Solan india. Patanjali ayurveda ltd. Haridwar India.
Somaraji	Liquid/oil	Applied topically to eczema, dermatitis	Shree baidyanath ayurved bhawan private ltd. Kolkata India.
Khadirarishta	Liquid	For treatment of skin diseases	Shree baidyanath ayurved bhawan private ltd. Kolkata India. Dabur

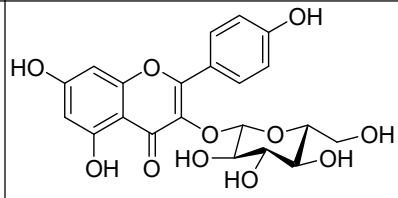
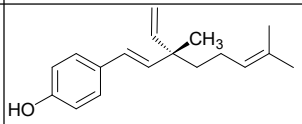
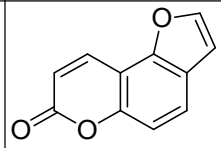
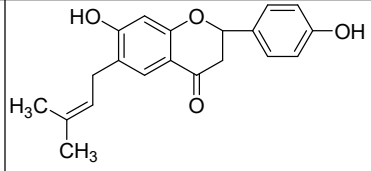
			India ltd. Solan, India.
Babchi	Liquid/oil	Used to treat vitiligo patches as well as grey hair.	Moksha lifestyle products, Delhi, India. Hamdard labortaries, India.
Psoralea and teasel combo	Powder	Dietary supplement	Hangzhou Hu Qing Yu Tang, pharmaceutical co. ltd., China.
Seven treasures for hair tea pills	powder	For alopecia, weak hair, and hair loss.	Qu Bao Mei Ran Wan, herbal dietary supplement, China.
Hakuchi	Powder form(seeds)	Bone diseases, skin diseases, men health	Nutra green biotechnology co. ltd. Shangai, China.
Bu Gu Zhi	Powder form (fruit)	Alpopecia, psoriasis, vitilago	Active herb technology, San Diego, California.
Pigmento	Tablets	Vitiligo	Charak pharma pvt. ltd. Mahalaxmi, Mumbai, India.
Bakuchighana	Cream	Skin diseases	Chaitanya pharmaceuticals pvt. ltd. Panchavati. Nasik
Kesh kanti-	Shampoo	Hair cleanser	Patanjali ayurveda ltd. Haridwar, India
Leucowin	Tablets	Vitiligo	Padmavati pharmaceuticals Thane, Mumbai, India.
Dermovita syrup	Syrup	Skin diseases, blood purifier	Ayurvita healthcare pvt. ltd. Mumbai, India
Zhuang Gu Xiao Ci Pian	Tablets	Increase bone strength	Active herb technology, San Diego, California

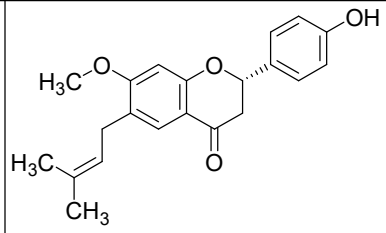
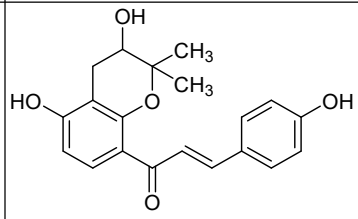
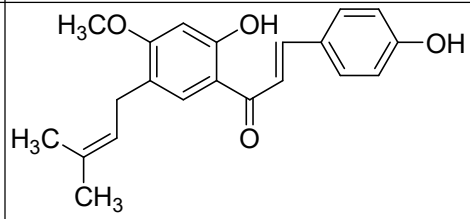
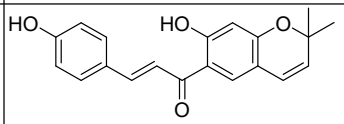
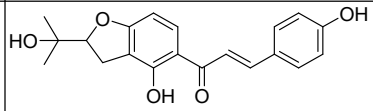
2.4.1.6 Bioactive constituents

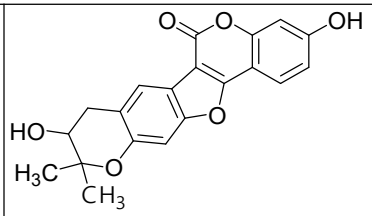
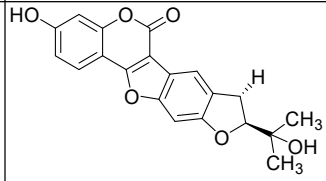
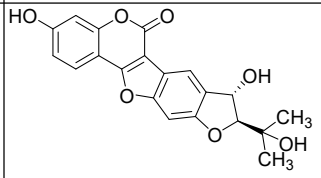
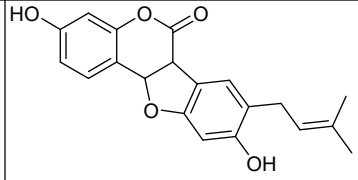
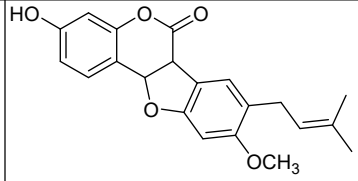
According to a survey of the scientific literature, 155 phytochemicals from several chemical classes, including flavonoids, chalcones, coumarins, terpenoids, lipids, and stigma steroids, have

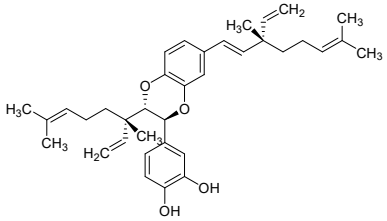
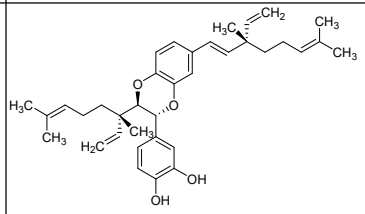
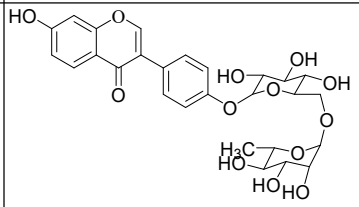
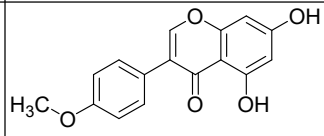
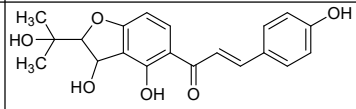
been isolated from *P. corylifolia* plant sections. Some of its important pharmacologically active constituents are bakuchiol **(2)**, corylin **(44)**, genistein **(65)**, isobavachalcone **(80)**, isopsoralen **(87)**, psoralen **(122)**, psoralidin **(128)**, which attributes to its anti-bacterial, anti-viral anti-inflammatory, anti-psoriasis, anti-vitilago, anti-carcinogenic and many other activities. A maximum number of the active constituents of this herb is found in the seeds. Traditional methods for separating and purifying psoralen and isopsoralen from plant sources are tiresome and usually need multiple chromatographic steps on silica gel. Extraction of psoralen and isopsoralen from *P. corylifolia* was performed using a supercritical fluid extraction technique under optimized reaction conditions, and the investigation revealed that combined yields of psoralen and isopsoralen were 2.5 mg/g of dry seeds. When these two components were separated by applying high-speed counter-current chromatography (HSCCC), the purity of the two compounds was more than 99.9% (Wang et al., 2004). Later on, in 2020, Khuranna et al. performed the extraction of Bakuchiol from *P. corylifolia* seeds using different extraction methods such as maceration, reflux, Soxhlet and ultrasonic assisted extraction (UAE). Five different solvents were used in the study. The authors emphasized that maximum extraction of bakuchiol (6.98 %, w/w) was observed when the UAE technique was applied in the extraction using petroleum ether as solvent. The bioactive compounds isolated from the extracts of PC, along with their tested/reported bioactivities, are described in **Table 2.12**.

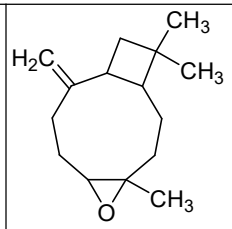
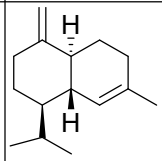
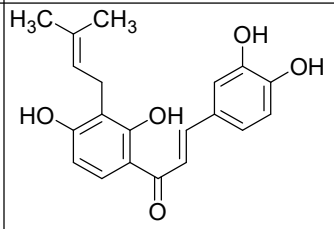
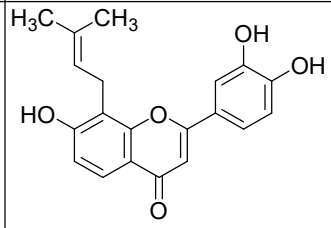
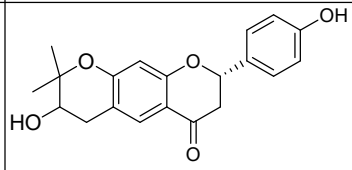
Table 2.12 Pharmacologically active compounds isolated from *P. corylifolia*

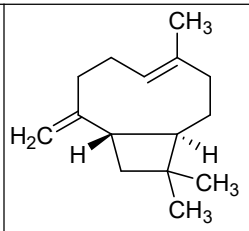
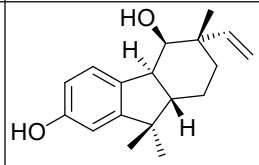
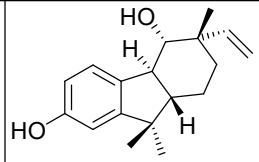
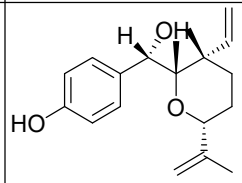
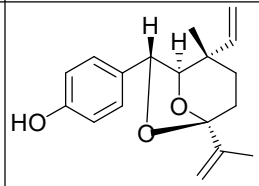
S.No.	Compound	Chemical structure	Chemical class	Plant part	Biological activity	Reference(s)
1	Astragalin		Flavonoid	Seed	Reduce inflammation, anti-oxidant, neuroprotective, cardioprotective, anti-carcinogenic	(Krishnamurthi et al., 1969; Riaz et al., 2018)
2	Bakuchiol		Meroterpene	Seed/Fruit	Anti-microbial, retinol functionality, anti-androgenic, anti-tumor, anti- aging, reduce inflammation, anti-helminthic	(Katsura et al., 2001; Adhakari et al., 2003; Bapat et al., 2005; Lin et al., 2007; Miao et al., 2013; Chaudhuri and Bojanowski, 2014; Lim et al., 2019; Bacqueville et al., 2020)
3	Bakuchicin		Furanocoumarin	Fruit	Cytochrome P450 (CYP1A) inhibition, induce vascular relaxation	(Li et al., 2011; Kim et al., 2016)
4	Bavachin		Flavonoid	Seed	Cholesterol acyltransferase inhibitor osteoblastic	(Wang et al., 2001; Yadava et al., 2005; Choi et al., 2008)

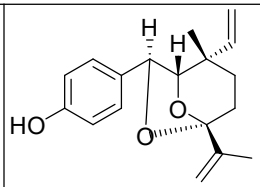
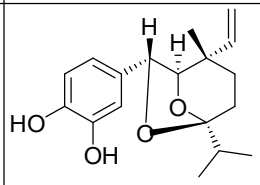
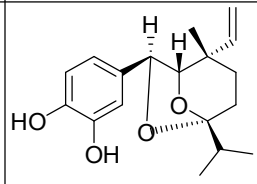
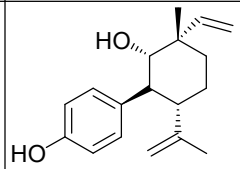
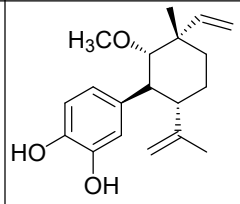
5	Bavachinin		Flavanone	Seed	Anti-inflammatory, Monoamine oxidase B inhibitor	(Lee et al., 2012; Chopra et al., 2013; Zarmouh et al., 2015)
6	Bavachromanol		Chalcone	Seed	Anti-fungal, anticancer	(Rastogi et al., 1999; Alam et al., 2018)
7	Bavachalcone		Chalcone	Seed	Reduce inflammation, osteoblastic	(Bhalla et al., 1968; Park 2008; Dang et al., 2015)
8	Bavachromene		Chalcone	Seed	Estrogenic activity	(Bajwa et al.,1972; Lim, 2011)
9	Bakuchalcone		Chalcone	Seed	Protein kinase inhibition	(Gupta et al., 1982)

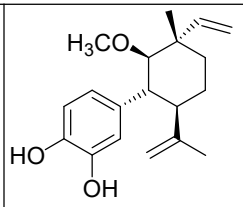
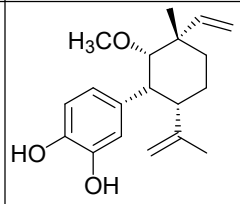
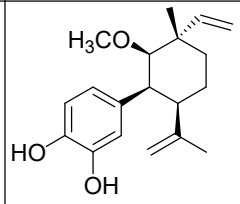
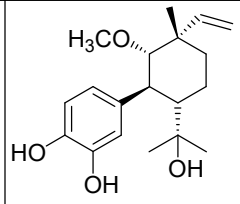
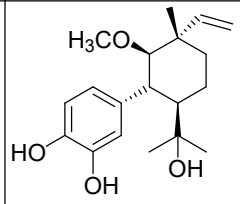
10	Bavacoumestans A		Coumestan	Seed	Antiviral, cytotoxic	(Rastogi et al.,1998; Abdelmohsen et al., 2021)
11	Bavacoumestan B		Coumestan	Seed	DGAT1 inhibitor	(Rastogi et al., 1998)
12	Bavacoumestan C		Coumestan	Seed	DGAT1 inhibitor	(Rastogi et al., 1998)
13	Bavacoumestan D		Coumestan	Seed	Anti- α- glucosidase	(Chai et al., 2019)
14	Bavacoumestan E		Coumestan	Seed	Not reported	(Zhu et al., 2018)

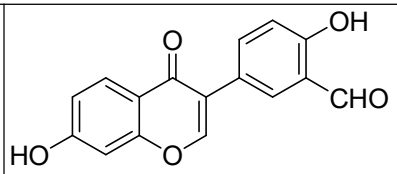
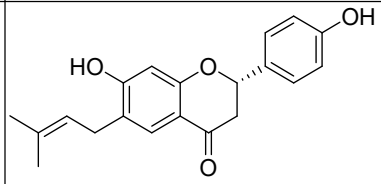
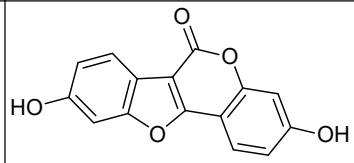
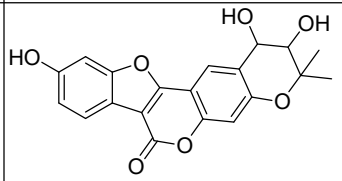
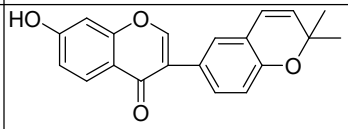
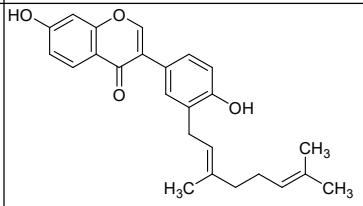
15	Bisbakuchiols A		Meroterpenoid	Seed	α- Glucoside inhibitor	(Wu et al.,2007; Mounika, 2015)
16	Bisbakuchiols B		Meroterpenoid	Seed	α- Glucoside inhibitor	(Wu et al., 2007; Mounika, 2015)
17	Bavadin		Isoflavone	Fruit	Not reported	(Yang et al., 2006)
18	Biochanin A		Isoflavone	Fruit	Monoamine oxidase B inhibitor	(Zarmouh et al., 2017)
19	Brosimacutin G		Benzofuran	Seed	Inhibits α-glucosidase	(Yin et al., 2004)

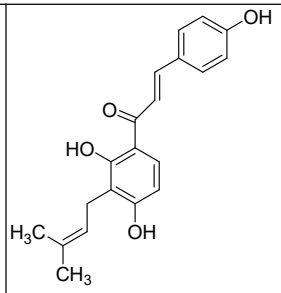
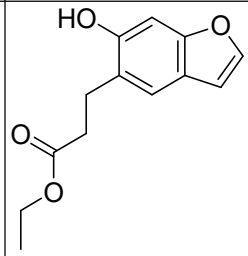
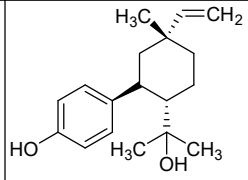
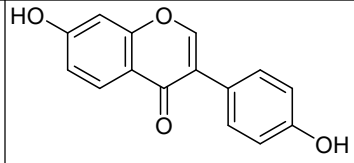
20	β-Caryophyllenoxide		Sesquiterpene	Seed Oil	Anti-inflammatory	(Kapoor, 2001)
21	γ-Cadinene		Sesquiterpenes	Seed	Larvicidal	(Rao et al., 2012)
22	Corylifol B		Prenylflavonoids	Seed	Anti-bacterial, blocks human carboxylesterase 2, protects against ionizing radiation injury	(Yin et al.,2004; Liet al. 2015; Du et al., 2019)
23	Corylifol C		Isoflavonoid	Seed	Protein kinase inhibitory, anti-oxidant	(Yin et al., 2004; Limper et al., 2013)
24	Corylifol H		Isoflavonoid	Fruit	Anti-inflammatory	(Liu et al., 2019)
25	β-Caryophyllene		Sesquiterpene	Seed	Reduce inflammation, anti-	(Mohamed et al., 2017;

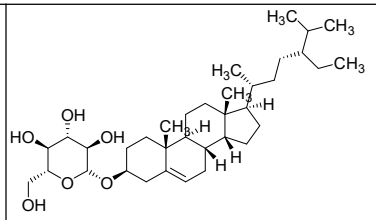
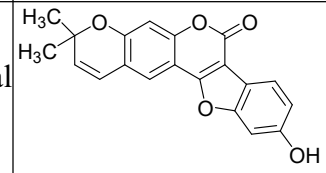
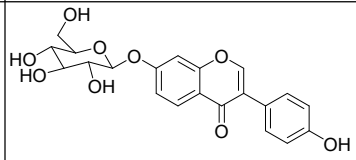
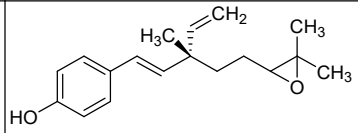
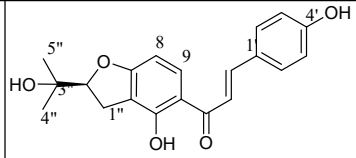
					carcinogenic, anti-microbial	Francomano et al., 2019)
26	Corypsoriol A		Meroterpenoid	Fruit	Helpful in muscle atrophy, Cytotoxic	(Han et al., 2020; Xu et al., 2020)
27	Corypsoriol B		Meroterpenoid	Fruit	Cytotoxic	(Xu et al., 2020)
28	Corypsoriol C		Meroterpenoid	Fruit	Cytotoxic	(Xu et al., 2020)
29	Corypsoriol D		Meroterpenoid	Fruit	Cytotoxic	(Xu et al., 2020)

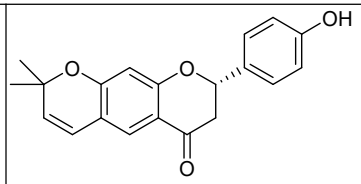
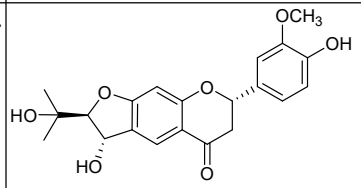
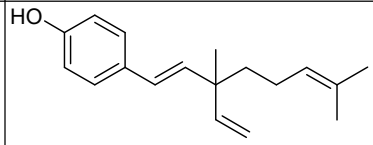
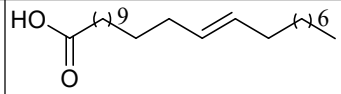
30	Corypsoriol E		Meroterpenoid	Fruit	Cytotoxic	(Xu et al., 2020)
31	Corypsoriol F		Meroterpenoid	Fruit	Cytotoxic	(Xu et al.,2020)
32	Corypsoriol G		Meroterpenoid	Fruit	Cytotoxic	(Xu et al., 2020)
33	Corypsoriol H		Meroterpenoid	Fruit	Cytotoxic	(Xu et al., 2020)
34	Corypsoriol I		Meroterpenoid	Fruit	Cytotoxic	(Xu et al., 2020)

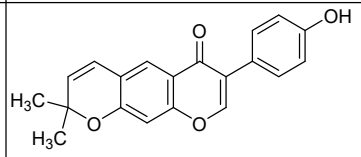
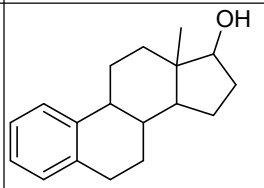
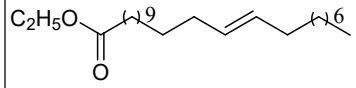
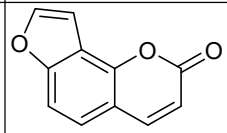
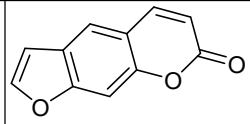
35	Corypsoriol J		Meroterpenoid	Fruit	Cytotoxic	(Xu et al., 2020)
36	Corypsoriol K		Meroterpenoid	Fruit	Cytotoxic	(Xu et al., 2020)
37	Corypsoriol L		Meroterpenoid	Fruit	Cytotoxic	(Xu et al., 2020)
38	Corypsoriol M		Meroterpenoid	Fruit	Cytotoxic	(Xu et al., 2020)
39	Corypsoriol N		Meroterpenoid	Fruit	Cytotoxic	(Xu et al., 2020)

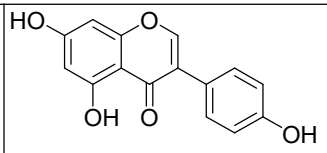
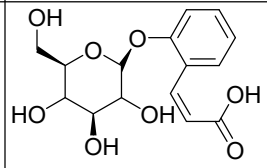
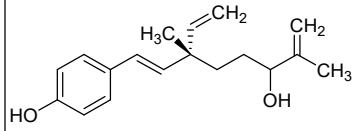
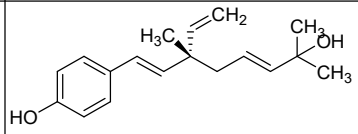
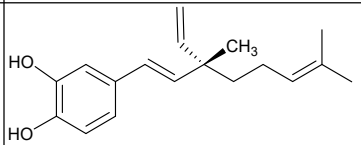
40	Corylinal		Isoflavone	Seed	Not reported	(Suri et al., 1978)
41	Corylifolin		Flavonoid	Leaves, seed	Anti- cancer	(Sun et al., 1998)
42	Coumesterol		Coumestan	Root	Estrogenic, Anti-cancer	(Rastogi et al., 2001; Liu et al., 2013)
43	Corylidin		Coumestrol	Fruits	Anti- microbial	(Gupta et al.,1977)
44	Corylin		Isoflavone	Fruit	Osteoblastic, anti-inflammatory	(Wanget al.,2001; Lee et al., 2012)
45	Corylinin (Corylifol A)		Isoflavone	Fruit	Effective in muscle atrophy, suppress IL-6-mediated STAT3 activation	(Ruan et al., 2007)

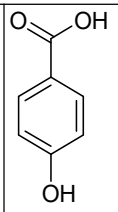
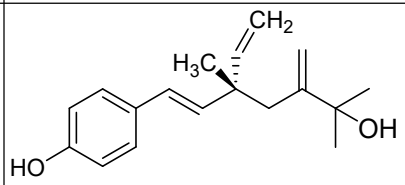
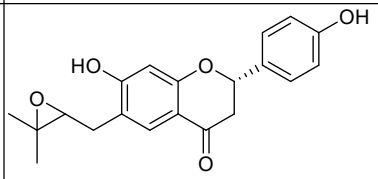
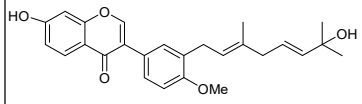
46	Corylifolinin		Flavonoid	Seed	Anti-microbial	(Wang et al., 2013)
47	Corylifonol		Benzofuran	Seed	Not reported	(Lin et al., 1992)
48	Cyclobakuchiol C		Meroterpene	Fruit	Inhibit influenza A virus	(Tan et al., 2015; Shojiet al., 2021)
49	Daidzein		Isoflavone	Fruit	Anti-oxidant, anti alcohol abuse action	(Zaheer et al., 2016)

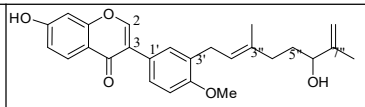
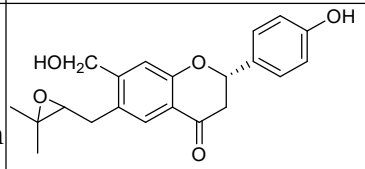
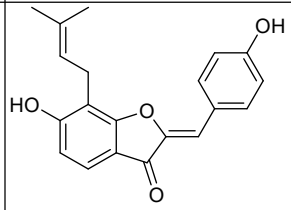
50	Daucosterol		Steroid	Seed	Anti-cancer	(Chopra et al., 2013; Zeng et al., 2017)
51	4", 5"-Dehydroisopsoralidin		Benzofurone	Seed	α- Glucosidase inhibition	(Qiu et al., 2011; Jiang et al., 2019)
52	Diadzin		Isoflavone	Fruit, Root	Anti-dipsotropic	(Rezvani et al., 2003; Yang et al., 2006)
53	12,13-Dihydro-12,13-epoxybakuchiol		Terpene	Fruit	Anti-inflammatory	(Chen et al., 2017)
54	5,4' –Dihydroxy-6,7-Furanbavachalcone		Flavonoids	Seed	Protein tyrosine phosphatase inhibition	(Ren et al., 2019)

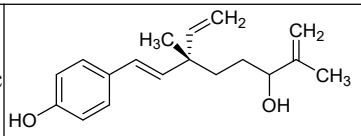
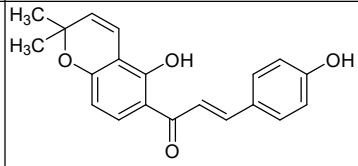
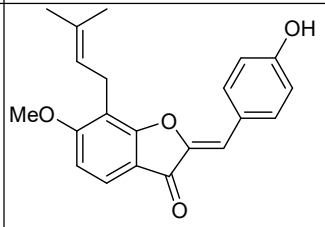
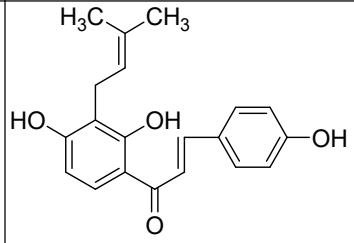
55	7, 8-Dihydro-8-(4-hydroxyphenyl)-2,2-dimethyl-2H,6H-benzo[1,2-*b*:5,4-*b*′]dipyran-6-one		Chromenoflavone	Seed	Inhibit Nitric oxide, anti-viral	(Yin et al., 2004; Lee et al., 2005)
56	4’,1”-Dihydroxy-3’-methoxy-6,7-furanflavanone		Flavonoid	Seed	Anti α-glucosidase	(Fei et al., 2020)
57	4-(3,7-Dimethyl-3-ethenylocta-1,6-dienyl]phenol		Phenol	Seed	Anti-bacterial	(Mohamed et al., 2017)
58	13-Docosenoic acid		Fatty acid	Seed	Effective in multiple sclerosis and has anti -alzheimer's effect	(Mohamed et al., 2017; Altinoz et al., 2019)
59	11-Eicosenoic	$CH_3(CH_2)_7CH=CH(CH_2)_9COOH$	Fatty acid	Seed	Anti-inflammatory	(Pereira et al., 2014);

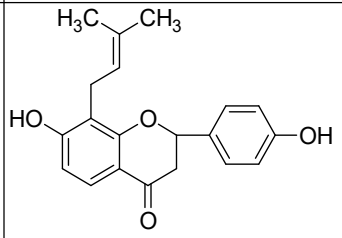
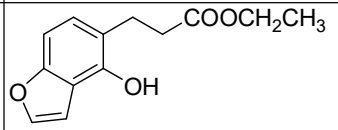
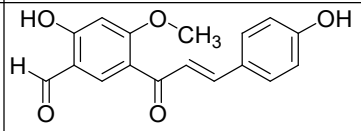
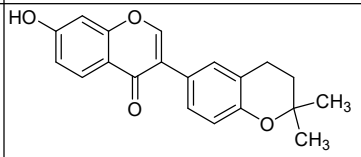
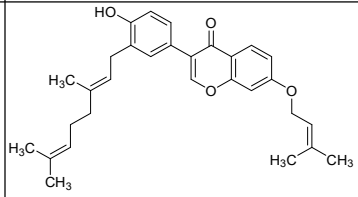
	acid					Mohamed et al., 2017)
60	Erythrinin A		Flavonoid	Seed	Antimicrobial	(Yin et al., 2004)
61	Estra-1,3,5 (10)-trien-17a-ol		Steroid	Seed	Anti-estrogenic activity	(Mohamed et al., 2017; Socrates et al., 2019)
62	Ethyl 13-docosenoate (ethyl erucate)		Fatty acid	Seed	Biochemical	(Mohamed et al., 2017)
63	2H-furo[2',3'-h][1]benzopyran-2-one		Coumarin	Seed	Anti-fungal	(Kiran et al., 2011b)
64	2H-furo[3',2'-g][1]benzopyran-2-one		Coumarin	Seed	Not reported	(Sah et al., 2006)

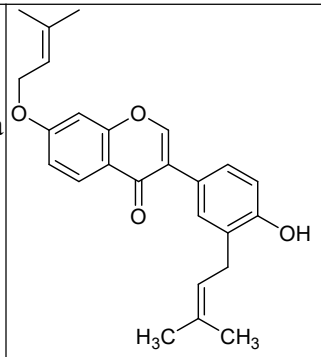
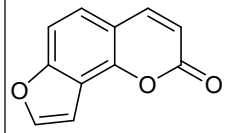
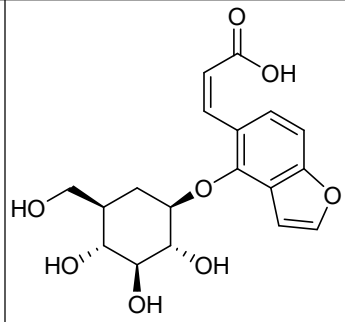
65	Genistein		Isoflavone	Fruit	Anti-oxidant, anti-cancer, cardio protectant	(Hsu et al., 2001; Ganai and Farooqi, 2015)
66	β-D-glucosyl-*cis*-O-hydroxycinnamic acid		Glucoside	Seed	Not reported	(Bourgaud et al., 2008)
67	3-Hydroxy-Δ^1-bakuchiol		Meroterpene	Fruit	Anti- microbial	(Matsuda et al., 2007; Zhang et al., 2016)
68	2-Hydroxy-Δ^3-bakuchiol		Meroterpene	Seed	Monoamine transporter inhibitors, anti- Parkinson	(Zhao et al., 2008; Zhao et al., 2009)
69	3-Hydroxybakuchiol		Meroterpene	Fruit	Putative ETC inhibitor	(Jana et al., 2013; Tan et al., 2015)

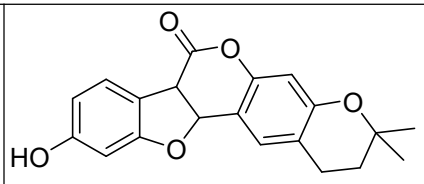
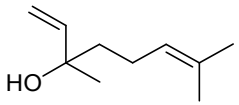
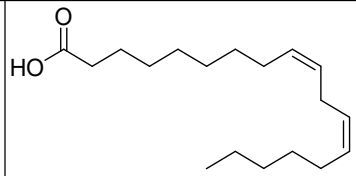
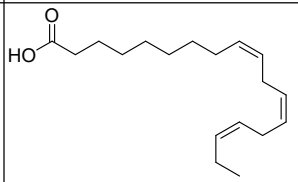
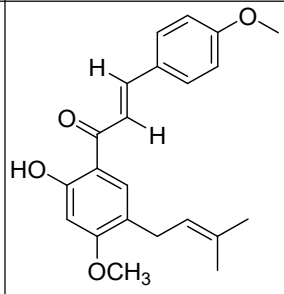
70	p-Hydroxybenzoic acid		monohydroxybenzoic acid	Seed	Anti-algal, anti-estrogenic, hypoglycemic, anti-inflammatory, anti-oxidant	(Krishnamurthi et al., 1969; Manuja et al. 2013)
71	Δ^{11}-12-Hydroxy-12-dimethyl bakuchiol		Meroterpene	Seed	Inhibits diacylglycerol acyltransferase (DGAT)	(Lin et al., 2018)
72	(2S)-7,4'-Hydroxyl-6-(2'',3''-epoxy-3''-methylbutyl)flavanone		Flavanone	Seed	α-Glucosidase inhibitory activity	(Liu et al., 2018)
73	7-Hydroxy-4'-methoxy-3'-(7''-hydroxy-3'',7''-dimethyl-octa-2'',5''-dienyl)-isoflavone		Isoflavones	Seed	Diacylglycerol acyltransferase inhibitory activity	(Wang et al., 2020)
74	7-Hydroxy-4'-		Isoflavones	Seed	diacylglycerol acyltransferase	(Wang et al., 2020)

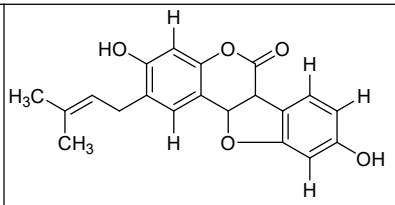
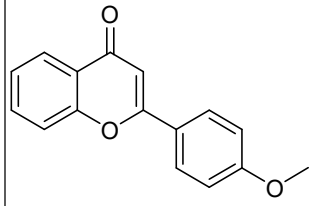
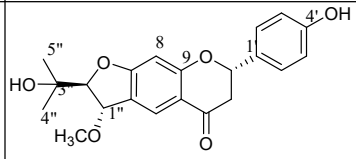
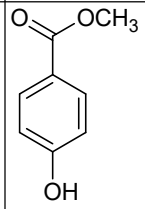
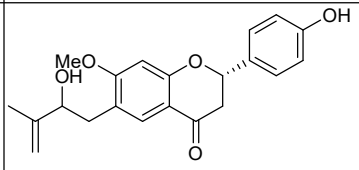
	methoxy-3'-(6''-hydroxy-3'',7''-dimethyl-octa-2'',7''-dienyl)-isoflavone				inhibitory activity	
75	(2*S*)-4′-Hydroxyl-7-hydroxymethylene-6-(2″,3″-epoxy-3″-methylbutyl)flavanone		Flavonoid	Seed	Anti- diabetic	(Zhu et al., 2018)
76	2*Z*)-2-[(4′-Hydroxyphenyl) methylene]-6-hydroxy-7-prenyl-3(2*H*)-benzofurane		Aurone	Seed	Anti- diabetic	(Zhu et al., 2018)

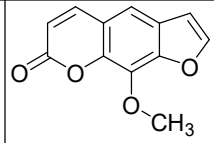
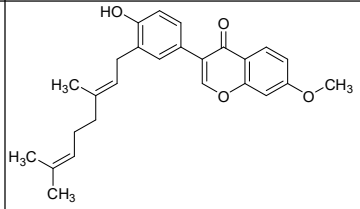
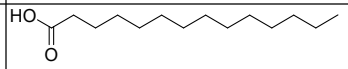
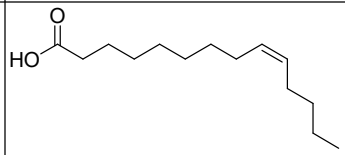
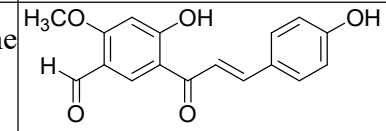
77	12-Hydroxyisobakuchiol		Meroterpene	Seed	Anti-cancer	(Yin et al., 2007; Madrid et al., 2015)
78	4-Hydroxylonchocarpin		Flavanone	Seed	Inhibit nitric oxide	(Lee et al., 2005)
79	(2Z)-2-[(4′-Hydroxyphenyl) methylene]-6-methoxy-7-prenyl-3(2H)-benzofurane		Aurone	Seed	Anti-cancer	(Liu et al., 2020)
80	Isobavachalcone		Chalcone	Seed	Anti-carcinogenic, effective in parkinson & alzheimer's disease, anti- bacterial and anti-fungal, inhibit pseudorabies virus	(Chen et al., 2010; Jing et al., 2010; Kuete et al., 2010; Lee et al., 2015; Wanget al., 2018; Wang et al., 2020)

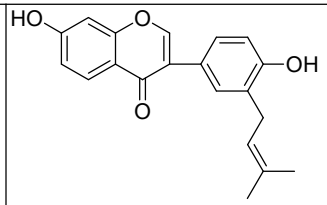
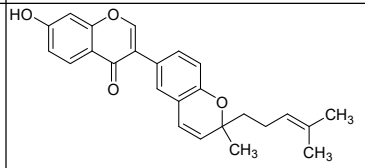
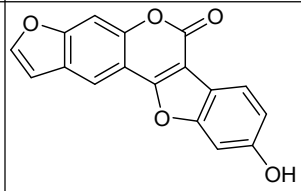
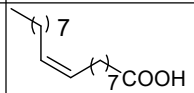
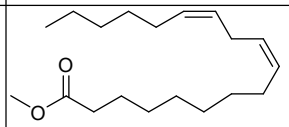
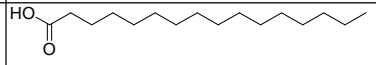
81	Isobavachin		Flavonoid	Fruit	Induceembryonic stem (ES) cells to neuronal cells, inhibit cytochrome P450s and UDP glucuronosyl transferases	(Wang et al., 2011; Chopra et al., 2013; Xing et al., 2020)
82	Isocorylifonol		Benzofuran	Seed	Not reported	(Lin et al., 1992)
83	Isoneobavachalcone		Chalcone	Seed	Not reported	(Dhar et al., 1980)
84	Isoneobavaisoflavone		Flavonoid	Seed	Anti-microbial	(Yinet al., 2004)
85	7-*O*-isoprenylcorylifol A		Flavanoid	Fruit	Anti-inflammatory	(Chen et al., 2017)

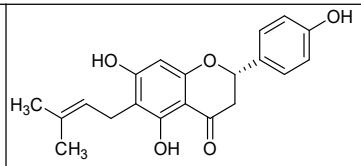
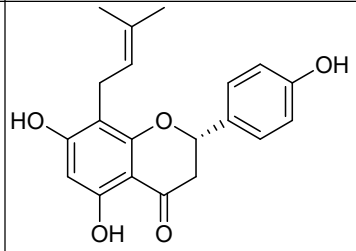
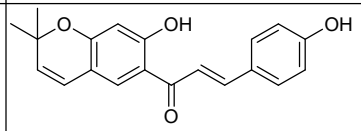
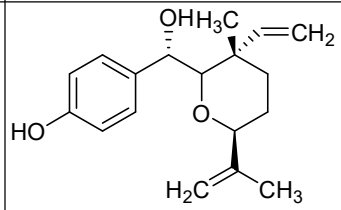
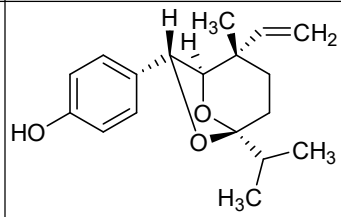
86	7-*O*-isoprenylneobavaisoflavone		Flavanoid	Fruit	Anti-inflammatory	(Chen et al., 2017)
87	Isopsoralen		Furocoumarin	Seed	Estrogenic, Anti-inflammatory, Anti- tumor, postmenopausal osteoporosis, Anti-bacterial, promote bone formation, Anti- viral	(Ding et al., 2004; Minget al., 2011; Wang et al., 2011; Cho et al., 2013; Li et al., 2017; Wei et al., 2016; Zhang and Na Ta, 2017; Li et al., 2018)
88	Isopsoralenoside		Benzofuran	Fruit	Estrogen-like activity, anti-tumor, anti-bacterial	(Qiao et al., 2006; Wang et al., 2014)

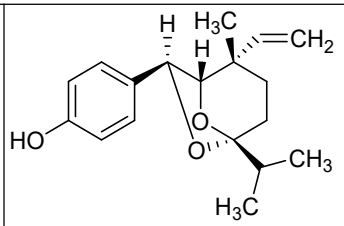
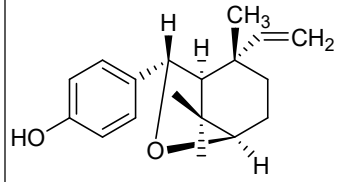
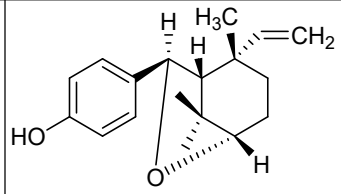
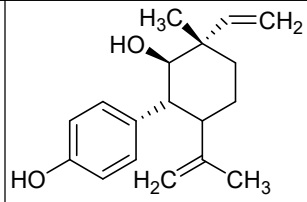
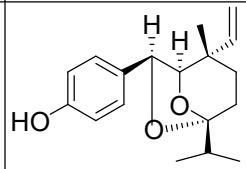
89	Isopsoralidin		Coumarin	Seed	Phosphatidylinositol 3-Kinase Inhibitors Phosphatidylinositol 3 kinase inhibitor	(Rastogi et al., 2004);
90	Linalool		Terpenol	Seed oil	Anti-microbial, anti-cancer, anti-oxidant	(Kamatou and Viljoen, 2008; Ahmed 2019)
91	Linoleic acid		Fatty acid	Seed oil	Skin moisturizer	(Parveen et al., 2020)
92	Linolenic acid		Fatty acid	Seed	Anti-microbial	(Dilika et al., 2000); Parveen et al., 2020)
93	4-O-methyl bavachalcone		Flavonoid	Seed	Inhibits tyrosinase	(Rastogi et al., 2001) Kim et al., 2010)

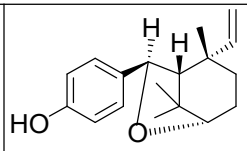
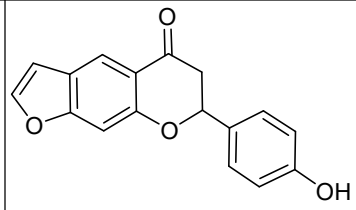
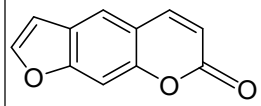
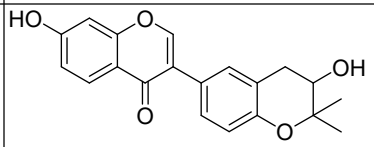
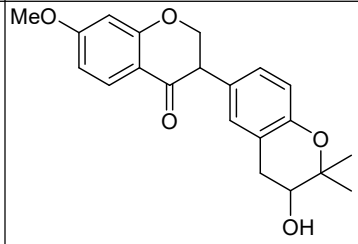
94	6-(-3-Methylbut-2-enyl)-6'-7-dihydroxycoumestan		Coumestan	Seed	Anti- insecticidal	(Khatune et al., 2002)
95	4′-Methoxyflavone		Flavone	Seed	Anti-dermatophytic	(Prasad et al., 2004; Bai et al., 2010)
96	1-methoxy-6,7 furanflavanone		Flavonoid	Seed	Protein tyrosine phosphatase inhibition	(Ren et al., 2019)
97	Methyl 4-hydroxybenzoate		Methyl ester	Seed	Anti-fungal	(Qiu et al., 2011)
98	(2*S*)-7-Methoxy-6-(2-hydroxy-3-methylbut-3-en-1-yl)-2-(4-		Flavonoid	Seed	Anti- diabetic	(Zhu et al., 2018)

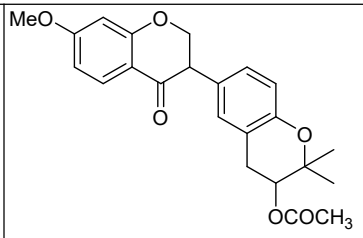
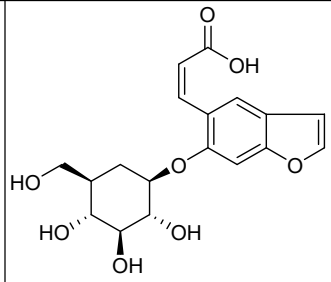
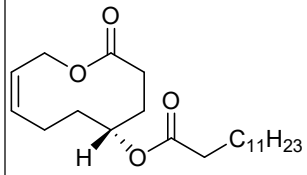
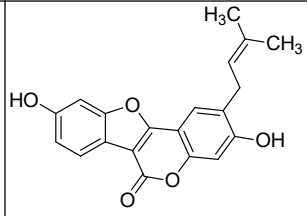
	hydroxyphenyl)chroman-4-one					
99	8-Methoxy psoralen		furocoumarins	Seed	PUVA	(Yurkow and Laskin, 1991; Chopra et al.,2013)
100	7-*O*-methylcorylifol A		Flavanoid	Fruit	Inhibit superoxide anion	(Chen et al., 2017)
101	Myristic acid		Fatty acid	Seed	Anxiolytic effect	(Parveen et al., 2020; Contreras et al., 2014)
102	Myristolic acid		Fatty acid	Seed	Anti-cancer	(Parveen et al., 2020)
103	Neobavachalcone		Chalcone	Seed	Not reported	(Gupta et al., 1977)

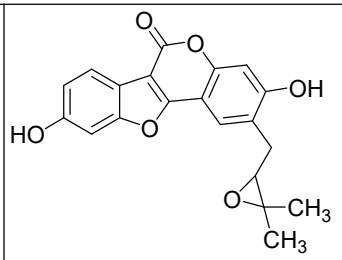
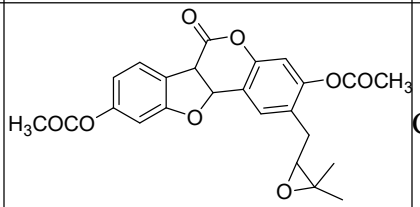
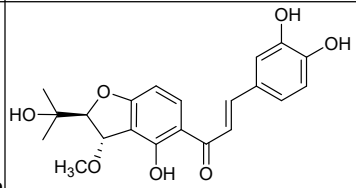
104	Neobavaisoflavone		Flavonoid	Seed	Inhibit platelet aggregation, Anti- inflammatory, estrogenic activity, Fatty acid amide hydrolase inhibitor	(Tsaiet al.,1996; Xin et al. 2009; Gao et al., 2015; Szliszkaet al.,2011; Chen 2021)
105	Neocorylin		Isoflavone	Seed	BACE 1 inhibitor	(Choi et al., 2008)
106	Neo-psoralen		Coumarin	Seed	Growth of skin cells in psoriasis	(Guoping et al.,1996)
107	Oleic acid		Fatty acid	Seed oil	Anti-microbial	(Dilika et al., 2000; Parveen et al.,2020)
108	9,12-Octadecadienoic acid (Z, Z)-, methyl ester		Fatty acid	Seed	Cytotoxic	(Mohamed et al., 2017)
109	Palmitic acid		Fatty acid	Seed oil	Increases levels of cholesterol and promote fat deposition	(Parveen et al., 2020)

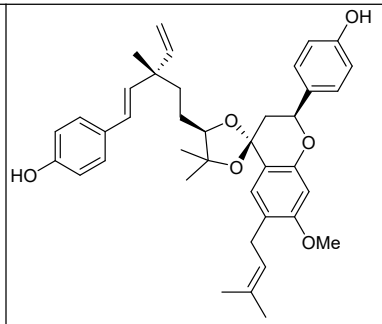
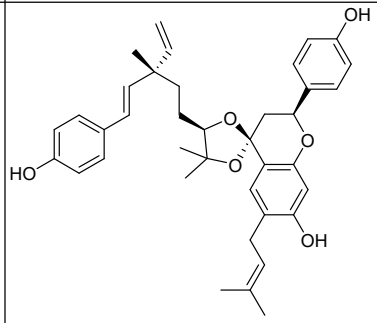
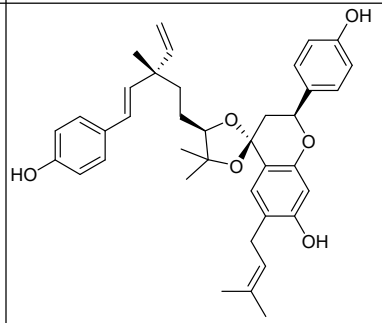
110	6-Prenylnaringenin		Flavanone	Seed	Aldose reductase inhibitory activity	(Shim et al., 2009)
111	8-Prenylnaringenin		Flavanone	Seed	Not reported	(Yin et al., 2004)
112	Psorachromene		Flavonoid	Seed	Anti- tumor	(Wang et al., 2019)
113	Psoracorylifol A		Phenol	Seeds	Active against *Helicobacter pylori*	(Yin et al., 2006)
114	Psoracorylifol B		Phenol	Seed	Anti-microbial	(Yin et al., 2006)

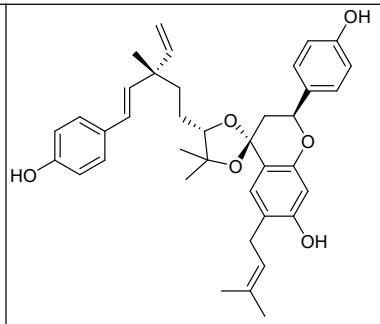
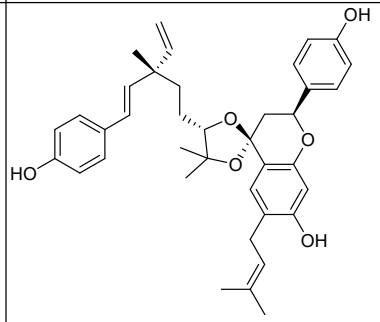
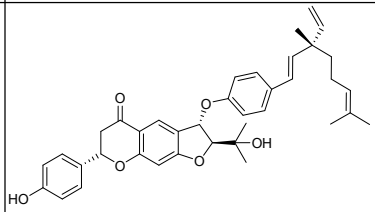
115	Psoracorylifol C		Phenol	Seed	Anti-microbial	(Yin et al., 2006)
116	Psoracorylifol D		Phenol	Seed	Lymphangiogenesis inhibition	(Yin et al.,2006; Jeong et al., 2013)
117	Psoracorylifol E		Phenol	Seed	Active against gram negative pathogen *H. pylori*	(Yin et al., 2006)
118	Psoracorylifol F		Meroterpene	Fruit	Inhibit NO production	(Xiao et al., 2012)
119	7*β*, 13*β*-Psoracorylifol B		Meroterpene	Seed	Diacylglycerol acyltransferase inhibition	(Wang et al., 2020)

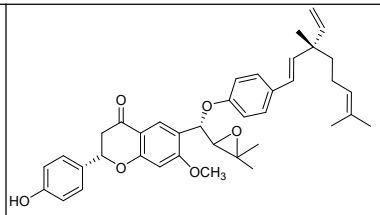
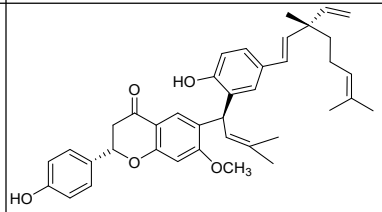
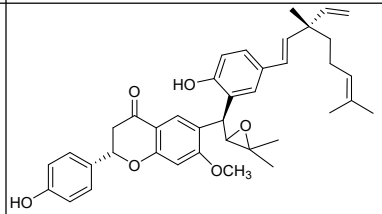
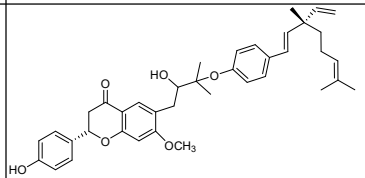
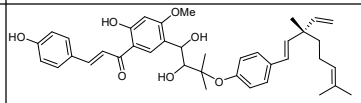
120	7*β*, 8*α*-Psoracorylifol D		Meroterpene	Seed	Diacylglycerol acyltransferase inhibition	(Wang et al., 2020)
121	Psoraleflavanone or furano (2", 3", 7, 6)-4'-hydroxyflavanone		Flavanoid	Seed	Not reported	(Liu et al., 2008)
122	Psoralen		Furanocoumarins	Seed, fruit	Anti- tumor Anti- microbial Anti-inflammatory photoinactivation of virus, osteoporosis, Anti-depressant	(Fendrick et al.,1984; Liu et al., 2005; Xu et al. 2008; Chiang et al., 2010; Wang et al., 2011; Chen et al., 2017; Li et al., 2017)
123	Psoralenol		Isoflavone	Seed	Not reported	(Suri et al., 1978)
124	Psoralenol methyl ether		Ether	Seed	Not reported	Suri et al. (1978)

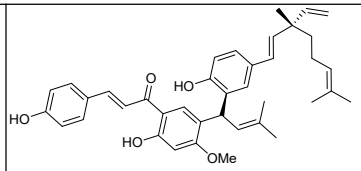
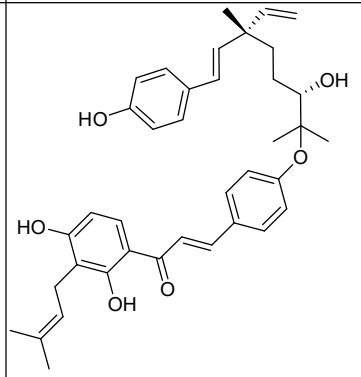
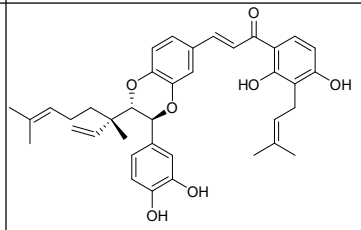
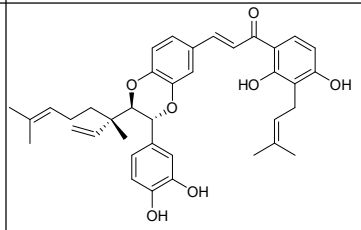
125	Psoralenol monomethyl ether monoacetate		Isoflavone	Seed	Not reported	Suri et al. (1978)
126	Psoralenoside		Benzofuran	Fruit	Inhibits HDAC1 - histone deacetylase 1	Qiao et al. (2006)
127	Psoralester		Chalcone	Seed	Not reported	Tewari et al. (2010)
128	Psoralidin		Phenolic coumarin	Seed, Leaves	Anti-protozoal, anti-bacterial, anti-depressant, anti-tumor, effective against viral infection, vasodilatory, neuroprotective	(Nazninet al., 2004; Jiangning et al., 2005; Yiet al., 2008; Xiaoet al., 2010; Szliszkaet al., 2011; Kim et al., 2014; Song et al., 2015; Kimet al., 2016; Gebremeskel et al., 2017)

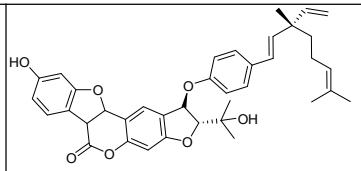
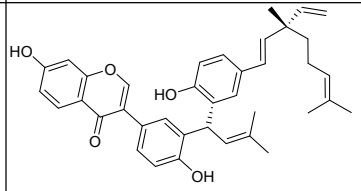
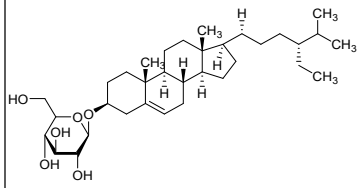
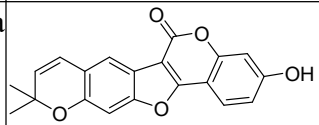
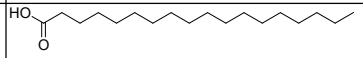
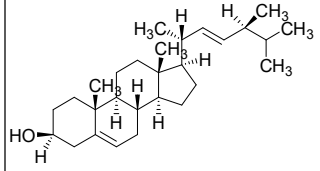
129	Psoralidin-2′, 3′-oxide		Coumastan	Seed	Not reported	(Chopra et al., 2013)
130	Psoralidin-2,3-oxide diacetate		Coumestan	Seed	Not reported	(Rastogi et al., 2001)
131	5,3’,4’-trihydroxy-1”-methoxy-6,7-furanbavachalcone		Flavonoid	Seed	Anti-α-glucosidase	(Fei et al., 2020)

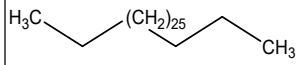
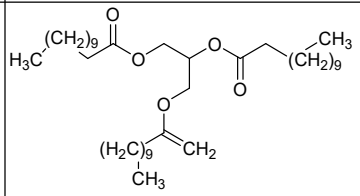
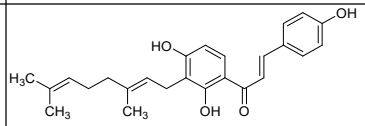
132	Psocorylin A		Meroterpene phenol	Fruit	Cytotoxic	(Xu et al., 2020)
133	Psocorylin B		Meroterpene phenol	Fruit	Cytotoxic	(Xu et al., 2020)
134	Psocorylin C		Meroterpene phenol	Fruit	Cytotoxic	(Xu et al., 2020)

135	Psocorylin D		Meroterpene phenol	Fruit	Cytotoxic	(Xu et al., 2020)
136	Psocorylin E		Meroterpene pheno	Fruit	Cytotoxic	(Xu et al., 2020)
137	Psocorylin F		Meroterpene pheno	Fruit	Cytotoxic	(Xu et al., 2020)

138	Psocorylin G		Meroterpene pheno	Fruit	Cytotoxic	(Xu et al., 2020)
139	Psocorylin H		Meroterpene phenol	Fruit	Cytotoxic	(Xu et al., 2020)
140	Psocorylin I		Meroterpene phenol	Fruit	Cytotoxic	(Xu et al., 2020)
141	Psocorylin J		Meroterpene phenol	Fruit	Cytotoxic	(Xu et al., 2020)
142	Psocorylin K		Meroterpene phenol	Fruit	Cytotoxic	(Xu et al., 2020)

143	Psocorylin L		Meroterpene phenol	Fruit	Cytotoxic	(Xu et al., 2020)
144	Psocorylin M		Meroterpene phenol	Fruit	Cytotoxic	(Xu et al., 2020)
145	Psocorylin N		Meroterpene phenol	Fruit	Cytotoxic	(Xu et al., 2020)
146	Psocorylin O		Meroterpene phenol	Fruit	Cytotoxic	(Xu et al., 2020)

147	Psocorylin P		Meroterpene phenol	Fruit	Cytotoxic	(Xu et al., 2020)
148	Psocorylin Q		Meroterpene phenol	Fruit	Cytotoxic	(Xu et al., 2020)
149	β-Sitosterol-D-glucoside		Steroid	Seed	Anti-helmintic	(Rastogi et al., 1999; Deepak et al., 2002)
150	Sophoracoumestan A		Coumestan	Fruit, Seed	Not reported	(Ruan et al., 2007)
151	Stearic acid		Fatty acid	Seed oil	Skin cleansing	(Parveen et al., 2020)
152	Stigmasterol		Sterol	Seed oil	Maintain the structure and physiology of cell membranes	(Rastogi et al.,1999)

153	Triacontane		Alkane	Seed	Anti-bacterial	(Rastogi et al.,1999)
154	Trilaurin		Triglyceride	Root	Used in cosmetic drugs	(Rastogi et al., 2001)
155	Xanthoangelol		Chalcone	Seed	Anti-cancer	(Limper et al., 2013)

2.4.1.7 Pharmacological activities

Since *P. corylifolia* has remarkable therapeutic potential, therefore it has grabbed the attention of the research community. **Figure 2.3** summarizes various health benefits associated with *P. corylifolia*.

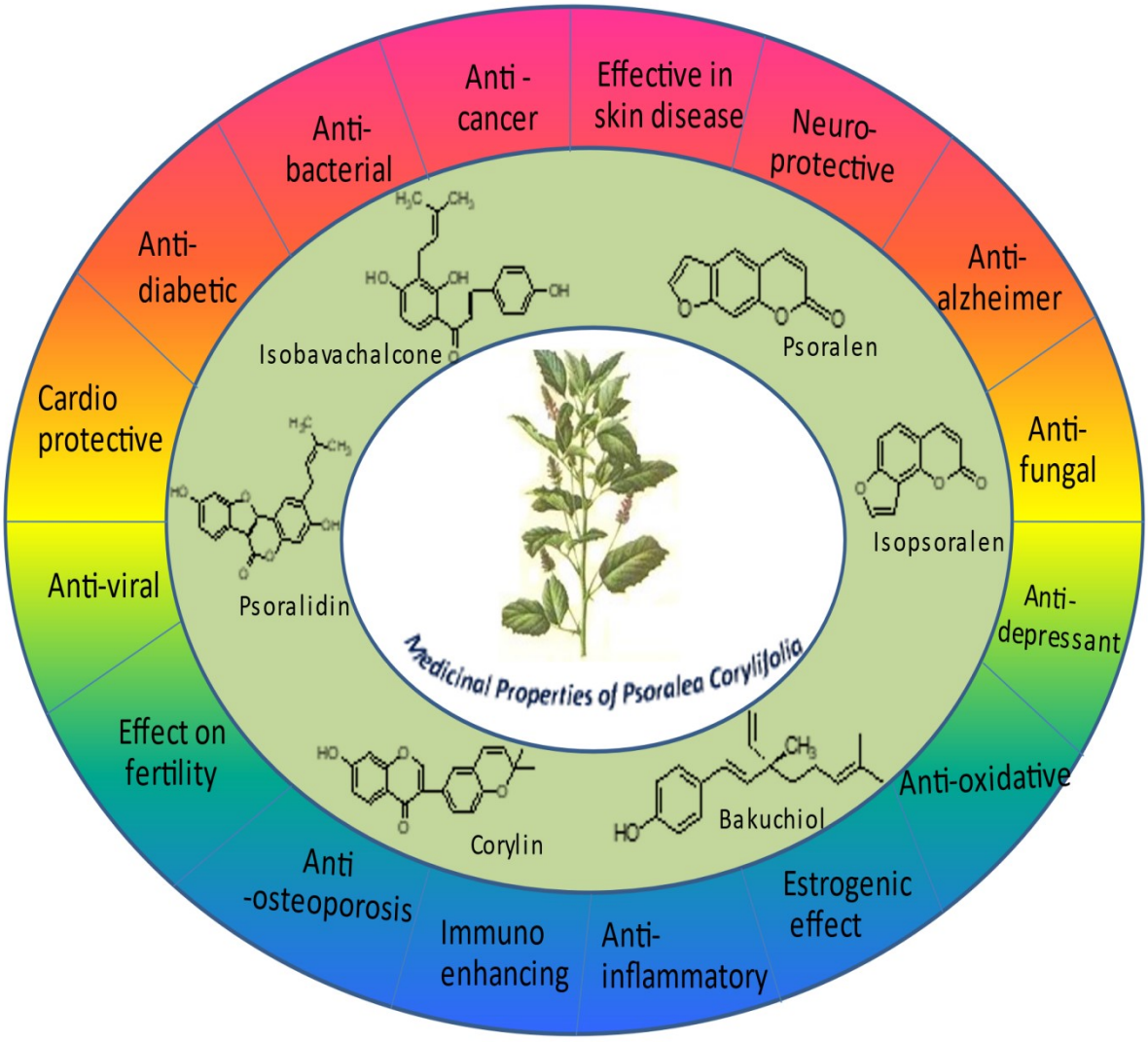


Figure 2.3 Pharmaceutical properties of *Psoralea corylifolia*

2.4.1.7.1 Anti-bacterial

Bakuchiol, an isolate from seeds of *P. corylifolia* inhibited oral microorganisms *Streptococcus mutans*, *S. sanguis*, *S. salivarius*, *S. sobrinus*, *Enterococcus faecalis*, *E. faecium*, *Porphyromonas gingivalis* etc. with the range of MIC value from 1 to 4 µg/ml (Katsura et al., 2001). Methanolic extracts (ME) of leaf and callus of *P. corylifolia* was disclosed to be effective against periodontitis causing organisms *Porphyromonas gingivilis*, *Prevotella intermedia*, *Camphylobacter rectus* and *Actinobacillus actinomycetemcomitans*. Leaf extract was effective at 30 mg/ml, and callus extract was effective at all the tested conc. (Moon et al., 2012). Thus, it was found that this plant had the potential to treat oral diseases.

It was reported by Acharya and his coworkers who tested the anti-microbial action of various Bakuchi preparations, including "Bakuchi taila," "Bakuchi gel," "Bakuchi siktha taila," and "Bakuchi ointment," that all of the dose forms reduced the development of *Staphylococcus aureus, Bacillus subtilis* (a gram-positive), *Escherichia coli*, and *Klebsiella pneumoniae* (a gram-negative) but the inhibition zone formed was highest in case of 'Bakuchi gel' against *Staphylococcus aureus*. Consequently, it can be utilised as an anti-microbial component in a variety of topical skin compositions (Acharya et al., 2015). Among all the tested fractions, the ME fraction of *P. corylifolia* seeds was found to be most active in inhibiting quorum sensing (QS), and biofilm formation in *Pseudomonas aeruginosa*, *Aeromonas hydrophila*, *Chromobacterium violaceum,* and *Serratia marcescens,* and the activity was found to be dose-dependent. The anti-QS action of bakuchiol, which was obtained from the ethanolic extract, was demonstrated by its ability to prevent the growth of biofilms and the synthesis of violacein in *C. violaceum*. According to molecular docking experiments, the compound's capacity to attach to the active pockets of LasR and RhlR (transcriptional activator proteins), both of which are involved in QS, is what gives it its therapeutic effects (Husain et al., 2018).

"Corylifolinin', one of the bioactive compounds of *P. corylifolia,* was reported to be active against a wide range of *Staphylococcus* species, and its anti-bacterial property is due to changes in bacterial morphology, alkaline phosphatase and extracellular soluble protein amount (He et al., 2018). Thus, *P. corylifolia* extracts exhibit anti-bacterial properties against a wide range of bacteria.

2.4.1.7.2 Anti-fungal

Bakuchiol, a phytochemical present in *P. corilifolia* extracts, possess anti-dermatophytic properties. In a study, it was reported that bakuchiol inhibited the growth of *Trichophyton mentagrophytes, Trichophyton rubrum* and *Paecilomyces variotii* with MIC values of 7.81, 15.6 and 15.6 g/ml, respectively (Lau et al., 2010). In another report, *P. corylifolia* extracts were tested against fungi *Aspergillus flavus oryzae* and *A. tamari*, the seed-borne fungi of maize. The highest antifungal activity was exhibited by petroleum ether extract against both fungi at a 2% concentration (Kiran et al., 2011 a). Later in 2012 (Srinivasan et al., 2012), again the seed extract of *P. corylifolia* has tested against the different species of fungi *Fusarium* and was found to inhibit all the tested fungi. A phenyl derivative of 'Pyranocoumarin' ($C_{27}H_{28}O_4$) was isolated from this extract, and it also had an anti-fungal activity with a MIC of 1 mg/ml. According to molecular docking studies, this compound prevents the acetylation capability of Trichothecene 3-O-Acetyltransferase by binding to it, leading to a decline in fungi. It was observed that Psc-AFP, a protein from *P. corylifolia* seeds, suppressed the mycelial growth of different fungus species at a conc. of 10 μM, and authors disclosed that Psc-AFP is a trypsin inhibitor protein and can be used against fungal pathogens (Yang et al., 2006). Later on, in 2020, Li et al. disclosed that ethanol extracts of *P. corylifolia* (seed) and *Sophora flavescens* (root) could inhibit *Phytophthora nicotianae*, the major pathogen of tobacco. The anti-fungal activity was due to restriction on sporangia development and zoospore release and was comparatively better in *P. corylifolia*. At conc. of 1.96 mg/ml and 0.98 mg/ml, respectively, the extract totally prevented the development of sporangium and the release of zoospores (Li et al., 2020).

2.4.1.7. 3 Hypoglycemic

The kinase 5′-AMP-activated protein kinase (AMPK), which plays a role in type 2 diabetes, is activated by ATP concentration in the cell. AMPK activation is required for the regulation of glucose transport and Akt kinase. In a study on adipocytes, activation of the Akt and AMPK pathways by bavachin, extracted from the EtOAc fraction of *P. corylifolia* seeds, has indeed been found to boost glucose intake via GLUT4 translocation (Lee et al., 2016). In mice treated with methylglyoxal (MGO), which causes insulin resistance, p-Akt and p-IRS1/2 expression were dramatically restored in the liver by *P. corylifolia* extract. Besides, enhanced glucose

tolerance and insulin sensitivity were observed at conc. of 200 and 500 mg/kg of *P. corylifolia* seed extract. Truong and co-workers, in 2019, reported that PCS extract greatly increased Akt and IRS-1/2 phosphorylation as well as glucose uptake in MGO-treated HepG2 cells, and there was a reduction in the formation of glycation ending products and ERK (kinase), p38, and NF-B phosphorylation (Truong et al., 2019). *P. corylifolia* is helpful in diabetes- nephropathy, a severe complication of diabetes mellitus. This protective function is due to the extract´s anti-fibrotic and anti-apoptotic properties as well as the activity of 'isopsoralen' and 'psoralen' against high glucose-induced mesangial cell injury. Thus *P. corylifolia* is a good candidate against complications of diabetes (Seo et al., 2017).

Some flavonoid molecules extracted from *P. corylifolia* seeds demonstrated hyperglycemic effect by inhibiting diacylglycerol acyltransferase. Inhibition of DGAT causes lesser damage to β–cells which secrete insulin (Zhu et al., 2018). Recently, Dong et al. revealed that 'isobavachalcone' recovers diabetic nephropathy by inhibiting the *NF-κB* pathway (Dong et al., 2020).

2.4.1.7.4 Skin diseases

There is strong evidence supporting that *P. corylifolia* seed extracts are effective in treating skin diseases.

2.4.1.7.5 Anti-vitiligo

A study involving twenty patients ranging in age from 18 to 60 years old was carried out to investigate the activity/role of 'bakuchiol' for vitiligo treatment. Once a day, the volunteers applied the hydrophilic ointment containing 10% *P. corylifolia* seed powder onto the white patches. A small portion of the affected part of the patient was taken as control. Five volunteers developed itching and were given betamethasone for application twice daily on the affected part. After a couple of days of ointment application and regular 2-3 h exposure to the sun, a change in colouration in the patch from white to red was observed. No relapse in skin colouration was observed even after 3 months of treatment. On the basis of these findings, it was concluded that *P. corylifolia* is beneficial in the treatment of white lesions of vitiligo (Hussain et al., 2016). In another report, 24 weekly studies were conducted on a 17-year-old male who had vitiligo. He

was orally administered 5 gm *P. corylifolia* seed powder, and a cream containing 5% hydroalcoholic seed extract was applied to the affected area once a day.

During day-to-day activities, the subject got exposed to sunlight each day. During the experiment/treatment, complete blood count (CBC), LFT and RFT remained normal, and skin colour changed from white to normal. In this report, the authors disclosed that no side effects or drug-induced reactions occurred. Thus, the oral and topical treatment with *P. corylifolia* seeds-powder-ointment can synergistically and effectively treat vitiligo (Hussain et al., 2019). Recently, Mir and coworkers reported the utilization of *P. corylifolia* in the management of vitiligo. The authors revealed that an experiment was carried out on sixty patients of dermatological and outpatients at NIH, India. The effects of three treatment regimes – (a) individualized homoeopathic medicines (IH), (b) *P. corylifolia* mother tincture external application, and (c) IHPC in the treatment of vitiligo were observed and analysed. Because this study was done on a pilot size, a clear conclusion cannot be formed based on the data, which demonstrated that all three regimes were equally useful and safe, with a modest direction of effect towards PC and IHPC (Mir et al., 2021).

2.4.1.7.6 Anti-Psoriasis

Bakuchiol salicylate (bakusylan), a synthesized molecule, was found to minimize the psoriasis-like phenotype and cytokines *IL-8* and *CXLC3* in skin substitutes. These cytokines are primarily responsible for adverse effects in case retinoids are used in treating psoriasis. The plausible mechanism involves keratinocyte desensitization to psoriatic cytokine by a reduction in the expression of STAT1 and STAT1-controlled genes (Ma et al., 2017). Psoriasis leads to keratinocyte proliferation and infiltration of immune cells. In a report, it was revealed that deposition of 8-MOP, isopsoralen, and bakuchiol at equal doses on pig ear skin was almost similar and was higher than that of psoralen and psoralidin. However, psoralidin and bakuchiol did not show penetration beyond the skin into the receptor. The antiproliferative activity of 8-MOP and isopsoralen against keratinocytes was greater than that of the other compounds. UVA exposure, along with topical applications of 8-MOP and isopsoralen, greatly reduced epidermal thickness. Isopsoralen, but not 8-MOP, reduced IL-6 expression in psoriasis. As a result, isopsoralen may be an appropriate candidate for PUVA therapy (Alalaiwe et al., 2018). Psoralen,

along with ultravioletA radiation, is an FDA endorsed drug/treatment in psoriasis, but its less permeability is the main problem with this treatment. The research group tried to increase the permeability by using psoralen loaded liposomal carriers and was successful. A five times increase in permeability of psoralen loaded with cationic or anionic liposomal carriers was observed. A decrease in psoriasis symptoms and cytokines such as IL-17 and IL-22 was caused by psoralen liposomal gels (Doppalapudi et al., 2017). Cyclodextrin based nanocarrier gel of 'babchi oil' (BO) was also tested for curing psoriasis using a mouse model. Anti-psoriatic efficiency was found to be twice in this nano-based BO gel as compared to Babchi oil alone. A positive change in psoriasis associated oxidative stress biomarkers was observed, which suggests that the nano gel of Babchi oil can be a reliable alternative in the management of psoriasis (Kumar et al., 2019).

2.4.1.7.7 Anti-wrinkle and skin whitening effect

A twelve-week, randomized, double-blind study on 44 patients was conducted in which they were asked to use two times a day either '0.5 % bakuchiol' or '0.5% retinol'. High-resolution images of patients were analyzed, and they were given a questionnaire to learn about the side effects. There was no statistical difference between bakuchiol and retinol in terms of minimizing wrinkles and hyperpigmentation. However, facial skin scaling and stinging were reported by retinol users only, and hence bakuchiol was better tolerated than retinol (Dhaliwa et al., 2018). Bakuchiol is a secure ingredient in cosmetics products and can be used by a person with sensitive skin (Draelos et al., 2020). Further, it can be used as an ingredient in skin creams due to its reliable anti-oxidant, anti-microbial and skin conditioning properties (Jafernik et al., 2020).

2.4.1.7.8 Anti-inflammatory

In both Lipopolysaccharide activated RAW 264.7 cells and murine peritoneal macrophages, 'corylin' from *P. corylifolia* decreased the TNFα, IL-6, and NO production. Furthermore, corylin suppressed *iNOS* and COX-2 expression, decreased PGE2 and HMGB1 production, blocked HMGB1 translocation from the nucleus to the cytosol, and decreased *MAPK* phosphorylation in LPS-activated RAW 264.7 cells. Moreover, liver and kidney damage marker levels returned to normal after corylin treatment. Thus, corylin possess anti-inflammatory property (Hung et al., 2017). The anti-inflammatory potential of 'bavachin' was investigated and

found to suppress LPS-induced inflammation and activation of *NLRP3* (NLR Family Pyrin Domain Containing 3) inflammasome in macrophages (Hung et al., 2019). In a report, Li et al. (2021) reported that 'psoralen' possesses anti-inflammatory and estrogen-like activity. In periodontitis, one of the oral problems, inflammation is the main issue caused due to *P. gingivalis*. With molecule docking studies, it was found that psoralen can combine better with *ERα* (Estrogen receptor alpha) than with *ERβ*. Also, it can decrease the expression of inflammatory cytokines which were secreted by human periodontal ligament cells due to *P. gingivalis.* Thus, psoralen can exert estrogen-like effects and block *TLR* (Toll like receptor) *4/NF κB* signaling pathway, lowering the levels of inflammatory cytokines (Li et al., 2021).

2.4.1.7.9 Cardio-protective

'Bakuchiol' has been reported to decrease ischemia-reperfusion damage (IRI) by stimulating the SIRT3 (Sirtuin 3)/PGC-1 signalling cascade and decreasing oxidative stress in mitochondria (Liu 2018). Wang et al. (2018) observed that bakuchiol inhibits the NF-κB signalling pathway and is helpful in cardiac hypertrophy in cardiomyocytes. In another study conducted by Ma et al., in 2020, bakuchiol played a significant part in heart hypertrophy, a disease common with diabetic patients. This activity can be attributed to the activation of the SIRT1/Nrf2 signalling pathway, which decreases ROS (Reactive oxygen species). Following treatment, there was a decline in cardiomyocyte-apoptosis and myocardial fibrosis (Ma et al., 2020).

2.4.1.7.10 Immunostimulant

Using the J774A.1 cell line, both the ethanolic extract of *P. corylifolia* and *Nigella sativa* showed immunostimulant properties. At 25 µg/ml, *N. sativa* had the highest relative activity of 138.77 per cent macrophage cell proliferation, and *P. corylifolia* had the highest relative activity of 80.70 per cent proliferation (Mohamed et al., 2017). Earlier, there were reports of immunomodulatory activities of *P. corylifolia* due to cell-mediated and humoral immune responses and an increase in NK (Natural killer) cells (Lathaa et al., 2000). Bakuchiol reduced delayed form hypersensitivity, antibody titre, and inflammatory cytokines in a concentration-dependent manner. It accomplishes this by inhibiting nuclear factor-κB, IκBα and p65 (Kumar et

al., 2021). PCp-I, a water-soluble heteroglycan from *Psoralea corylifolia*, was discovered to have an immunomodulatory effect on RAW264.7 cells through the NF-B/MAPK signalling pathway (Wang et al., 2021).

2.4.1.7.11 Neuroprotective effect

N-methyl-D-aspartate receptor plays a significant part in memory by modulating immediate early genes (IEGs) expression and hence controlling long-term potentiation (LTP). In the present study, a library of 436 natural compounds was screened to check their effect on IEGs, and MAPK signaling by Erk1/2 phosphorylation was done. Psoralidin was found to be the most active compound and at 5 μM concentration caused a 1.7–2-fold increase in Erk1/2 phosphorylation but did not have any effect on phospho-Akt at Serine473. Psoralidin increased the expression of *IEGs* like *Arc*, *c-fos* and *Egr-1* in a conc.-dependent manner. Further, it was revealed that psoralidin cause structural change in excitatory synapses, such as their density and area, which was conciliated due to the activation of NMDA receptor and MAPK signaling (Hwang et al., 2018). A study on mice was conducted to estimate the effect of bakuchiol on subarachnoid haemorrhage (SAH), which involves early brain injury. Bakuchiol decreases the death rate of mice and assuages damage to the blood-brain barrier. A reduction in brain edema, superoxide amount and neuron damage were also observed (Liu et al., 2020).

2.4.1.7.12 Hepatoprotective activity

By using bioassay-guided fractionation of the aqueous extract of *Psoralea corylifolia* seeds in tacrine-induced cytotoxicity in human liver-derived Hep G2 cells, one hepatoprotective molecule, bakuchiol, as well as two moderately active chemicals, bakuchicin and psoralen, were identified. Compounds bakuchiol, bakuchinin and psoralen have EC 50 values of 1.0, 47.0, and 50.0 mg/ml respectively (Cho et al., 2001). Bakuchiol treatment decreases activated hepatic stellate cells *in vivo* after liver injury induction, and *in vitro* it induced caspase-3 dependent apoptosis in activated hepatic stellate cells and myofibroblasts by activating c-Jun NH2-terminal protein kinase (JNK) signaling. *P. corylifolia*, seed water extract, reduces the generation of ROS and mitochondrial dysfunction brought on by oxidative stress in hepatocytes, and bakuchiol is primarily accountable for this protective activity (Zhang et al., 2016).

2.4.1.8 Biotechnological interventions

P. corylifolia has high demand due to its versatile properties, but overexploitation and low seed germination capability lead to the depletion of *P. corylifolia* in its natural environment. Biotechnological applications can be used in the conservation of such valuable plants having a commercial applications. In a study, *P. corylifolia* root segments that can form embryogenic calluses were introduced to MS media containing Naphthaleneacetic acid (NAA) (1.34mM) and benzyl adenine (BA) (2.2-8.8mM) and the calluses were regenerated (Chand et al., 2002). Gamma radiation at 20 kGy increased the content of psoralen in *P. corylifolia* seeds by up to 7.56%. (Jan et al., 2011). Jasmonic acid at a concentration of 10 M boosted "daidzin-synthesis" in the hairy root culture of *P. corylifolia* by 7.3-fold after the 10th week, and acetylsalicylic acid at a concentration of 25 M increased it by 2.3-fold (Zaheer et al., 2016). Psoralen amount increased to 13.6 and 10.2-fold, respectively, when methyl jasmonate (MeJA) or salicylic acid (SA) was used as elicitors along with precursor *trans*-cinnamic acid (CA) in *P. corylifolia* leaf-callus suspension culture. However, when both these elicitors were added in combination, there wasn´t any enhancement in psoralen production (Kumar et al., 2018). It is reported that *the* isoflavone synthase (IFS) gene having a cDNA of 1,563 bp, is expressed in all parts of *P. corylifolia* and responds to methyl jasmonate, salicylic acid and wounding (Misra et al., 2010).

2.4.1.9 Toxicity trials

The safety and efficacy of *P. corylifolia* extracts have been verified through time and research. However, still, there is scope for standardization of the dosage of bioactive compounds reported in this plant. In a few of the studies, it has appeared to be associated with some negative impacts. A study on HepG2 cells found that psoralen results in liver injury by inducing ER stress-mediated apoptosis (Yu et al., 2019). Previous reports revealed that bakuchiol at a high dose is hepatotoxic (Jiang et al., 2010). An in-vivo study on rats was performed to test this, and it was discovered that the hepatotoxicity of bakuchiol is caused by a change in the lipid metabolism of HMG-CoA via the *RohA* pathway and *PPAR* via the induction of *LXR* (Liver X Receptor) expression (Li et al., 2017). A 60-year-old patient with a persistent cough admitted to the hospital was given TCM (Traditional Chinese Medicine) herbal footbaths containing 5g *Psoraleae fructus*. Two weeks after her discharge, she went on with her footbaths and sat under

sunlight, and developed painful blebs on her feet. The blebs are probably a phototoxic side-effect (Bachmeier et al., 2019).

Although there are several reports of safe trials with some mild or negligible toxicity, therefore there is scope for standardization of the dosage of bioactive compounds reported in *P. corylifolia.* From the literature review, it is clear that *P. corylifolia* has a high biological potential according to traditional knowledge and advanced scientific studies. The effectiveness and efficiency of the bioactive chemicals found in *P. corylifolia* have been time-tested based on their safe use. Indeed, more clinical trials have to be conducted to bolster its restorative properties. While it is understandable that not all extracts or compounds developed from *P. corylifolia* based research would end up as a possible medicine, others may not even be suitable owing to toxicity. But it has indeed been used for a long time to prevent or cure ailments with varying degrees of success. In recent years, there has been a revival of curiosity in rediscovering traditional health-promoting uses of *P. corylifolia*. Bioactive constituents found in *P. corylifolia* have several applications and are available commercially are bakuchiol (CAS 10309-37-2), psoralen (CAS 66-97-7), corylin (CAS 53947-92-5), genistein (CAS 446-72-0), psoralidin (CAS 18642-23-4), 8-Methoxypsoralen (CAS 298-81-7), isopsoralen (CAS 523-50-2), daidzein (486-66-8) and many more. Considering the immense medicinal advantage and commercial requirement, bulk cultivation and extraction for the discovery of new pharmaceutically active chemicals should be a priority for further study. So, this plant should be conserved for future generations.

Chapter 3

3.0 Statement of the problem/ research gap:

Obesity prevalence has sharply increased in recent years, which has major implications for global public health. Obese people have more risk of developing diabetes (type 2), gallstone disease, hypertension, heart disease, inflammatory bowel condition, neurological diseases and cancer.

Rheumatoid arthritis is an autoimmune disease that has a 1% prevalence and is linked to severe morbidity and elevated mortality. Though there have been a variety of anti-obesity and anti-arthritic medicines accessible in the market, it is still not advisable to use them over an extended period of time because they have various negative side effects associated with them.

Any region's medicinal plants act as a natural pharmacy and are a blessing because they may rapidly and efficiently treat a variety of illnesses. The widely used medicinal herb *Psoralea corylifolia* has estrogenic, anti-inflammatory, carcinogenic, antioxidant, antibacterial, antidepressant, hepatoprotective, osteoblastic and many more medicinal properties associated with its extracts and components. According to reports, PCSE has anti-obesity properties also. Moreover, its compound corylin and neobavaisoflavone are known to possess anti-osteoporosis activity.

To the best of my knowledge, there is hardly any description of the anti-obesity and anti-arthritic activity of bakuchiol, isopsoralen and psoralen, some of the secondary metabolites of PC.

Chapter 4

4.0 Research objectives:

Herbal research continues to be a key source of cutting-edge pharmaceutical products, and it also serves as one of the greatest sources for the synthesis of novel structure-based bioactive compounds. Nearly 30% of the medications that are now being used derive from natural sources, and another 20% are structural modifications of naturally occurring lead compounds. *Psoralea corylifolia* L. (PCL), a well-known herbal remedy for orthopaedic problems, is frequently utilised in traditional Chinese medicine. Anti-obesity action has been discovered in its seed extract. Keeping all this in view, the objectives of the present work are:

1. To collect, identify and successive solvent extraction of powdered seeds of *Psoralea corylifolia*
2. To isolate compounds from extracts and their structural elucidation by spectroscopic techniques
3. To evaluate anti-arthritic/anti-inflammatory activity of selected compound *in vivo*
4. To evaluate anti-obesity activity of the selected compound *in vitro*

Chapter 5

5.0 Material and Method:

5.1 Plant Collection and processing:

Four fundamental strategies are typically used when choosing a plant for pharmacological activities (Suffness and Douros, 1979):

a) A random selection of plant species
b) A selection based on ethnomedical usage
c) A review of the literature on the use of the species already in existence
d) A choice based on chemotaxonomic techniques, etc.

The plant selected for the study is ***Psoralea corylifolia.*** The selection of plants was based on ethnobotanical and ethnopharmacological literature, i.e., by following criteria 2nd and 3rd. The plant product selected for the present study is seeds of *Psoralea corylifolia*, which were received from CSIR- IIIM Jammu. The specimen of the seeds of PC has been deposited in the Crude Drug Repository (CDR) of Janaki Ammal Herbarium at IIIM Jammu. The CDR accession no. CDR 4242 has been assigned to the submitted voucher specimen.

5.1.1 Grinding of plant material:

Grinding of the 100 g of seeds was done with an electric grinder to get powder. It was afterwards macerated repeatedly at ambient temperature with several organic solvents in accordance with the sequence of increasing polarity Zang et al., (2018). At 4°C, various extracts were stored in the dark.

5.2 Chemicals and Reagents:

Dichloromethane, hexane/petroleum ether, methanol, dimethyl sulphoxide, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide, p-nitrophenyl butyrate, acetonitrile, formic acid, Lipopolysaccharide, porcine pancreatic lipase, Dexamethasone and 3-isobutyl-1-methylxanthine were obtained from Sigma Aldrich Co. Dulbecco's Modified Eagle Medium, Fetal Bovine Serum, Penicillin-Streptomycin and Phosphate Buffered Saline were procured from

Lonza Inc. ELISA kits for IL-1β, TNF-α and PGE2 were obtained from Invitrogen. All the chemicals were of analytical grade and were either Sigma or Merk chemicals.

Mouse 3T3-L1 preadipocytes utilized in the present work were acquired from National Centre for Cell Science (NCCS), Pune, India.

5.3 Preparation of crude plant extracts:

5.3.1 Maceration-

In this study, for the extraction process maceration method was used. Solvent extraction is a diffusion process. This technique has the benefit of yielding more compounds in the extract due to the preservation of all thermolabile and thermostable components. Even though a lot of solvents is required and the extraction process takes a long time, maceration is beneficial for extracting unknown components to investigate their biological activity.

Principle: The powdered plant material used in this process is immersed in a specific solvent at room temperature in a closed container. To speed up the extraction process, stirring may be applied intermittently or continuously. Until equilibrium is reached, the compounds come out from the plant cells into the extract. Filtration is used to separate the marc (residue) from the extract. To get the highest possible extraction yield, a fresh unused solvent is again added to the residue, and the process is repeated again. All extract-containing filtrates are collected after the exhaustion point is reached, and the solvent is then evaporated to get the crude extract (Abubakar et al., 2020). In the maceration, frequent agitation improves diffusion and eliminates concentrated solution from the sample's surface by adding more solvent to the menstruum to enhance extraction efficiency. The solvents used for the preparation of extracts, along with their polarity, are shown in **Table 5.1**.

Table 5.1 Solvents used and their polarity

S. No.	Solvents used in extraction	Polarity
1	Petroleum ether	0.117

2	Dichloromethane	0.309
3	Methanol	0.762

5.3.2 Preparation of Petroleum ether extract (PEE):

For PEE preparation, powdered seed material was immersed into the petroleum ether solvent (500 ml) and stirred on a magnetic stirrer for forty-eight hours at room temperature Filtrate from the mixture was collected after filtering. Following that, 500 ml of new solvent was added to the remaining plant material, and the process was repeated again. On the fifth day, all of the filtrates was combined, producing a 1000 ml solvent that included the extracted metabolites. The solvent was removed by evaporation to get the petroleum ether extract.

5.3.3 Preparation of dichloromethane extract (DCME):

The residue obtained while preparing petroleum ether extract was stirred at room temperature for 48 hours with 500 ml of dichloromethane. The mixture was filtered, and the extraction procedure was carried out once more using the residue. The filtrate was all combined and kept for evaporation at room temperature to get dichloromethane extract.

5.3.4 Preparation of methanol extract (ME):

The residue obtained in the previous step was stirred at room temperature with half a litre (500 ml) of methanol for 48 hours. The mixture was filtered, and the methanolic layer was allowed to dry in the air. The extraction procedure was carried out once more using the residue.

After combining the filtrates, the solvent was evaporated under a vacuum to produce the ME.

The number of recovered extracts was then computed and represented using the formula below as a percentage of the original plant sample:

$$\text{Percentage of extract recovered} = \frac{Weight\ of\ concentrated\ extract}{Weight\ of\ sample\ utilized} \text{ X } 100$$

5.4 Phytochemical screening for various seed extracts of *P. corylifolia:*

It is usually observed that plants with a variety of phytochemical classes have greater biological activity. Therefore, it may be concluded that a plant can demonstrate a wider range of biological activities the more diverse these chemical groups it has. Generally, the seed extract in a test tube was mixed with the necessary chemicals and reagents to conduct tests to check the presence of several kinds of phytochemicals using the procedures listed below. The presence or absence of several types of phytochemicals was confirmed by changes in the solution's appearance, as the case may be.

A preliminary phytochemical study of *P. corylifolia* seed extracts was carried out by standard methods (Banu and Cathrine, 2005). Phytochemical constituents such as alkaloids, flavonoids, phenols, glycosides, and terpenoids were qualitatively analyzed in this study.

5.4.1 Alkaloid:

Mayers test: 2- 3 ml of extract was taken in a test tube. “To it, 1-2 drops of Mayers reagent (Obtained by dissolving a mixture of $HgCl_2$ (1.36 g) and of KI (5 g) in 100 ml of water) was added along the sides of the test tube. The appearance of creamy white or yellowish precipitate confirms the presence of alkaloid”.

5.4.2 Flavonoid:

Alkaline reagent test: To 1ml of the extract, first of all, 2ml of 2% NaOH and then a few drops of dilute HCl was added. On addition of dil. HCl, an intense yellow colour, becomes colourless, representing the presence of flavonoids.

5.4.3 Carbohydrate:

Benedict′s test: To 1 ml of the extract, add an equal volume of benedicts reagent (“complex mixture of sodium citrate, sodium carbonate, and the pentahydrate of copper (II) sulfate”). Boil in a water bath for 2 minutes. The appearance of yellowish or red precipitate gives a positive result for carbohydrates.

5.4.4 Phenols:

Ferric Chloride test: To a small amount of extract, add a few drops of 5% $FeCl_3$ solution. The presence of phenol is confirmed by the colour, which is bluish-black.

5.4.5 Glycosides:

Borntragers test: 2 ml of the chloroform few drops of ammonia solution (10%) were added to a small amount of plant extract. The development of a pink tint denotes the existence of glycosides.

5.4.6 Terpenoids:

Salkowski test: To the extract, carefully add 2 ml of the chloroform and 3 ml of conc. H_2SO_4 to form a layer. The formation of red-brown colour at the interface represented a positive result for terpenoids.

5.5 Isolation of major constituents from seed extract using Column Chromatography:

The numerous compounds that make up a plant extract have unique physical, chemical, and biological characteristics. If the lead ingredient is present in a mixture of different compounds derived from a natural source, it must be isolated and purified. How simple it is to separate and purify the active principle depends significantly on the chemical's structure, stability, and quantity. There are two basic methods for finding novel bioactive compounds from natural sources: either random material collection and screening or the utilization of ethnopharmacological knowledge in the selection process (Lahlou et al., 2013). One of the most effective and economic/time-saving methods for the isolation of compounds from the mixture and their structural elucidation is the combination of nuclear magnetic resonance spectroscopy and chromatographic separation techniques (Srivastava et al., 2021).

The dichloromethane extract was subjected to column chromatography (CC), utilizing silica gel as a stationary phase for separating/isolating major constituents from *P. corylifolia* seeds.

5.5.1 Preparation of Column: A vertical glass column constructed from the borosilicate was used for the fractionation. The column was thoroughly cleaned with water, rinsed with acetone and dried before adding the stationary phase. A cotton plug was set up at the base of the column. Hexane was used to create the silica slurry, which was then poured into the top two-thirds of the column.

5.5.2 Adding the Sample to the Column:

9.46 grams of the extract was placed onto a column filled with silica gel of 60–120 mesh size and was gradually eluted first with pure n-hexane and then with 1–15% (vol/vol) EtOAc in n-hexane. Each chromatographic fraction having 15-20 ml of the eluent was collected and examined using thin-layer chromatography (TLC). The separated compound was detected using TLC silica gel 60 F_{254} sheets under the UV Fluorescence Analyzing Cabinet (Sunstar).

5.5.3 Recovering the Constituents:

The solvent continued running till the bands were eluted out separately. TLCs were evaluated, and fractions having similar R_f values were combined together/pooled and allowed to evaporate.

5.6 Ultra-fast liquid chromatographic studies (UFLC):

In natural product chemistry, extract clean-up/work-up and identification are still mostly accomplished using chromatographic methods like column chromatography and thin layer chromatography (TLC). Moreover, the analytical work of effective detection and characterization of natural compounds is crucial.

One of the primary concerns with the development of herbal medications is the need for standardization. Due to its high-pressure limit, selectivity, and sensitivity, the UFLC was the most significant method for standardizing herbal medicines compared to HPLC. The main drawback of HPLC is that it has a higher sample and reference standard requirements than RP-UFLC. When compared to HPTLC, RP-UFLC procedures deliver the most affordable, reliable validated results (Ramaswamy et al., 2021).

5.6.1 Instrumentation:

The dichloromethane extract and the compounds PC1, PC2 & PC3 isolated from the DCM extract were analysed using a UFLC system of Shimadzu company, outfitted with an automatic sampler (SIL-20A HT), a column oven (CTO-10ASvp), a gradient solvent quaternary pump (LC-20 AD), and a PDA SPD-M20A detector.

5.6.2 Chromatographic conditions:

The column Merck RP-18e LiChrospher, with particle size 5 µm and L x I.D. 25 cm x 4.6 mm, was used to isolate the analytes. The overall run time was 50 minutes with gradient elution using ACN (A) and 0.1% vol/vol HCOOH in H_2O (B) at a flow rate of 0.75 ml/minute. The gradient followed in the current experiment study was (in terms of vol/vol% of B): 0−0.01 minutes, 90 %; 0.01-15 minutes, 90 to 70 %; 15-25 minutes, 70 to 40 %; 25-35 minutes, 40 to 0 %; 35-40 minutes, 0 %; 40-45 minutes, 0 to 90 %; and 45-50 minutes, 90 %.

5.7 Structural Elucidation of isolated compounds:

The process of revealing a compound's chemical structure is known as structural elucidation in the drug development industry. Nuclear magnetic resonance spectroscopy is one of the most remarkable techniques for analyzing the structure and physicochemical characteristics of molecules.

Principle:

In NMR, the interaction between radiofrequency electromagnetic radiation and atom nuclei is studied. The magnetic field, called magnetic moment, is created by a charged particle like a proton or nucleus when it spins about its own axis. Without an external magnetic field, the nuclear spins are randomly oriented. The interaction of the magnetic moment occurs when an atom's nucleus is exposed to an external magnetic field. The magnetic moment must be in line with or opposed to the surrounding field. If a nucleus is aligned with the magnetic field or against it, it is said to be in the α -spin state and the β -spin state, respectively.

There is a discernible energy difference between the two spin states; their energies are not equal. An energy gap between spin states is created by the external magnetic field. Nuclear magnetic resonance is the process by which the α -spin state absorbs energy and flips to the β -spin state when the difference in energy between the two states is within the ranges of radiofrequency radiation.

A local magnetic field that counters the external magnetic field is created in the existence of an external magnetic due to the movement of the electron density around the nucleus. Diamagnetic is the name given to this phenomenon. This phenomenon is known as the name shielding effect. While certain nuclei/protons have a larger electron density surrounding them, making them more shielded, other nuclei/protons have a lower electron density, making them less protected or de-shielded. Because of this, protons and nuclei in various chemical environments have varying energy gaps between the α-spin state and the β-spin state, which causes them to absorb radiofrequency radiation at various frequencies (Atta-Ur-Rahman, 2012).

5.7.1 Determination of spectra for structural elucidation:

The samples, whose spectra were to be determined, were firstly dried in a high vacuum pump thoroughly so that no organic solvent or water remained. After that, the sample was dissolved in about 1.5 ml of $CDCl_3$ solvent and transferred to an NMR standard tube for further assessment. Utilizing a Bruker FT-NMR 400 MHz instrument, ^1H NMR spectra were obtained. Proton chemical shift measurements are presented downfield from tetramethyl silane in parts per million (ppm), and in reference to the chemical shift value of proton in the deuterated solvent (e.g., $CDCl_3$, 7.26 ppm). At 125 MHz, a Bruker FT-NMR spectrometer was used to record carbon-13 nuclear magnetic resonance spectra (13C NMR). Similarly, with reference to the solvent's carbon resonance, chemical shift information/data for carbons are presented in ppm downfield from $(CH_3)_4Si$.

Obtained values were compared with the available data (Jiangning et al., 2005), which helped in predicting the structure of the isolated compounds.

5.8 Anti-obesity activity studies:

Lipids are a vital part of human nutrition. One of the most common anti-obesity mechanism of herbs is by inhibiting lipase enzyme or adipogenesis. In this study, anti adipogenesis assay was done using 3T3-L1 cell lines.

5.8.1 Cell viability assay:

The MTT (3, (4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) tetrazolium assay is also described as a colorimetric assay for the measurement of cell viability, cell proliferation and cytotoxicity assay was utilized in the present work. As reported in numerous articles published in the past, the MTT tetrazolium assay has been widely adopted and is still popular in academic labs.

Principle: The reduction of MTT to formazan product provides the basis for this MTT assay. So, by using a plate reader to measure formazan concentration as reflected in optical density (OD), it is possible to determine whether the quantity of viable cells has increased or decreased. In order to check the sensitivity of the drug, the optical density values of wells having cells that have been subjected to compounds are compared to the optical density values of wells having cells that have not been treated.

Assay: In order to check cell viability, the toxicity of the extract, as well as isolates, was tested in 3T3-L1 cell line using the MTT assay (Khare et al., 2019). In a 96-well culture plate, undifferentiated 3T3-L1 cells were plated at a seed density of 1×10^4 cells/ml.

After 24 h, the medium was replaced with one containing DCME or isolates at various concentrations. For 24 hours, the cells were incubated at 37 °C in a CO_2 (5%) atm.

The test solutions were withdrawn from the wells after incubation, and 10 µl of MTT (5 mg/ml MTT in PBS, pH 7.4) was added to each well. After that, the plates were incubated for 4 hours at 37°C. In order to dissolve the formazan that was created, 100 µl of DMSO was added to the plates after the supernatant was removed. A microplate reader was used to measure absorbance at 570 nm. For a control, undifferentiated pre-adipocytes were treated with 1% DMSO.

The percentage growth inhibition value was calculated as:

$$\% \text{ Inhibition} = \frac{\text{Mean Test OD}}{\textit{Mean Non-treated OD}} \text{X } 100$$

5.8.2 Anti-adipogenesis assay and Cell culture:

Adipocyte cells have lipid reserves, and a rise in the accumulation of lipid droplets within cells may indicate obesity.

Assay: Dulbecco's Modified Eagle Medium (DMEM) containing bovine calf serum (10 %) and penicillin-streptomycin (1%) was used to cultivate mouse 3T3-L1 preadipocytes in a 250 ml culture flask.

In a humidified incubator with a constant flow of 95% oxygen and 5% carbon dioxide, the whole cell culture was kept at 37 degrees Celsius (Mangal et al., 2017).

"Post-confluent cells were induced to differentiate after being incubated for 48 hours in a mixture containing basal media (DMEM) with 10 per cent bovine calf serum, 1 per cent penicillin-streptomycin solution, and IDM cocktail (1 g/ml insulin, 0.1 M dexamethasone, and 0.1 mM 3-isobutyl-1-methylxanthine). After two days, the media was changed to the maintenance medium, which included DMEM supplemented with 10 per cent bovine calf serum, 1 per cent penicillin-streptomycin solution, and 1 g/ml insulin over the following ten days. For a total of 12 days, isolated compounds and DCME underwent treatment in both differentiation and maintenance mediums. The untreated cell lines that had been cultured in maintenance medium after being differentiated medium-grown were the control group".

5.8.3 Oil Red 'O' staining:

The production of lipid components by adipose cells begins after differentiation stimulation. Oil Red O, a dye which is based on the solubility of the dye in the lipid material, was used to stain the lipid droplets.

Staining: Using a method that has been previously described, lipid deposition was measured (Kim et al., 2010). Plant extracts and/or chemicals were given to 3T3-L1 cells during an adipogenesis experiment. Cells were rinsed in PBS, fixed with 3% formaldehyde in PBS for 30 min at room temperature and then rehydrated before being incubated with Oil Red 'O' solution (0.5 per cent Oil Red 'O' dye in isopropyl alcohol:water:: 60:40). The cells were washed with H_2O to remove any dye that might have remained after the excess dye solution was removed.

An inverted microscope (Leica DMI 6000 B, Germany) was utilized for taking images of the treated cells, and absorbance at 520 nm was measured with an ELISA plate reader.

5.8.4 Pancreatic lipase (PL) inhibition assay:

Principle:

The idea behind the pancreatic lipase (PL) inhibition assay is that it prevents triglycerides from being broken down into fatty acids, therefore preventing the digestion and metabolism of lipids and causing anti-obesity and anti-hyperlipidemic effects.

Assay: With a few minor modifications, the PL inhibition assay was performed in agreement with the method of Sridhar et al., 2018. Prior to usage, the enzyme solution was made by adding 25 mg of PPL in 5 millilitres of the Tris HCl buffer, having pH of 7.4. Centrifugation was then performed at 4000 rpm (18 °C) for 10 minutes. The supernatant was taken out and put to use. Different conc. (100 µg/mL, 50 µg/mL, and 25 µg/mL) of DCM extract, the separated compounds, and the orlistat (as positive control) were produced for each in Tris HCl buffer containing 1% DMSO. The final reaction mixture of 1000 µl is composed of a pre-incubated mixture (5 minutes at 37°C) comprising of 875 microlitre buffer, 100 microlitre enzyme, and 20 microlitre DCM extract/isolates/orlistat of various conc., which is then added with 5 µl of the substrate (4-nitrophenyl butyrate, 10 millimolar in ACN).

After 5 minutes at 405 nm, the final mixture's absorbance was measured using a microplate spectrophotometric reader (Spectramax i3 Molecular Devices, USA). The experiment was run in triplicate, and the formula for calculating the percentage of inhibition is as follows:

$$\% \text{ Inhibition} = \frac{AE - AT}{AE} X100$$

Where AT shows the difference in absorbance of the test sample with and without substrate, and AE represents the absorbance of the enzyme control (without inhibitor).

5.8.5 In Silico studies:

Using virtual screening and docking tools, such as SWISSADME, AutoDock vina and PyMOL, an in-silico study of anti-obesity activities was conducted.

5.8.5.1 Extraction of receptor from PDB:

The 3-D X-Ray diffraction crystallographic structure of the Peroxisome proliferator-activated receptor gamma (PPAR-γ) with PDB code 6L8B **(Figure 5.1)** was acquired from the Research Collaboratory for Structural Bioinformatics of Protein Data Bank (https://www.rcsb.org/).

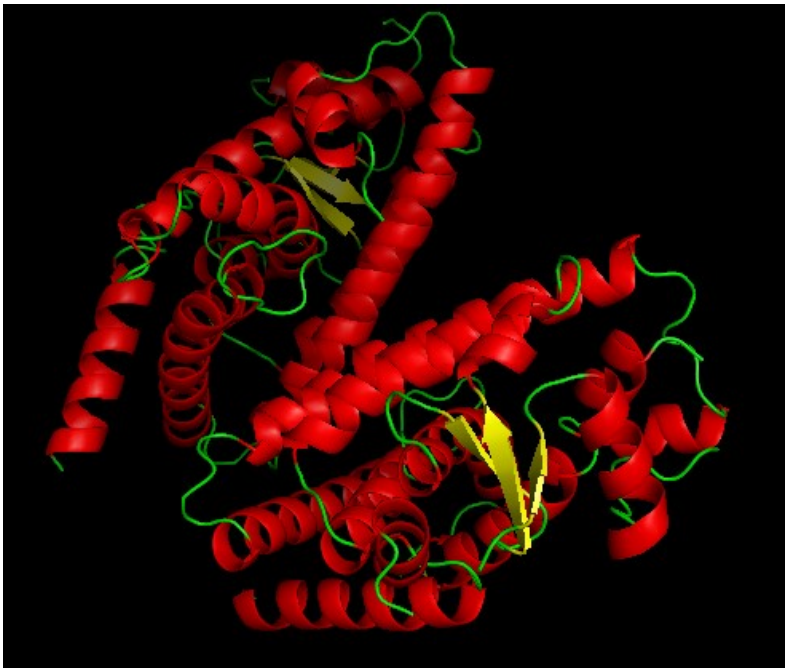


Figure 5.1 Structure of the target protein in three dimensions (3D) (PPAR-γ)

5.8.5.2 Extraction of ligand:

The three phytocompounds from *Psoralea corylifolia* were used in the current work as ligand molecules and were obtained from NCBI PubChem (https://pubchem.ncbi.nlm.nih.gov/) in SDF files format. These were converted to PDB files to obtain 3D structure using the PYMOL software **(Figure 5.2).**

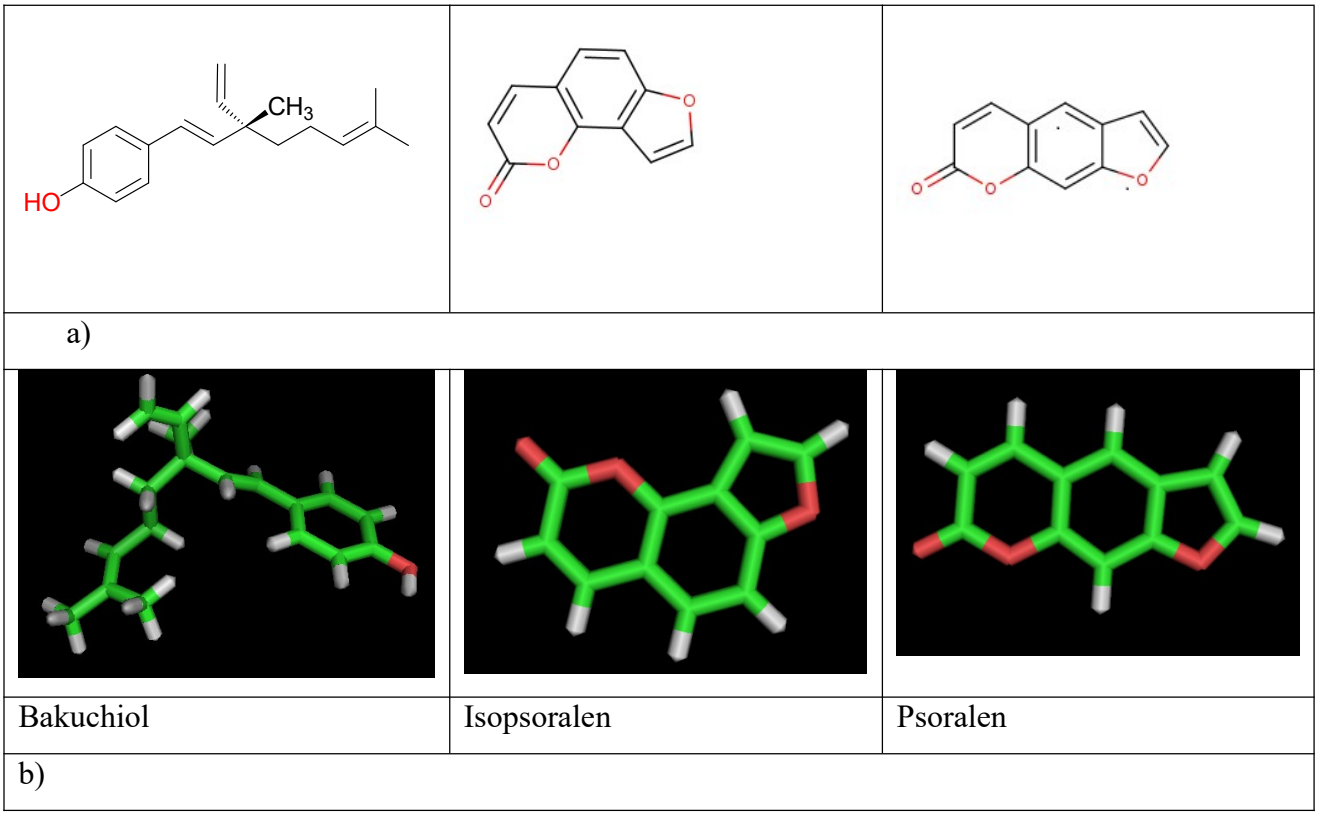


Figure 5.2 2D (a) and 3D (b) structure of the ligand molecules

5.8.5.3 Drug-likeness properties and ADME prediction by Computational Analysis:

The selected *P. corylifolia* compounds were subjected to in silico testing to see whether or not the compounds met the requirements or necessary criteria for drug-likeness. A molecule is not necessarily a promising choice just because it is highly bioactive and has minimal toxicity. It must have a better pharmacokinetic profile. To avoid wasting time or resources, it is crucial to review the ADMET profile of compounds as soon as possible. Consequently, we anticipated the ADMET characteristics of our compounds. Chemoinformatics is frequently used to forecast the relative qualities of molecules, i.e. Absorption, Distribution, Metabolism, and Excretion (ADME) in order to improve their pharmacokinetic features by removing those that would not make suitable therapeutic candidates.

Additionally, a drug must meet other requirements in order to be effective and bio-available. It must adhere to Lipinski's guidelines (Isyaku et al., 2020). Lipinski's rule of five is

helpful throughout the pre-clinical stage of developing drugs. It claims that a medicine is considered impermeable or poorly absorbed if it fails to meet more than two of the criteria (Mol. mass< 500, number of H-bond donors ≤ 5, number of H- bond acceptors ≤ 10, Calculated Log p ≤ 5). Numerous research has suggested that all of the compounds could be pharmacologically active if they do not breach more than two of Lipinski's rule of five (RO5) criteria (Umar et al., 2021), (Hajji et al., 2022, Thakur and Pande, 2021). The ADMET results demonstrate the physicochemical properties of the ligands, including the RO5 (Mol. mass, CLogP, H-Bond acceptors and H-Bond donors) and other characteristics, including the total polar surface area (TPSA), the number of rotatable bonds (ROTBs), aromatic rings and the alerts for unfavourable substructures (alerts such as PAINS #alert and Brenk #alert) (Isyaku et al., 2020). Three methods-ESOL, (ALI) logS, and (SILICOS-IT) logs-predict that molecules with drug-like properties will have strong aqueous solubility. Bioavailability radar was used to measure bioactive substances' drug-likeness based on various metrics. Bakuchiol, isopsoralen and psoralen ligands were assessed for ADME characteristics and drug-likeness using the free online tool SwissADME.

5.8.5.4 Docking of receptor and ligand:

First of all, the crystal structure of the ligand was processed by removing water molecules. The Autodock tools were then used to add Kollman charges and polar hydrogen to the protein. The molecular docking tool Autodock vina was used to predict the molecular binding affinity of the protein-ligand complex.

5.9 Anti- Arthritic studies:

The current experiment was designed to evaluate the anti-inflammatory and anti-arthritic effect of isolated compounds of *P. corylifolia* using the lipopolysaccharide (LPS) induced mouse macrophages (RAW 264.7) *in vitro* and Carrageenan/CFA-mediated arthritis model *in vivo*.

5.9.1 *In vitro* studies: "The RAW 264.7 cell line was employed to investigate the anti-inflammatory effects of the isolated compounds. RAW 264.7 macrophages were procured from

the American Type Culture Collection, Rockville, Maryland, USA. GIBCO's Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10 per cent fetal bovine serum (FBS), 100 U/ml penicillin, and 100 µg/ml streptomycin was used to culture the cells." After that, the cells were then incubated at 37°C in 5% CO_2. For all of the studies listed below, cells between the third and fourth passages were employed.

5.9.1.1 MTT Assay:

The method of Mosmann (1983) for MTT was followed to verify the viability of RAW264.7 cells in the presence of compounds. $1x10^5$ cells of RAW264.7 cell lines were plated in 96 well tissue culture plates. The cells were incubated with medium alone and different concentrations of isolated compounds. The cells were incubated for 24 hours following treatment. Following the incubation period, the cells were washed with PBS and incubated with a PBS-prepared 20µl MTT solution (2.5 mg/ml), pH 7.4. The cells were then washed with PBS, and the reduced formazan was solubilized in 100µl of 100% DMSO. The absorbance at 570 nm was measured using an ELISA plate reader Synergy Mx microplate reader (Bio-Tek, USA) after 10-minute incubation at room temperature.

The cells' survival was found from the below-mentioned formula.

$$Percentage\ Cell\ viability = \frac{\text{Absorbance of treated cells}}{\text{Absorbance of untreated cells}} \text{ X } 100$$

5.9.1.2 Nitric oxide production inhibition:

Principle:

Nitrite (NO_2), one of the nitric oxide (NO) breakdown products that are stable and non-volatile is measured by the Griess Reagent System.Sulfanilic acid reacts with nitrite in an acid solution to quantitatively transform it into a diazonium salt. N-(1-naphthyl)ethylenediamine and the diazonium salt were then mixed to create an azo dye that can be detected spectrophotometrically.

Assay: The protocol by Khajuria et al., 2018 was followed with some modifications. At 37°C and 5% CO_2 in a humid incubator, RAW 264.7 cells were grown to confluence in Dulbecco's

Modified Eagle's Medium (DMEM) containing 10 per cent fetal calf serum (FCS) and 5 µg/mL Penicillin. In 96-well microtitre plates, cells (2×10^5 cells per well) were seeded. After 12 hours, the fully adhered cells were treated with 50 µl of the test compound. After 2 hours of incubation with the test compound at 37°C, 50 µl of LPS (5 µg /ml) in Dulbecco's Modified Eagle's Medium was supplemented in all wells and incubated for another 24 hours. Using the colorimetric Griess reaction protocol, the supernatants from the cells were recovered after 24 hours of incubation. A 96-well plate was incubated at room temperature for 10 minutes with 100 µl of cell supernatant and an equal volume of Griess reagent (1 per cent sulphanilamide in 5 per cent phosphoric acid and 0.1 per cent N-(1-napthyl) ethylene diamine in distilled water; purchased from Sigma) The absorbance was measured at 540 nm on a SpectraMax ABS Plus plate reader. Absorbance readings measured above were averaged, and Nitrite concentration was calculated using a standard curve created with $NaNO_2$ standard solution. The assay was carried out three times for each concentration.

The NO % inhibition was computed as:

$$NO\ inhibition\ (\%) = \frac{NO\ Control - NO\ sample}{NO\ Control} \text{X } 100$$

Control is the LPS-induced group without any treatment.

5.9.1.3 Reactive oxygen Species (ROS) inhibition:

Principle:

The assay uses the cell-permeable fluorogenic probe dichloro-dihydro-fluorescein diacetate (DCFH-DA). When DCFH-DA enters cells, cellular esterases deacetylate it into a non-fluorescent substance DCFH, which, when exposed to ROS, transforms into the intensely fluorescent 2′,7′-dichlorofluorescein (DCF). Therefore, this method is widely used to evaluate the intracellular redox status.

Assay:

Reactive oxygen species (ROS) generation within cells was determined using the fluorogenic probe DCFH-DA (Invitrogen, USA). RAW264.7 cells were plated at the conc. of $1X10^6$ per well

and treated with 1µg/ml LPS for 24 hours and then incubated with or without compounds for 1hr. After washing thrice with 1X phosphate-buffered saline, cells were incubated for 30 min at 37°C in the dark with 10 mM DCFH-DA. Again, cells were washed two times with 1X PBS. By using a fluorescent microscope (Nikon Corporation), the production of fluorescence caused by the conversion of dichloro-dihydro-fluorescein diacetate (DCFH- DA) to dichloro-dihydro fluorescein (DCFH) by non-specific cellular esterases and the successive oxidation of DCFH by peroxides was examined.

The corrected total cell fluorescence (CTCF) was calculated for the cells captured in each image using the Image J software.

$$\text{CTCF} = \text{Integrated Density} - (\text{Area of selected cell } X \textit{ Mean fluorescence of background readings})$$

The results are articulated as arbitrary units.

5.9.1.4 TNF-α inhibition assay:

96-well plates containing RAW 264.7 cells were plated at a seeding density of 5 X 10^5 cells per ml and were left overnight for acclimatization (37°C, 5-6% CO_2). Then treatment for 1 h, with dexamethasone (a positive control) or various concentrations of bakuchiol, isopsoralen, psoralen (1.25 µM, 2.5 µM and 5 µM), were given. Furthermore, cells were exposed to LPS (1 g/ml) for 24 hours to promote the release of pro-inflammatory cytokines, i.e., TNF-**α**. With the help of competitive enzyme immunoassay kit (Invitrogen, USA), the presence of cytokine TNF-**α** was measured in the supernatant. The manufacturer's instructions for the ELISA assay were followed. Each sample's optical density was measured, and a standard curve was employed to quantify the production of cytokine TNF-**α**. By contrasting the treated group with the LPS-induced group as the control, the per cent inhibition by bakuchiol, isopsoralen or psoralen was computed.

5.9.2 *In vivo* studies:

Test material preparation: Accurately weighed quantity of **BAK** (bakuchiol) and **ASA** (Acetyl salicylic acid) used as standard test drugs were dissolved to prepare doses whose conc. were **BAK-**1.0, 4.0 and 8.0 mg/kg and ASA- 250 mg/kg in 1% DMSO.

Animals:

In this investigation, female Wistar albino rats were utilised. They were maintained in a standard laboratory environment with the following parameters: 23 ±1 °C temperature, 55± 10 per cent relative humidity, 12/12 hr dark/light cycles, and unrestricted admittance to H_2O and standard pellet diet (Lipton India Ltd.). In every experiment, a control group was kept (given a vehicle) while the other group was administered a standard medication for comparison and to ensure the test's validity. The institutional animal ethics board gave its approval and licence to all of the experiment's protocols, and all of the animals used in it were treated humanely.

5.9.2.1 Carrageenan mediated paw edema: A well-known paradigm of acute inflammation for determining/testing anti-inflammatory properties of compounds is carrageenan-induced paw edema in mice. Hence, to evaluate the test compound's anti-inflammatory properties, carrageenan-induced paw edema was envisaged as a practical option (Winter et al. 1962).

Experimental procedure: 5 groups of rats, having 6 animals in each group, were used. Paw volume of all the animals kept in different groups was measured by utilizing a digital plethysmometer in ml, i.e., edema causing water displacement. Rats were pretreated orally by test substance at different concentrations 1h before the injection of carrageenan (1%, 0.1 ml in 0.9 % sterile NaCl). Carrageenan was administered in the plantar region of the right hind paw of a rat. 3-5 hour after the carrageenan injection, paw volume was determined again, and the effect of test material on inflammation was checked.

$$\text{Percentage inhibition of paw edema} = \frac{Vc - Vt}{Vc} \times 100$$

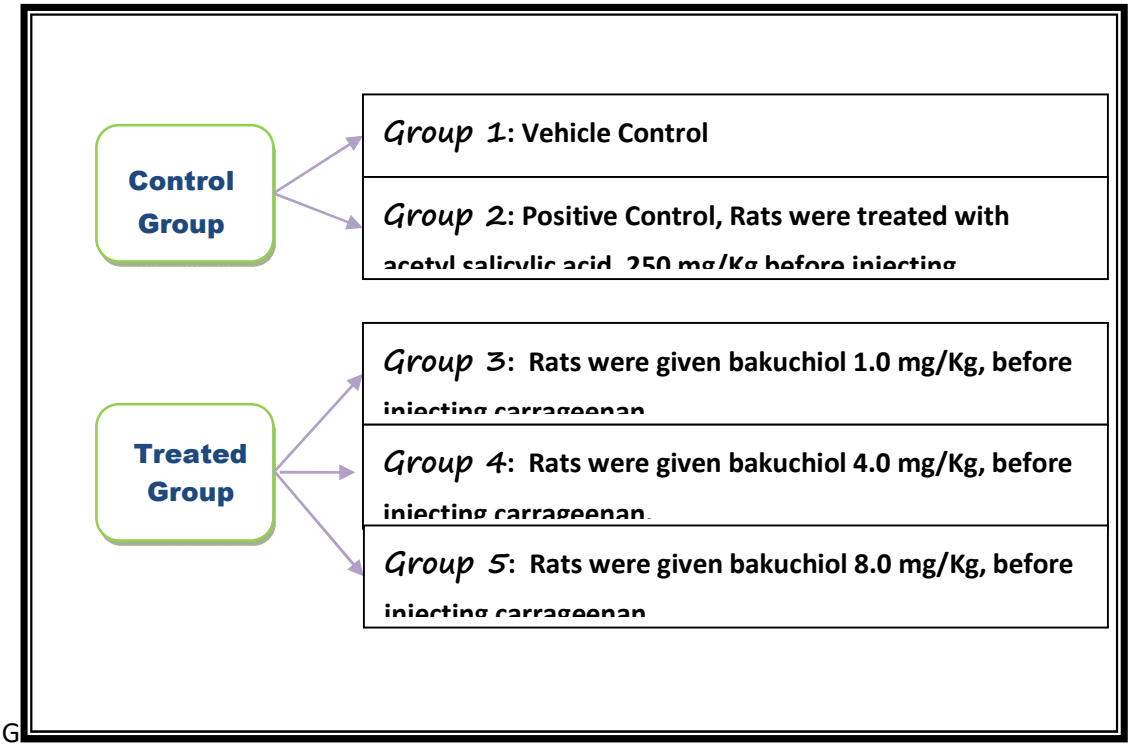
where,

Vc is the volume that the control group has displaced,

Vt is the volume that the treated group has displaced.

5.9.2.2 Adrenalectomy:

Under ether anaesthesia, rats underwent bilateral adrenalectomy (Schultzer, 1935). Two dorsal incisions were made to remove both adrenal glands. The adrenalectomized rats were given normal saline to consume in place of water. The Carrageenan-induced acute edema test, according to Winter et al. approach, was used to evaluate the effect two days following the surgery (1962).



G

Figure 5.3 Control and bakuchiol treated groups in carrageenan induced inflammation

5.8.2.3 Effect of BAK on CFA-mediated arthritis in rats:

Anti-arthritic activity: CFA induced arthritis (Bani et al. 2009)

Adjuvant-induced arthritis in mice-

In order to produce adjuvant arthritis in mice, a subplantar injection of 0.02 millilitre of CFA (Difco, USA) was injected in the left hind footpad. The test compound was administered orally in different doses 1 hour prior to the injection and then once daily for 13 days. On the first

and thirteenth days, the paw's volume was measured, and the percentage inhibition was then calculated. A plethysmometer was used to measure the paw's volume.

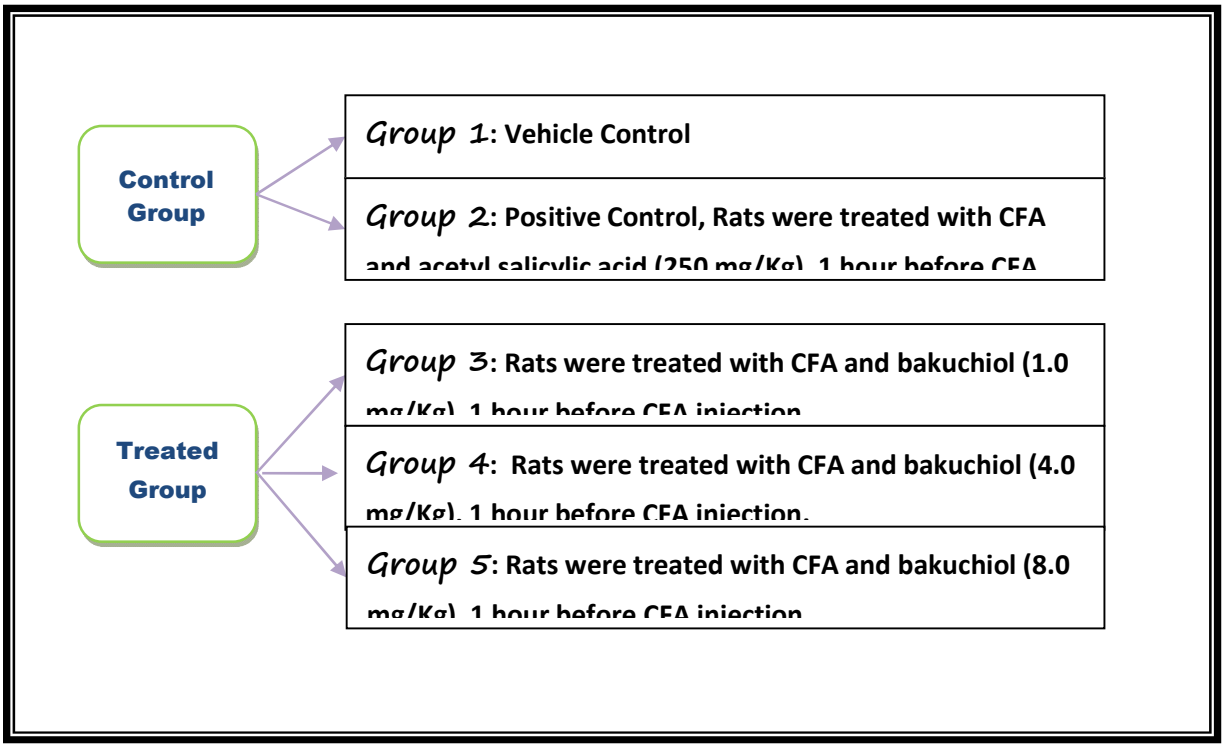


Figure 5.4 Control and bakuchiol treated groups in CFA induced arthritis

5.9.2.4 Quantification of TNF-α, IL-1β, PGE2 in serum samples of arthritic mice:

Using commercially available kits of the ELISA technique (R&D Systems, MN, USA), samples of the serum taken on the 14th day from several assemblies of animals were evaluated for cytokines such as TNF-**α**, IL-1**β**, and PGE2 in accordance with the manufacturer's instructions. On an ELISA plate reader, colorimetric measurements at 450 nm were used to determine all cytokine concentrations.

5.10 Statistical Analysis:

In order to reduce experimental error, the values were presented as the mean plus the standard error of the mean. Software from Microsoft Excel and GraphPad Prism was used to conduct statistical analysis. For group comparison one way ANOVA was used, followed by Tukey's posthoc test.

Chapter 6

6.0 Results and Discussion:

6.1 Plant material:

The starting material of *P. corylifolia* was the seeds, which were received from IIIM Jammu. These were air-dried, grinded into a fine powder and packed into airtight container before further use. The physical characteristics of the seeds were blackish brown in color with an oily texture, and that of the powder was a dark brown coloration with a pungent smell. Physically the selected plant material appeared as depicted in **Figure 6.1**. This fabaceae plant, *P. corylifolia*, is a popular therapeutic plant used in India and China as well as Southeast Asian nations. This plant possesses an extensive range of pharmacological activities. Keeping in view its excellent activity, as demonstrated in the literature available (Alam et al., 2017), this plant was chosen. In order to increase the surface area for proper mixing of powdered materials with the solvents used (Srivastava et al., 2021), the powdered seed material was used for extraction.

a) Seeds of *Psoralea corylifolia* b) Powder of *Psoralea corylifolia* seeds

Figure 6.1: Physical appearance of plant material of *Psoralea corylifolia* selected for the present work

A lot of health-related issues and disorders can be solely treated using herbs, according to ancient scholars. They tested and conducted in-depth research to reach reliable results on the effectiveness of various herbs with medicinal properties. The majority of the medications developed in this way do not have any negative effects or responses. This is the cause of the global rise in the popularity of herbal medicine. These medicinal herbs offer realistic solutions for the treatment of numerous ailments that are otherwise thought to be challenging to cure. *P. corylifolia* is an imperative herbal medicine that has been utilized for several hundreds of years

in therapy. For the management of a wide range of illnesses, including leucoderma and other skin disorders, cardiovascular issues, nephritis, osteoporosis and cancer, *P. corylifolia* has been comprehensively employed in many conventional Ayurvedic and Chinese medicine formulae (Zhang et al., 2016).

6.1.1 Extract yield:

The choice of solvent for the extraction process depends on a number of variables, including the polarity of the phytochemicals to be extracted, the speed of extraction, the convenience of handling the extracts, the variety of compounds to be extracted, and the toxicity of the compounds (Tiwari at al., 2011). In solvent-based extractions, the literature review revealed that DCME displayed more antiinflammatory activity when compared with PEE and ME of *Psoralea* species (Backhouse et al., 2001); however, the anti-obesity potential was reported in the alcoholic extract of seeds of *Psoralea corylifolia* (Liu et al., 2019). Moreover, the yield of major constituents present in *P. corylifolia* was reportedly found to be higher in extracts prepared using non-polar solvents like petroleum ether, dichloromethane etc., suggesting that non-polar solvents are the most effective choice for the extraction/separation from the seeds of *P. corylifolia* (Krishna et al., 2022). Hence, keeping into consideration the findings of the literature scan and poor solubility of major constituents in water, solvents such as petroleum ether, dichloromethane, and methanol were chosen for the preparation of extracts through sequential extraction in the present study than using water extract.

After sequential extraction with solvents of different polarity, which were used in the order: of petroleum ether, then dichloromethane and after that, methanol, different amounts of PEE, DCME and ME were recovered, depending upon the kind of solvent system utilized. Extraction was carried out by immersing the seed powder in a specific solvent at room temperature in a closed container. To speed up the extraction process, stirring was applied intermittently/continuously, as depicted in **Figure 6.2**.

Figure 6.2: *Psoralea corylifolia* seed extract

The yield percentage of the various extracts obtained was calculated as being the ratio between the mass of the extract and that of the dry matter subjected to the extraction. The result of the percentage yield/ extractive value is presented below in **Table 6.1**. From the table itself, it is clear that the extractive value of DCME is the highest, i.e. 9.64%, whereas the extractive value of ME and PEE is 7.62 and 5.29%, respectively. Thus, the extractive value of dichloromethane extract is more than that of petroleum ether extract (**Figure 6.3**). In a report published by Khuranna and coworkers, the seed extracts of *P. corylifolia* using different solvents were prepared, and their extractive percentage value was found to be 13.71%, 14.20%, 11.22%, 12.71% and 12.90 % with methanol, ethanol, petroleum ether, acetone and DCM solvents respectively (Khuranna et al., 2020).

Table 6.1: Extractive value of different extracts prepared

S.No.	Name of the *P. corylifolia* Seed extract	Extractive value (%)
1	Petroleum Ether Extract (PEE)	5.29
2	Dichloromethane Extract (DCME)	9.64
3	Methanolic Extract (ME)	7.62

The serial extraction method is considered exhaustive since it is one of the key procedures used to ensure that the phytocompounds are extracted to their maximum potential. Serial extraction was used in this investigation since different compounds have varied polarity values and are likely to be eluted under the range of polarity of their solvent. The employment of various solvents in a single extraction procedure is an exhaustive strategy to recover numerous compounds from the same plant/plant parts because a sole solvent cannot be depended upon to achieve the complete extraction of all molecules from the plant (Bimakr *et al.*, 2011).

The extract yield depends upon the varying polarity levels of solvents and their ability to dissolve a variety of compounds from the samples (Harborne, 1973). Along with the solvent used, the extraction yield also depends on factors such as extraction time, temperature, the solvent ratio, the chemical nature of the compounds etc. (Iloki-Assanga et al., 2015).

Extraction can be followed by further processing, by the fractionation to separate certain chemical compounds, like the isolation of modern medications such as ajmalicine, hyoscine, and vincristine were done earlier. Thus, the ultimate quality of the herbal medication is significantly impacted by the standardization of extraction techniques (Srivastava et al., 2021).

6.1.2 Screening of the crude extract constituents:

Chemicals found in plants called phytochemicals are surely responsible for the effectiveness of plants against chronic conditions like cancer, hyperglycemia, obesity etc. It has been demonstrated that extracts with potential against diseases have particularly high

concentrations of polyphenols and flavonoids. To determine the promising phytochemicals present in each preparation, a preliminary study of the components of the crude extracts is helpful. The crude extract seems to be more likely to be a combination of substances that are extracted from the matrix of a plant (Heendeniya et al., 2020). These compounds are either essential for the physiological functions of plants or are merely secondary metabolites.

A biochemical assay was done to check the presence of various phytoconstituents in the different extracts of seeds of *P. corylifolia,* which were prepared during sequential extraction. A total of six phytochemical tests were gone to verify the presence of Alkaloids, Flavonoids, Carbohydrates, Phenols, Glycosides, Terpenoids etc.

From the results, it is clear that flavonoids, carbohydrates, phenols, glycosides and terpenoids were present, but alkaloids were absent (**Table 6.2**). Flavonoid was present in the petroleum ether extract and dichloromethane extract but was absent in the methanolic extract. Flavonoids are a type of polyphenolic compound that occurs naturally. Flavonoids are regarded as dietary supplements that promote health and fight against diseases. According to epidemiological, clinical, and animal findings, flavonoids might have a protective influence against a number of disease conditions, such as cancer and cardiovascular disease. In addition to it, flavonoids have antibacterial, antiviral, hypolipidemic and anti-inflammatory properties (Babu and Liu., 2009). Our study is supported by the study of Li et al., 2016 in which it was reported that *P. corylifolia* has secondary metabolites belonging to classes flavonoids, coumarins, phenols, benzofurans, terpenoids, steroids and others.

Carbohydrate was found in the methanolic extract and absent in the PEE and DCME. The phytochemical screening depicted that phenol and glycoside were found in all the seed extracts of *P. corylifolia* seeds, whereas terpenoid was found in all except ME.

Table 6.2 Phytoconstituents which are present (+) or absent (-) in different extracts of *Psoralea coryliolia* seeds

Phytoconstituent	**Test**	**Petroleum ether extract (PEE)**	**DCM extract (DCME)**	**Methanol extract (ME)**

Alkaloid	Mayer′s test	-	-	-
Flavonoid	Alkaline reagent test	+	+	-
Carbohydrate	Benedict′s test	-	-	+
Phenols	Ferric Chloride test	+	+	+
Glycoside	Borntrager's test	+	+	+
Terpenoid	Salkowski test	+	+	-

In *P. corylifolia* extract, a varied number of bioactive substances such as flavonoids, coumarins, meroterpenes, and benzofuran glycosides have been found, which serve as the molecular basis for its effect. Pandey et al., in 2013, revealed that in the petroleum ether extract of *P. corylifolia* seeds, alkaloids, carbohydrates, glycosides, flavonoids, and saponins were absent, but steroids and triterpenoids were present. In another report by Borate et al. in 2014, alkaloid, carbohydrates, flavonoids, glycosides and saponins presence was revealed in the methanolic extract of *P. corylifolia* seeds, but steroids, as well as terpenoids, were not present in the same extract. So, the present study is in agreement with previous reports with few variations. There can be any of the three possible causes for the difference in the phytochemical test results. First could be the geographical area where the plant material was grown. This can be brought on by variations in environmental factors, such as soil composition, average temperature etc., that have an impact on secondary metabolic pathways and the composition of the extract. The method used to extract the crude extracts can be the second potential contributing element. The accuracy of the tools or techniques used for phytochemical research can be the third plausible element influencing the variability of the composition of the plant extracts (Nakweti et al., 2013).

6.1.3 Column Chromatography (CC):

The plant extract is a concoction of compounds with various physical, chemical and biological characteristics. As it is one of the potential natural product sources, separating secondary metabolites from plants is typically difficult and time-consuming work. Therefore, these procedures might be simplified with appropriate initial sample preparation. One of the simplest sample preparation techniques is filtration, which can be used to remove particles and

insoluble components using a variety of filtration techniques, such as filter paper and specific membranes with specified pore sizes. Then the next step, i.e. fractionation, aims to divide the extracted components into discrete fractions (Lahlou, 2013).

A method used for removing/isolating a single chemical substance from a mixture is column chromatography (CC). Depending on the unequal adsorption of substances to the adsorbent as they move through the column at varying speeds, it separates substances into fractions. Therefore, this technique can be used to purify substances on a small or large scale.

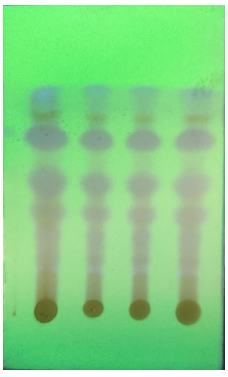
Modern drug discovery and development have greatly benefited from the application of secondary metabolites originating from natural sources (plants, microorganisms, and animals). It follows that rigorous examination of these natural resources is necessary in order to find unidentified metabolites that could one day form the basis for the discovery and development of novel medications. Such exhaustive examinations necessitate the use of numerous chromatographic techniques (Rahman, 2018).

In the present work, DCME from *P. corylifolia* seeds was chosen for secondary metabolite isolation and characterization. The CC technique was employed for the separation of three prominent molecules from the dichloromethane extract of *P. corylifolia* seeds. Utilizing pure hexane, the slurry pack method was employed in loading 9.6 g of extract into the column.

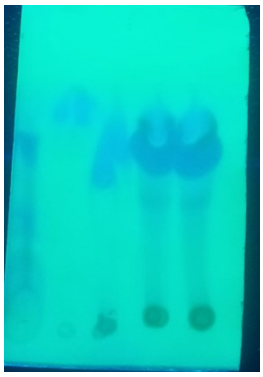
The elution of the sample started with 100 % n-hexane only, and then the ethyl acetate (EtOAc) concentration in n-hexane was gradually raised from 1 to 15% (vol/vol). The thin-layer chromatography (TLC) technique was used to examine each chromatographic fraction. Many solvent systems were tried to separate the compounds. The best result was obtained with 20% EtOAc in n-hexane, as visualized through TLC in the UV chamber. From the TLC image of DCM extract, it was clear that this extract had a large number of compounds. That's why a smear was obtained. The TLC of the first fraction was revealed to have oils in it. The elution process was continued till the first compound was visible in the fraction (**Figure 6.4c**). The R_f value of the first compound, which was obtained from fractions 7-19 [solvent system: up to 5% (vol/vol) ethylacetate in n-hexane], was found to be 0.65. Under vacuum, the collective organic solvent from various flasks was allowed to evaporate to produce compound 1 (0.6 g), which is a brown

oily substance. Then by further fractionation and TLC examination of fractions, it was found that in fractions 20 to 34 [solvent system: up to 13% (vol/vol) ethylacetate in n-hexane) a single compound marked as compound 2 was obtained. The R_f value of compound 2 as observed from TLC studies carried out at 20 % EtOAc in n-hexane, was found to be 0.45. The melting point of compound 2 (0.09 g), which was produced by vacuum-evaporating the combined organic solvent from the various fractions, was found to be 136 ^oC. In the next fractions, compounds 2^{nd} and 3^{rd} get eluted out in the same flask. In order to recover the third compound from the mixture, fractions 35–45, as obtained above, are mixed together, and the sub-fraction so obtained was evaporated under vacuum. The residue so obtained was then submitted to repeated column chromatography utilizing gradient elution with 0–15% ethylacetate in n-hexane to get compound 3 (0.04 g) as a white solid. The R_f value of compound 3, as determined through TLC, was found to be 0.31. The melting point of compound 3, as determined by Labwan's digital melting point apparatus, was found to be 163-164^oC. TLC analysis of different fractions obtained by column chromatography was done side by side to check whether a fraction with the pure compound was obtained (**Figure 6.4**).

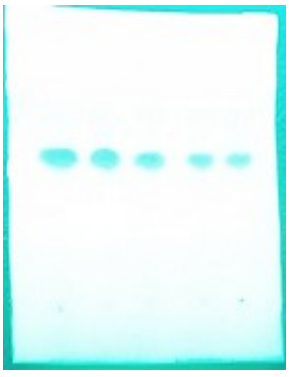
TLC for visualization of different fractions collected from column chromatography



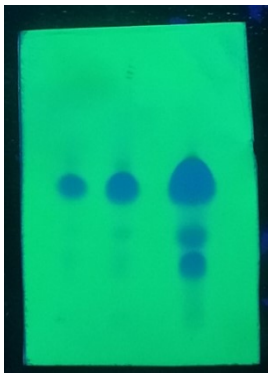
TLC of DCM extract (a)



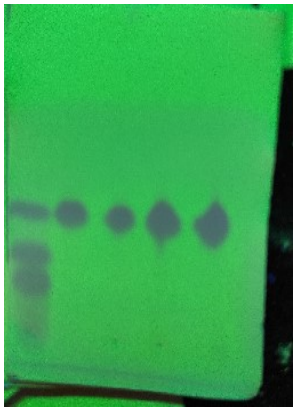
TLC of first fraction (b)



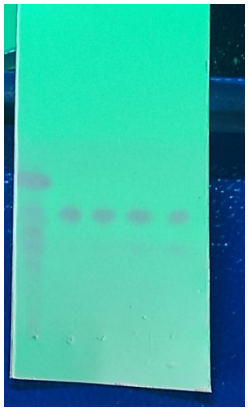
TLC of PC 1(c)



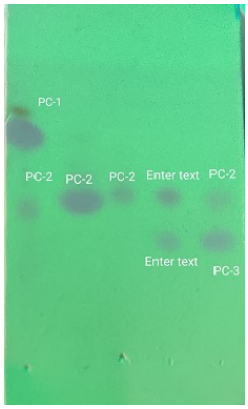
TLC of PC1 and mixture (d)



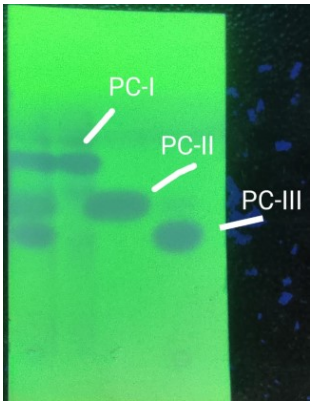
TLC of PC 1, along with a mixture



TLC of PC 2, along with a mixture



TLC of PC 1, PC 2 and PC 3



TLC of mixture (spot 1), PC 1 (spot 2), PC 2 (spot 3) & PC 3 (spot 4)

Figure 6.4 TLC chromatograms of DCM extract and different fractions obtained during column chromatographic separation, visualized using UV chamber

Table 6.3 R_f value, Solubility, TLC solvent system and nature of isolated compounds

S.No.	Compound	Rf value	Solvent system for TLC	Solubility	Nature of the compound
1	PC-1	0.65	20% EtOAc in n-hexane	Dichloromethane, Ethyl acetate, Methanol, Dimethylsulphoxide	Oily
2	PC-2	0.45	20% EtOAc in n-hexane		Solid
3	PC-3	0.31	20% EtOAc in n-hexane		Solid

The current study agrees with previous research where column chromatography was used for the separation of compounds (Baig 2022). In a report by Chopade et al. in 2019, three compounds were isolated from *P. corylifolia* green seeds. These compounds were Psoralen, Bakuchicin and

Bakuchiol, having R_f values of 0.37, 0.48 and 0.63. The R_f value of psoralen was reported to be 0.32 ± 0.02 (Ali et al., 2008). These results are in agreement with the present study.

In the present study, PC1 had R_f value 0.65, and PC3 had R_f value of 0.31 which are comparable to the values of bakuchiol and psoralen, respectively. While doing fractionation, solvents were shifted from non-polar to more polar. Since PC1 was the first compound which got eluted out, it means PC1 is of a more non-polar nature as compared to the rest of the two isolated compounds. Between PC2 and PC3, PC2 is of more non-polar nature than PC3.

Because of the complexity of naturally existing chemical constituents in plants, the separation and purification of pure natural compounds have been a bottleneck in the whole process of discovering therapeutic active molecules. Very frequently, natural product isolation which is a time-consuming and tedious separation and purification process results in a small quantity of compound with a moderately low level of purity at the end. Studies have been done on the purity-activity connections of natural products, and it has been concluded that biological activity is generally steady at purity levels above 90% without experiencing a major influence from contaminants (Jaki et al., 2008).

Generally speaking, the chromatography approach is split into two groups depending on how complicated the procedures are. Conventional procedures, such as TLC, CC, preparative thin layer chromatography (PTLC) and flash chromatography (FC), are considered classical chromatographic techniques. Whereas recent chromatography techniques involve High-Performance thin-layer chromatography (HPTLC), Ultrafast liquid chromatography (UFLC) and High-Performance Liquid Chromatography (HPLC). In addition to establishing a robust mobile phase system for the best resolution of chromatographic separation, analytical HPLC provides a wide-ranging evaluation of the relative proportion of the components in the crude extract (Sarker et al., 2006). The physical and chemical characteristics of a known molecule, viz molecular weight, stability, solubility, charge, and acid-base properties, can be used to choose the isolation techniques for that molecule. If the target compound is an unidentified molecule, it would be more challenging to find such features. In this situation, it is crucial to take into account the nature of the crude extracts, particularly the solvents used. Analytical chromatography techniques should be used in order to establish an overall profile for the extracts.

6.1.4 Liquid Chromatographic Studies/ Analysis of purity of the isolated compounds:

Determining the purity of a pharmaceutical molecule is crucial, particularly when one biological activity is proclaimed as the drug's therapeutic property. However, isolating pure chemicals from a mixture of crude plant extracts has always been difficult. Before continuing with the determination of molecular structure and subsequent testing of biological activity, the purity of the separated compounds desires to be assessed. In fact, the purity level has a significant impact on the process of identifying and characterizing the molecular structure.

The use of UHPLC/UFLC for analytical separations has significantly increased due to better speed and improved performance. Higher pressure improves the separation effectively. Longer high-resolution separations are what UHPLC was first designed for, and this is where it really excels (Kaplitz et al., 2019). One of the most commonly used techniques for pharmaceutical analysis is liquid chromatography, and its most recent type, known as "ultra-fast liquid chromatography," has a number of advantages over older methods, including quicker analysis times, better resolution, higher peak capacities, and minimal solvent consumption. Impurities in a sample can be estimated using UFLC. This technique can be regularly used to check for adulteration in pharmaceutical formulations (Nagavi. and Gurupadayya, 2016). There are numerous uses for UFLC, including the detection of iodiconazole in microdialysis samples, the analysis of podophyllotoxin in rat dermal and blood microdialysis samples, the simultaneous analysis of fluoroquinolones and xanthenes derivatives in serum, the evaluation of isoflavones in soy, and the investigation of catechins in green tea (Bhati et al., 2022).

For determining the major constituents and purity of isolates, a UFLC study of the extracts was performed. The analysis of the DCM and methanolic extract using UFLC eluted with acetonitrile and 0.1 % vol/vol formic acid in water on a C-18 reverse phase column depicted that the extract contains a complex mixture of compounds. Results revealed that in both the extract, there were three major components present at the retention time of 28.03, 28.55 and 40.28 minutes, respectively, along with some minor components (**Figure 6.5**). DCM extract was chosen for further isolation of these components. Column chromatography technique was used for the

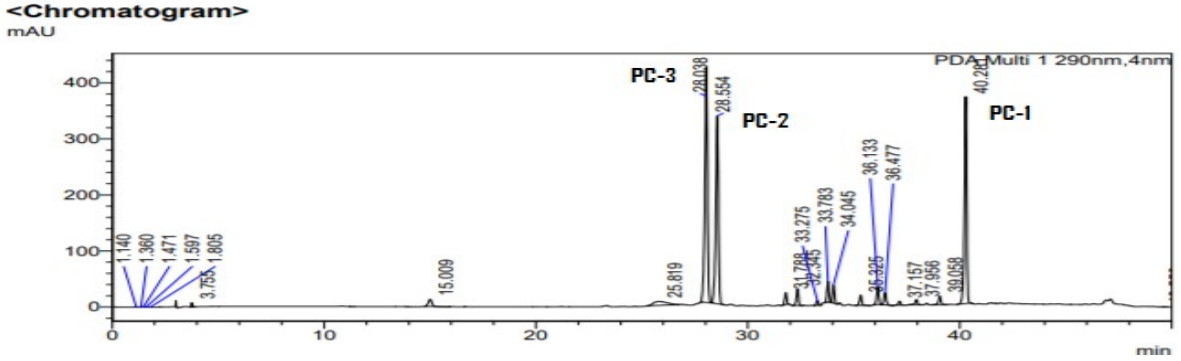
purification process, and the isolates designated as PC-1, PC-2 and PC-3 were further subjected to UFLC to confirm the purity of isolates.

SHIMADZU LabSolutions **Analysis Report**

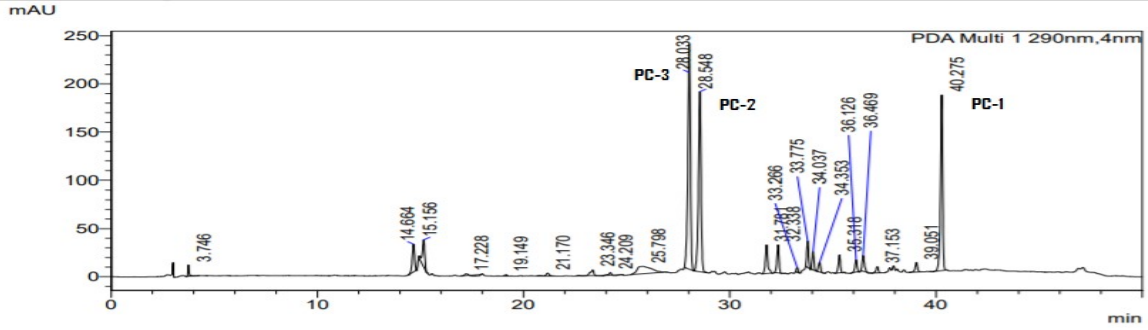
<Sample Information>

Sample Name	: PC-DCM		
Sample ID	: PC-DCM		
Data Filename	: 29-09-21AMIT054.lcd		
Method Filename	: AMIT NM.lcm		
Batch Filename	: amit.lcb		
Vial #	: 1-82	Sample Type	: Unknown
Injection Volume	: 5 uL		
Date Acquired	: 30-09-2021	Acquired by	: Amit Kumar

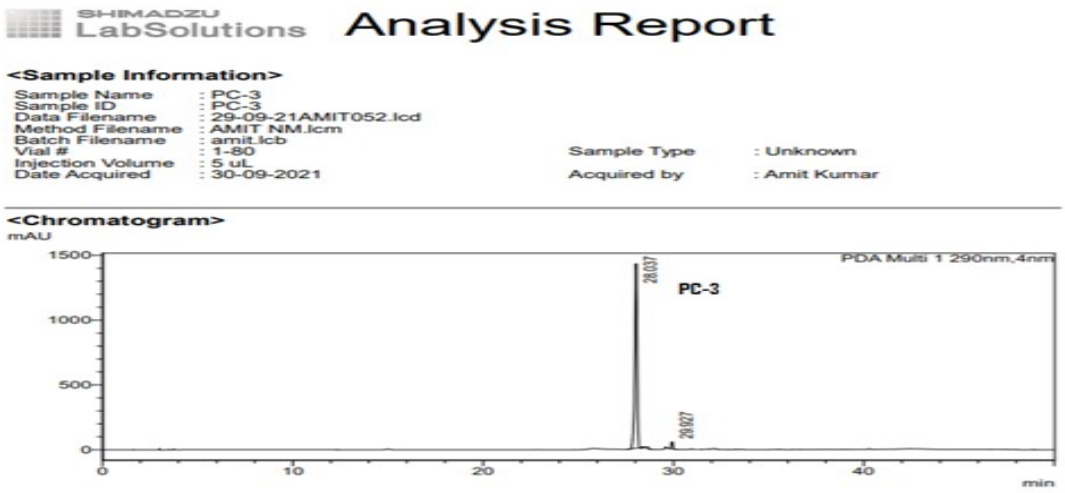
<Chromatogram>



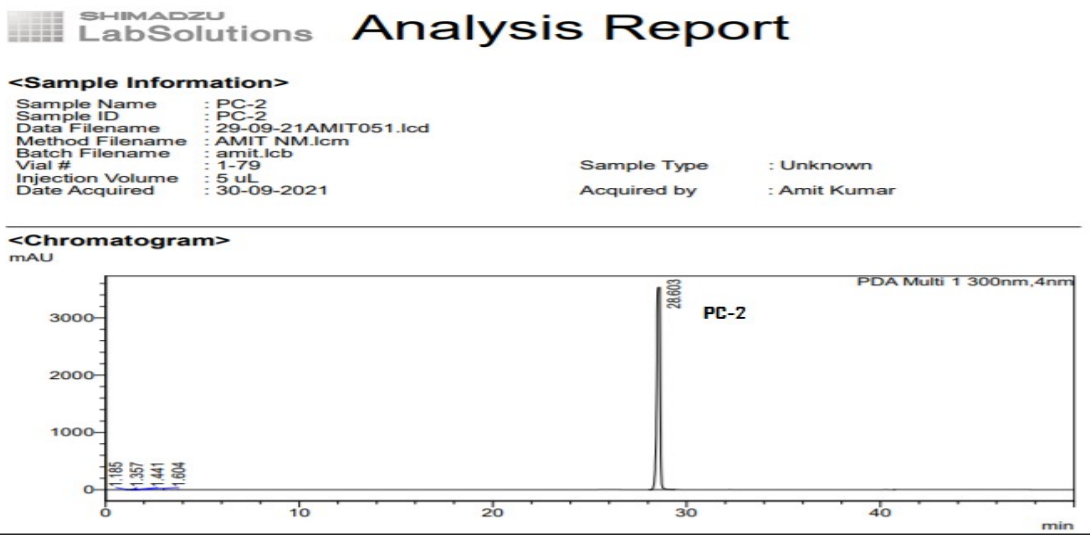
Chromatogram of DCM extract of *P. corylifolia*



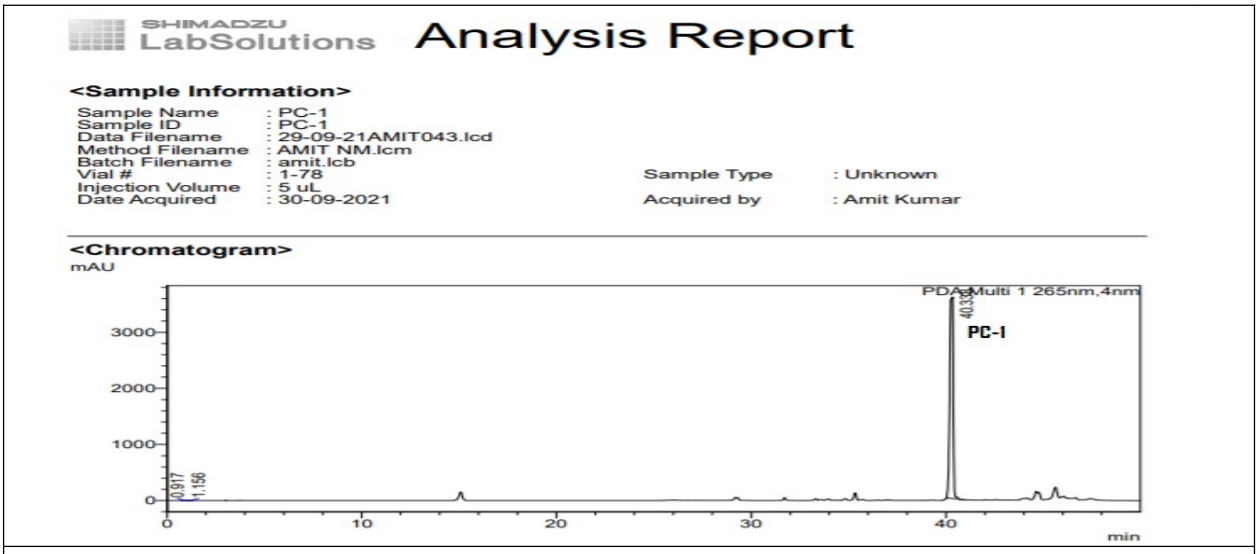
Chromatogram of methanolic extract of *P. corylifolia*



Chromatogram of PC-3 isolated by column chromatography from DCM extract of *P. corylifolia*



Chromatogram of PC-2 isolated by column chromatography from DCM extract of *P. corylifolia*



Chromatogram of PC-1 isolated by column chromatography from DCM extract of *P. corylifolia*

Figure 6.5 Chromatograms of extracts and isolated compounds from *P. corylifolia*

UFLC analysis of the extract as well as of isolates was performed with gradient elution using acetonitrile (ACN)(A) and 0.1 % vol/vol formic acid in H_2O (B). The gradient used was (0−0.01 min, 90% A in B; 0.01-15 min, 90 to 70% A in B; 15-25 min, 70 to 40% A in B; 25-35 min, 40 to 0% A in B; 35-40 min, 0% A in B; 40-45 min, 0 to 90% A in B; and 45-50 min, 90% A in B. The UFLC chromatogram of PC1, PC2 and PC3, as presented in **Figure 6.5**, depicted a single peak on each chromatogram with the retention time of 40.28, 28.55 and 28.03 minutes, respectively, confirming the purity of the isolates.

6.1.5 Structure elucidation of the isolated compounds by NMR:

NMR is a cutting-edge analytical method with numerous applications in the field of pharmaceuticals. It has been known as a structural biology tool for revealing the molecular features of bio-macromolecular systems and carries the responsibility of knowing the basic chemical and biological processes. It can reveal the atomic-level molecular interactions and structure of pharmacological substances (Li et al., 2021). NMR is the most potent of the four spectroscopic techniques because it can generate very informative two-dimensional (2D) spectra using just sub-milligram quantities of amorphous products. It offers extensive details on the

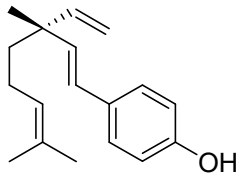
bonding, stereochemistry, and local chemical environment of specific hydrogen and carbon atoms (Van, 2021). Information on the chemical shifts of many metabolites is available in several pioneering reports.

So, elucidation of the structure of the isolated compounds was done by using a spectroscopic technique such as Nuclear Magnetic Resonance (NMR). The sample, whose spectra were to be predicted, was first dried in a high vacuum pump thoroughly so that no organic solvent or water remained. After that, the sample was dissolved in about 1.5 ml of $CDCl_3$ solvent and transferred to an NMR standard tube for further assessment. ^1H NMR spectra were recorded on Bruker FT-NMR 400 MHz instrument. Chemical shift measurements are presented in parts per million (ppm) downfield from tetramethylsilane (TMS). ^{13}C NMR was noted at a 125 MHz spectrometer. Chemical shift data for carbons are reported in parts per million (ppm, δ scale) downfield from TMS. Coupling constants (J) are quoted in Hz. Structure elucidation of two polysaccharides isolated from *P. corylifolia* was conducted by analyzing ^1H and ^{13}C-NMR spectra recorded with Bruker Nuclear Magnetic Resonance spectrometer (Yin et al., 2019). In analogy to that, the structures of compounds isolated in the present work were predicted with ^1H and ^{13}C-NMR spectra obtained with Bruker spectrometer.

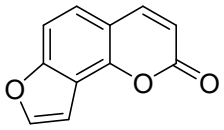
The ^{13}C-NMR data of PC-1 indicated the presence of 18 C-atoms comprising three CH_3 groups, two -CH_2 groups, one =CH_2 group, eight CH groups, and four quaternary C-atoms. TheCH_3 groups resonated at δ (H) 1.14 (s, 3H), 1.52 (s, 3H), 1.59 (s, 3H), CH_2 groups at 1.42 (t, 2H), 1.87 (q, 2H), and CH at 5.03 (t, 1H), 5.80 (dd, 1H), 5.97(d, 1H), 6.17 (d, 1H), 6.68 (d, 2H), 7.16(d, 2H) in the 1H NMR indicated that the compound PC-1 is Bakuchol. Similar NMR studies of PC-2 and PC-3 were also carried out, and it was observed that PC-2 is isopsoralen while PC-3 is Psoralen.

^{1}HNMR and ^{13}CNMR studies, when compared with available literature (Jiangning et al., 2005), also suggest that PC-1 is Bakuchiol, PC-2 is Isopsoralen, and PC-3 is Psoralen (**Table 6.4**).

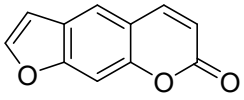
Structures of Bakuchiol (PC-1), isopsoralen (PC-2) and psoralen (PC-3), as confirmed by NMR studies are given in **Figure 6.6**.



Bakuchiol



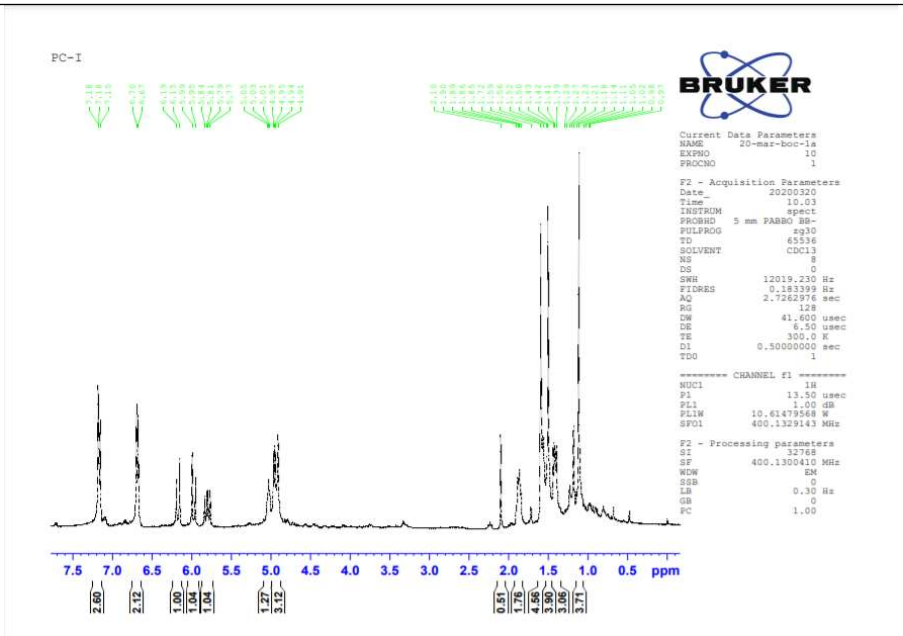
Isopsoralen



Psoralen

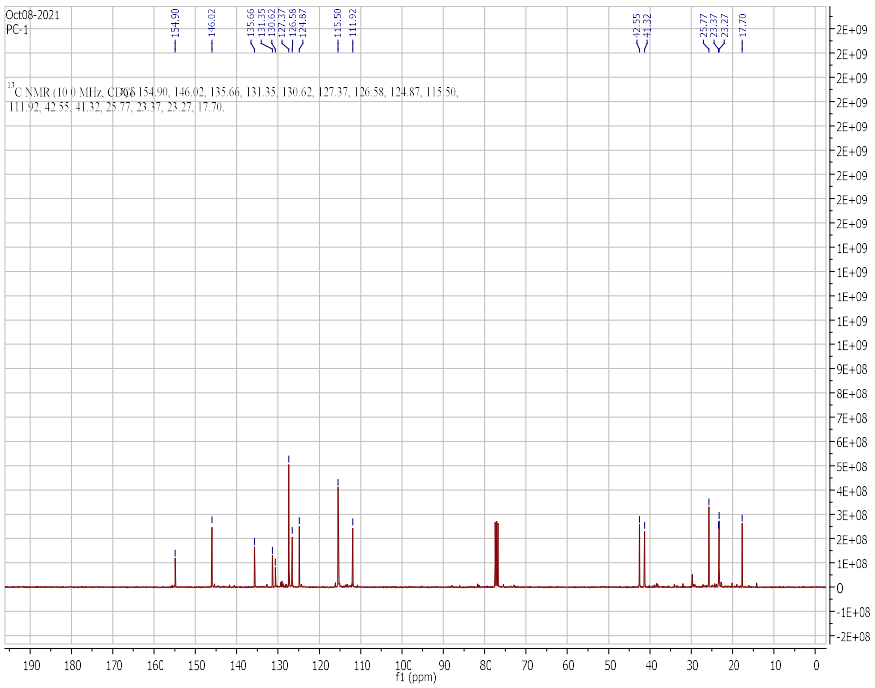
Figure 6.6: Chemical structures of PC-1, PC-2 and PC-3

6.1.6 Spectra of Pure CompoundsPC-1, PC2 and PC-3:

Compound	Spectra
PC 1 **^{1}H NMR**	

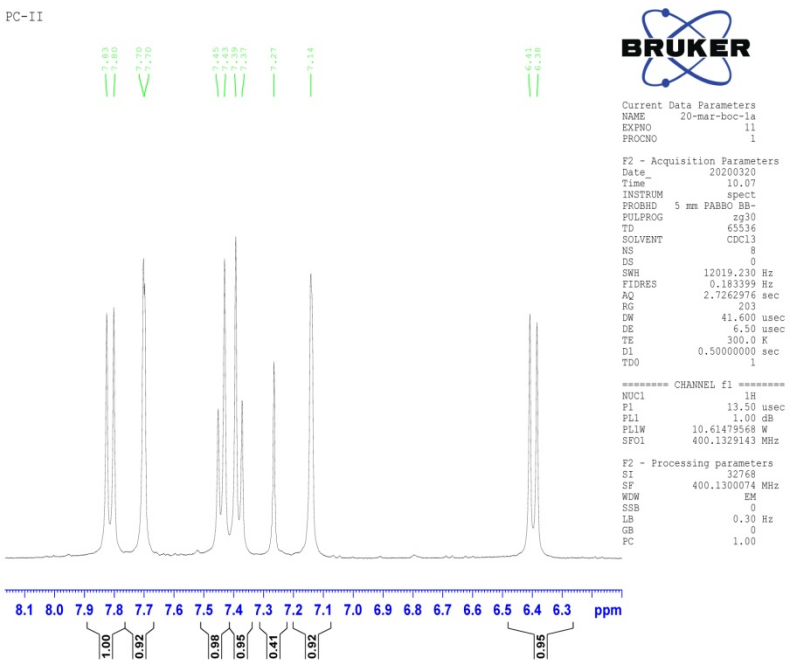
PC-1

^{13}C NMR



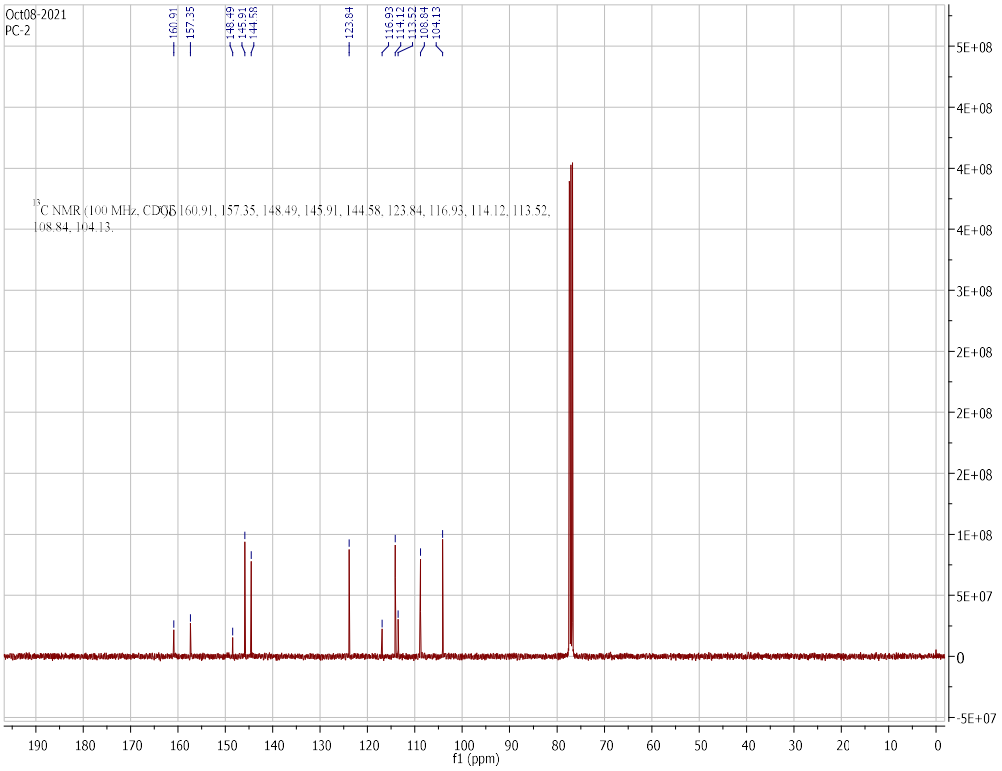
PC 2

^{1}H NMR



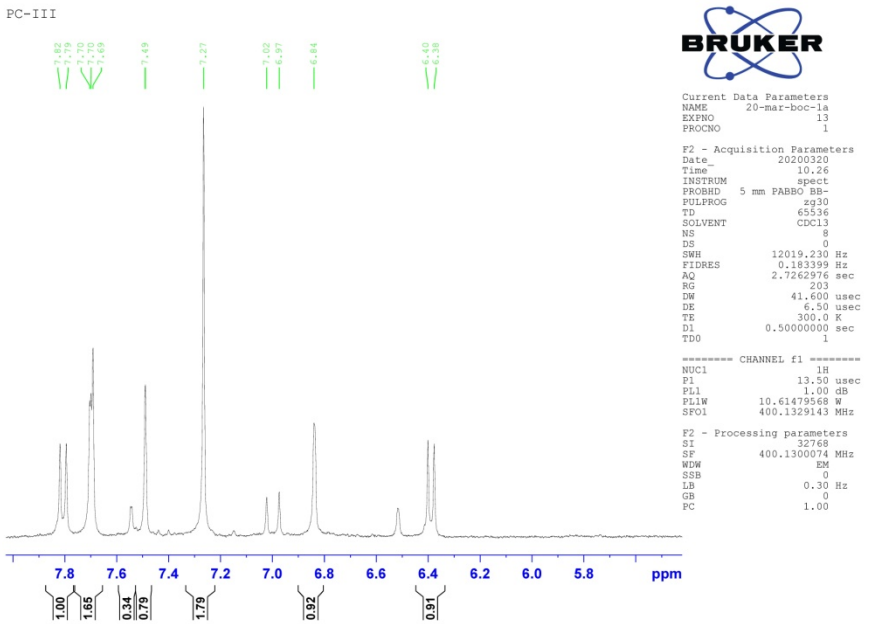
PC-2

^{13}C NMR



PC 3

^{1}H NMR



PC 3

^{13}C NMR

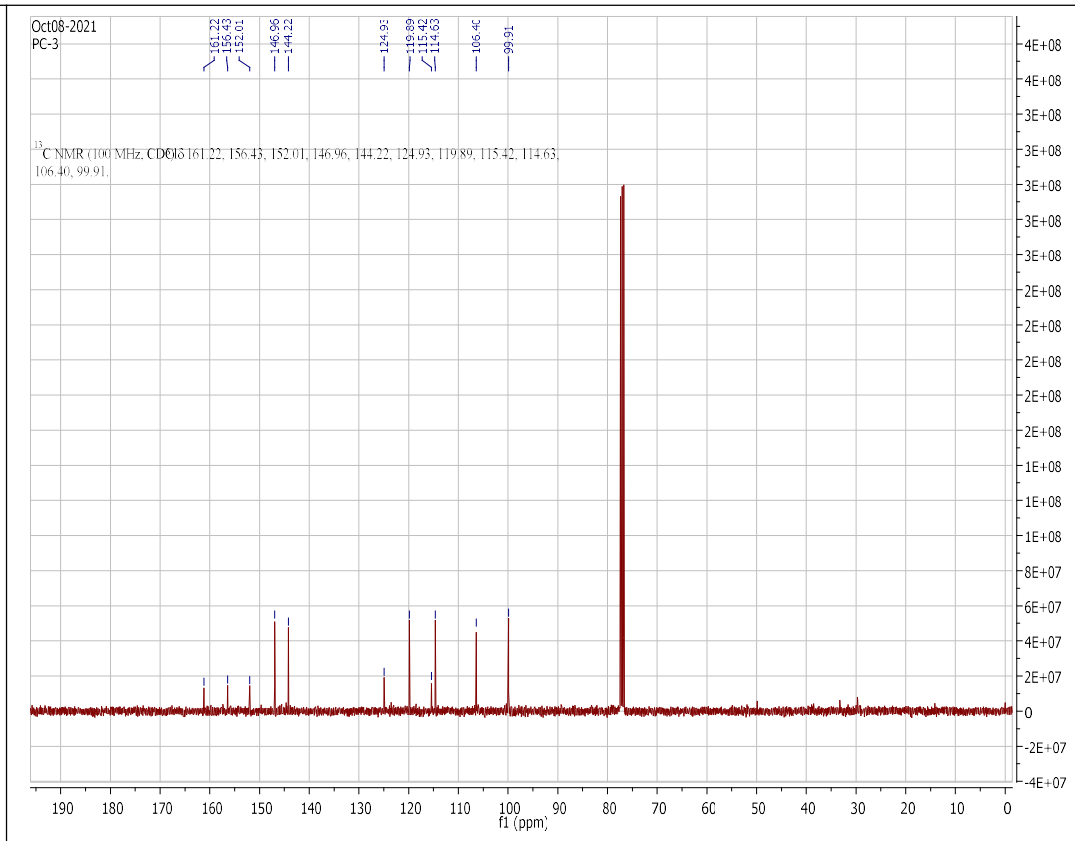


Figure 6.7 H^1 NMR and C^{13}NMR of different isolates, isolated by column chromatography from DCM extract of *Psoralea corylifolia*

Table 6.4 Spectral and melting point (MP) data of the major constituents isolated from DCM extract of *Psoralea corylifolia* L.

Compound	**Data of spectra**		**MP (°C)**	**Literature Values (Jiangning et al. 2005)**	
	^{1}H NMR (400 MHz) TMS as internal standard	**^{13}C NMR (100 MHz, CDCl3) δ: TMS as internal standard**		**^{1}H NMR (400 MHz) Tetramethyl silane (TMS) as internal standard**	**^{13}C NMR (100 MHz) Tetramethyl silane (TMS)as internal standard**
Bakuchiol	1.14 (s, 3H), 1.42 (t, 2H), 1.52 (s, 3H), 1.59 (s,3H), 1.87 (q, 2H), 5.03 (t, 1H), 5.80 (dd, 1H), 5.97 (d, 1H), 6.17 (d, 1H), 6.68 (d, 2H), 7.16 (d, 2H).	154.90, 146.02, 135.66, 131.35, 130.62, 127.37, 126.58, 124.87, 115.50, 111.92, 42.55, 41.32, 25.77, 23.37, 23.27, 17.70.	--	"1.19(s, 3H), 1.46 (t, 2H), 1.52 (s, 3H), 1.63 (s, 3H), 1.96 (q, 2H), 5.10 (t, 1H), 5.89 (q, 1H), 6.05 (d, 1H), 6.20 (d, 1H), 6.70 (d, 2H), 7.20 (2H), 9.37 (s, 1H)"	"156.7, 145.9, 134.0, 130.5, 128.4, 127.2, 126.6, 124.8, 115.4, 111.82, 42.2, 41.08, 40.53, 25.61, 23.03, 17.61"
Isopsoralen	6.39 (d, 1H), 7.14 (m, 1H), 7.38, d, 1H), 7.44 (d,1H), 7.70 (m, 1H), 7.82 (d, 1H)	160.91, 157.35, 148.49, 145.91, 144.58, 123.84, 116.93, 114.12, 113.52, 108.84,	136 (lit. value 137-138)	"6.40 (d, 1H), 7.14 (m, 1H), 7.38 (d, 1H), 7.44 (d, 1H), 7.70 (m, 1H),7. 82(d,1H)"	"160.8, 114.1, 144.4, 123.8, 108.8, 157.4, 116.9, 148.5, 113.5, 145.8, 104.1"

		104.13.			
Psoralen	6.39 (d, 1H), 6.84 (d, 1H), 7.49 (s, 1H), 7.69 (s, 1H), 7.70 (d, 1H), 7.80 (d, 1H)	161.22, 156.43, 152.01, 146.96, 144.22, 124.93, 119.89, 115.42, 114.63, 106.40, 99.91.	161-162 (Lit. value 162-163)	"6.36 (d, 1H), 6.84 (d, 1H), 7.48 (s,1H),7.68 (s, 1H), 7.70 (d, 1H), 7.80 (d, 1H)"	"160.8, 114.5, 144.0, 119.8, 124.8, 156.3, 99.6, 151.9, 115.3, 106.3, 146.8"

6.2 Biological Evaluation:

Natural products have been used for a very long time as pharmaceuticals, pharmaceutical precursors, and/or adjuvants in complementary medicine. The best chemist is nature, which has produced countless natural products with diverse structures and distinctive diversity. For ages, people have found, researched, and used resources made from plants, microorganisms, and animals. After the determination of structure, new techniques and pharmacological targets can be widely used to evaluate the biological functions of active ingredients found in conventional medicine (Zhu et al., 2022). Biological activity or pharmacological activity in pharmacology refers to the positive or negative effects of a compound on living things. This activity is exhibited by the active ingredient or pharmacophore when a pharmaceutical is a complex chemical mixture, albeit it can be altered by the other ingredients. Pharmacological/biological activity is one of the many characteristics of chemical compounds. Chemical substances, however, may exhibit some unfavourable and toxic consequences, which may preclude their usage in the medical field. In general, dose affects activity. Furthermore, switching from low to high doses of a chemical frequently results in consequences that can be both positive and negative. Therefore, it is crucial to monitor drug concentrations and effects while developing new medications (Longuespee et al. 2021). Meeting the ADME standards is thus essential for activity. In order to be a successful drug, a chemical

must not only be active against a target but also have the required ADME (Absorption, Distribution, Metabolism, and Excretion) properties (Pathak et al., 2020).

6.2.1 *In vitro* anti-obesity activity of extracts/isolated compounds of *Psoralea corylifolia:*

Both in developed and emerging economies, obesity has recently reached alarming levels. Although there is increasing interest in herbal medicines around the world, their use is being constrained by a lack of sufficient systematic investigations and scientific information on plants and herbs. Therefore, more in-depth investigations of herbs are required.

Before determining the anti-obesity activity of extracts/isolated compounds of *P. corylifolia*, first of all, the toxicity of extracts/compounds was determined by MTT (dimethyl thiazol diphenyl tetrazolium bromide) assay. After that, anti-obesity activity was determined by anti-adipogenesis and anti-lipase activity. The hallmark events of obesity are thought to be lipid droplet formation and preadipocyte differentiation into mature adipocytes (Rayalam et al., 2008).

6.2.1.1 Cell viability assay:

To determine the effects of isolated compounds or DCME on cell viability and for estimation of safe and toxic concentration levels, an MTT cell viability assay was done on 3T3-L1 cells treated with 12.5 µM - 50 µM concentration of isolated compounds or 100 µg/ml- 400 µg/ml of DCME and for non treated non-differentiated cells. Data were collected after 2 days. The percentage of cells that survive in comparison to control cells is how the results of cell viability are expressed, which was calculated on the basis of O.D. measurement of the purple colour of the formazan product, which was formed due to the action of viable cells on MTT. The experiment was performed in triplicates. At concentrations up to 100 µg/ml, the DCM extract of *P. corylifolia* did not exhibit any discernible toxicity to 3T3-L1 cells. At 100 µg/ml, 125µg/ml,150 µg/ml, 175 µg/ml, 200 µg/ml and 400 µg/ml extract concentration, the percentage cell viability was 97.02%, 78.54%, 65.25%, 60.59%, 58.47% and 52.25% respectively. So, at a concentration greater than 100 µg/ml, DCM extract significantly reduced the viability of 3T3-L1

cells (**Figure 6.8**). As a result, a safe concentration of DCM extract for additional studies was found to be up to 100 µg/ml.

Cell viability studies on isolates were also carried out, and it was observed that at concentrations up to 25 µM, none of the isolates exhibited any appreciable toxicity to 3T3-L1 cells. At a concentration higher than 25 µM, the viability of 3T3 -L1 cells was decreased (**Figure 6.9**). Cell viability when treated with PC1 at a concentration of 12.5 µM, 25 µM and 50 µM was 96.23%, 93.97% and 85.10%, respectively, PC2 at a concentration of 12.5µM, 25 µM and 50 µM was 96.77%, 91.90% and 87.25% respectively whereas cell viability percentage of 3T3 -L1 cells which were treated with PC3 at a concentration of 12.5µM, 25 µM and 50 µM was 95.43%, 94.11% and 88.20% respectively. As a result, safe doses of PC-1, PC-2, and PC-3 were established as up to 25 µM for future research.

S.No.	Sample	% Cell Viability
1	Control	100
2	Extract 100	97.02
3	Extract125	78.54
4	Extract150	65.25
5	Extract 175	60.59
6	Extract 200	58.47
7	Extract 400	52.25

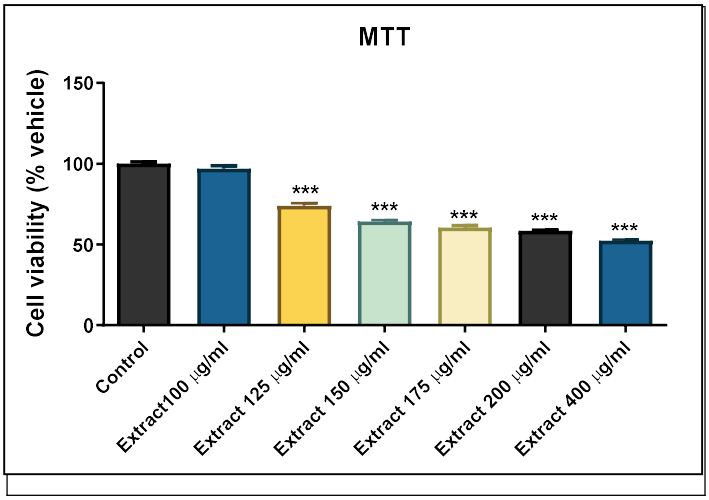


Figure 6.8 Viability percentage of 3T3L1 cell line after the addition of DCME at different concentrations. DCME (100µg/ml, 125µg/ml, 150 µg/ml, 175 µg/ml, 200 µg/ml and 400 µg/ml) were added to various wells with the same number of cells. Cells that weren't treated served as the control group. After adding the MTT reagent, the absorbance of the dissolved formazan crystals in DMSO was measured to determine the cell viability for each group. Safe /toxic conc. were determined. Data is presented as mean ± SEM. (* $p < 0.05$, ** $p < 0.01$ *** < 0.001as compared to control).

SNo.	Treatment	% cell viability
1	Control	100
2	PC1 12.5µM	96.2313
3	PC1 25 µM	93.9759
4	PC1 50 µM	85.1084
5	PC2 12.5µM	96.7710
6	PC2 25 µM	91.9036
7	PC2 50 µM	87.2578
8	PC3 12.5µM	95.4313
9	PC3 25 µM	94.1108
10	PC3 50 µM	88.2024

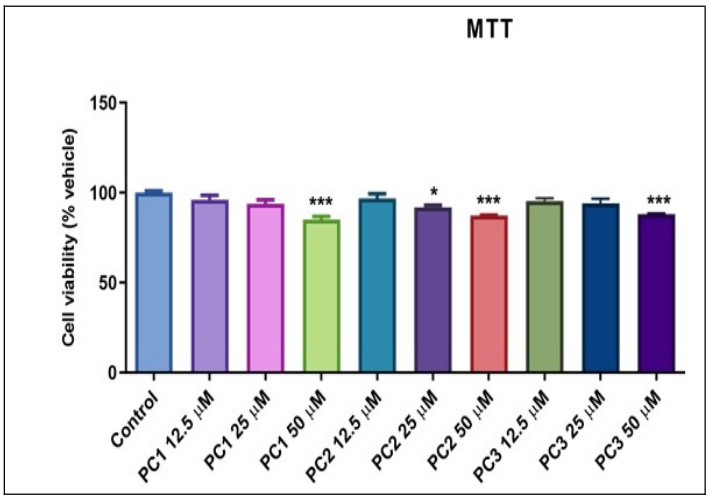


Figure 6.9 Viability percentage of 3T3L1 cell line after the addition of PC1, PC2 and PC3 at different concentrations. Isolated compounds (at 12.5 µM, 25 µM & 50 µM concentrations) were added to different wells containing the same number of cells. Cells that weren't treated served as the control group. After adding the MTT reagent, the absorbance of the dissolved formazan crystals in DMSO was measured to determine the cell viability for each group. Safe and toxic concentrations were determined. Data are presented as mean ± SEM. (* $p < 0.05$, ** $p < 0.01$ *** < 0.001as compared to control).

Some previous studies have already established that plant compounds' cytotoxicity can be found by performing an MTT assay. The MTT assay has been used for about 40 years to assess cell viability and proliferation, drug cytotoxicity, and mitochondrial activity (Stockert et al., 2018). In an earlier study, when adrenal phaeochromocytoma (PC12) cells were treated with water extract of *P. corylifolia* seeds at 1–100 μg/ml for 24 h, no cytotoxic effects were detected (Lee et al., 2018). This study supports our study of MTT assay on extracts where up to 100 μg/ml, no significant reduction in cell viability was found. Madrid et al. reported that bakuchiol isolated from *Psoralea glandulosa* reduced cell viability to 50% at 29.3 ± 0.4 µM concentration as checked by MTT assay (Madrid et al., 2015). Recently, Kumar et al. studied the cell viability with Bakuchiol and disclosed that no toxicity was observed on RAW cell lines when checked at 10, 30, and 100 µM concentrations of bakuchiol and incubated for 48 h (Kumar et al., 2021). In another study, eleven different compounds named bakuchalkone, bavachromene,

corylifol C, corylin, 7,2′,4′-trihydroxy-3-arylcoumarin, isowighteone, 6-prenylnaringenin, psorachalcone A, psoracoumestan, wighteone and xanthoangelol were isolated from *P. corylifolia* seeds. The cytotoxicity of the compounds was analyzed by MTT assay using three mammalian cell lines, rat H4IIE hepatoma and C6 glioma and human Hct116 colon carcinoma. Most compounds displayed only mild cytotoxicity (IC50 value >50 M) (Limper et al., 2013). Thus, MTT assay is a good choice to check the cytotoxicity of the isolated compounds.

6.2.1.2 Anti adipogenesis assay:

A reliable and often used model for studying adipogenesis and the processes that take place in mature adipocytes is the 3T3-L1 murine cell line. Hormones can cause fibroblast-like cells to develop into mature adipocytes. With the emergence of the global "obesity issue," the usage of the 3T3-L1 adipose differentiation model has recently increased. The adipocyte is not merely a fat store but a crucial metabolic regulator that produces cytokines, substrates for metabolism, and adipokines, exerting control over metabolism both locally and systemically. Between 24 and 48 hours into the 3T3-L1 adipocyte's development, it commits to differentiate, and then the lipid-accumulation process begins (Roberts et al. 2009).

By introducing hormonal inducers such as insulin, 1-methyl-3-isobutyl xanthine (IBMX) and dexamethasone, preadipocytes (3T3-L1) begin to differentiate *in vitro*. While the synthetic glucocorticoid IBMX increases the glucocorticoid pathways and activates CCAAT/enhancer-binding protein beta (C/ebpβ) gene expression, dexamethasone increases the expression of the C/ebpδ gene.

The beginning of adipogenesis is indicated by the expression of both C/EBPβ and C/EBPδ. Peroxisome proliferator-activated receptor gamma (PPARγ) is a protein that is expressed during the second day of differentiation. PPARγ, in turn, increases the expression of C/ebpα. Observable lipid increases in the cells can be seen (Jakkawanpitak et al., 2021).

The increase in Oil Red O-stained cells and fat accumulation are related to the development of pre-adipocytes into mature adipocytes. 3T3-L1 cells were treated for two days (24 hours) with differentiation media plus 100 µg/ml dichloromethane extract of *P. corylifolia* seeds or 25µM bakuchiol/isopsoralen/psoralen to realize if *P. corylifolia* influences adipogenesis.

In addition, for 10 days, the cells were moved to basal medium with 1μg/ml insulin (maintenance medium), with medium change every other day. Extracts/isolated compounds were added to the differentiation and maintenance medium containing 0.1% DMSO for up to twelve days as part of the anti-adipogenesis experiment. Cells treated with differentiation medium and maintenance media serve as the control. Cells were observed under an inverted microscope on day 12^{th} after being stained with an Oil Red O stain. When examined under a microscope, the Oil Red O stain showed a significant decrease in the amount and magnitude of lipid droplets gathered in the cells treated with extract/isolates. Hence, dichloromethane extract, as well as isolated compounds treatment, led to significant suppression of adipogenesis (**Figure 6.11a-e**). OD detection was done after image capture. According to the findings, treatment with the extract or isolates significantly reduced OD as measured by the percentage of lipid accumulation (**Figure 6.10**). Specifically, treatment with dichloromethane extract (100 μg/ml), bakuchiol (25 μM), isopsoralen (25 μM) and psoralen (25 μM) led lipid accumulation to 75 ± 0.003%, 78.06 ±0.002%, 80.91 ± 0.004% and 80.91 ± 0.001% when compared with control respectively. Shaikh et al. stated in their investigation that due to the effect on the adipocyte life cycle, obesity-related low-grade inflammation, and oxidative stress, *P. corylifolia* extract possesses anti-obesity and anti-diabetic properties (Shaikh et al., 2021) supporting our work on DCME to have anti-obesity property. The degree of inhibition was highest in cells treated with DCM extract as compared to the isolated compounds. The fact that DCME demonstrated greater action than the isolated compounds demonstrated in the present study is intriguing. This may be because the *P. corylifolia* seed extract contains several substances known to have anti-obesity potential, such as genistein (Kim et al., 2006; Grossini et al., 2018), bavachin (Lee et al., 2016), isobavachalcone (Lee et al., 2018), and corylin (Chen et al., 2021).

Compound (Conc.)	% Lipid accumulation
PC1 (25µM)	78.06 ± 2.67
PC2 (25µM)	80.91 ± 4.69
PC3 (25µM)	80.91 ± 1.86
DCM extract (100µg/ml)	75 ± 3.57

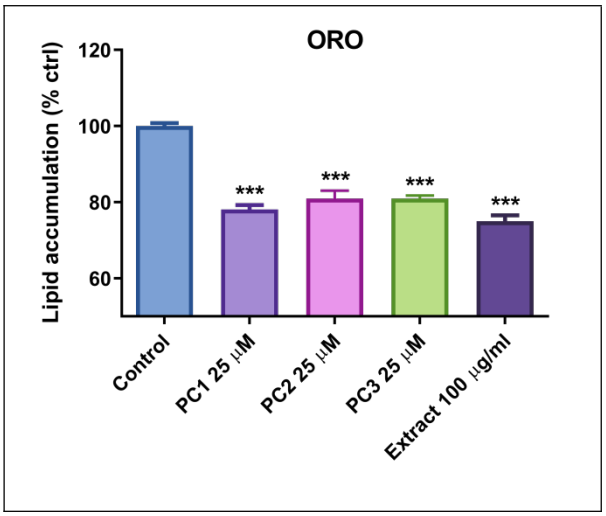
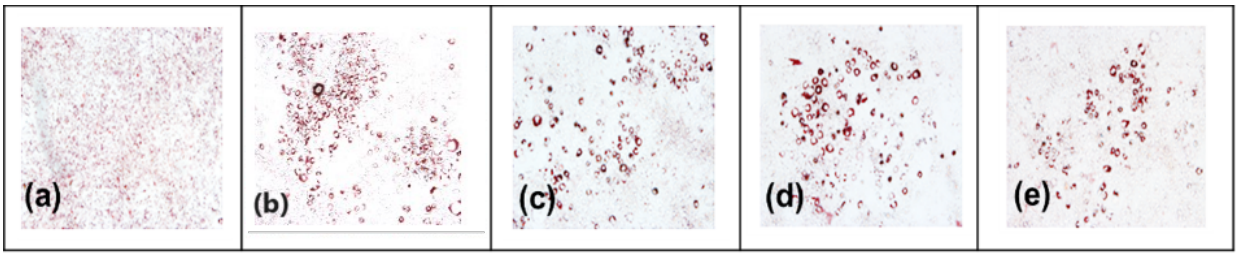


Figure 6.10 Percentage of lipid accumulation after treatment with DCM extract /Isolates of *Psoralea corylifolia.* Inhibition of fat accumulation in 3T3-L1 via suppression of adipogenesis by DCM extract/isolated compounds from *Psoralea corylifolia* as determined by Oil O Red staining. 3T3-L1 pre-mature cells were allowed to proliferate in DMEM media in control and DCM extract/isolates (PC-1, PC-2 & PC-3) treated groups. The Oil Red O solvent was used to stain the lipid droplets that had accumulated in mature differentiating 3T3-L1 cells, and the number of droplets was determined by measuring the absorbance with the use of a micro titer plate reader spectrophotometer. Data are presented as mean ± SEM (n=5). (* < 0.05, ** < 0.01 and *** < 0.001 versus control group). 100% lipid accumulation was assumed to have occurred in the control group, which consisted of normal adipocytes supplemented with 1% DMSO.

Photographs after Oil Red O staining



(a) Control, (b) PC1 treated, (c) PC2 treated, (d) PC3 treated, (e) DCM extract treated

Figure 6.11 Inverted microscope images of cells treated with extract/isolates. Red spots in images represent areas stained by Oil Red O Dye, i.e., fat deposits

Observations made in the present study revealed that phytochemicals separated/isolated from *P. corylifolia* seed extract have an anti-obesity effect. In contrast to psoralen/isopsoralen, which are coumarins, bakuchiol is a mero terpenoid (phenolic substance). A terpenoid compound named crypto tanshinone isolated from *Salvia miltiorrhiza,* when administered, decreased the body weight of obese mice in a dose-dependent manner (Kim et al. 2007). It was found that this compound could prevent the differentiation of white adipocytes into lipid-accumulating cells by controlling the expression of adipogenesis-related genes (such as PPAR$^{\gamma}$, C/EBPα, and FABP4) via the STAT3 signalling pathway (Rahman et al. 2016). Furthermore, crypto tanshinone was found to stimulate mitochondrial biogenesis in C3H10T1/2 Mesenchymal stem cells (MSCs) and commitment to brown adipocytes via AMPK and p38-MAPK signalling pathways. Over the last few years, phenolic compounds' ability to treat non-communicable diseases like cancer, obesity and their comorbidities has been thoroughly established. According to Wang et al. (2014), consuming polyphenols can reduce food intake, reduce lipogenesis, increase lipolysis, stimulate fatty acid (FA) β-oxidation, limit adipocyte differentiation and proliferation, attenuate inflammatory reactions, and reduce oxidative stress, which could be the possible mechanism against obesity (Wang et al., 2014). The present findings support the anti-obesity potential of terpenoids and phenols.

The current results provide additional evidence for prior research that has shown that *P. corylifolia* extract may be a candidate for the development of novel drugs; this plant extract has previously been shown to have anti-obesity properties. According to one study, PCS extract administration dramatically decreased serum lipid and hepatic triglyceride levels as well as lipid buildup in the liver and adipose tissue (Seo et al., 2016). This study supports our decision to evaluate *P. corylifolia* for its anti-obesity properties. Over time, excess energy is retained as triglycerides in the adipose tissue when energy intake surpasses energy disbursement. Increased adipose tissue mass can be brought about by the rise in cell size, cell number, or both (Spiegelman and Flier, 1996). *In vitro* models of adipogenesis, such as the 3T3-L1 cell lines, are being utilised to study pre-adipocyte differentiation, which has recently become a focus of intense research (Marrelli et al., 2020).

6.2.1.3 Porcine Pancreatic lipase Assay:

Plants are a wonderful source of many natural compounds that can be investigated and developed as therapeutic medicines for the control of obesity and its complications. In the current work, *P. corylifolia* was investigated as a potential source of pancreatic lipase inhibitors. This study aims to treat and manage obesity by changing lipid metabolism by inhibiting dietary fat absorption using a digestive enzyme, pancreatic lipase, which is an intriguing, comparatively safer, and less explored approach towards the development of an anti-obesity drug. This was done keeping in mind the potential direct and indirect harmful effects of excess fat accumulation in the body.

In order to examine the inhibitory effect of PC1, PC2, and PC3 as well as dichloromethane extract of *P. corylifolia* at a concentration of 25, 50, and 100 µg/ml on pancreatic lipase, an *in vitro* PPL inhibition assay was conducted to measure the amount of p-nitrophenol (yellow colour) obtained after hydrolysis of p-nitrophenyl butyrate (p-NPB). A dose-dependent reduction in pancreatic lipase activity was observed. At 100 µg/ml concentration level, bakuchiol, isopsoralen, and psoralen suppressed pancreatic lipase by 24.2 ± .037, 22.69 ± .026 and 22.24 ± .057%, respectively. DCME and orlistat (positive control) at 100 µg/ml suppressed lipase enzyme to 26.02 ± .041and 40.93 ± .024 %, respectively. At 50 µg/ml, PPL inhibition percentage by PC1, PC2, PC3, DCME, and orlistat was 21.06%, 16.52%, 17.53%, 24.62% and 40.09%, respectively, and at 25 µg/ml concentration PPL inhibition percentage was 13.80%, 14.98%, 17.79%, 24.02% and 31.69% respectively (**Table 6.5, Figure 6.12**). Thus, DCME has the highest inhibition percentage as compared to the isolated compounds.

Table 6.5: Porcine Pancreatic lipase inhibition percentage of PC1, PC2, PC3, DCM extract and orlistat at different concentration

S. No.	Treatment		Porcine Pancreatic lipase inhibition (%)
	Compound	Concentration	
1	PC1	100 µg/ml	24.20 ± 0.37

2	PC1	50 µg/ml	21.06 ± 0.02
3	PC1	25 µg/ml	13.80 ± 0.37
4	PC2	100 µg/ml	22.69 ± 0.26
5	PC2	50 µg/ml	16.52 ± 0.30
6	PC2	25 µg/ml	14.98 ± 0.14
7	PC3	100 µg/ml	22.24 ± 0.57
8	PC3	50 µg/ml	17.53 ± 0.42
9	PC3	25 µg/ml	17.79 ± 0.42
10	DCME	100 µg/ml	26.02 ± .041
11	DCME	50 µg/ml	24.62 ± 0.56
12	DCME	25 µg/ml	24.02 ± 0.30
13	Orlistat	100 µg/ml	40.93 ± 0.24
14	Orlistat	50 µg/ml	40.09 ± 0.49
15	Orlistat	25 µg/ml	31.69 ± 0.39

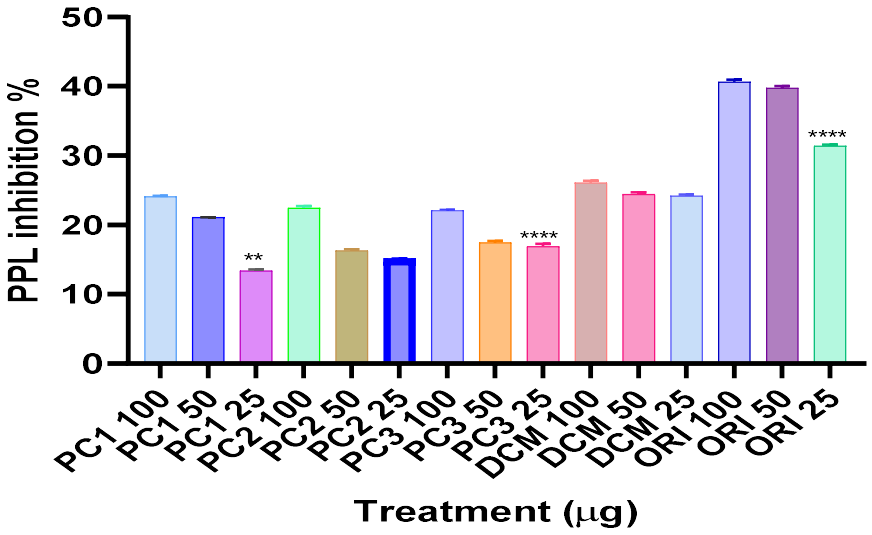


Figure 6.12 PPL inhibition percentage of bakuchiol, isopsoralen, psoralen, DCM extract and orlistat at different concentrations. Values are represented as mean ± SEM (n=3). A statistically significant difference was obtained using one-way ANOVA followed by Tukey's post-hoc test. Orlistat (Ori) was used as a positive control.

In a recent study, chloroform, ethanol and acetone extract from leaves of *P. corylifolia* displayed anti-lipase activity (Karale et al., 2022). This study has revealed that *P. corylifolia* exhibited anti-lipase activity with different solvent extracts. Moreover, pancreatic lipase has been seen to be inhibited by a variety of extracts from herbs and spices, including *Camellia sinensis*, *Syzygium anisatum*, *Glycyrrhiza uralensis*, and *Vitis rotundifolia,* which are rich in polyphenolic compounds. Individual polyphenolic substances have also been reported to inhibit pancreatic lipase (Martinez et al., 2017). Hence, the presence of terpenoids, flavonoids and polyphenols in the DCME extract of *P. corylifolia* supports pancreatic lipase inhibitory action and anti-obesity effects of *P. corylifolia*.

Pancreatic lipase was strongly inhibited by Bavachalcone, corylifol, and isobavachalcone isolated from Fructus *Psoraleae* with IC50 < 10 mol l^{-1}. Based on docking simulations, both isobavachalcone and bavachalcone were found to be capable of forming hydrophobic and hydrogen-bonding with the important residues in the catalytic pocket of pancreatic lipase (Hou et

al., 2020). Thus, our work favours the report of Hou et al. which claims that *Psoralea* is a potential anti lipase agent.

After PPL inhibition assay and anti-adipogenesis studies, our next endeavour was to identify the specific target responsible for the anti-obesity potential of isolated compounds from *P. corylifolia*. For that, insilico studies with peroxisome proliferator activated receptor gamma (PPAR-γ), a transcriptional factor which is a key regulator of obesity and metabolism, were chosen as a practical option. The results of docking studies are depicted in section 6.2.2.

6.2.2 Docking studies of isolated compounds:

The development of new drug candidates is a labour and money-intensive process. In the pre-clinical stage of drug discovery, computational methods can be used for the accomplishment of this task. Well over 70 commercialized medications have been discovered so far by using some sort of computational approach. A relatively effective and affordable approach used in drug development is virtual screening, which allows researchers to systematically look for new small compounds with biological activity, typically against a target protein molecule. In order to speed up the search process, particularly when a protein 3D structure is known, molecular docking has been widely employed in virtual screening. Predicting a ligand's bound conformation within a receptor's binding site is the main goal of docking. The two virtual screening tools AutoDock and GLIDE, are reported to be used the most frequently, with AutoDock taking the top spot. The third most popular software program is GOLD (Sabe et al., 2021).

Due to the speed and accuracy of docking, Auto Dock Vina is frequently suggested as the first preferred tool in molecular docking among all the other available tools of Autodock. In AutoDock Vina, the scoring function that sums the intramolecular energy and the intermolecular energy (ligand-receptor) is used to determine the binding affinity (Tang et al., 2022).

An understanding of binding geometries and interaction is necessary to fully comprehend the structural characteristics that govern how strongly a ligand binds to its receptor. The molecular docking program Autodock and Vina, when used along with the graphical software PyMOL, enable the study of molecular combinations to visualize and hence analyze the structure-based drug discovery efforts (Rauf et al., 2015).

In the present investigation, Autodock vina was used for docking studies and SwissADME tool for ADME studies. Different binding poses are produced during docking, and the best ones are chosen based on their binding energy, binding score, and other free energies. There are several reports supporting the use of these tools for such types of studies.

6.2.2.1 ADME Studies - The most important and difficult step in the discovery and development of new pharmaceuticals is conducting drug metabolism and pharmacokinetics (DMPK) studies, also known as ADMET, and it is responsible for the failure of over 60% of all treatments in the clinical phases. The SMILES nomenclature (**Table 6.6**) was used to introduce the ligands' molecular structures into the ADME/Tox web tools SwissADME.

Table 6.6 List of SMILES and PubChem IDs of ligands

Ligand	Canonical SMILES	PUBCHEM ID
Bakuchiol	C=C[C@@](/C=C/c1ccc(cc1)O)(CCC=C(C)C)C	5468522
Isopsoralen	O=c1ccc2c(o1)c1ccoc1cc2	10658
Psoralen	O=c1ccc2c(o1)cc1c(c2)cco1	6199

The role of a molecule in the development of drugs is determined by its ability to be absorbed. A radar plot was used to investigate the physicochemical characteristics influencing the absorption of the three ligands under study. Lipophilicity, size, polarity, solubility, flexibility, and saturation were the six physicochemical characteristics that were taken into consideration. The "oral drug-like" limitations for the six qualities are indicated by the pink area of the plot. To be considered a drug-like molecule, the radar plot of the molecule must totally fall into a pink area on each axis

representing a physicochemical range**.** Predicted bioavailability radar makes it possible to evaluate drug likeness quickly. Our results revealed that for all three ligands, except for one parameter rest five parameters of the radar fall within the pink area. **Figure 6.13** represents the bioavailability radar of bakuchiol, isopsoralen and psoralen.

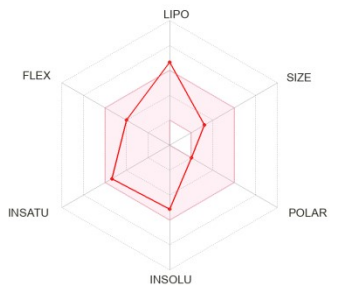
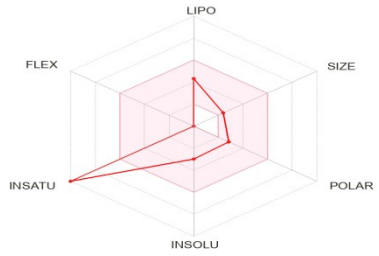
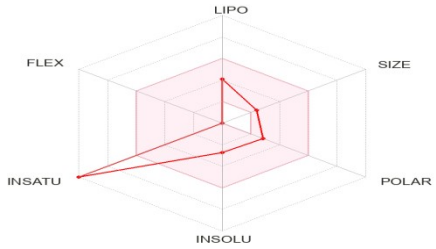
Bakuchiol	**Isopsoralen**	**Psoralen**
		

Figure 6.13 Physicochemical characteristics radar for oral bioavailability of bioactive compounds from *Psoralea corylifolia.* The ideal range for each property is represented by the pink area.

The most extensively used and acknowledged method for assessing the pharmacological efficacy of various chemicals from various sources is Lipinski's rule of five (RO5). If the compounds' number of hydrogen bond acceptor and the donor is less than 10 and 5, respectively (HBA < 10 and HBD < 5), and the average molecular weight of less than 500, it means that the compound satisfied all Lipinski's rule requirements. Our findings revealed that the investigated ligands' molecular weights (MW) fall between the range of 186-257. The conformational flexibility of the molecule, defined by the number of rotatable bonds, is a critical factor in determining the effectiveness of binding to receptors/channels and optimal bioavailability. A sufficient number of rotatable bonds is 10 or less.

According to Veber's guidelines, a molecule is more likely to have an acceptable oral bioavailability when it has 12 or fewer hydrogen donors and acceptors, at least 10 rotatable bonds, and a polar surface area of at least 140 (Smelcerovic et al., 2017). The evaluated ligands

i.e bakuchiol, isopsoralen and psoralen have hydrogen bond acceptor 1, 3, 3 and Hydrogen bond donors 1, 0, 0, respectively, which are within the acceptable limits. Moreover, the number of rotatable bonds is in the range of 0-6, and the polar surface area is in the range of 20.23- 43.35A, hence it can be predicted that they have a good level of bioavailability **(Table 6.7)**. The compounds have n-octanol and water partition coefficient (cLogP) values in the range of 2.12-4.92 (cLogP< 5). It means all the compounds are lipophilic in nature. A compound's lipophilicity in drug development is represented either as a partition coefficient or logP. Lipophilic substances frequently penetrate biological membranes more easily than lipophobic substances, increasing their oral bioavailability via improved gastrointestinal absorption (**Table 6.8**).

ESOL, (ALI) logS, and (SILICOS-IT) studies predicted all three ligands to be moderately soluble to soluble. Oral dosage formulations of poorly water-soluble drugs typically have low bioavailability **(Table 6.9).**

Table 6.7 Physicochemical properties of the ligands/bioactive compounds of *Psoralea corylifolia*

Compound	Molecular Weight	Heavy Atoms	Aromatic Heavy Atoms	Fraction Csp3	Number of Rotatable bonds	HB Acceptor	HB Donor	Molar Refractivity	TPSA
Bakuchiol	256.38	19	6	0.33	6	1	1	85.42	20.23
Isopsoralen	186.16	14	13	0	0	3	0	52.26	43.35
Psoralen	186.16	14	13	0	0	3	0	52.26	43.35

Table 6.8 Lipophilicity of the tested compounds/ Ligands

Molecule	iLOGP	XLOGP3	WLOGP	MLOGP	SILICOS-IT	Consensus Log Po/w
Bakuchiol	3.54	6.12	5.24	4.59	5.1	4.92
Isoporalen	2.03	2.08	2.54	1.48	2.91	2.21
Psoralen	2.01	1.67	2.54	1.48	2.91	2.12

.

Table 6.9 Water solubility prediction values for the Ligands/Compounds

Ligand	ESOL				Ali				SILICOS-IT			
	Log S (ESOL)	Solubility		Class	Log S	Solubility		Class	Log S (ESOL)	Solubility		Class
		mg/mL	mol/L			mg/mL	Mol/L			mg/mL	Mol/L	
Bakuchiol	-5.12	1.93E-03	7.54E-06	Moderately soluble	-6.33	1.21E-04	4.71E-07	Poorly soluble	-4.47	8.65E-03	3.37E-05	Moderately soluble
Isopsoralen	-2.99	1.90E-01	1.02E-03	Soluble	-2.62	4.47E-01	2.40E-03	Soluble	-4.5	5.87E-03	3.16E-05	Moderately soluble

Psoralen	-2.73	3.44E-01	1.85E-03	Soluble	-2.19	1.19E+00	6.39E-03	Soluble	-4.5	5.87E-03	3.16E-05	Moderately soluble

Table 6.10: Pharmacokinetic Parameters of the Ligands

Ligand	**GI absorption**	**BBB permeantt**	**P-gp substrate**	**CYP1A2 inhibitor**	**CYP2C19 inhibitor**	**CYP2C9 inhibitor**	**CYP2D6 inhibitor**	**CYP3A4 inhibitor**	**Log Kp (cm/s)**	**AMES Toxicity**	**Carcinogenic**
Bakuchiol	High	Yes	No	No	Yes	Yes	No	No	-3.52	Non-Toxic	Non-Carcinogen
Isopsoralen	High	Yes	No	Yes	No	No	No	No	-5.96	Non-Toxic	Non- Carcinogen
Psoralen	High	Yes	No	Yes	No	No	No	No	-6.25	Non-Toxic	Non- Carcinogen

Determination of the probable route of administration, whether the substances would be absorbed orally, and if they would be able to pass through the blood-brain barrier (BBB) without having any negative effects are all things that are determined through ADME investigations (Albohy et al., 2022). All of the investigated compounds were predicted to pass the BBB**.** To assess active efflux through biological membranes, such as from the brain or from the gastrointestinal wall to the lumen, one must be aware of whether the drug would be a substrate or non-substrate of the P-glycoprotein (P-gp). P-gp serves a number of important roles in the central nervous system's (CNS) protection against xenobiotics. It was predicted that none of the ligands, i.e. bakuchiol, isopsoralen and psoralen P-gp substrates. It has been proposed that the processing of small compounds is done by both CYP and P-gp to enhance the defence of tissues and organisms (Daina et al., 2017).

Clinical pharmacology places special emphasis on the metabolism of most medicines and xenobiotic chemicals in order to accurately predict how pharmaceuticals will be metabolized in patients. The primary enzyme family in the liver and intestine that may metabolize or oxidatively biotransform the majority of pharmaceuticals and lipophilic xenobiotics is called cytochromes 450 (CYPs). In this study, in silico prediction took into account five key CYP isoforms (CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4). All three ligands were neither CYP3A4 or CYP2D6 inhibitors. Over 50% of medications are metabolised by the enzyme CYP3A4, which is mostly found in the liver and small intestine (Tao et al., 2019). It was predicted that bakuchiol was CYP2C19 and CYP2C9 inhibitor, whereas isopsoralen and psoralen are inhibitory to CYP1A2.

The use of various natural compounds derived from plants, animals, and marine sources as rejuvenators, nutraceuticals, and agents for disease prevention is on the rise right now. When administered internally without scientific support, therapeutic herbs with a variety of pharmacological properties may exhibit adverse effects. It is essential to assess the toxicological profile of various chemicals utilized in medication development. In order to determine if the ligands were mutagenic or not, the AMES toxicity test was used. Since neither of the ligands tested positive for mutagenicity nor for toxicity in the AMES toxicity test, they were all nonmutagenic and non-toxic. The ligands were non-carcinogenic, according to the carcinogenic profile **(Table 6.10).**

In terms of bioavailability, drug-likeness assesses qualitatively the possibility that a molecule might transform into an oral drug. Five distinct rule-based filters, each with a different set of property types, help in determining a molecule to be drug-like. Drug likeness parameter was predicted to be high as the ligands followed Lipinski, Verber, Ghose, Egan and Muegge's rule, with all of them having a bioavailability score of 0.55 **(Table 6.11).**

Table 6.11 Drug likeness profile of the Ligands

	Drug likeness					
Ligand	**Lipinski Violation**	**Veber Violation**	**Ghose Violation**	**Egan Violation**	**Muegge Violation**	**Bioavailability score**
Bakuchiol	Yes 1 MLOGP>4.15	0	0	0	2 XLOGP3>5, Heteroatoms<2	0.55
Isopsoralen	0	0	0	0	1 MW<200	0.55
Psoralen	0	0	0	0	1 MW<200	0.55

Table 6.12 Medicinal Chemistry Ligand Properties

Ligand	**Pains**	**Brenk**	**Leadlikeness**	**Synthetic accessibility**
Bakuchiol	0 alert	1 alert	No, 1 alert	3.13
Isopsoralen	0 alert	1 alert	1 alert, MW<250	3.07
Psoralen	0 alert	1 alert	1 alert, MW<250	3.06

The goal of the current study was to evaluate the pharmacological characteristics of the bioactive compounds that were extracted from *P. corylifolia*. These substances underwent in silico study using the Swiss Institute of Bioinformatics' SwissADME web tool to confirm the pharmacokinetic and pharmacodynamic features and assess each substance's absorption, distribution, metabolism, and excretion (ADME). When *P. corylifolia* compounds' ADME parameters were evaluated using their physical-chemical characteristics and pharmacokinetics, the results revealed that all of the compounds that were chosen had the properties that were essential for the medication development, including brenk, pains and lead likeness. Moreover, synthetic accessibility was in the range of 3.06–3.13 **(Table 6.12).**

6.2.2.2 Molecular docking of bakuchiol, isopsoralen and psoralen as ligand and PPAR gamma as target:

Molecular docking plays a significant role in novel drug discovery. Molecular docking studies assist medicinal chemists in quickly and inexpensively identifying new medicines. It accurately forecasts a compound's experimental binding properties and mechanism involved in the binding to the target proteins. The ligand molecules bakuchiol, isopsoralen and psoralen were docked with 6L8B protein using Autodockvina software. The ligand was docked into the relevant protein's functional sites one at a time, with the docking energy analyzed to determine the lowest value. All three ligands were predicted to have negative binding energy values. This result revealed that their interactions with the PPAR-γ active location were favourable. Good affinity represented by binding energy is shown in **Table 6.13.**

Finding the best protein-ligand pairing with the minimum amount of energy was done using the docking method. **Figure 6.14(a)** showed that Bakuchiol binding with PPAR-γ involves pi-sigma interaction with His^{266}, Alkyl interaction with Phe^{264}, Ile^{341} $Met^{348,}$ as well as pi-Alkyl interaction with Arg^{288} and Leu^{330}. However, isopsoralen also binds strongly to the PPAR-γactive site and stabilizes it through three hydrogen bonds (His323, His449 and His473). While Cys285 established an Amide pi-stacked association, the 2D (two-dimensional) graphic shows that more residues were seen to be connected through the Vander Waals interaction (**Figure 6.14(b**)). Psoralen binds through three conventional hydrogen bonds (Ser^{289}, His^{323}, Tyr^{473}) and one pi-

Alkyl interaction with Leu[469], beside these other interactions are also found to bound through pi-pi T-shaped, Amide-pi stacked and Vander Waals interaction described in 2D (two dimensional) plot (**Figure 6.14(c)**). On comparison of the binding affinity energy of the ligands, as revealed in **Table 6.13,** it can be said that Bakuchiol and Psoralen have the uppermost negative binding energy with -7.8 kcal/mol and -7.7 kcal/mol followed by Isopsoralen with -7.6 Kcal/mol. Thus, bakuchiol has the highest binding affinity as compared to isopsoralen and psoralen.

Table 6.13 Binding energy and inhibition constant of bakuchiol, isopsoralen and psoralen

Sr. No.	Docked Complex	Protein ID	Inhibition constant (micromolar)	Binding Energy (kcal/mol)
1	Bakuchiol	6L8B	1.8764	-7.8
2	Isopsoralen	6L8B	2.6312	-7.6
3	Psoralen	6L8B	2.2220	-7.7

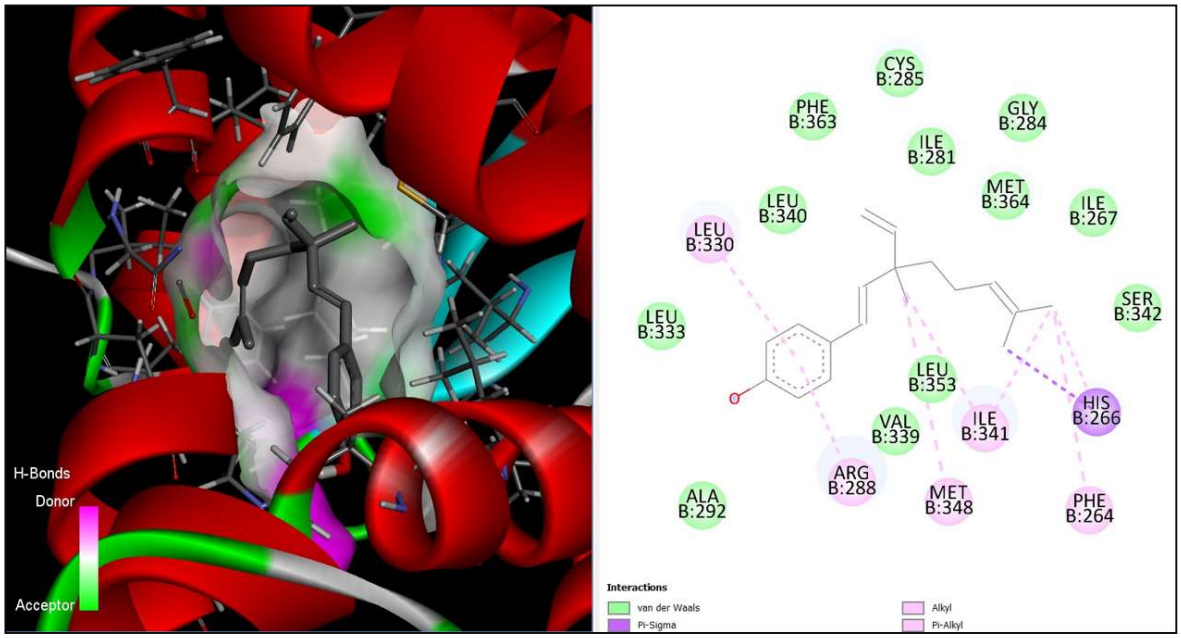


Figure 6.14 (a) Bakuchiol and 6L8B (2D) binding sites.

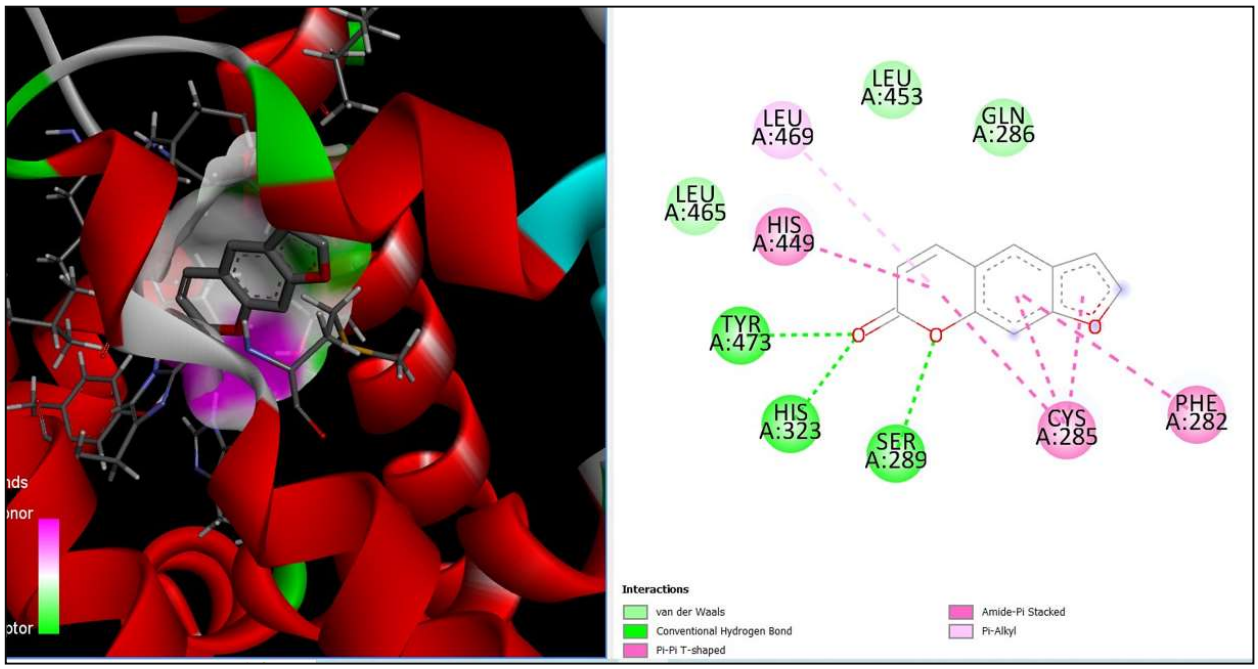


Figure 6.14(b) Isopsoralen and 6L8B(2D) binding sites.

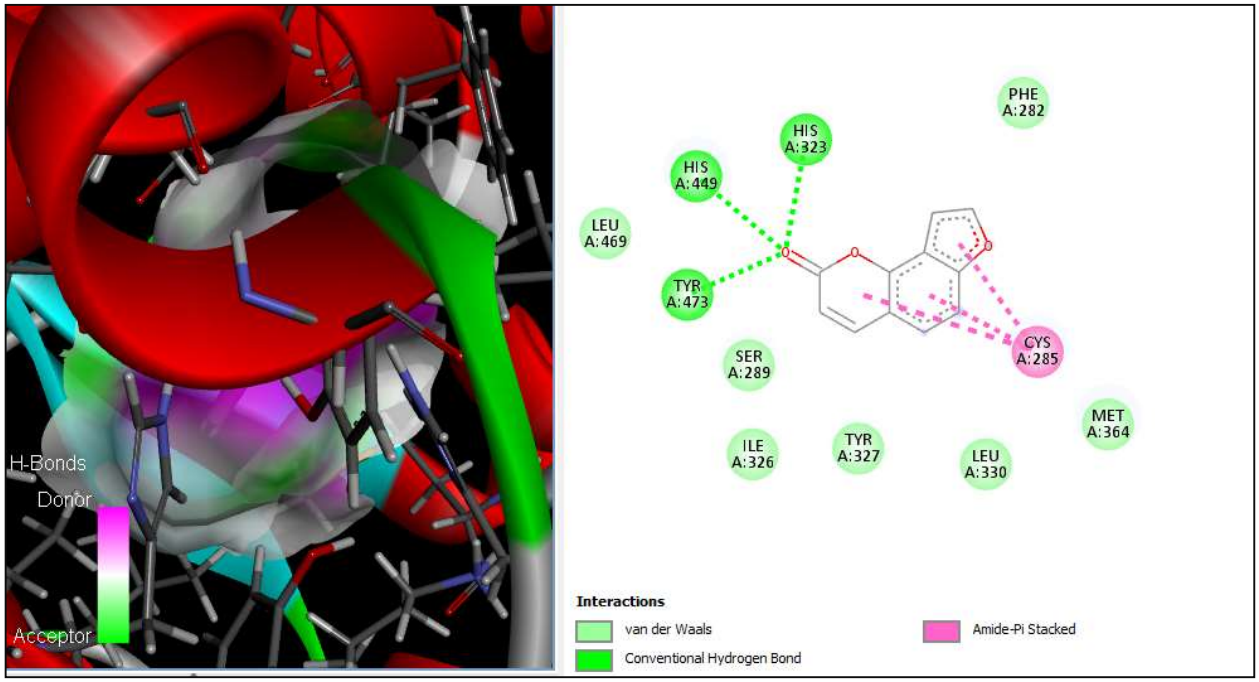
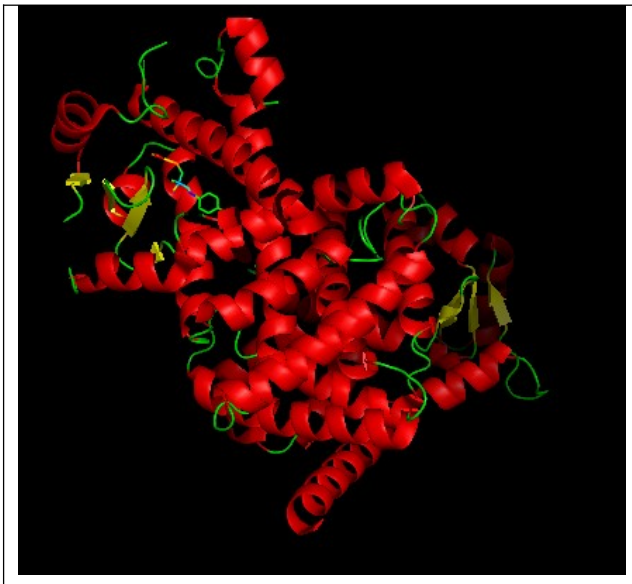
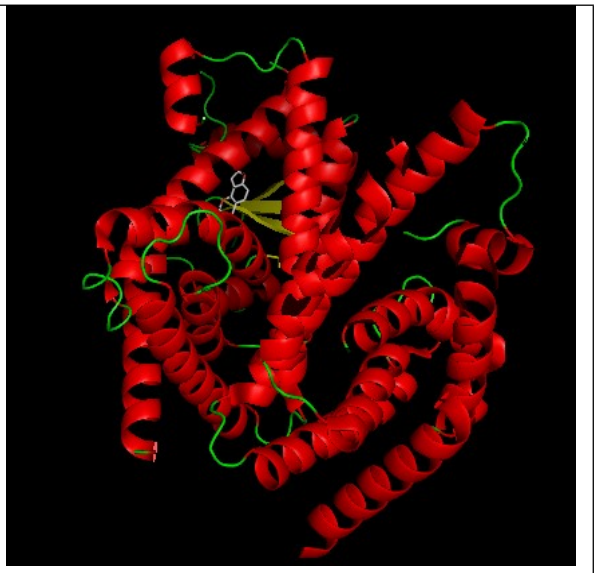


Figure 6.14 (c) Psoralen and 6L8B(2D) binding sites.

Figure 6.14 2D images. Docking of compounds bakuchiol, isopsoralen and psoralen with **6L8B**



Bakuchiol and 6L8B



Isopsoralen and 6L8B

Psoralen and 6L8B

Figure 6.15 3D images. Docking of compounds bakuchiol, isopsoralen and psoralen with **6L8B**

In today's life, obesity poses a serious risk to everyone's metabolic health. The need for new therapeutic agents is evidenced by the ongoing global hunt for anti-obesity therapeutics as the

disease condition continues to afflict the world's population. Our aim was to determine in silico, the specific receptor responsible for the anti obesity potential of bakuchiol, isopsoralen, and psoralen. Due to its potential use in the evaluation of bioactive compounds physicochemical and pharmacokinetic properties, the usage of in-silico models has come to be recognized in recent decades as being of vital importance in the study field of research and development of pharmaceuticals (R&D). As a result of in silico modeling, a more effective and efficient new era of drug design has been created (Rodrigues et al., 2020). PPARγ has been considered an important target for treating metabolic disorders. In this study, structure-based drug designing was performed by molecular docking in order to investigate the effects of bakuchiol, isopsoralen, and psoralen, phytochemicals of *P. corylifolia* on suppressing fat storage and adipogenic differentiation by PPAR gamma protein. If the molecule violates more than two rules, it may be predicted that it will not be well absorbed by the intestines. Likewise, molecular weight, hydrophobicity and hydrophilicity of drug molecules influence their physiochemical characteristics. Owing to its low bioavailability, the majority of active biomolecules fail in clinical trials, making this prediction of drug-likeness very crucial (Mandar et al., 2021). Three *P. corylifolia* bioactive compounds were projected to have positive drug-likeness scores in the current investigation, which suggests their oral bioavailability and systemic absorption, where they can interact with PPARγ involved in disease pathogenesis. ADMET properties further predicted that the compounds of *P. corylifolia* were non-toxic, non-carcinogenic and had a good probability of absorption through the human intestine.

Hydrogen bonds and other weak intermolecular interactions, such as hydrophobic interactions, stabilize a ligand at the binding site in the protein structure energetically (Mazumder et al., 2017). The binding process of bakuchiol, isopsoralen, and psoralen at the binding site of human PPARγ was investigated using molecular docking techniques. The results of the docking clearly show that the ligands have a high affinity for the human PPARγ protein **(Figure 6.15)**. According to the docked conformations, the chosen ligands engage in interaction with PPARγ in the ligand binding domain (LBD). Vander walls, pi interactions, and hydrophobic interactions all contribute together and stabilize the ligands' docking conformation in the PPAR binding site. PPARγ is mostly involved in the regulation of fat. An *in vitro* study where it was observed that Psoralen

reduced the protein expression levels of PPARγ (Li et al., 2019) supported our in-silico work for psoralen to bind to PPARγ. So, the findings of the present work study should spur further investigation into the features of the phytochemicals from *P. corylifolia*, followed by a pharmacological evaluation of these in-silico findings in suitable models. Thus, it may be inferred that *P. corylifolia* is a significant PPAR gamma (6L8B) protein inhibitor that limits the deposition of fat and the differentiation of adipocytes.

Low-grade inflammation and obesity go together, and obesity is known to increase one's risk of developing a number of well-known conditions, such as certain rheumatic diseases, metabolic syndrome disorders, cardiovascular diseases, and malignancies. Evidence shows that higher birth weight is linked to the later onset of RA (Baker et al., 2011). Hence, in continuation of our study to explore the antiobesity potential, our next endeavour was to investigate the anti arthritic/anti inflammatory potential of major constituents isolated from *P. corylifolia*.

6.2.3 *A*nti-arthritic activity of extracts/isolated compounds of *P. corylifolia*

A number of autoimmune and inflammatory diseases have been linked to excess body weight. Adipose tissue is thought to play a role in modifying physiological and pathological processes related to inflammation and immunity. Leptin is one of the adipokines that adipose tissue manufactures and secretes, along with cytokines like tumour necrosis factor-α (TNF-α), interleukin-1 (IL-1), IL-6, and monocyte chemotactic protein-1. Increased levels of proinflammatory adipokines are linked to obesity, which is characterised by an excessive buildup of adipose tissue. Numerous epidemiological studies have suggested that obesity may increase the chance of developing Rheumatoid arthritis. According to reports, between 18% and 31% of RA patients are obese. Free fatty acids and adipokines, which are more prevalent in obese people, can lead to the activation of macrophages and are hence responsible for chronic inflammation (OM et al., 2018). According to some preliminary studies, persons who are diagnosed with RA and are obese or overweight may have a lower chance of experiencing a satisfactory response to the drug. Additionally, it's been established that obesity decreases the likelihood that anti-tumour necrosis factor (anti-TFN) treatments will work. Also, patients using Disease-modifying

antirheumatic drugs **(**DMARDs) (86% methotrexate) had a lower likelihood of achieving excellent disease control if they were overweight (Abuhelwa et al., 2020).

6.2.3.1 *In vitro* studies:

Traditional Chinese medicine makes extensive use of the fruit of *Psoralea corylifolia* L. (PCL) as a well-known herbal remedy for orthopaedic problems. Studies on its impact on rheumatoid arthritis, however, are limited (Pai et al., 2021). The main aim of the study was to understand the effect and mechanisms underlying *P. corylifolia's* effect on rheumatoid arthritis in order to get some understanding for the potential creation of new drugs to treat the condition in the future. The anti-arthritic activity of the isolated compound/s of *P. corylifolia* was determined by performing studies on RAW cell lines and on rats.

Before determining the anti-arthritic activity of isolated compounds of *P. corylifolia* by using cell lines, the dimethyl thiazol diphenyl tetrazolium bromide (MTT) experiment was conducted to determine the toxicity of the compound. The cells used in the study were macrophages, RAW 264.7 (**Figure 6.16**). This cell line is frequently used as communicated in the literature, and is responsive to LPS. The murine macrophage cell line RAW264.7 is used to create *in vitro* inflammatory models. Previous research has shown that RAW264.7 cells release significant quantities of pro-inflammatory cytokines such TNF-α, IL-6, and IL-1 in response to LPS-mediated inflammation (Han et al., 2019).

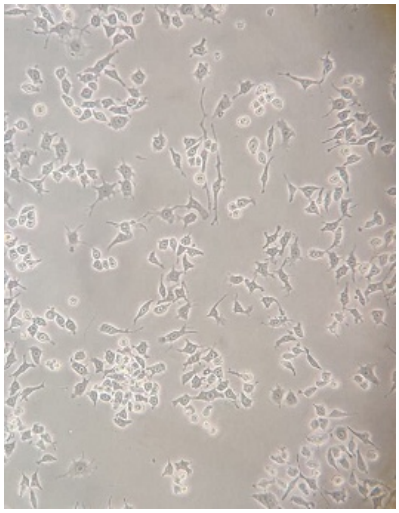


Figure 6.16 RAW cell line (At 10X)

6.2.3.1.1 Result of MTT assay for determining cell viability:

In this study, the isolated compounds of the chosen plants were subjected to toxicity tests to determine their level of risk or lack thereof and to determine whether they should be employed or chosen for further research. It is always advised to employ *in vitro* techniques to analyze a compound's toxicity profile in order to reduce the usage of laboratory animals and define an initial dosage (NIEHS, 2001). The MTT cell viability assay was used in this study to evaluate the toxicity of the test compounds. The MTT test, which measures the activity of the mitochondrial succinate dehydrogenase enzyme, is frequently used to assess the metabolic activity of cultured cells. This assay is frequently used to assess the cytotoxic effects of medicinal herbs *in vitro*. For this assay, RAW 264.7 cells (10^4 – 10^6 cells) were subjected to treatment with bakuchiol/ isopsoralen / psoralen at the concentration range from 1.25µM- 5 µM. No significant reduction in cell viability was observed in the RAW cell line after treatment with the isolates. PC1 at 5µM, 2.5 µM and 1.25 µM conc. displayed cell viability in RAW cells to 86.4%, 88.5% and 90.3%, respectively, whereas PC2 at 5µM, 2.5 µM and 1.25 µM conc. displayed cell viability percentages to 87.4, 90.5 and 91.5, respectively. However, RAW cells treated with PC3 at 5µM, 2.5 µM and 1.25 µM conc. displayed cell viability to 90.3%, 91.5% and 92.4%, respectively (**Figure 6.17**). Camptothecin which was used as a positive control, displayed cell viability of 61.7% and 36.56% at 1 µM and 10 µM conc. respectively.

When the effect of bakuchiol on cell viability by MTT test was studied, similar outcomes were reported by Pae et al. (2001). It was found that the viability of RAW 264.7 cells was unaffected when treatment was given with bakuchiol, in the dose range of 2-10 µM.

In a study, the effect on cell viability of α-(1 → 3)-glucan, isolated from *Fomitopsis betulina,* was studied, and it was found that this compound did not show any cytotoxicity effect on the HT-29 cell line. The positive control used was camptothecin which is the same positive control used in our study. There are several other reports on the use of camptothecin as a positive control (Czerwonka et al., 2019).

SNo.	Treatment	% cell viability
1	Camptothecin 10µM (Positive control)	36.56
2	Camptothecin 1µM (Positive control)	61.7
3	PC1 5 µM	86.4
4	PC1 2.5 µM	88.6
5	PC1 1.25 µM	90.3
6	PC2 5 µM	87.4
7	PC2 2.5 µM	90.5
8	PC2 1.25 µM	91.5
9	PC3 5 µM	90.3
10	PC3 2.5 µM	91.5
11	PC3 1.25 µM	92.4

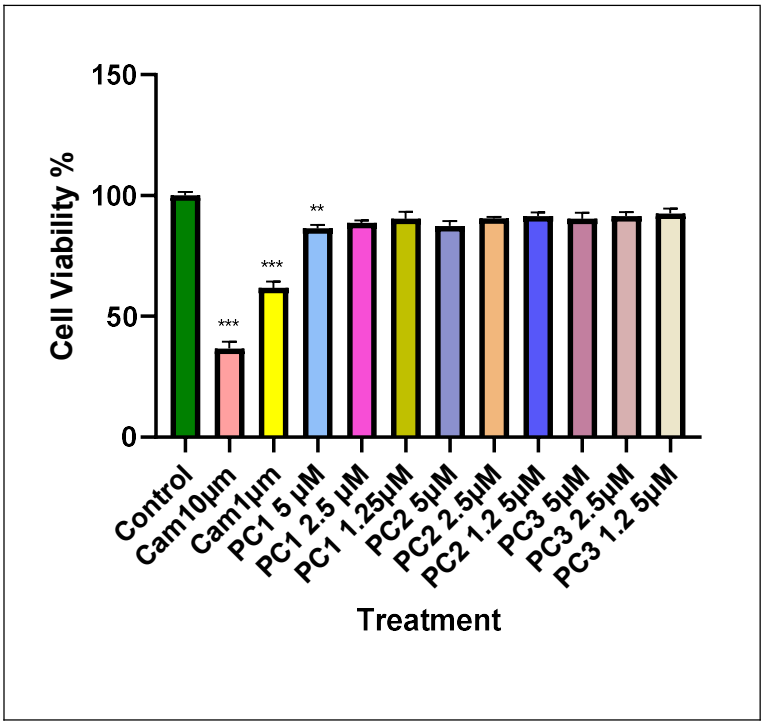


Figure 6.17 Viability of RAW cells under various concentrations of isolated compounds PC1, PC2, PC3 as determined by MTT assay. To various wells containing the same number of cells, isolated chemicals (5 M, 2.5 M, and 1.25 M) were introduced. Cells that weren't treated served as the control group. Camptothecin (Cam) was taken as positive control.

6.2.3.1.2 Nitric oxide production inhibition:

Nitric oxide is regarded as a pro-inflammatory mediator that causes inflammation in abnormal situations due to overproduction and is involved in the pathogenesis of inflammatory disorders such as rheumatoid arthritis. An *in vitro* nitric oxide (NO) test was conducted to evaluate the anti-inflammatory effects of the compounds bakuchiol, isopsoralen and psoralen. Nitric oxide assays are widely applied to measure nitrate/ nitrite levels in experiments studying the scavenging of free radicals, the anti-cancer, anti-aging, and anti-inflammatory properties. (Kagoo and Chellathai 2014). NO levels were determined as an indicator of inflammation. To induce inflammation and NO generation, a certain number of cells were first given LPS treatment. Next, the test compounds were respectively given to the cells, and the cells were incubated to respond to the compound (level of NO produced). Then, to determine the anti-

inflammatory effects of the treatment, the NO levels produced by the treated cells were compared to untreated (control) cells using colorimetric measurement. Being an unstable free radical, nitric oxide immediately interacts with oxygen to produce nitrites (NO_2^-) and nitrates (NO_3^-). We assessed the amounts of nitrite in the cell culture media using by Griess reagent. A purple azo colour is produced when nitrite ions and Griess reagent react. In order to calculate the unknown nitrite concentration in the culture media, a standard curve, as depicted in **Figure 6.18,** was created. Absorbance value in the culture media having cells treated with bakuchiol, isopsoralen and psoralen was measured. The linear equation was then applied to determine the associated nitrite concentration produced by cells treated with compounds by comparing their absorbance value to the standard curve.

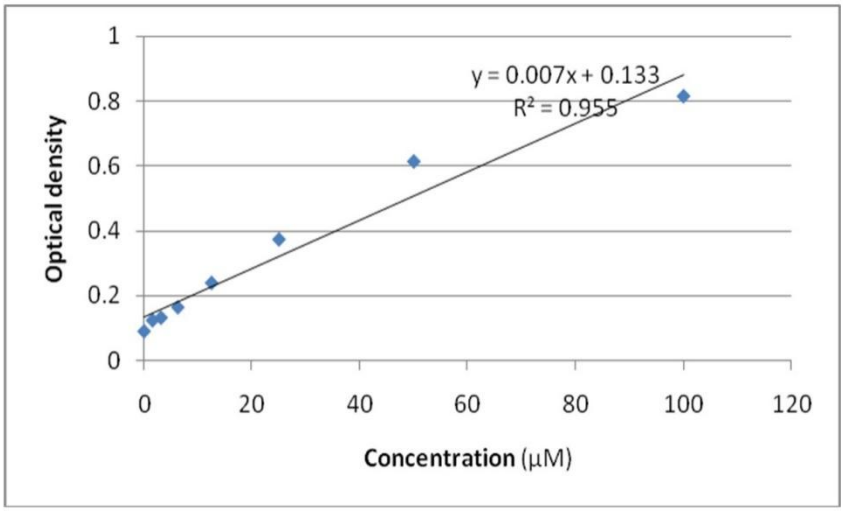


Figure 6.18: Standard curve used to quantify the concentration of nitrites present in the culture

Table 6.14 Percentage suppression of nitric oxide after treatment with various concentrations (5 µM, 2.5 µM &1.25 µM) of PC1, PC2, PC3 in LPS-stimulated RAW cells

S. No.	Treatment		Nitric oxide % age inhibition
	Compound	Concentration	
1	PC1	5 µM	21.33 ± 1.4

2	PC1	2.5 µM	17.23 ± 2.6
3	PC1	1.25 µM	16.48 ± 1.9
4	PC2	5 µM	20.19 ± 3.0
5	PC2	5 µM	16.46 ± 7.6
6	PC2	1.25 µM	16.23 ± 1.7
7	PC3	5 µM	21.30 ± 6.3
8	PC3	2.5 µM	16.37 ± 1.4
9	PC3	1.25 µM	14.62 ± 0.8
10	L-Nitro-Arginine Methyl Ester (L-NAME) (Positive control)	100 µM	44.31 ± 6.6

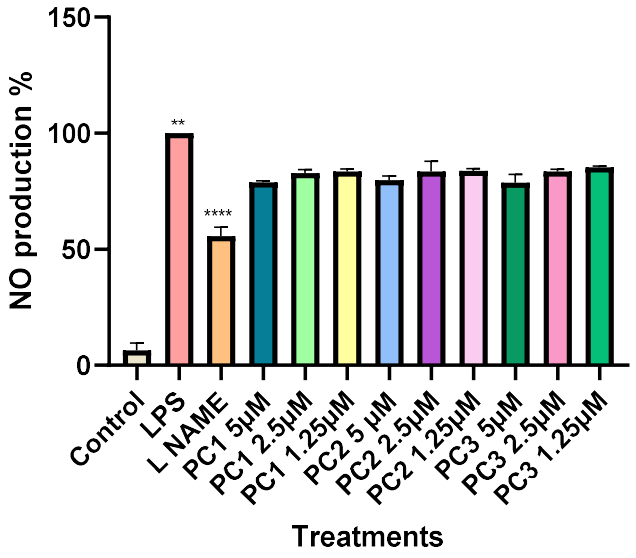


Figure 6.19 Percentage NO concentration produced by RAW 264.7 cells treated with bakuchiol/ isopsoralen/ psoralen after applying LPS. Each value corresponds to the mean ± S.E.M (n=3). (* < 0.05, ** < 0.01 and *** < 0.001, ****<.0.0001)

By using the Griess reagent assay, all three isolated compounds were evaluated for their potential to reduce the formation of nitric oxide (NO) in LPS-induced RAW 267.4 cells. The NO level was 16-fold higher after LPS treatment. Contrarily, the administration of bakuchiol, isopsoralen and psoralen reduced the level of nitrite in LPS-activated macrophages in a concentration-dependent manner (**Figure 6.19**). At 5µM concentration, bakuchiol, isopsoralen, and psoralen reduced NO levels to 21.23553%, 20.1908% and 21.30367%, respectively, but at 1.25 µM concentration reduction was16.48873%, 15.23893% and 14.6265% respectively. **Table 6.14** represents the NO inhibition by bakuchiol, isopsoralenand psoralen. From the graph, it is clear that at low concentrations, PC1 (bakuchiol) displays more NO reduction potential as compared to the other two (isopsoralen and psoralen), although the difference is not very much significant. In the present study, G-Nitro- l-Arginine Methyl Ester (L-NAME) was used as a positive control, which at 100 µM concentration displayed NO inhibition of 44.31%.

The level of statistical significance was determined by one-way analysis of variance (ANOVA) followed by Turkey's multiple comparison test using Graph Pad Prism-8 software and the results revealed that no significant reduction in the level of nitric oxide is seen after

treatment with bakuchiol, isopsoralen & psoralen when compared to the positive control (**Figure 6.9)**. This might be due to low affinity of the these metabolites for iNOS gene which causes NO production in RAW cell lines. Although the compounds show anti inflammatory effects but the detail mechanistic study is still in the early stages for any conclusion and required to be carried out as future work.

To release proinflammatory mediators such as cytokines, NO and PGE2 during inflammation, macrophages follow a number of steps. These chemical entities/ cytokines attract additional immune cells to inflammatory areas. Therefore, one effective method for combating inflammatory illnesses is to prevent the production of these entities (Payne et al., 2010). In the present investigation, we found that bakuchiol, isopsoralen, and psoralen treatment of LPS-activated RAW 264.7 murine cells caused NO production to be downregulated in a concentration-dependent manner. These findings were in agreement with earlier research that indicated NO was being inhibited. Bakuchiol, a compound isolated from the plant *Ulmusdavidiana* var. *japonica*, has been studied for its anti-inflammatory potential. Prostaglandin E2 (PGE2) and nitrogen oxide generation caused by lipopolysaccharide (LPS) were significantly reduced by bakuchiol in RAW 264.7 macrophages without causing any cytotoxicity (Jafernik et al., 2020). Neobavaisoflavone, one of the bioactive compounds of *P. corylifolia* , significantly reduced NO generation in RAW264.7 macrophages activated by LPS and IFN. Similarly to our results, Pae and coworkers reported inhibitory activity of bakuchiol on NO production. This inhibition is due to a decrease in the expression of iNOS mRNA due to the inactivation of nuclear transcription factor-kB (Pae et al., 2001). In a previous study, Bakuchiol was reported to strongly inhibit the production of nitrogen oxide and prostaglandin E2 (PGE2) which was induced by lipopolysaccharide (LPS), in RAW 264.7 macrophages cell lines, without showing any cytotoxicity (Choi et al., 2010). From the fruits of *P. corylifolia*, Xiao and the research team isolated three meroterpenes psoracorylifol F, A and bakuchiol and observed that these strongly block LPS-induced NO generation in RAW 264.7 cells with IC50 values ranging from 7.71 to 27.63 M (Xiao et al., 2012). Inducible nitric oxide (iNOS) gene regulation or direct interference with iNOS activity could both be contributing factors to this inhibition (Debprasad et al., 2012).

Also, research on *Cullen corylifolium* (L.) Medik (a synonym of *Psoralea corylifolia* L.) revealed that its two newly isolated compounds, corylifol H and epi-bavacoumestan C, decreased the ability of LPS-activated RAW 264.7 macrophages to produce nitric oxide. All this previous research provided support for our study on the NO inhibition potential of *P. corylifolia* (Liu et al., 2021).

So, according to the findings, nitrite overproduction may be inhibited by seeds of *P. corylifolia* which may have therapeutic benefits in the management of inflammatory diseases.

6.2.3.1.3 Reactive Oxygen Species Inhibition:

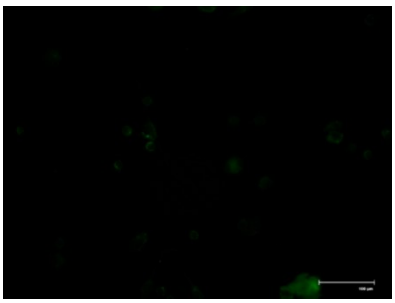
In cell lines, the 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) approach for ROS detection is frequently utilized (DCFH is formed after DCFH-DA is hydrolyzed in the cell, which is then oxidized by ROS into a fluorescent product, and afterwards identified, e.g., by fluorescence microscopy) (Gomes et al., 2021).

Since it is challenging to determine how much 2′, 7′-dichlorodihydrofluorescein diacetate is taken up by cells from microscopic pictures, an empirical parameter [i.e., corrected total cell fluorescence (CTCF)] representing fluorescent intensity was used for indirect quantification of 2′, 7′-dichlorodihydrofluorescein diacetate within the cell. After eliminating the intensity of the background from the cells that fluoresced, CTCF was calculated.

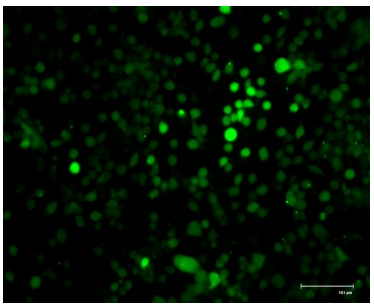
So, we investigated the efficiency of bakuchiol, isopsoralen and psoralen on inhibition of ROS production in RAW 264.7 macrophages treated with LPS. After 24 hours, LPS treatment increased the intracellular ROS levels in macrophages. In contrast, treatment with bakuchiol/ isopsoralen / psoralen at 5µM concentration considerably reduced LPS-induced ROS production.

Figure 6.21 depicts the corrected total cell fluorescence (CTCF) obtained after applying Image J software to the images of bakuchiol, isopsoralen, and psoralen treated RAW cells. Fluorescent microscopic images of cells treated with isolates are shown in **Figure 6.20**. It is evident from the

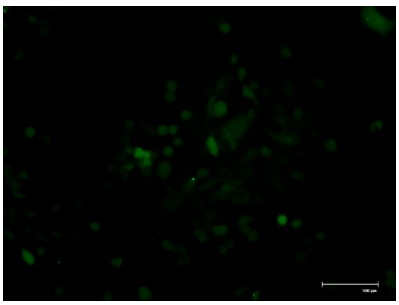
image that a significant decrease in fluorescence intensity was observed after treatment with bakuchiol, isopsoralen and psoralen.



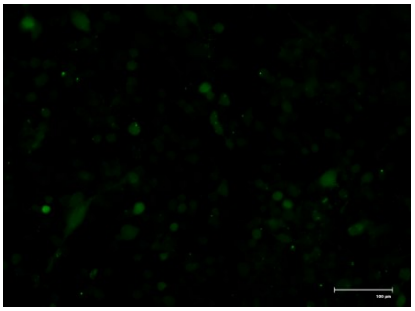
Control



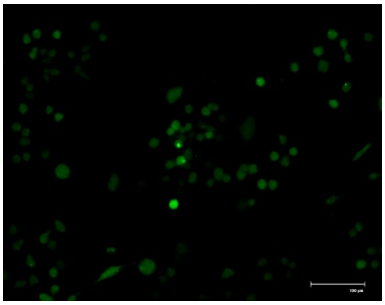
LPS



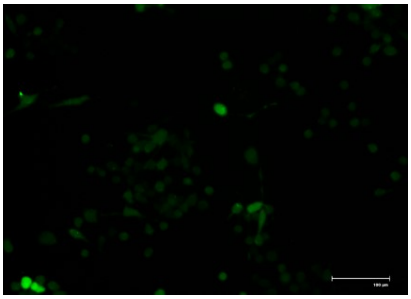
LPS + Methotrexate



LPS+Bakuchiol



LPS+Isopsoralen



LPS+ Psoralen

Figure 6.20 Effect of bakuchiol, isopsoralen and psoralen on the generation of reactive oxygen species (ROS) in LPS-stimulated RAW264.7 cells by fluorescence microscopy. Cells were stained with DCFH-DA for visualization.

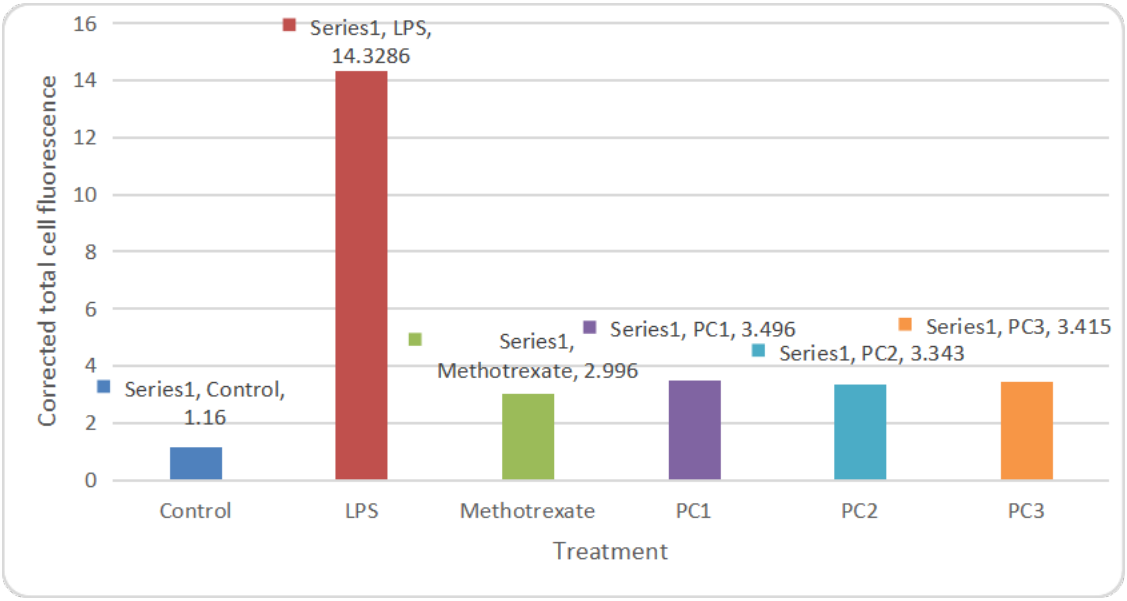


Figure 6.21 Fluorescence intensity measurement. Using Image J, the fluorescence intensity was measured and depicted as corrected total cell fluorescence (CTCF) a.u

Corrected total cell fluorescent (CTCF) of LPS treated RAW cells was 14.32 (a.u), while that of PC1 (bakuchiol), PC2 (isopsoralen) and PC3 (psoralen) treated RAW cells was 3.49, 3.34 and 3.41 (a.u) respectively. CTCF of methotrexate treated cells which was used as a positive control, was 2.99 (a.u). On the basis of calculated corrected total cell fluorescence values, a reduction of fluorescent intensity was observed after the given treatment with the isolates.

ROS and oxidative damage are crucial factors in inflammation and inflammatory diseases. Oxidative stress and free radicals are well-known to be induced by LPS in activated macrophages. As a result, we studied how bakuchiol, isopsoralen or psoralen affected intracellular ROS in LPS-activated RAW264.7 cells. ROS levels are elevated as a result of LPS induction (Kumar et al., 2022). The findings of the immunofluorescence investigations showed that bakuchiol, isopsoralen or psoralen treatment had decreased the amount of ROS that LPS-activated macrophage cells produced. Our findings are supported by a study in which the production of ROS caused by palmitate or H_2O_2 was considerably inhibited by PCS extract. In this report, it was revealed that bakuchiol obtained from the seed extract of *P. corylifolia* exhibited hepatoprotective activity by inhibiting the production of reactive oxygen species (ROS) and malfunctioning of the mitochondria in human diploid fibroblast (HDF) (Seo

et al., 2013). Additionally, PCS extract elevated superoxide dismutase and substantially lower intracellular ROS in the primary hepatocytes of aged mice. Hence, it can be concluded that bakuchiol, isopsoralen and psoralen have the potential for significant ROS reduction.

6.2.3.1.4 TNF-α inhibition:

Cytokines are crucial components of the immune system. Inflammatory diseases can arise from any problem with the regulation of cytokines. Rheumatoid arthritis is one of several inflammatory diseases that are associated with the overproduction of TNF-α cytokine. Clinically, TNF-α inhibitors are helpful in the treatment of rheumatoid arthritis; however, the inhibitors that are now available are expensive biological medications that must be injected and are not always effective. TNF-α is therefore considered to be a promising target for the creation of novel drugs to treat chronic inflammatory diseases, and the current demand is for the identification of drugs that may act as antagonists of this cytokine by oral route (Henriques et al., 2016).

The effect of bakuchiol, isopsoralen and psoralen on TNF-*α* release by LPS-stimulated RAW cells was assayed at three different concentrations. The amounts of TNF-α secreted by RAW 264.7 cells which were treated with isolated compounds or positive control dexamethasone were evaluated from the standard curves as depicted in **Figure 6.22**. According to the results shown in **Table 6.15**, TNF-α expression levels increased significantly after LPS stimulation. However, treatment with bakuchiol, isopsoralen or psoralen leads to a decrease in the concentration of TNF-α in a concentration dependent means. The release of TNF-α was reduced by up to 55.33% ±6.28, 39.74% ±7.1and 43.76%±1.5 respectively, by bakuchiol, isopsoralen and psoralen at 5 µM treatment as compared to LPS stimulated control cells. The outcomes showed that the inhibitory activity of bakuchiol, isopsoralen, or psoralen on the TNF-*α* level was more evident at a concentration of 5 µM.

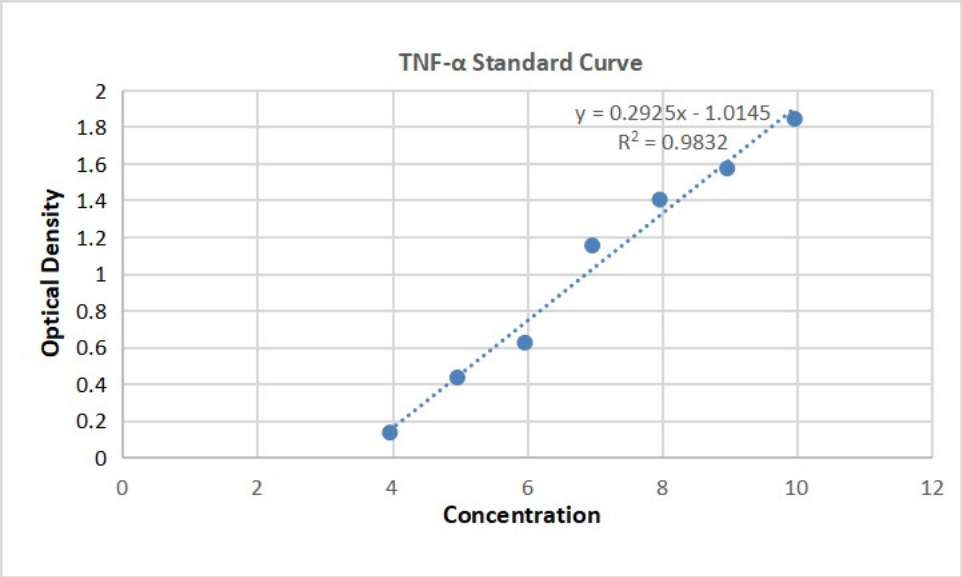


Figure 6.22 Standard curve used to quantify the concentration of TNF-α. (here, optical density was plotted against logrithmic value of concentration (pg/ml))

Table 6.15 Percentage inhibition of TNF-α when LPS stimulated RAW cells were given treatment with different concentrations (5 µM, 2.5 µM, 1.25 µM) of PC1, PC2, PC3

S. No.	Treatment		% age inhibition
	Compound	Concentration	
1	DEXA	DEXA	73.44 ± 6.28
2	PC1	5 µM	55.33 ± 6.28
3	PC1	2.5 µM	38.73 ± 5.3
4	PC1	1.25 µM	19.61 ± 4.5
5	PC2	5 µM	39.74 ± 7.1
6	PC2	2.5 µM	34.71 ± 0.8

7	PC2	1.25 µM	28.17 ± 0.8
8	PC3	5 µM	43.76 ± 1.5
9	PC3	2.5 µM	39.23 ± 1.5
10	PC3	1.25 µM	30.68 ± 5.7

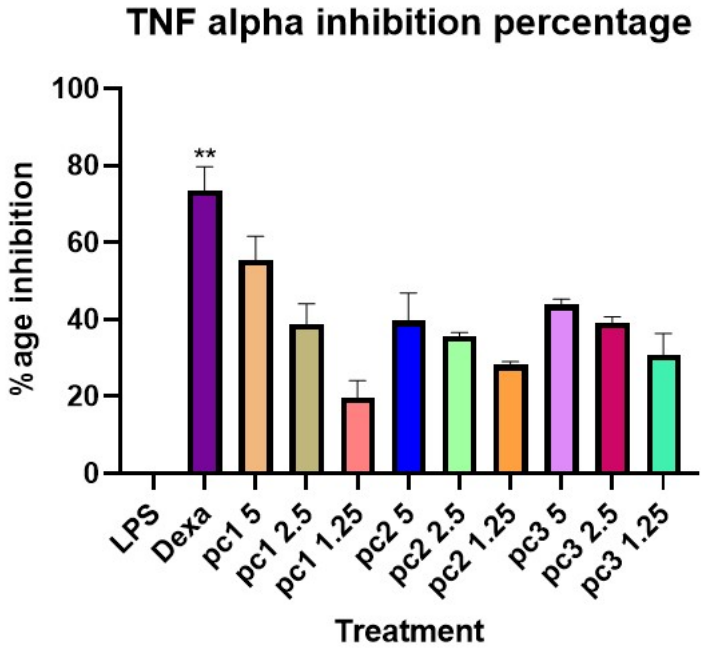


Figure 6.23 Effect of bakuchiol (PC1), isopsoralen (PC2) and psoralen (PC3) in LPS-induced RAW-264.7 cells on TNF-α levels. Every result is the average standard deviation across three separate studies. Turkey's test was employed after one-way ANOVA to establish statistical significance. *p <0.05; **p <0.01; p***<0.001; and ****p <0.0001 versus untreated LPS group.

After being activated by LPS, macrophages secrete TNF-α, which causes a variety of physiological changes, such as inflammation, septic shock, and cytotoxicity. According to the findings of this investigation, Bakuchiol, Isopsoralen, or Psoralen lowered TNF-α production in a manner similar to dexamethasone (**Figure 6.23**). Recent studies have demonstrated that the *P. corylifolia* compounds bavachin and bakuchiol has preventive effects against bone loss in

osteoporosis (Weng et al., 2015). Our results confirmed previous findings that *P. corylifolia* decreases the expression of TNF-α.

Biomedical researchers use a variety of techniques to develop treatments for human diseases and disorders, including computational simulations, tissue and cell cultures, experimental animals, and clinical studies. Each of the aforementioned methods has pros and cons of its own. Because it allows researchers to assess how human, animal, or microbial cells respond when grown in culture, *in vitro* testing is an essential component of biological research. *In vivo* models can be used to advance drug development studies after a drug candidate proves its efficacy in a series of *in vitro* experiments. Animals are typically used in these preclinical studies to assess the safety, effectiveness, and delivery of a drug candidate. (Saeidnia et al., 2015).

For further *in vivo* studies, bakuchiol was chosen. One reason for the selection is the high amount that was obtained during isolation with column chromatography. Another reason was it's little bit more activity which was observed during *in vitro* studies. For the *in vivo* studies, first, ethical approval was obtained. The study was approved in the 80th institutional animal ethics committee meeting on 25th January 2022 at CSIR, IIIM Jammu (Approval No. 285/80/2/2022).

6.2.3.2 *In vivo a*nti-arthritic study of extracts/isolated compounds of *P. corylifolia*:

Due to a lack of safe and efficient medications, it's still difficult to treat chronic inflammatory conditions like inflammatory bowel illnesses and rheumatoid arthritis. Numerous animal models have been used to assess compounds with anti-inflammatory activities. Products originating from plants seem to be a significant basis of pharmaceuticals and are being assessed as a pharmacological candidates for anti-inflammatory effects in recent years. The concept of using phytoconstituents to test for anti-inflammatory properties in in-vivo animal models has been proven, and this method has been a cornerstone of drug development programmes. Despite the availability of several in-vivo and in-vitro models for the development of anti-inflammatory drugs, it is challenging to use animal models in drug discovery and development since proper animal model selection is always required (Patil et al., 2019).

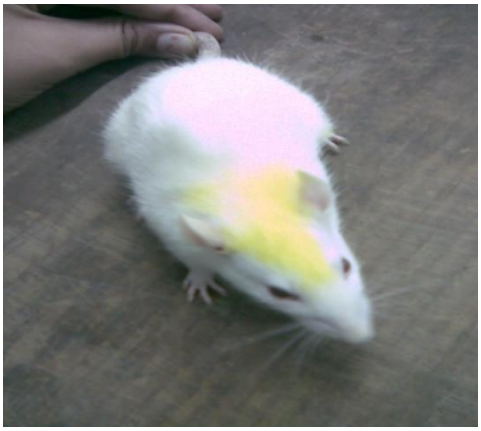
- 

Figure 6.24 Wistar rat

6.2.3.2.1 Carrageenan mediated paw edema in rats

One of the most common tests used to check for antiinflammatory efficacy is carrageenan-induced paw edema. It has long been recognized as a reliable model for investigating novel anti-inflammatory medications since it is a highly sensitive and reproducible test for nonsteroidal anti-inflammatory medicines. An immediate and localized inflammatory reaction brought on by the injection of carrageenan results in the formation of edema. Histamine, serotonin, and bradykinin are the first mediators participating in the early phase (0–1 h), while prostaglandins and a number of cytokines, including IL–1, IL–6, IL–10, and TNF-α are active in the second phase (Dzoyem et al., 2017). For determining the proper dose of bakuchiol in *in vivo* experiments, toxicity studies were conducted by Kumar et al. in 2021, and the findings revealed that no death was observed for up to 14 days when Bakuchiol was administered orally in six animal groups in a single dose of 2000 mg/kg (Kumar et al., 2021). The results of this earlier study were taken into account.

Female Wistar rats (**Figure 6.24**) were used. Five groups of six rats each were formed. The experimental work started with the measurement of paw volumes by using a plethysmometer (**Figure 6.25**). Group Ist was controlling, and group IInd received acetyl salicylic acid (250 mg/kg) and groups IIIrd, IVth and Vth were given Bakuchiol in doses of 1, 4 and 8 mg/kg, resp. Effect on paw volume was confirmed 4 h post-carrageenan injection. Both the bakuchiol treated group and acetyl salicylic acid-treated group show significantly lower paw volume as compared to the control group. **Figure 6.23** illustrates the findings of the anti-inflammatory impact of bakuchiol,

which prevented the inflammation brought on by carrageenan. Animals given bakuchiol showed the greatest reduction in paw volume at 4 mg/kg.

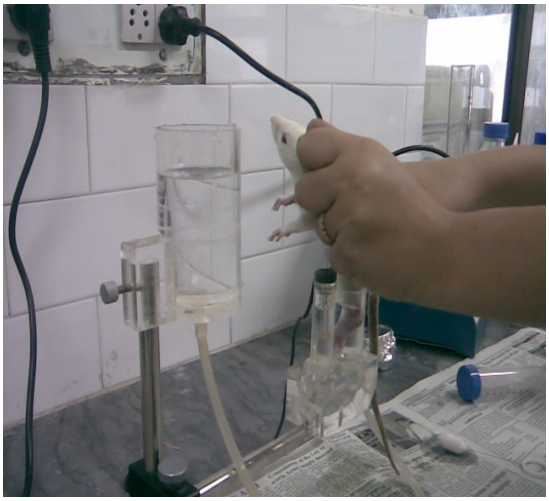


Figure 6.25 Paw volume measurement with plethysmometer

Moreover, Bilateral adrenalectomy in Female Wistar mice was also carried out. After two days of surgical removal of adrenal glands, the assessment was made by Carageenan-mediated acute edema test. As depicted in **Figure 6.26**, both intact and adrenalectomized rats had their paw volume drastically reduced after receiving varying dosages of BAK. BAK at a dose of 4 mg/kg showed a significant decrease in edema with percentage inhibition of 35.65% and 34.05% for intact and ADX rats, respectively, when a comparison was made with control animals. The findings obtained from this work suggest that bakuchiol can efficiently reduce inflammatory edema caused by carrageenan, and this anti-inflammatory action is not due to the release of cortisol from adrenal glands, as evidenced by its effectiveness in experimental rats.

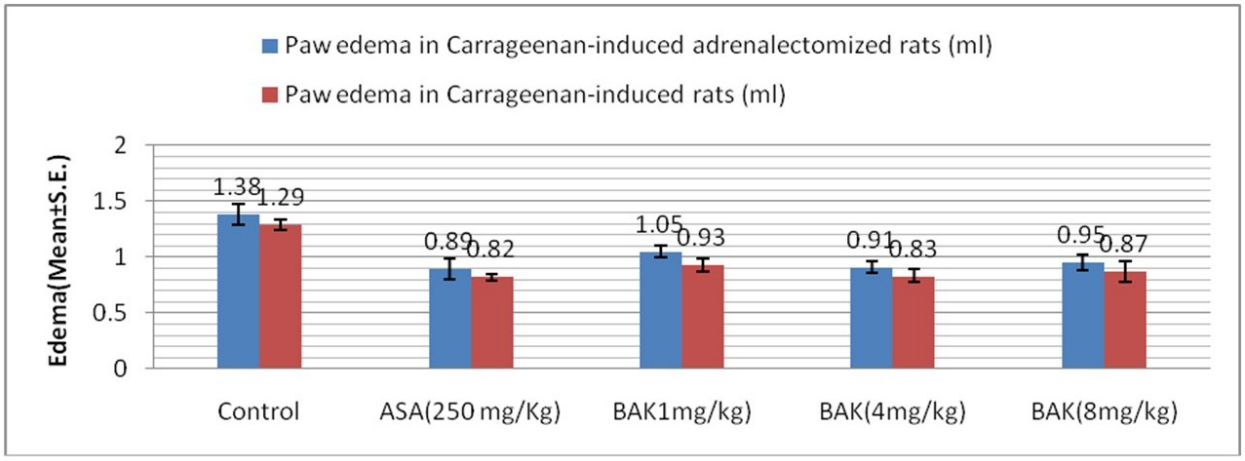


Figure 6.26 Anti inflammatory activity of BAK / PC1 in *Carageenan* induced inflammatory arthritis in the intact and adrenalectomized rats

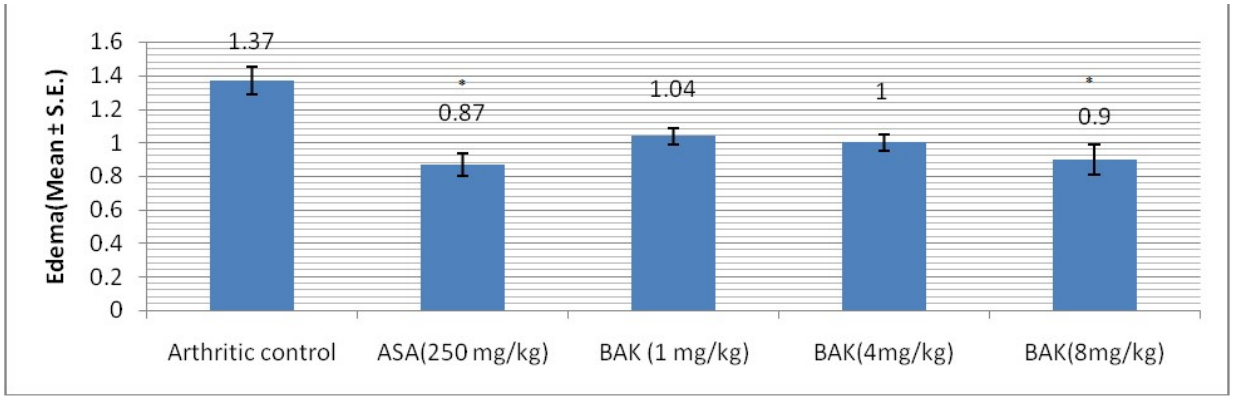
Joint swelling is a key clinical sign of rheumatoid arthritis and is a reflection of synovial membrane inflammation. In the current study, we reported the antiinflammatory effect of bakuchiol, one of the compounds of *P. corylifolia*. These observations were in agreement with previous studies, which reported that the n-hexane extract of *P. corylifolia* when tested for anti-inflammatory effects in albino rats using a carrageenan-induced rat paw oedema assay, displayed strong anti-inflammatory activity (44%) as compared to the standard diclofenac sodium (Gidwani et al., 2010). In another study, one of the compounds of *P. corylifolia* Bakuchiolin, a phenolic monoterpene isolated from the hexane extract of *P. corylifolia* reduced carrageenan-induced rat paw edema (Cui et al., 2015). Our work supports both of these earlier reports, which claim *P. corylifolia* to play a significant role in the reduction of inflammation.

6.2.3.2.2 Effect of BAK on CFA-induced arthritis in rats:

Experimental rats with arthritis elicited by CFA exhibit persistent inflammation with synovial enlargement and a number of systemic alterations. The primary alterations seen in RA include significant leukocyte infiltration, elevated levels of chemokines and cytokines such as IL-1 and TNF-α, the generation of ROS, the deterioration of cartilage and bone, edema, and deformation. Ligaments and joint capsule swelling are brought on by CFA injection into the rat's footpad. At the beginning of the inflammation, edema caused by CFA grows gradually and

becomes persistent after 14 days. The reduction in CFA-induced paw inflammation is an indicator of the test drug's anti-inflammatory action. Measurements of injected and non-injected paw edema, in addition to estimates of antioxidants, the visual arthritis scoring system, the determination of nitrite content, haematological and biochemical evaluations, as well as radiological and histopathological studies, all aid in determining the most likely mechanisms underlying the analgesic and anti-inflammatory effects of the compounds under study (Patil et al., 2019).

CFA induced arthritic model was examined to demonstrate the anti-arthritic response of bakuchiol, as depicted in **Figure 6.27**. Rats were given the drug (at a dose of 1,4, and 8 mg/kg) one day before the induction of arthritis and were given the drug till the 13th day of the experiment. On alternate days, the paw's volume was measured, and on the thirteenth day, the percentage of inhibition was calculated. It has been observed that bakuchiol displayed a dose-dependent reduction of swelling. Blood was taken from these animals on the 14th day of the experiment for the estimation of cytokines like TNF-α, IL-1β, and PGE_2. The standard drug Acetyl Salicylic Acid (ASA) was used as a positive control.



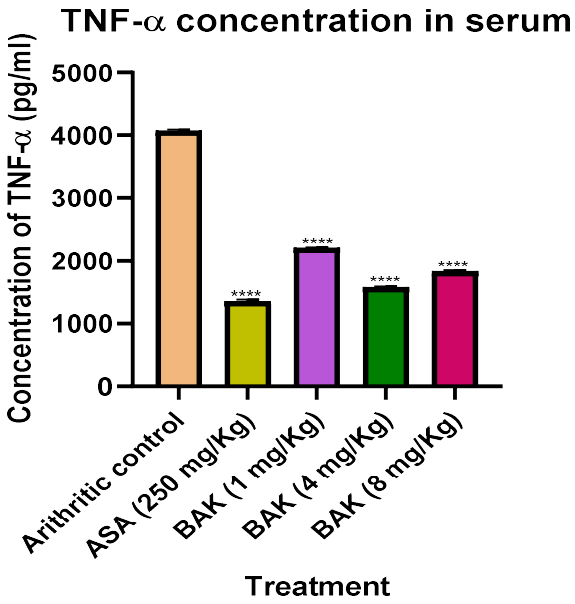
p values < 0.05

Figure 6.27 Anti inflammatory response of BAK in CFA mediated inflammatory arthritis in rats.

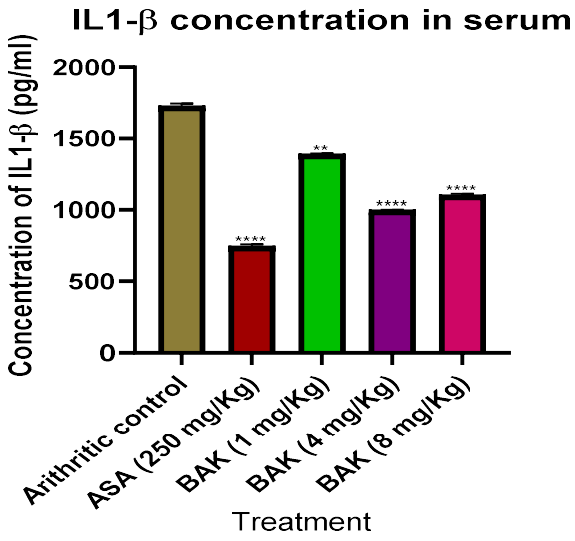
6.2.3.2.2 Bakuchiol effect on serum proinflammatory cytokine levels in CFA induced chronic arthritis:

TNF-α, IL-6, and IL-1, which are produced by macrophages, are thought to play a significant role in inflammatory responses related to the pathogenesis of RA, from the peri-articular phase of autoimmunity to joint tissue damage and chronic inflammation of synovitis (Scott et al., 2010).

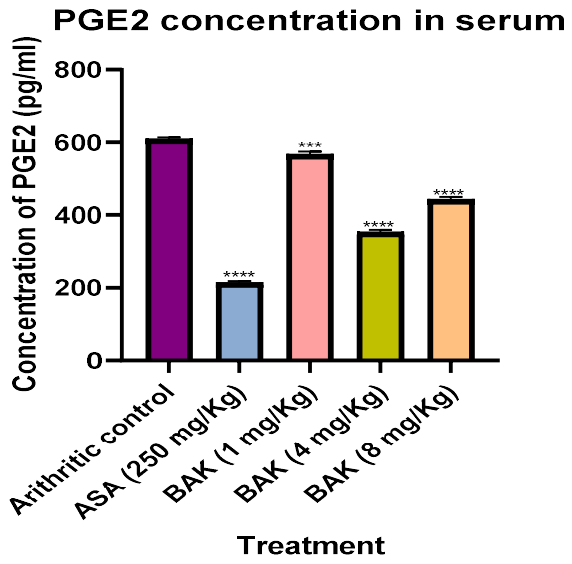
In the CFA investigation, the serum taken on day 14 underwent further processing for the evaluation of cytokine mediators. The cytokines tumour necrosis factor-alfa (TNF-α), interleukin-1β (IL-1β), and prostaglandin E2 (PGE2) were quantified in line with the manufacturer's instructions using commercially accessible kits based on the Sandwich and Competitive ELISA technique. On an ELISA plate reader (Multiskan, USA), colorimetric measurements at 450 nm were used to determine all cytokine concentrations. The result of serum TNF-α, IL-1β and PGE_2 levels by ELISA are presented in **Figure 6.28**. Bakuchiol considerably decreased TNF-α, IL-1β, & PGE_2 levels as compared to control at higher dose levels of 4 & 8 mg/kg.



a)



b)



c)

Figure 6.28 Effect of BAK on concentrations in pg/ml of a) TNF-α, b) IL-1β & c) PGE_2 produced in serum of arthritic animals.

We investigated the effect of bakuchiol, a compound of *P. corylifolia*, a traditional Chinese herbal remedy, to treat RA in CFA induced arthritic model. Inhibition of inflammation

was found in the Bakuchiol (BAK)) treated group during the entire examination time, thus obviously representing the inhibitory activity of BAK against various cytokines. TNF-α, a proinflammatory factor, induces leucocytes which leads to the synthesis of mediator PGE_2, and the suppression of PGE_2 with Bakuchiol in 4 and 8 mg/kg concentration is in agreement with a significant decrease in infiltrated leucocytes. In the present investigation, it was found that bakuchiol extensively lowers the effects of cytokines viz TNF-α, IL-1β and mediators PGE2. The observations made in the present work provide the scientific substantiation to support and rationalize the conventional use of *P. corylifolia* and its isolate bakuchiol for the therapy of RA and other inflammatory disorders.

Bakuchiol, a bioactive component of *P. corylifolia*, was shown to have strong anti-inflammatory and anti-arthritic action in the current study using carrageenan-induced paw edema and CFA-induced arthritis models. This activity was concentration-dependent. The most common model for evaluating anti-inflammatory potential is carrageenan-induced paw edema in rats, which is strongly interrelated with the initial granulomatous phases of inflammation. Following subplantar injection, there is a sharp increase in paw size that is associated with the histamine and serotonin-induced increase in vascular permeability. Our results indicate that BAK can effectively inhibit carrageenan-induced inflammatory edema and that this anti-inflammatory effect does not require adrenal activity, as evidenced by its effectiveness in adrenalectomized (ADX) rats.

Studies showed that supplementation with BAK significantly inhibited the progression of arthritis in treated rats. It is evident that BAK has inhibitory efficacy against a variety of autocoid mediators because the dose-dependent suppression of inflammation was seen in the BAK group throughout the observation period. This experimental model is thought to be the most accurate representation of rheumatoid arthritis in humans.

Cytokines executes a pivotal role in the endogenous and invasive events in rheumatoid arthritis. Proinflammatory factors like TNF-α induce leucocytes to produce PGE_2, and substantial inhibition of PGE_2 by BAK (4 and 8 mg/kg) is consistent with a significant decrease in infiltrating leukocytes. In the present investigation, it is observed that bakuchiol significantly

lowers the levels of cytokines TNF-α, IL-1β and mediators PGE_2. Thus bakuchiol is effective in cytokine neutralization which is a driving force for the inhibition of arthritic inflammation. When cytokines are inhibited, the host immune system is modulated, which raises the possibility that this process could be used as a therapeutic approach to treat arthritis.

It is suggested that Bakuchiol may possibly be developed as a curative agent in the management of arthritis due to its noticeable safety over long-term administration. Our present findings regarding *P. corylifolia* are in agreement with the earlier reports in which it was observed that *P. corylifolia* herbal (PCL) preparation considerably reduced the clinical symptoms and paw swelling as compared to starch treated group. The degradation of synovial tissues and infiltration of lymphocytes were both lessened by PCL, according to the histopathology of the hind paws (Pai et al., 2021). It was also reported earlier that the compounds bavachin and bakuchiol prevent bone loss in osteoporosis (Weng et al., 2015). More research should be done to determine the significance of this revelation in humans.

In the end, it can be demonstrated that nowadays, there is an increasing demand for plant-based drug candidates as they are more free from side effects as compared to synthetics. In this context, our motive was to look for plant-based molecules having anti-arthritic/anti inflammatory and anti obesity potential. Anti-obesity potential of this plant can be partly explained by the reduction of pancreatic lipase activity and decrease in lipid accumulation capacity of 3T3-L1 adipocytes. As evidenced by Oil O Red staining and then absorbance measurement, compounds bakuchiol, isopsoralen and psoralen when added to the differentiation and maintenance medium of adipocytes, cause a reduction in the lipid accumulation by adipocytes. Moreover, when the isolated compounds were incubated with pancreatic lipase, a reduction in the release of p-nitro phenol was observed, which strongly suggests that bakuchiol, isopsoralen and psoralen have pancreatic lipase inhibitory activity. Insilico studies demonstrated that three compounds isolated from *P. corylifolia,* viz. bakuchiol, isopsoralen and psoralen, have an affinity for PPAR gamma, a regulator of obesity. However, these three ligands, when subjected to ADME studies, were predicted to have drug-likeness characteristics and were found to be non-toxic and non-carcinogenic.

In individuals with RA, oxidative stress plays a significant role in the pathophysiology and aetiology of joint tissue injury and chronic inflammation, which can result in connective tissue breakdown and joint and periarticular abnormalities. The risk of autoimmune diseases has been thought to be enhanced by reactive oxygen species (ROS). ROS are significant intracellular signalling molecules in immune system cells that boost the inflammatory-proliferative response in the synovium. Tumor necrosis factor-alpha (TNF-α) can induce higher oxidative stress by initiators of the nuclear factor kappa B activation cascade. Additionally, anti-TNF-therapy can lessen oxidative stress in RA patients (Costa et al., 2016). Nitric oxide, a sign of oxidative stress, has been observed to increase significantly in the plasma of RA patients (Mateen et al., 2016). Joint destruction that occurs in RA patients is associated with an increased level of IL-1β, IL-6, tumour necrosis factor alpha TNF- α, prostaglandins (PG), reactive oxygen species (ROS) and nitric oxide (NO) at sites of inflammation (Abbas, M & Monireh, 2008).

The present investigations, which involve anti inflammatory/anti arthritic study of major constituents isolated from *P. corylifolia* through *in vitro* and *in vivo* experiments, showed Bakuchiol to possess slightly more anti- inflammatory/anti-arthritic tendency than isopsoralen and psoralen. Bakuchiol, isopsoralen and psoralen decreased the level of some important inflammatory molecules like Nitric oxide, reactive oxygen species and TNF-α. Moreover, BAK had reduced the edema in carrageenan mediated inflammatory model and completed Freund's adjuvant (CFA) - mediated arthritis animal models. In the injected paw, there was substantial dose-dependent inhibition of paw edema. In arthritic animals, BAK greatly inhibited the expression of TNF -α and IL-1β levels, which are potent triggers involved in leukocyte migration. TNF- α induces leucocytes to synthesize PGE2, and substantial inhibition of PGE2 by BAK (4 and 8 mg/kg) correlates with a significant reduction in infiltrated leukocytes.

Chapter 7

7.0 Summary and conclusion:

7.1 Summary:

The use of alternative and complementary therapies is higher than ever today, and it presents the special potential for the production of natural medicine. The creation of newer medicinal compounds greatly benefits from the traditional wisdom of the people of the past. The separation of the compounds that are responsible for the mode of action and therapeutic benefits in various types of illnesses is greatly aided by earlier investigations. The current lifestyle changes are a cause for the rise in ailments, including obesity, inflammation, arthritis, and other related conditions. As the fifth most common cause of death worldwide, obesity is regarded as a serious public health issue. Numerous chronic diseases, including cancer, diabetes mellitus, metabolic disorders, and cardiovascular disease, are influenced by being overweight or obese. Reports revealed that an estimated 27.8% of Indians are predicted to be overweight, and 5.0% of them will be obese by 2030. The treatment and prevention of obesity remain complex. In the past three decades, the US Food and Drug Administration (FDA) has approved only a few drugs to treat obesity. However, some of the anti-obesity medications that were approved and sold have since been withdrawn as a result of side effects that have been reported. Numerous natural substances have been found to affect fat storage, weight loss and prevent diet-induced obesity.

Rheumatoid arthritis (RA) is an autoimmune illness that causes persistent inflammation and swelling in the joints and irreparable joint deformity, disability, and a decreased quality of life. Inflammation and immunity are thought to be regulated physiologically by adipose tissue, and it has been proposed that obesity, as assessed by the BMI, is linked to a number of autoimmune and inflammatory diseases. The immune system's reaction to potentially dangerous stimuli, including viruses, damaged cells, poisonous substances, or radiation, is inflammation. The inflammatory reaction needs to be as short-lived as possible in order to prevent any worsening of its unfavourable circumstances, in contrast to its beneficial role as a protection for cellular physiology. Inflammatory diseases are an assemblage of clinical disorders that are distinguished by chronic inflammation, which is associated with some diseases such as rheumatoid arthritis. Rheumatoid arthritis is categorised as an inflammatory poly arthropathy, a disease affecting the connective tissue and musculoskeletal system. Arthritis is the second most frequently reported chronic health condition and one of the nation’s most common causes of

disability. The key characteristic of rheumatoid arthritis (RA) is immune system dysfunction, in which there is an abnormal increase in autoreactive CD_4^+ T cells, pathogenic B cells, macrophages, inflammatory cytokines, chemokines, and autoantibodies. The initial inflammation occurs in the synovial lining of diarthrodial joints.

Plentiful plant genera are well-known for their contributions to conventional and contemporary medication. One of them is the genus *Psoralea. P. corylifolia*, an Indian native and one of the most well-known *Psoralea* plants in the world, deserves special notice for its historical role in medicine. It has been widely used to treat a variety of skin conditions, including leprosy, vitiligo, eczema, psoriasis, and other illnesses. *P. corylifolia* has a number of beneficial characteristics, including those that are anti-vitiligo, anti-microbial, oestrogenic, anti-tumor, anti-depressant, anti-diabetic, anti-inflammatory, anti-oxidant, and immunomodulatory. Due to these reasons, this plant has been considered valuable for research to uncover potential active components that contribute to these traditional tribal medicines.

Previous work has shown that a hundred or so bioactive compounds have so far been isolated from seeds and fruits of *P. corylifolia*, and the most significant ones belong to the coumarins, flavonoids, and meroterpenes groups. So far, chemical constituents of *P. corylifolia* have been isolated from petroleum ether extract, methanolic extract, ethanolic extract and water extract. A long-term answer to the threat that both obesity and rheumatoid arthritis pose to people worldwide may be found by evaluating the effectiveness of plant extracts or isolated compounds against these diseases by several methods.

In the present investigation, *P. corylifolia* seeds were collected, processed and extracted by sequential extraction with petroleum ether, then dichloromethane and after that with methanol solvent. Phytochemical analysis of all three extracts was done, and it was found that there were flavonoids, carbohydrates, phenols, glycosides and terpenoids in the extracts, but alkaloids were absent in all the extracts studied. Later three compounds named PC1, PC2 and PC3 were isolated from the dichloromethane extract of seeds of *P. corylifolia* by using column chromatography.PC1 had R_f value of 0.65, PC2 had R_f value of 0.45, and PC3 had R_f value of 0.31. The structure of the isolated compounds had been elucidated by ^{1}HNMR and ^{13}CNMR, and UFLC studies was conducted for both the isolates as well as for DCME. A comparison of data

with available literature values revealed that the three compounds were bakuchiol (PC1), isopsoralen (PC2) and psoralen (PC3).

For the evaluation of the anti-obesity potential of DCME as well as isolated compounds viz PC1, PC2 and PC3, *in vitro* studies (enzymatic and cell lines) and in silico studies were conducted. MTT cell viability assay was also done on 3T3-L1 cell lines. Treatment was given with 12.5 µM - 50 µM concentration of isolated compounds or 100 µg/ml- 400 µg/ml of DCME for the estimation of safe and toxic concentration levels. Results revealed the viability of 3T3-L1 cells when treated with varied conc. of DCME was decreased at conc. above 100 µg/ml, and when treated with different concentrations of isolated compounds, the viability decreased at conc. > 25 µM. At 100 µg/ml of DCME the percentage cell viability was 97.02%, whereas at 25 µM conc. of PC1, PC2 and PC3 the percentage cell viability was 93.97%, 91.90% and 94.11% respectively. Thus, safe conc. of DCM extract for additional studies were found to be up to 100 µg/ml, and that for PC1, PC2 and PC3 was found to be 25 µM. DCM extract was found to display anti-adipogenesis characteristics, 75 ± 0.003% lipid accumulation, when compared with control at 100 µg/ml dose observed. Moreover, Bakuchiol (PC1), isopsoralen (PC2) and psoralen (PC3) alsoreduced the lipid accumulation in 3T3-L1 preadipocytes. At a 25 µM dose ofPC1, PC2, & PC3,lipid accumulation of 78.06 ± 0.002%, 80.91 ± 0.004%, and 80.91 ± 0.001%, respectively, was observedin contrast to control.In continuous to our study regarding the exploration of anti-obesity potential of *P. corylifolia*, the dichloromethane extract and isolated compounds were then screened for anti-lipase potential also. The maximum anti-lipase property was recorded with DCME (26.02 ± .041% inhibition) at 100 µg/ml, while, among the isolated compounds, bakuchiol exhibited a higher activity (24.2 ± 0.037% inhibition) followed by isopsoralen and psoralenat 100 µg/ml concentration.

Additionally, in silico studies were also conducted to explore the target involved in the anti-obesity potential of the isolated compounds. In order to know about the pharmacokinetic and pharmacodynamic features like absorption, distribution, metabolism, and excretion of bakuchiol, isopsoralen and psoralen, in silico studies were conducted using the SwissADME tool. All three ligands were predicted to exhibit suitable drug-like characteristics and be physiochemically biocompatible. According to in silico ADMET predictions, all three ligands were discovered to

be nontoxic and to have good absorption and solubility qualities. The ligand molecules bakuchiol, isopsoralen and psoralen were docked with receptor Peroxisome proliferator-activated receptor gamma (PPAR-γ)6L8B protein using Autodockvina software. Bakuchiol and psoralen, were predicted to have higher negative binding energy with -7.8 kcal/mol and -7.7 kcal/mol, followed by isopsoralen with -7.6 Kcal/mol.

The anti-arthritic activity of the isolated compound/s of *P. corylifolia* was then determined by performing studies on RAW 264.7 cell lines and on Wistar rats. Before determining the anti-arthritic activity of isolated compounds of *P. corylifolia* by using RAW cell lines, the MTT experiment was conducted to determine the toxicityof compounds. For this assay, RAW 264.7 cells were subjected to treatment with bakuchiol/ isopsoralen / psoralen at the concentration range from 1.25µM- 5 µM. No significant reduction in cell viability was observed in RAW cell line after treatment with the isolates.

By using the Griess reagent assay, all three isolated compounds were evaluated for their potential to reduce the formation of nitric oxide (NO) in LPS-induced RAW 264.7 cells. At 5µM concentration, bakuchiol, isopsoralen, and psoralen reduced NO levels to 21.33%± 1.4, 20.19% ±3.0 and 21.30% ±6.3, respectively. The efficiency of bakuchiol, isopsoralen and psoralen for inhibition of Reactive oxygen species (ROS) production in RAW 264.7 cells treated with LPS was also investigated. Image J software, when applied to the fluorescent images, depicted the corrected total cell fluorescent (CTCF) of LPS treated RAW cells to be 14.32 (a.u), while that of PC1 (bakuchiol), PC2 (isopsoralen) and PC3 (psoralen) treated RAW cells was 3.49, 3.34 and 3.41 (a.u) respectively at 5µM conc. Treatment with bakuchiol, isopsoralen or psoralen leads to a decrease in the concentration of TNF-α in a concentration dependent means. The release of TNF-α was reduced by up to 55.33% ±6.28, 39.74% ±7.1and 43.76%±1.5 respectively, by bakuchiol, isopsoralen and psoralen at 5 µMtreatment as compared to LPS stimulated control cells. So, we can say that bakuchiol, isopsoralen and psoralen exerts anti-inflammatory effects via suppression of ROS and NO and TNF-α.

Further, *in vivo* studies were conducted on Wistar rats with bakuchiol (BAK)/(PC1). To determine anti-inflammatory potential, the carrageenan-induced paw edema model was studied, and bakuchiol was given in doses of 1, 4 and 8 mg/kg. After 4 h of carrageenan injection, it was

found that bakuchiol treated group displayed significantly lower paw volume as compared to the control group. BAK at dose of 4 mg/kg showed a significant decrease in edema with percentage inhibition of 35.65% and 34.05% for intact and ADX rats, respectively, when compared with control animals. CFA induced arthritic model was examined to demonstrate the anti-arthritic response of bakuchiol. Rats were given the drug (at a dose of 1,4, and 8 mg/kg) one day before the induction of arthritis and were given the drug till the 13^{th} day of the experiment. It was observed that bakuchiol treatment resulted in a reduction of swelling in arthritis-induced rats. Further, Bakuchiol considerably decreased TNF-α, IL-1β, & PGE_2 levels as compared to control at higher dose levels of 4 & 8 mg/kg.

Based on the findings, the compounds found in *P. corylifolia* dichloromethane seed extract can be used as one of the greatest remedies and alternatives against the existing issues of obesity and rheumatoid arthritis. It can also be utilized to conduct more analyses and tests to certify them in producing modern pharmaceuticals that can be employed against the aforementioned ailment, which could greatly aid in the fight against the existing problem.

7.2 Conclusion:

Obesity and rheumatoid arthritis are regarded as the major public health problems in the world. The present study is the first reported study for anti-obesity and anti-arthritic potential of bakuchiol, isopsoralen and psoralen, major constituents of *P. corylifolia*. The present research was undertaken to find the solution to these two diseases by evaluating the extract or isolated compounds from the seeds of the medicinally important plant *Psoralea corylifolia*. Three compounds bakuchiol, isopsoralen and psoralen were isolated from the dichloromethane extract of *P. corylifolia* seeds. The mechanisms involved in obesity can be partly explained by the reduction of pancreatic lipase activity and decrease in lipid accumulation capacity of 3T3-L1 adipocytes. Moreover, these three compounds were found to have an affinity for PPAR gamma, a regulator of obesity.

Furthermore, our investigation provides evidence for the anti-inflammatory and anti-arthritic activity of *P. corylifolia* as bakuchiol, isopsoralen, and psoralen decreased the level of some important inflammatory molecules like Nitric oxide, reactive oxygen species and TNF-α. Anti -

arthritic tendency was confirmed for this plant as bakuchiol had reduced the edema in carrageenan mediated inflammatory model and completed Freund's adjuvant(CFA) - mediated arthritis models. Also, a decrease in the levels TNF-α, IL-1β, & PGE_2 in CFA- mediated arthritis models supports our findings regarding anti-inflammatory/anti-arthritis potential of *P. corylifolia*.

7.3 Key findings:

Following the research conducted with the seeds of *P. corylifolia* against obesity and arthritis, the key findings made are mentioned below:

- Bakuchiol, isopsoralen and psoralen are some major constituents present in dichloromethane extract of seeds of *P. corylifolia*.
- DCME and isolates viz., bakuchiol, isopsoralen and psoralen have anti-lipase as well as anti-adipogenesis activity.
- Toxicity studies with DCME, bakuchiol, isopsoralen and psoralen by MTT assay revealed that DCME is safe upto dose of 100 µg/ml whereas viability reduced at concentrations greater than 25 µMin cases of isolates in 3T3 – L1 cell lines.
- The isolated bioactive compounds which were used as ligands have drug-like properties as predicted by *in silico* analysis by the ADME study.
- Bakuchiol, isopsoralen and psoralen have a high affinity for Peroxisome proliferator-activated receptor gamma, an important regulator of adipogenesis.
- Bakuchiol, isopsoralen and psoralen have anti-inflammatory potential due to their capability to reduce levels of Nitric oxide, reactive oxygen species and TNF-α in RAW 264.7 cell lines.
- Cell viability with isolates was also carried out, and the results revealed that no significant toxicity was observed at a concentration of up to 5 µM in the case of Raw 264.7 cell lines.
- Bakuchiol successfully reduced edema in the carrageenan mediated inflammatory model and complete freund's adjuvant(CFA) - mediated arthritis models.
- Bakuchiol decreased the levels of TNF-α, IL-1β,& PGE_2 in CFA- mediated arthritis models

Thus, this investigation offered an evidence-based analysis of *P. coylifolia* as a treatment for arthritis and obesity. Improving disease control and reducing expenses and side effects of the present conventional drugs may enhance the quality of life and total healthcare for obese and arthritis patients. Additionally, this experiment identified some crucial *P. corylifolia* quality traits that the herbal medicine industry and pharmaceutical organizations could use to create high-quality, effective *P. corylifolia* products.

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Appendices 1

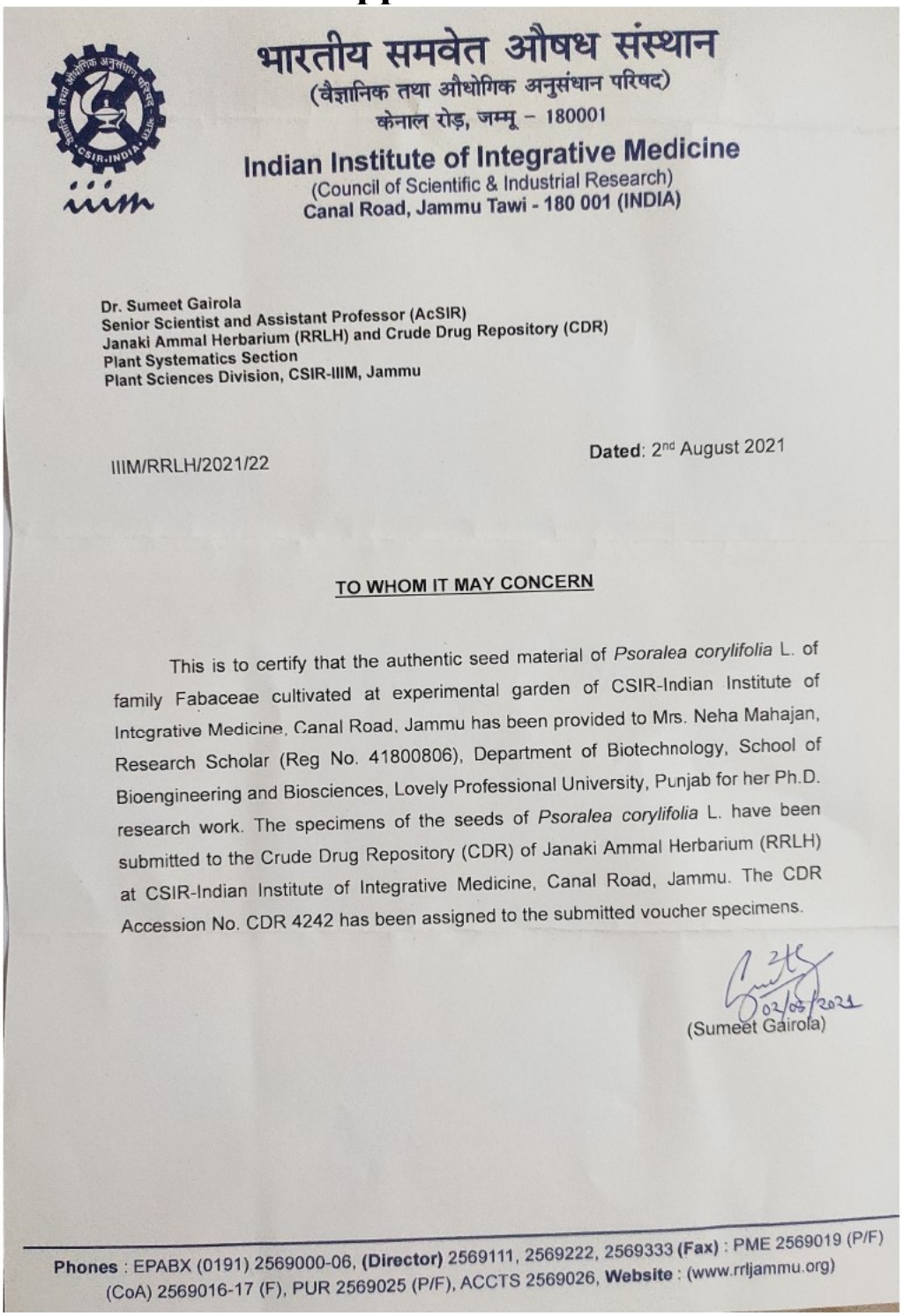
List of Abbreviations:

Abbreviation	Meaning
ACPAs	Anticitrullinated protein antibodies
ADME	Absorption, Distribution, Metabolism and Excretion
ADX	Adrenalectomy
ASA	Acetyl salicylic acid
BAK	Bakuchiol
BBB	Blood Brain Barrier
CC	Column chromatography
CDR	Crude drug repository
CFA	Complete freunds adjuvant
CGs	Cyanogenic Glycosides
COX-2	Cyclooxygenase-2
Conc.	Concentration
CTCF	Corrected total cell fluorescence
DCFH	dichloro-dihydro-fluorescein
DCME	Dichloromethane Extract
Dil.	Dilute
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethyl sulfoxide
ELISA	Enzyme linked immunosorbent assay
EMA	European Medicines Agency
FA	Fatty acid
FBS	Fetal bovine serum
FDA	Food and Drug Administration

G	Gram
H	Hour
HPTLC	High performance thin layer chromatography
Hz	Hertz
IL	Interleukin
Kg	Kilogram
LPS	Lipopolysaccharide
M	Meter
ME	Methanolic Extract
MIC	Minimium inhibitory concentration
Ml	Milli litre
MSCs	Mesenchymal stem cells
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide,
MW	Molecular weight
NMR	Nuclear magnetic resonance
NO	Nitric oxide
OD	Optical density
P. corylifolia	*Psoralea corylifolia*
PC1	Bakuchiol
PC2	Isopsoralen
PC3	Psoralen
PCS	*Psoralea corylifolia* seed
PCSE	*Psoralea corylifolia* seed extract
PDB	Protein data bank
PEE	Petroleum Ether Extract
PGE 2	Prostaglandin E2
P-gp	P-glycoprotein
PPAR	Peroxisome proliferator-activated receptors

PPL	Porcine pancreatic lipase
PTPN	Protein tyrosine phosphatase nonreceptor
QR	Quinone reductase
QS	Quorum sensing
QS	Quorum sensing
RA	Rheumatoid arthritis
Rf	Retention factor
RO5	Rule of 5
ROS	Reactive oxygen species
r.t.	Room temperature
SF	Synovial fluid
SMILES	Simplified molecular-input line-entry specification
TKDL	Traditional Knowledge Digital Library
TLC	Thin layer chromatography
TNF α	Tumour necrosis factor α
UFLC	Ultra fast liquid chromatography
µM	Micro mole

Appendices II



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IIIM/RRLH/2021/22

Dated: 2nd August 2021

TO WHOM IT MAY CONCERN

This is to certify that the authentic seed material of *Psoralea corylifolia* L. of family Fabaceae cultivated at experimental garden of CSIR-Indian Institute of Integrative Medicine, Canal Road, Jammu has been provided to Mrs. Neha Mahajan, Research Scholar (Reg No. 41800806), Department of Biotechnology, School of Bioengineering and Biosciences, Lovely Professional University, Punjab for her Ph.D. research work. The specimens of the seeds of *Psoralea corylifolia* L. have been submitted to the Crude Drug Repository (CDR) of Janaki Ammal Herbarium (RRLH) at CSIR-Indian Institute of Integrative Medicine, Canal Road, Jammu. The CDR Accession No. CDR 4242 has been assigned to the submitted voucher specimens.

02/08/2021
(Sumeet Gairola)

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Appendices -III

Publications:

1. Mahajan Neha, Koul B, Kaur J, Bishnoi M, Gupta P, Kumar A, Shah BA, Mubeen I, Rai AK, Prasad R, Singh J. Antiobesity Potential of Bioactive Constituents from Dichloromethane Extract of Psoralea corylifolia L. Seeds *Biomed Research International* Volume 2022, Article ID 9504787, 10 pages https://doi.org/10.1155/2022/9504787 scopus indexed
2. Mahajan, Neha, Koul, B., Gupta, P., Shah, B.A. and Singh, J., *Psoralea corylifolia* Linn: Panacea to several maladies *South African Journal of Botany* vol 149, 28, 963-993. Scopus indexed

Conferences:

1. Presented a paper entitled "*Porcine pancreatic lipase inhibitory studies of the secondary metabolites isolated from Psoralea corylifolia seeds***"** in National Conference on Recent trends in Agriculture, Biosciences, Computer Applications, Environment & Humanities organized by Government Degree College, Billawar on 24th March 2022
2. Presented a paper entitled "*Exploring the biopotential of Psoralea corylifolia: a well known traditional medicinal plant*" in International conference on Plant Physiology and Biotechnology(ICPPB) held from 10th to 12th September 2021 organized by Department of Molecular Biology and Genetic Engineering School of Bioengineering and Biosciences under the aegis of Lovely Professional University Punjab.
3. Presented a paper entitled "*Biological evaluation of Natural and Natural derived molecules from some important medicinal plants of Jammu Region*" in International conference on Applied Biology held from 4th to 6th November 2019 Shri Mata Vaishno Devi University Katra J&K.

Awards

1. Received best poster award for presentation of paper entitled "*Biological evaluation of Natural and Natural derived molecules from some important medicinal plants of Jammu Region*" in International conference on Applied Biology held from 4th to 6th November 2019 Shri Mata Vaishno Devi University Katra J&K.