Exploring the Potential of *Tribulus terrestris* in Pancreatic Adenocarcinoma: A Network Pharmacological Approach

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CANDIDATE'S DECLARATION

I Bharmjeet, Roll Number: 2K21/MSCBIO/09 student of M.Sc. Biotechnology, hereby declare that the project dissertation titled - "**Exploring the Potential of *Tribulus terrestris* in Pancreatic Adenocarcinoma: A Network Pharmacological Approach"** which is submitted by me to the Department of Biotechnology**,** Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Science, is original and not copied from any source with proper citation. This work has not previously formed the basis for the award of any degree, Diploma Associateship, fellowship or other similar title or recognition.

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CERTIFICATE

I hereby certify that the Project Dissertation titled "**Exploring the Potential of *Tribulus terrestris* in Pancreatic Adenocarcinoma: A Network Pharmacological Approach"** which is submitted by Bharmjeet, Roll No.: 2K21/MSCBIO/09, Department of Biotechnology**,** Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Science, is a record of the project work carried out by the student under my supervision. To the best of my knowledge, this work has not been submitted in part or full for any Degree or Diploma to the University or elsewhere.

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ABSTRACT

Pancreatic cancer, one of the deadliest tumors, has a low chance of survival because there aren't many effective therapies available. Pancreatic adenocarcinomas, the most common kind of pancreatic cancer, account for around 90% of cases. Currently available therapeutic options include surgery, chemotherapy, and radiation therapy; however, these treatments typically have limited efficacy and substantial side effects. Therefore, its imperative to find alternate treatments that might provide pancreatic cancer patients with better outcomes. Research from the past has demonstrated that *Tribulus terrestris* has anticancer capabilities against several different forms of cancer including breast, colon, and prostate. However, the therapeutic potential of *T. terrestris* for pancreatic cancer has mainly remained unexplored. The present study's goal is to assess *T. terrestris* potential for treating pancreatic cancer. This study makes use of a range of computer methods to predict the effects of phytochemicals from fruits of *T. terrestris* on the molecular processes connected to the spread of pancreatic cancer. The work investigates the main objectives for developing precision medicine. By concentrating on the molecular pathways that lead to the development of cancer, the study's results demonstrate that *T. terrestris* greatly inhibits the growth of pancreatic cancer cells. *T. terrestris* was primarily shown to cause apoptosis and inhibit pancreatic cancer cells growth. These effects were achieved by downregulation of critical proteins that regulate cell growth and survival, including MMP9 and MAPK1. The study's findings imply that *T. terrestris* may be used in addition to other medications to treat pancreatic cancer. Natural supplements, such as *T. terrestris,* are a safe and effective alternative to conventional cancer treatments. The study also emphasizes how important it is to examine the possible use of natural substances to treat cancer from a molecular perspective.

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CHAPTER 1: INTRODUCTION

1.1 GENERAL INTRODUCTION

The pancreas is an abdominal glandular organ that is located below the stomach. It is essential for digestion as well as blood sugar regulation. The cells lining the pancreatic ducts give birth to pancreatic adenocarcinoma, often known as pancreatic cancer, which is a very fatal and aggressive kind of cancer.

Pancreatic adenocarcinoma, which accounts for majority of instances, account for 90% diagnoses of pancreatic cancer [1]. Rapid tumor development, early metastasis, and resistance to standard cancer therapies are its defining characteristics. The disease often has a bad prognosis and a high fatality rate since it is typically detected at an advanced stage [2]. There is a higher chance of getting pancreatic adenocarcinoma due to a number of causes. The majority of occurrences affect those over the median age of 60, making aging a key risk factor [3]. Pancreatic cancer risk is also significantly elevated by cigarette smoking [4]. A family history of pancreatic cancer, and certain genetic disorders such as hereditary pancreatitis, chronic pancreatitis, Lynch syndrome, and long-term diabetes are additional risk factors [5]. Fig 1.1 (A) displays the prevalence of pancreatic cancer in various geographic areas, whereas Fig 1.1 (b) displays the crude rate and age-standardized rate (ASR) of pancreatic cancer in 2020 per 100000 according to GLOBOCAN [6].

Early-stage pancreatic cancer sometimes exhibits vague symptoms, which delays detection. Jaundice, stomach discomfort, unexpected weight loss, digestive issues, and exhaustion are the frequently witnessed symptoms [7]. The disease has frequently progressed to surrounding organs or distant places by the moment symptoms manifest, making curative therapy difficult. In order to see the pancreas and identify any anomalies, a variety of imaging techniques, including magnetic resonance imaging (MRI), computed tomography (CT) scans, or ultrasound, are used in the diagnosis of pancreatic adenocarcinoma. To confirm the existence of cancer cells, a biopsy is required, which entails collecting tissue samples for lab investigation.

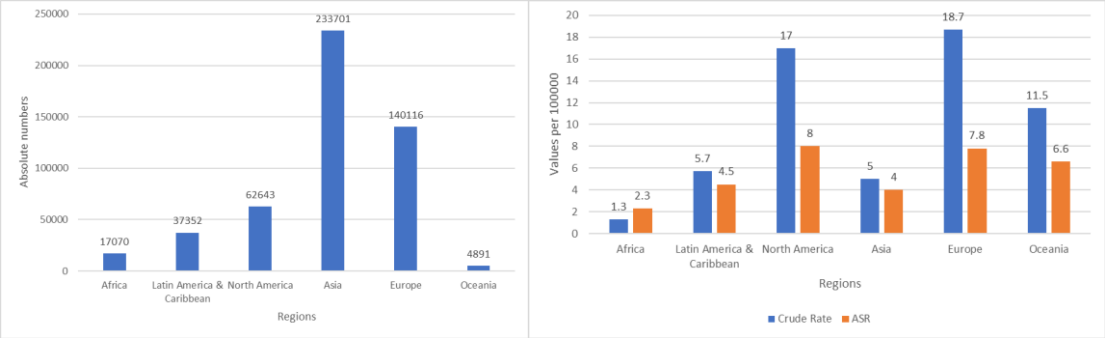


Fig 1.1 (a) Incidence of pancreatic cancer in 2020. (b) Crude rate and Age-standardized rate (ASR) of pancreatic cancer in 2020 per 100000 (Data source – GLOBOCAN 2020)

The pancreatic adenocarcinoma therapy choices rely on the cancer's stage and the patient's general condition. If the tumor is small and resectable, surgery, such as a Whipple technique (pancreaticoduodenectomy) or distal pancreatectomy, may be used to remove it [8]. However, because of their severe illnesses, the majority of patients are not surgical candidates. Cancer patients may benefit from chemotherapy, radiation treatment, and targeted therapy medications to reduce tumor size, ease symptoms, and prolong life. Despite improvements in cancer research and therapy, pancreatic adenocarcinoma still poses a significant threat. The aggressiveness of the illness, late-stage detection, and few available treatment choices all contribute to pancreatic cancer's poor five-year survival rate [9]. The molecular mechanisms underlying the onset and spread of pancreatic cancer are still being studied in an effort to create better early detection techniques, more potent therapies, and early detection tools.

The high rates of recurrence and resistance to standard therapy in pancreatic adenocarcinoma are two major problems [10]. By focusing on various cancer cell routes, processes, or vulnerabilities, alternative therapeutic methods may be able to solve this problem. We may be able to avoid or overcome the resistance mechanisms displayed by pancreatic cancer cells by investigating cutting-edge treatment modalities like immunotherapy, gene therapy, or targeted therapeutics targeting certain molecular targets.

Tribulus terrestris is a plant species that has been utilized for millennia in traditional medical systems, notably in Ayurveda and Traditional Chinese Medicine [11]. It is also known as puncture vine or Gokshura. It is indigenous to several places, including Asia, Europe, and Africa, and has drawn a lot of interest because of its possible therapeutic benefits [12]. It has a long history of usage in folk medicine to cure a variety of illnesses, such as cardiovascular diseases, urinary and reproductive issues, and to improve athletic performance. Exploring the pharmacological characteristics and possible medical uses of *T. terrestris* has been the subject of this scientific study.

Various bioactives, such as flavonoids, steroidal saponins, alkaloids, and phenolic compounds, may be found in *T. terrestris* fruit [13]. These elements are believed to contribute to the role in pharmacological effects and medicinal potential of the plant. *T. terrestris* has also been shown to have antioxidant and anti-inflammatory properties [14]. Its antioxidant qualities aid in scavenging dangerous free radicals and shielding cells from oxidative stress, which is linked to the emergence of a number of illnesses, including cancer. The possibility of *T. terrestris* having anticancer effects has been researched. Studies have shown that it has the power to stop the development and division of cancer cells in a different cancer types, such as prostate, breast, and liver cancer [15]. Its antioxidant, anti-inflammatory, and apoptosis-inducing qualities are credited with its anticancer actions.

In this thesis, I use a network pharmacological strategy to investigate the potential of the medicinal plant *T. terrestris* in the treatment of pancreatic adenocarcinoma. I am attempting to determine the possible targets and pathways affected by *T. terrestris* and clarify its modes of action in pancreatic cancer by combining bioinformatics, computational biology, and pharmacological study. This study shows potential for identifying new therapy approaches and advancing the study of pancreatic cancer.

1.2 OBJECTIVES

1. To analyze *Tribulus terrestris* fruit's therapeutic potential for the management of pancreatic adenocarcinoma (PAAD).
2. To assess the possible synergistic effects of several active phytonutrients and anti-PAAD core targets found in *Tribulus terrestris* fruit.
3. To conduct molecular docking studies to evaluate the affinity of *Tribulus terrestris* fruit's active phytonutrients for the main anti-PAAD targets.
4. To pinpoint the molecular processes, biological mechanisms and cellular elements related to the *Tribulus terrestris* -affected gene targets.
5. To find the KEGG pathways that could be important for understanding the molecular mechanism of *Tribulus terrestris* in treating PAAD.

CHAPTER 2: REVIEW OF LITERATURE

2.1 Pancreatic cancer

Pancreatic cancer is one of the primary diseases linked to the pancreas. Pancreatic cancer is a malignant tumor that develops in the tissues of the pancreas. It is a dangerous and frequently aggressive kind of cancer that metastasizes quickly to other body areas. Since early-stage pancreatic cancer is difficult to diagnose, it is typically discovered at more advanced stages, when the number of available treatments is constrained [16].

Depending on the type of cells involved, there are many forms of pancreatic cancer. The most typical varieties include:

1. **Pancreatic adenocarcinoma:** With 90% of cases being this kind, it is the most typical kind of pancreatic cancer [17]. It develops from the exocrine pancreatic cells, which are responsible for producing the digesting enzymes. The ducts of the pancreas are where pancreatic cancer generally begins before spreading to other tissues and organs. Adenocarcinomas of the pancreas are often aggressive, have a poor prognosis, and are frequently discovered at advanced stages when there are few curative options. Because pancreatic adenocarcinoma doesn't initially present with any particular symptoms, early identification is difficult [18]. For people with pancreatic adenocarcinoma, routine medical exams, understanding of risk factors and prompt evaluation of alarming symptoms can aid in earlier diagnosis and possibly improve treatment outcomes. The tumor's growth and spread can cause a number of symptoms, such as:

- Jaundice: When the tumor obstructs the bile duct and prevents the passage of bile, the skin and eyes turn yellow. This may result in itching, pale stools, and dark urine.
- Abdominal pain: As the tumor develops and affects neighboring tissues, nerves, or organs, dull or acute discomfort in the upper abdomen or back may develop.

- Unexplained weight loss: Pancreatic adenocarcinoma patients frequently have significant and unplanned weight loss as a result of a number of reasons, including decreased appetite, changes in metabolism, and cachexia brought on by the malignancy.
- Digestive problems: Symptoms of pancreatic adenocarcinoma that affect digestion include nausea, vomiting, indigestion, and changes in bowel habits.
- Diabetes: Because of the tumor's effect on insulin production, pancreatic adenocarcinoma can occasionally cause new-onset diabetes or aggravate pre-existing diabetes.

Pancreatic adenocarcinoma typically exhibits the following characteristics and features:

❖ Location: About 60–70% of instances involve the head of the pancreas, where the tumor is often detected. Additionally, the body or tail of the pancreas may be affected.

❖ Histological appearance: Pancreatic adenocarcinoma shows as infiltrating, insufficiently differentiated malignant cells creating glandular structures under a microscope. The tumor cells can infect nearby tissues and organs and frequently have an aggressive development pattern.

❖ Metastasis: Adenocarcinomas of the pancreas are more susceptible to early metastasis. It frequently invades adjacent lymph nodes and has the potential to migrate to distant organs such the liver, lungs, and peritoneum.

❖ Genetic alterations: Pancreatic adenocarcinoma may have various genetic changes, including mutations in the KRAS, TP53, CDKN2A, and SMAD4 genes. These genetic alterations aid in the onset and development of the illness.

❖ Diagnosis and staging: Imaging techniques including CT scans, MRIs, and endoscopic ultrasonography (EUS) may be used to diagnose pancreatic adenocarcinoma, combined with a biopsy to validate the cancer cells presence. Staging is done to assess the severity of the disease and inform treatment choices.

Pancreaticoduodenectomy or distal pancreatectomy surgery, radiation therapy, chemotherapy, immunotherapy, targeted therapy, or a combination of these approaches may be used as treatment options.

2. **Pancreatic neuroendocrine tumors (PNETs):** The endocrine cells of the pancreas are the source of PNETs. It is also known as pancreatic islet cell tumors or pancreatic neuroendocrine neoplasms (PanNENs). PNETs are quite uncommon and typically develop more slowly than pancreatic adenocarcinoma, which is more prevalent. PNETs can also be divided into functional and non-functional tumors according to whether or not they produce hormones into the circulation that cause certain symptoms.

A. Functional PNETs: Hormones produced by these tumors have been linked to certain clinical disorders. Examples include gastrinomas, which secrete too much gastrin and cause stomach ulcers, glucagonomas, which produce too much glucagon and cause symptoms resembling diabetes, and somatostatinomas, which produce too much somatostatin and cause hormonal imbalances.

B. Non-functional PNETs: These tumors don't make hormones, and because of their size or local spread, they are frequently found accidentally or when they start to cause symptoms.

3. **Acinar Cell Carcinoma:** Acinar cells of the exocrine pancreas are the source of the uncommon pancreatic cancer known as acinar cell carcinoma. Digestive enzyme production and secretion are carried out by these cells. Only a tiny portion of pancreatic tumors are acinar cell carcinomas, which have a distinctive histological appearance. Under a microscope, acinar cell carcinoma tumors often have a solid or glandular appearance and are well-defined. They frequently manifest as sizable tumors inside the pancreas, and they have the capacity to spread to neighboring lymph nodes or distant organs. Acinar cell carcinoma has a somewhat better prognosis than the more frequent pancreatic adenocarcinoma and may react differentially to various therapeutic modalities.

4. **Adenosquamous Carcinoma:** An aggressive form of pancreatic cancer with both glandular (adenocarcinoma) and squamous cell carcinoma components are adenosquamous carcinoma, also known as adenoacanthoma. Both glandular structures and squamous

differentiation are present inside the tumor in this kind of malignancy. Compared to pancreatic adenocarcinoma, adenosquamous carcinoma has a worse prognosis [19]. It spreads and grows fast, which makes therapy more difficult. Due to the aggressiveness of the condition, surgical resection may not always be an option; in these cases, chemotherapy, radiation therapy, or targeted therapy may be explored as alternative treatment methods.

5. **Squamous Cell Carcinoma:** A very uncommon kind of pancreatic cancer that develops from the squamous cells lining the pancreatic ducts. The lining of the digestive and respiratory systems often contains these cells. It is believed that pancreatic squamous cell carcinoma arises from metaplasia or the conversion of the healthy pancreatic ductal epithelium into squamous cells [20]. Comparing squamous cell carcinoma to other forms of pancreatic cancer, the prognosis is frequently considered to be worse. With a larger risk of metastasis at the time of diagnosis, it has a tendency to be more aggressive. Squamous cell carcinoma of the pancreas may be treated with surgery, chemotherapy, radiotherapy, or a combination of these methods.

Given the rarity of these subtypes, research into and knowledge of the best treatment approaches are still developing, and therapy choices are frequently based on the unique circumstances of each individual patient. For patients with these uncommon pancreatic cancer forms, consultation with a multidisciplinary team of healthcare specialists skilled in pancreatic cancer management is essential for choosing the best course of therapy.

2.2 Current treatment options for Pancreatic Adenocarcinoma

The current choices for treating pancreatic adenocarcinoma rely on various parameters, including cancer's stage, tumor's resectability, and the patient's general health, Table 2.1 summarizes the currently available treatments and their limitations. The therapeutic methods comprise:

❖ Surgery: The most efficient cure is surgery. The pancreaticoduodenectomy, also known as the Whipple surgery, is a frequent surgical treatment that entails the bile duct, the gallbladder, and a portion of the small intestine removal [21]. If the tumor is found in the pancreatic tail, a distal pancreatectomy may be performed. Surgery seeks to completely resect the tumor by removing it together with any adjacent lymph nodes.

❖ Chemotherapy: Before and after surgery, chemotherapy is frequently used. Cancer cells are targeted and killed with anticancer medications. When administered alone or in conjunction with other medications like nab-paclitaxel or 5-fluorouracil (5-FU), gemcitabine has been a mainstay chemotherapy treatment for pancreatic cancer [22]. Chemotherapy may slow the growth of tumors, stop the spread of illness, and increase survival rates.

❖ Radiation Therapy: High-energy X-rays are used in radiation treatment to target and kill cancer cells. It may be used internally or externally. Radiation therapy is often used in combination with chemotherapy, either before or after surgery, to enhance treatment outcomes.

❖ Targeted Therapy: Targeted treatments are created with the explicit goal of destroying the molecular anomalies and signaling networks that support the development and spread of cancer. Erlotinib and cetuximab are two examples of medications that block EGFR, which are used in targeted therapy for pancreatic cancer [23]. Clinical studies are also being conducted for other targeted medications, such as poly(ADP-ribose) polymerase (PARP) inhibitors and immune checkpoint inhibitors.

Although there are therapeutic possibilities, pancreatic cancer still poses a serious threat. Chemotherapy is frequently ineffective against cancer. The poor five-year overall survival rate for pancreatic cancer highlights the need for more potent treatments. Current research focuses on creating novel therapeutic modalities, such as immunotherapy, molecular profiling-based personalized medicine, and targeted medicines targeting certain genetic mutations or disrupted signaling pathways. Novel therapeutic approaches and combinations are being studied in clinical trials to enhance patient outcomes and lengthen survival.

Table 2.1 The limits of the current PAAD therapy options.

Treatment	Examples	Limitations
Chemotherapeutics	Monotherapy - - 5-fluorouracil - Gemcitabine	- Limited efficacy - Development of drug resistance - Incomplete symptom relief - Lack of disease modification - Patient variability - Lack of flexibility
	Combinatorial Therapy - - Gemcitabine + capecitabine - Gemcitabine + capecitabine - Gemcitabine + oxaliplatin - Gemcitabine + irinotecan - Gemcitabine + pemetrexed - Gemcitabine + nab-paclitaxel - Gemcitabine + cetuximab - Gemcitabine + bevacizuab	- Increased risk of side effects - Higher cost - Potential drug interactions - Difficulty in determining optimal combinations - Increased risk of non-adherence to complex regimens. - Variability in treatment response among patients

	- Gemcitabine + ablifercept - Gemcitabine + axtinib	
Targeted therapy	- Cetuximab (anti-EGFR) - Tipifarnib - AZD6244 - Axitinib - Allogenic tumor vaccine	- High cost of production and treatment - Challenges in drug delivery and administration - Complex manufacturing and regulatory processes.

Pancreatic adenocarcinoma continues to provide considerable problems in terms of treatment efficacy and patient outcomes despite developments in medical research and treatment techniques. The available therapies for pancreatic cancer have a number of drawbacks and difficulties. These consist of:

❖ Late-stage diagnosis: Oftentimes, pancreatic adenocarcinoma is discovered in an advanced stage, when the tumor has already spread or invaded other tissues. The prognosis of patients is strongly affected by late-stage diagnosis and the possibility of curative therapy choices.

❖ Limited surgical resectability: In cases with pancreatic adenocarcinoma, surgery gives the best chance for long-term survival. Only a tiny portion of patients, however, are qualified for surgery because of things like tumor location, blood vessel involvement, and the existence of far-off metastases. The illness has a generally dismal prognosis due to the restricted surgical resectability.

❖ Chemoresistance: Chemotherapy is notoriously ineffective against pancreatic cancer. The pancreatic cancer cells' resistance to frequently used chemotherapeutic drugs is influenced by the tumor microenvironment, genetic changes, and molecular features.

Chemotherapy's efficacy is compromised, as is the capacity to slow the spread of the disease.

- Radiation therapy limitations: The pancreas is a very sensitive organ, making it difficult to deliver a suitably high radiation dosage to the tumor while minimizing harm to the surrounding healthy tissues. Radiation therapy is used to treat pancreatic adenocarcinoma. Radiation treatment can also be ineffective for some pancreatic tumors because of this resistance.
- Toxicity and side effects: Chemotherapy, Radiation therapy and surgery are the available therapeutic options for pancreatic adenocarcinoma, although all of them have considerable toxicity and adverse effects. Negative side effects from chemotherapy medications might include myelosuppression, tiredness, nausea, and vomiting. Complications from surgery and organ damage from radiation treatment are also possible.
- Lack of effective targeted therapies: While targeted medicines have transformed the way that some malignancies, such lung and breast cancer, are treated, it has been difficult to create efficient targeted treatments for pancreatic adenocarcinoma. It is challenging to pinpoint particular molecular targets that can be successfully addressed by medications because of the variety and hereditary complexity of pancreatic tumors.
- Limited treatment options for advanced stages: Overall survival rates for advanced-stage diseases continue to be poor, and current medicines have difficulty slowing the disease's course.
- Lack of predictive biomarkers: Personalizing therapy and enhancing patient outcomes would be made possible by biomarkers that can direct treatment decisions and forecast treatment response.

For individuals with pancreatic adenocarcinoma, improvements in targeted medicines, immunotherapy, and early detection methods provide hope for better outcomes. Clinical trials and collaborative research are crucial for advancing the field and removing the present barriers to pancreatic cancer treatment.

2.3 *Tribulus terrestris*

Tribulus terrestris is a plant that is indigenous to many parts of the world, including Europe, Asia, and Africa [24]**.** It is often referred to as puncture vine or bindii. Fig 2.1 is showing a picture of the *T. terrestris* plant and its fruit. It's been used for a very long time in traditional medical practices. Due to its medicinal qualities and possible health advantages, the fruit of *T. terrestris* is very significant in medicine [25]**.**

Fig 2.1 *Tribulus terrestris* plant and its fruit

- Therapeutic Properties: The medicinal qualities of *T. terrestris* are a result of a various bioactives found in the plant. These consist of flavonoids, alkaloids, glycosides, steroidal saponins (such as protodioscin), and other phytochemicals [26]. These elements provide the plant with its pharmacological effects and therapeutic advantages.
- Sexual Health and Libido Enhancement: *T. terrestris* has grown in popularity because of its ability to increase libido and sexual wellness. By boosting the release of luteinizing hormone (LH), which in turn increases the creation of testosterone in males, it is thought to raise testosterone levels [27]. Due to this characteristic, it has long been used as an aphrodisiac and is now found in many products intended to improve sexual health and performance.
- Anti-inflammatory and Antioxidant Effects: *T. terrestris* contains phytochemicals, mainly flavonoids, which have been proven to have antioxidant and anti-inflammatory effects [28].
- Adaptogenic and Stress-Reducing Effects: As an adaptogenic plant, *T. terrestris* may aid in the body's ability to cope with stress and support a healthy physiological

response [29]. It may lessen the damaging effects of stress on the body by assisting the adrenal glands and modulating stress hormone levels.

- Cardiovascular Health Benefits: *T. terrestris* may assist the heart, according to certain research. By lowering LDL cholesterol levels and raising HDL cholesterol, it may aid in lowering blood pressure and lipid profiles [30]. These effects support cardiovascular health and might have consequences for people with high blood pressure or cholesterol.
- Other Potential Benefits: *T. terrestris* has been studied for its possible impact on a number of medical issues, including diabetes, renal health, and immune system performance [31]. To determine the scope of these advantages and their methods of action, more study is necessary.

Due to its medicinal qualities, *T. terrestris*, and notably its fruit, has importance in medicine. Its traditional use and early scientific research point to possible advantages for cardiovascular health, stress reduction, anti-inflammatory effects, antioxidant activity, sexual health, and other health-related uses. To completely comprehend the mechanisms of action and effectiveness of *T. terrestris* in various health scenarios, additional study is necessary.

T. terrestris has been researched for its possible anticancer qualities, however there has only been a small amount of work done in this field, most of which has been done on animals or in lab settings. The following are some crucial details regarding *T. terrestris*' potential anticancer abilities:

- Anti-inflammatory and Antioxidant Effects: Flavonoids and steroidal saponins, two phytochemicals with antioxidant and anti-inflammatory effects, are found in *T. terrestris*. These could prevent the start and spread of cancer by reducing inflammation, fighting oxidative stress, and protecting cells from harm [32].
- Apoptosis Induction: According to several studies, *T. terrestris* extracts or isolated chemicals may cause cancer cells to undergo apoptosis [33]. This process may prevent the development and division of cancer cells while fostering their eradication

❖ Anti-proliferative Effects: In several cancer cell lines, *T. terrestris* has shown antiproliferative actions that prevent the growth of tumors. Cell signaling pathways involved in growth and multiplication, modification of cell cycle regulators, and interference with angiogenesis (the creation of new blood vessels to feed tumors) are all possible explanations for these effects [34].

❖ Immune Modulation: According to some research, *T. terrestris* may improve immune function by boosting the activity of immune cells. The ability of immune system to fight off cancer cells may be enhanced by this immunological modulation, which may help with cancer monitoring.

It has been discovered that *T. terrestris* affects testosterone levels among other hormones. While this characteristic is frequently connected to its historic usage to improve male sexual health, it may also have consequences for diseases that are sensitive to hormones, such as breast and prostate cancer. However, further research is needed to determine how *T. terrestris* affects the development of cancer and tumors linked to hormones.

2.4 Review of existing research on *Tribulus terrestris* and its potential anti-cancer properties

The medicinal herb *T. terrestris*, which is frequently employed in conventional medical practices, has drawn interest recently due to its conceivable anti-cancer capabilities. Numerous research, including in vitro, in vivo, and clinical examinations, have examined the impact of *T. terrestris* and its bioactive components on various cancer types. Here, we examine the research that has been done so far on *T. terrestris* and its conceivable anti-cancer effects.

T. terrestris extracts and chemicals have been shown in several studies to prevent the growth of cancer cells. Table 2.2 summarizes the studies done on cancer using *T. terrestris*. *T. terrestris* extract, for instance, was shown in a study on breast cancer cells to reduce cell growth and cause cell cycle arrest. Likewise, *T. terrestris* extract suppressed prostate

cancer cells, indicating its potential as an anti-cancer drug [35]. It has been noted that *T. terrestris* causes certain cancer cells to undergo apoptosis, or programmed cell death [36].

Table 2.2 Summary of studies on cancer using *Tribulus terrestris*

Study	**Cancer**	**Plant part**	**Findings**	**References**
Kim HJ et. al. (2011)	Liver cancer	Fruit	- Strong HepG2 cell growth inhibitory activity. - Suppression of the expression of NF-KB subunit p50 and NF-KB dependent reporter gene expression. - Suppression of invasion-related gene transcription, anti-apoptosis genes, and cell cycle regulation.	[37]
Pourali M. et. al (2016)	Prostate & colon	Fruit	- Extract triggered 74% and 46% apoptosis respectively.	[38]
Wei S et. al. (2014)	Prostate	Whole plant	- Strongly suppresses prostate cancer cell growth. - Induced apoptosis & cell cycle arrest in	[39]

			PC-3 cells. - shown induced apoptotis & inhibited angiogenesis in xenograft tumor cells.	
Han R. et. al (2021)	Liver	Whole plant	- Suppressed proliferation, migration & invasion of HCC in Hep38 & SNU-449 cells - Inhibited CA XII expression & modulated E2F/CASP3 axis.	[40]
Abbas MW et. al. (2022)	Ovarian	Whole plant	- IC50 values are 6.0 µg/ml & 8.2 µg/ml	[41]
Patel A. (2019)	Breast	Seed & leaves	- Inhibited MCF-7 breast cancer cells by upregulation of p53, Caspase 3, Bax, caspase 8 & FADD and downregulation of Bcl-2 expression	[42]

2.5 Exploration of the network pharmacology approach and its relevance in studying complex diseases like cancer

In order to comprehend the interactions between medications, targets, and biological networks at a systems level, network pharmacology blends concepts from pharmacology, systems biology, and network analysis [43]. In the study of complicated diseases, this method has shown to be quite beneficial, providing information on the following topics: disease processes, drug targets, and treatment approaches. Fig 2.2 conceptualizes the network pharmacology approach for the present study.

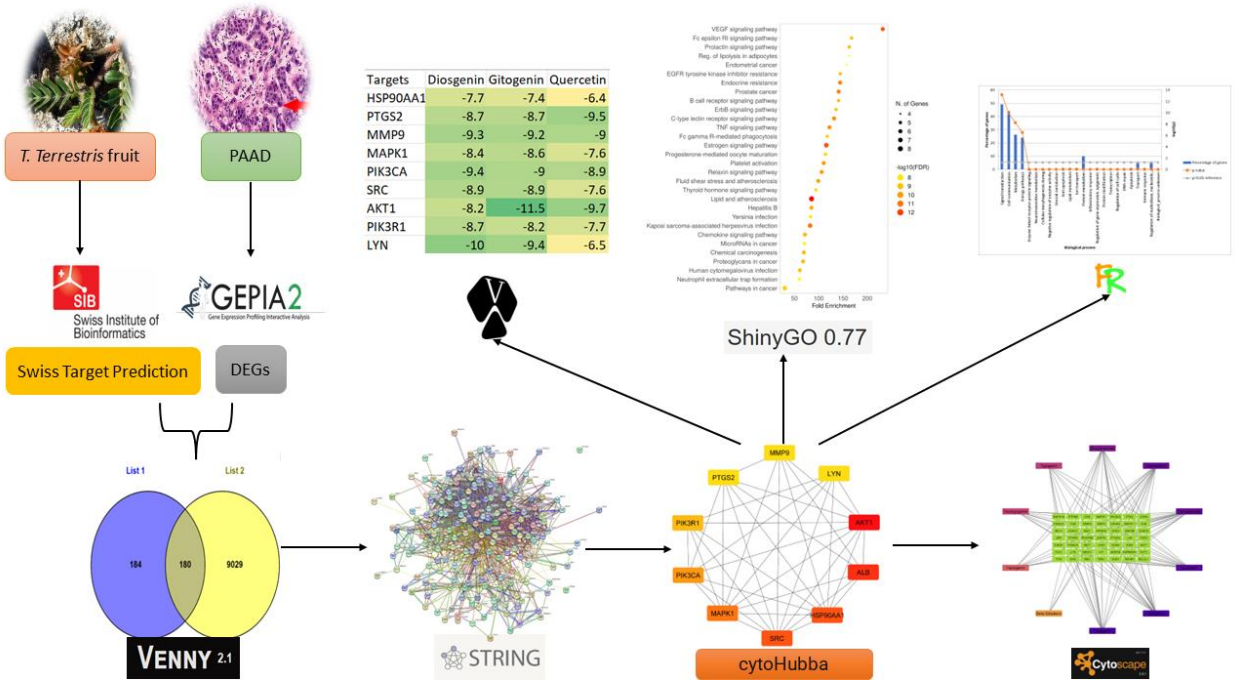


Fig 2.2 Conceptual representation of the network pharmacology approach

❖ <u>Understanding disease mechanisms:</u> Complex molecular connections and the deregulation of several pathways frequently play a role in complex illnesses. The creation and study of illness-specific networks are made possible by network pharmacology, which captures the interactions between the molecules involved in disease processes. Network pharmacology offers a comprehensive understanding of disease processes by combining omics data, drug-target interactions, and protein-protein interaction networks. This

thorough understanding makes it possible to pinpoint specific molecular actors, signaling pathways, and crucial nodes for therapeutic approaches.

- Polypharmacology and combination therapy: Multiple targets and pathways may be dysregulated in many illnesses, which are multifactorial in nature. The term "polypharmacology" refers to a drug's capacity to interact with several targets at once, and it is recognized by network pharmacology. Network pharmacology facilitates the development of multi-target medications or drug combinations that can more effectively modify complicated disease networks by studying drug-target networks. This strategy creates opportunities for personalized medicine and combination treatments that are specifically designed to address the disease's unique molecular profile and network dynamics in a given patient.
- Predicting adverse drug reactions: The development of new drugs and patient safety are both seriously affected by adverse drug reactions (ADRs). Network pharmacology offers insights into the underlying processes of ADRs by comprehending the network context of drug-target interactions. It may also help create safer medications by optimizing and modifying drug structures or doses.
- Systems pharmacology and personalized medicine: The idea of systems biology is compatible with network pharmacology. Network pharmacology allows a systems-level knowledge of illnesses and medication responses by combining data from genomes, proteomics, metabolomics, and clinical information. With the use of this strategy, personalized medicine may be developed, allowing for more precise and successful treatment options based on each patient's unique genetic profile and disease network features.

The network pharmacology approach, which offers a comprehensive understanding of disease mechanisms, enables drug repurposing, makes it easier to identify drug targets, encourages the idea of polypharmacology and combination therapies, forecasts adverse drug reactions, and supports personalized medicine initiatives, has revolutionized the study of complex diseases. Our approach to investigating illnesses has been completely changed by its combination of pharmacology, systems biology, and network analysis. It also has the potential to lead to future drug discovery and development methods that are more effective.

CHAPTER 3: MATERIALS AND METHODOLOGY

Physical materials are not required because the task is done *in silico* using software. Utilizing computational methods and models, the analysis is carried out within the software itself.

3.1 TOOLS & SOFTWARES

3.1.1 IMPPAT

One may access the user-friendly online interface of the IMPPAT 2.0 database at IMPPAT | IMPPAT: Indian Medicinal Plants, Phytochemistry And Therapeutics (imsc.res.in) [44], [45] to retrieve plant phytochemical information. The open-source CSS framework Bootstrap 4.1.3 was used to build the website's front end, which was then customized with internal HTML, PHP, CSS, jQuery scripts, and JavaScript. Additionally, the jQuery plug-in Data Tables and Cytoscape.js [46] are used to visualize networks and display tables, respectively.

3.1.2 PUBCHEM

The U.S. National Institutes of Health (NIH), houses the public chemical database PubChem [47], [48]. In addition to researchers, patent agents, and students, PubChem is a well-liked resource with millions of monthly users. Importantly, PubChem data is frequently used in several studies on machine learning and artificial intelligence. A data aggregator called PubChem gathers chemical data from thousands of sources. While siRNA, miRNA, lipids, carbohydrates, and biopolymers that have undergone chemical modification make up the majority of the compounds in PubChem, it also includes other chemical substances like siRNA and miRNA [49], [50]. This data is arranged into several data collections, such as Substance, Compound, BioAssay, Gene, Protein, Taxonomy, Pathway, Cell Line, and Patent.

While Compound includes distinctive chemical structures that were derived from Substance, Substance archives depositor-provided chemical descriptions [49], [51].

BioAssay stores the depositor's descriptions and test results for biological assay investigations. Information on the substances, proteins, genes, and taxa referenced in each patent filing is available in the Patent Collection.

3.1.3 UNIPROT

The vast database UniProt offers details about proteins. Protein sequences, functional annotations, structural details, interactions, and other data are all available through UniProt [52].

The UniProt database is divided into several sections:

1. UniProtKB (Knowledge Base): It is the main area of UniProt and has an extensive database of protein sequences and functional details. Moreover, UniProtKB is separated into two sections:
2. UniProtKB/Swiss-Prot: Entries for proteins in this subsection have been curated and annotated by hand. Experts examine the data in Swiss-Prot to guarantee its quality and correctness.
3. UniProtKB/TrEMBL: It includes protein sequences that have been computationally predicted or automatically annotated. Compared to Swiss-Prot, TrEMBL entries offer a wider coverage of protein sequences but may have less thorough annotations.
4. UniRef (UniProt Reference Clusters): Proteins are grouped by UniRef based on sequence similarity to decrease duplication and boost computational performance. UniRef offers representative sequences, grouped collections of protein sequences, and related functional details.
5. UniParc (UniProt Archive): All publicly accessible protein sequences are kept in this extensive collection, even if they have been dropped or combined with sequences from other UniProt sections. Protein sequence information is archived in UniParc.

In addition to these primary parts, UniProt also provides supplementary information and tools, including variant sites for UniProtKB/Swiss-Prot, UniProtKB terms and controlled

vocabulary, cross references to other databases, tools for protein annotation, and more.

3.1.4 Swiss Target Prediction

The Swiss Institute of Bioinformatics (SIB) offers a computer tool called Swiss Target Prediction that forecasts probable targets or interactions for small molecules like pharmaceuticals or chemical compounds. In order to calculate the likelihood that a given molecule would bind to particular protein targets, it combines a number of techniques and algorithms [53].

A ligand-based methodology is used by the Swiss Target Prediction programme to compare a compound's chemical structure to a database of known ligands and their related protein targets. Additionally, a target-based strategy is used to identify probable binding sites and interactions by contrasting the chemical characteristics of the substance with those of known protein structures.

The predictions and estimates provided by Swiss Target Prediction are based on computational methods and existing data, but experimental validation is typically needed to validate the actual interactions between a chemical and its targets. As a result, the tool is useful for developing hypotheses and looking into possible study topics in the disciplines of drug discovery and associated studies.

3.1.5 GEPIA2

A web-based application called GEPIA 2 (Gene Expression Profiling Interactive Analysis 2) is used to examine gene expression data in different cancer types [54].

GEPIA 2's main attributes and capabilities include the following:

1. Gene Expression Analysis: The levels of expression of certain genes in distinct cancer kinds or healthy tissues may be looked up and compared by users. Users may build

interactive graphs using the tool that displays gene expression profiles and provide statistical data.

2. Differential Expression Analysis: The genes for a certain cancer type that exhibit differential expression in tumor and normal samples can be located by GEPIA 2 users. Volcano graphs and lists of genes that have undergone significant up- or down-regulation, together with their statistical significance, are provided.
3. Survival Analysis: Using Kaplan-Meier survival graphs and log-rank testing, GEPIA 2 assesses the significance of gene expression in relation to overall survival or disease-free survival.
4. Correlation Analysis: Using the scatter plots and correlation coefficients it provides, researchers can identify potential co-regulated genes or evaluate the relationship between gene expression and clinical variables.
5. Customizable Visualization: Users may alter the colors, data range, and plot kinds to create bespoke plots using the tool's many visualization choices. Additionally, it enables the creation of heatmaps to display the patterns of gene expression in various samples.

3.1.6 Venny 2.1

A web-based tool for creating Venn diagrams is called Venny 2.1. Venn diagrams are visual depictions that show the connections between several sets or groupings of objects. They are often used to visualize the overlaps and distinctive components across numerous sets in a variety of disciplines, including biology, statistics, and data analysis [55].

With its user-friendly interface, Venny 2.1 makes it simple to create Venn diagrams with up to six groups. Here are some of the main attributes and capabilities of Venny 2.1:

1. Set Input: Users have the option of entering data as lists or sets. Items inside sets may be separated by commas, spaces, or line breaks, and each set may be labeled.
2. Venn Diagram Generation: Venny 2.1 creates an interactive Venn diagram when the sets are entered, graphically illustrating the crossings and differences between the sets. The figure shows the areas that overlap as well as the distinctive components that are exclusive to each group.

3. Data Visualization: For customizing the Venn diagram's appearance, use Venny 2.1. Users may adjust the visual representation to suit their tastes by changing the colors, text sizes, and line thickness.
4. Set Operations: Users of the tool may execute operations on sets including union, intersection, and relative complement (difference) between sets. Based on the relationships between sets, these procedures may be used to further analyze and extract certain elements.
5. Data Export: In order to use the created Venn diagram in presentations, reports, or publications, users of Venny 2.1 can export it as an image file (PNG format).

An effective tool for visualizing and analyzing set connections is Venny 2.1. Researchers and data analysts that work with overlapping data sets and wish to understand the similarities and contrasts between those sets may find it particularly helpful.

3.1.7 STRING-DB

Protein-protein interactions (PPIs), functional connections, and networks are all covered in the bioinformatics database and online resource STRING-DB (Search Tool for the Retrieval of Interacting Genes/Proteins). It combines information from numerous sources to build an extensive network of both known and anticipated protein interactions [56].
Here are some of STRING-DB's main attributes and features:

1. Protein Interaction Data: STRING-DB gathers and combines information on protein interactions from the literature, computational predictions, and experimental data. Additionally, functional relationships derived from many sources, such as co-expression, co-occurrence, and common domains, it also incorporates physical interactions.
2. Interaction Network Visualization: Users of STRING-DB can view networks of protein-protein interactions. The network view gives a visual representation of nodes (proteins) and edges (interactions), giving a summary of the connections between relevant proteins. By changing variables like confidence scores and interaction kinds, users may personalize the visualization.
3. Functional Enrichment Analysis: STRING-DB provides functional enrichment analysis

tools to find biological processes and pathways that are overrepresented in a set of proteins. Understanding the functional context of a protein family and how they could be involved in particular biological processes might be aided by this information.

4. Protein Annotation and Details: The database offers comprehensive details on specific proteins, such as functional annotations, domain structures, information about protein families, and references to other databases. It can help in gaining a thorough grasp of the features and purposes of certain proteins.
5. Integration with External Resources: To give further details on proteins, pathways, and functional annotations, STRING-DB interfaces with a number of external databases and resources, including UniProt, KEGG, and GO (Gene Ontology).

In order to analyze protein interactions, comprehend functional linkages, and look into the underlying molecular mechanisms in diverse biological processes and disorders, researchers in the domains of molecular biology, systems biology, and related subjects frequently utilize STRING-DB.

3.1.8 FUNRich

A software program called FUNRich (Functional Enrichment analysis tool) is used to analyze gene or protein lists for functional enrichment. By detecting overrepresented gene ontology (GO) concepts, biological pathways, and functional categories, it is intended to offer insights into the functional traits and biological relevance of a particular set of genes or proteins [57].

Here are some key features and functionalities of FUNRich:

1. Functional Enrichment Analysis: By comparing the supplied gene/protein list against a reference database, such as the Gene Ontology or pathway databases, FUNRich does enrichment analysis. In the input set, it finds highly enriched functional words or pathways that describe the bodily functions, molecular processes, or cellular elements connected to the input genes/proteins.
2. Gene Ontology Analysis: Gene ontology analysis, which is a component of FUNRich,

entails classifying genes and proteins into distinct functional words such as biological processes, molecular functions, and cellular components. It aids in comprehending how the genes and proteins in the input list work.

3. Pathway Analysis: By plotting the supplied gene/protein list into recognised biological pathways or signaling networks, the application also does pathway analysis. It reveals overrepresented biological pathways and sheds light on those that may be connected to the input genes or proteins.
4. Visualization of Results: To display the findings of the functional enrichment study, FUNRich provides graphics such as bar charts and heatmaps. Effective interpretation and communication of the expanded functional concepts and pathways are made possible by these visual representations.
5. Export and Integration: Users of the program may export the results in a number of formats, including Excel or text files, for use in other applications or for additional analysis.

Genomic, transcriptomic, and proteomic researchers frequently utilize FUNRich to acquire functional insights into their gene or protein lists and to identify the underlying biological processes and pathways linked to their data. In addition to providing a framework for additional biological interpretation and hypothesis creation, it aids in the identification of important functional annotations.

3.1.9 Cytoscape

Popular open-source software platform Cytoscape is employed for the visualization, analysis, and modeling of complicated networks. It offers a full range of instruments and plugins for network research, visualization, and interaction with different kinds of data. Network biology, systems biology, and bioinformatics studies all frequently employ Cytoscape [58].

Here are some key features and functionalities of Cytoscape:

1. Network Visualization: The construction and personalization of eye-catching network visualizations is made possible by Cytoscape. Users may import network data and portray

it as nodes and edges. To organize the network parts, the tool offers a variety of layout methods, and modification of colors, sizes, and styles is possible to draw attention to certain properties of the nodes and edges.

2. Network Analysis: To explore and analyze network features, Cytoscape offers a wide variety of network analysis tools and plugins. In addition to performing clustering, network motif identification, community detection, and topology analysis, users may also compute network centrality metrics. Understanding the structure, connectivity, and functional characteristics of complex networks is aided by these methods.
3. Data Integration and Visualization: Users may combine network data with several other biological data types, such as gene expression data, protein annotations, or functional annotations, using the Cytoscape software. Through this integration, additional network data, like as expression levels, functional annotations, or route details, may be visualized.
4. Plugin System: Cytoscape has a robust ecosystem of plugins that increase its capability. To add more analytic methods, visualization designs, and data connection capabilities, users may pick from a variety of plugins. For certain research requirements, these plugins increase Cytoscape's adaptability and breadth.
5. Network Modeling and Simulation: Tools for modeling and simulating network dynamics are available through Cytoscape. Dynamic models may be built and altered by users to simulate how network behavior evolves over time. Understanding how network features affect biological processes and analyzing biological systems makes advantage of this property.

Cytoscape is a potent tool for network visualization, analysis, and modeling because of its attractive user interface, rich feature set, and vibrant community support. It makes it easier to explore and analyze complicated networks, which helps researchers better comprehend the make-up, operation, and dynamics of biological systems.

3.1.10 Cytoscape-MCODE

The MCODE (Molecular Complex Detection) plug-in for Cytoscape is an effective tool for finding clusters or sections of a biological network that are highly related to one another.

In protein-protein interaction networks or other kinds of molecular networks, it aids in the discovery of putative functional modules or complexes [59].

Here are the key features and functionalities of the MCODE plug-in:

1. Cluster Detection: Based on the idea of "vertex-weighted density," MCODE locates clusters or highly linked areas within a network. It looks for areas with a lot of nodes and gives these clusters ratings.
2. Scoring and Ranking: Each recognised cluster receives a cluster score from MCODE depending on the size and density of the cluster. Users may concentrate on the most important and meaningful clusters in the network since the clusters are sorted according to their scores.
3. Cluster Visualization: The nodes and edges inside each cluster are highlighted by MCODE as it creates visual representations of the discovered clusters. It offers choices to alter the visualization's appearance, including modifying the node size, edge thickness, and color coding.
4. Cluster Analysis: MCODE offers a number of tools for examining the characteristics of found clusters. The functional annotations, enrichment of particular gene ontology concepts or pathways inside the clusters, and other characteristics related to the cluster nodes may all be examined by users. Understanding the links and probable functions of the proteins inside the clusters is made easier by this study.
5. Interactive Exploration: Users of MCODE can interactively browse the network visualization's discovered clusters. To get more specific information and carry out additional analysis, users may zoom in and out, pan the image, and choose certain clusters or nodes.

When analyzing large-scale molecular networks, the MCODE plug-in is frequently used to find physiologically significant modules or complexes. It helps in the identification of biological systems' regulatory components, possible protein complexes, and functional connections.

3.1.11 Cytoscape-CytoHubba

An effective tool for network analysis and locating key hubs or nodes within a biological network is Cytoscape's CytoHubba plug-in. For determining node centralities, ranking nodes according to their topological relevance, and locating important nodes in the network, it offers a variety of algorithms and techniques [60].
Here are the key features and functionalities of the CytoHubba plug-in:

1. Node Ranking Algorithms: CytoHubba provides a number of node centrality calculation techniques, including degree, betweenness, proximity, and eigenvector centrality. These methods evaluate many facets of node relevance using the connection and topology of the network.
2. Node Ranking and Selection: Users may find the most significant or influential nodes in the network by using CytoHubba, which ranks nodes in the network according to their centrality scores. To determine and rank node centralities, users can use a single ranking method or a mix of many.
3. Hub Detection: Hub nodes are strongly linked nodes that are recognised by CytoHubba and serve crucial roles in network connection and communication. Key proteins or genes with significant functional significance in biological systems are frequently represented as hub nodes.
4. Multiple Scoring Methods: CytoHubba also supports edge-based approaches (edge percolated component and maximal clique centrality) and neighborhood-based methods (k-core and maximal clique percolated component), in addition to centrality-based rankings.
5. Customizable Analysis: By changing parameters and settings, such as the number of top-ranked nodes to show, filtering criteria, and selecting the scoring technique or combination of methods, CytoHubba users may tailor the analysis.
6. Result Visualization: The top-ranked nodes in the network and their connections are visualized using CytoHubba. The detected hub nodes, their connections, and their placements within the network may all be explored and visualized by users.

Network biology research frequently employs the CytoHubba plug-in for Cytoscape to locate significant nodes, hub proteins, or crucial regulatory components within biological networks. It helps in deciphering the functional and regulatory functions of certain components in biological systems, comprehending the network architecture, and finding crucial nodes.

3.1.12 DAVID

A web-based bioinformatics application called DAVID (Database for Annotation, Visualisation, and Integrated Discovery) is used for functional annotation and enrichment analysis of gene or protein lists. To comprehend the biological significance of a particular set of genes or proteins, it offers an extensive collection of functional annotation methods and resources [61].

Here are the key features and functionalities of the DAVID tool:

1. Functional Annotation: By putting biological annotations on the input gene or protein list, DAVID carries out functional annotation. The input is annotated with functional keywords using a variety of databases and resources, including Gene Ontology (GO) terminology, protein domains, pathway details, illness connections, and protein-protein interactions.
2. Functional Enrichment Analysis: To find overrepresented functional keywords or pathways in the input gene or protein list, DAVID performs functional enrichment analysis. It provides data reflecting the enhanced functional categories and estimates statistical significance using the relevant statistical techniques. Understanding the biological procedures, molecular operations, and cellular elements connected to the input genes or proteins is made easier by this approach.
3. Data Visualization: DAVID offers methods for visualizing the outcomes of functional enrichment analysis. To show the statistical importance of the functional terms or pathways as well as the enrichment scores for each, it creates interactive graphical representations like bar charts and scatter plots. This aids in the understanding and dissemination of the results of the enrichment analysis.
4. Gene Set Analysis: Users can examine gene sets or gene clusters for functional enrichment

using DAVID. It allows for the comparison of functional annotations between various gene sets or the discovery of functional words that are common or exclusive among various gene sets.

5. Gene-Term Associations: Information on the relationship between genes and functional words is provided by DAVID. Users can investigate the genes linked to one particular functional phrase and vice versa. Understanding the genes involved in particular biological processes or pathways is made easier thanks to this capability.
6. Data Export and Integration: Users of DAVID can export the analysis findings in a number of formats, including Excel or text files, for use in other applications or for additional investigation. Additionally, it offers choices for integrating the outcomes with other databases or visualization software.

Genomic, transcriptomic, and proteomic researchers frequently utilize DAVID to acquire functional insights into their gene or protein lists. It helps with high-throughput data analysis, biological pathway identification, and the identification of underlying biological processes linked to the input genes or proteins.

3.1.13 RCSB PDB

A comprehensive and well-known tool for investigating the three-dimensional structures of biological macromolecules is the RCSB PDB (Research Collaboratory for Structural Bioinformatics Protein Data Bank). It gives users access to a sizable library of complicated biomolecular structures, that have been determined experimentally [62].
Here are some key features and functionalities of RCSB PDB:

1. Structure Database: The RCSB PDB is a central location for storing and exchanging the three-dimensional structures of biological macromolecules that have been determined via experimentation. It includes several proteins, nucleic acid, and complex structures that have been identified using analytical high-throughput methods.
2. Structure Search: The RCSB PDB provides a number of search tools to browse the database. Users can do searches utilizing sophisticated search criteria, protein or nucleotide

sequences, keywords, authors, and PDB IDs. According to their interests or study requirements, this enables researchers to locate certain structures or subsets of structures.

3. Structure Visualization: Powerful visualization tools are available in the RCSB PDB to visualize and examine protein structures. Structures can be seen by users in a variety of forms, including ribbon, wireframe, and surface models. The viewer enables interactive exploration, zooming, rotation, and close examination of particular areas of the building.
4. Structure Analysis: A variety of tools are available in the RCSB PDB for examining and comprehending protein structures. Secondary structure components, binding sites, ligand interactions, and protein-ligand interactions are among the attributes that users may compute and visualize. Additionally, it offers resources for comparing and superimposing various architectures.
5. Functional Annotations: Functional annotations and details on the biological context of the structures are included in the RCSB PDB. Users get access to data on articles relating to protein functions, ligands, ligand-binding sites, and protein-protein interactions. This aids in comprehending the biological implications and functional significance of the structures.

Researchers working in structural biology, bioinformatics, and drug development can benefit greatly from the RCSB PDB. It offers a greater comprehension of the roles, relationships, and structural features of protein and nucleic acid structures through exploration, analysis, and visualization

3.1.14 AutoDock Vina

A well-known and extensively used molecular docking program called AutoDock Vina is used to forecast the affinities and binding patterns of small molecules (ligands) to protein targets. It performs flexible docking simulations by combining evolutionary algorithms and empirical scoring methods [63].

Here are the key features and functionalities of AutoDock Vina:

1. Ligand-Protein Docking: Through the prediction of the binding affinities and poses of small molecules to protein targets, AutoDock Vina executes molecular docking

simulations. It predicts the most advantageous binding orientations and energies by exploring the conformational space of both the protein and the ligand.

2. Protein Preparation: Tools for getting the protein target ready for docking simulations are provided by AutoDock Vina. It entails operations like producing rotatable bonds and torsional information, assigning atom kinds and charges, and inserting missing hydrogen atoms. By designing flexible side chains or flexible sections of the protein, protein flexibility may also be introduced.
3. Ligand Preparation: By creating 3D conformations, inserting missing hydrogen atoms, and giving partial charges, the programme aids in the production of ligands. It can work with ligands in a variety of formats, including SDF, PDB, and MOL2.
4. Genetic Algorithm-Based Docking: The ligand-protein interactions of AutoDock Vina are enhanced by exploring the conformational space using a genetic algorithm-based search technique. It quickly and effectively searches for the optimum binding poses while taking the required protein and ligand flexibility into account.
5. Scoring and Ranking: To assess the binding affinity of various ligand poses, AutoDock Vina uses an empirical scoring algorithm. For identifying the most advantageous binding modes, it ranks the postures according to the projected binding energy.
6. Visualization and Analysis: Tools for visualizing and analyzing the docking results are provided by AutoDock Vina. Users may examine protein-ligand interactions, visualize projected binding poses, and extract crucial structural data for additional research.

   Virtual screening studies and computer-aided drug discovery frequently employ AutoDock Vina. It helps in predicting ligand binding affinities, understanding how small molecules bind to protein targets, and designing new therapeutic candidates.

3.1.15 Open Babel GUI

The Open Babel programme, a potent and adaptable toolkit for chemical informatics, computational chemistry, and molecular modeling, has a graphical user interface (GUI) called Open Babel. The Open Babel GUI offers a simple user interface for navigating and using Open Babel's features [64].

Here are the key features and functionalities of Open Babel GUI:

1. Chemical File Conversion: Chemical file formats may be converted by using the Open Babel GUI. It supports a broad variety of formats, including chemical markup languages like SMILES and InChI, molecular file formats like PDB, SDF, XYZ, and MOL2, and several additional formats used in molecular modeling and cheminformatics.
2. Structure Editing and Visualization: The GUI offers editing and visualizing tools for chemical structures. Using tools like bond formation and deletion, atom and bond editing, and 3D structure visualization, users may construct, change, and control molecular structures. Users may now construct and alter molecular models as necessary.
3. Molecular Descriptor Calculation: Users may compute numerous chemical descriptors and attributes using the Open Babel GUI. To compute descriptors like molecular weight, LogP, hydrogen bonding, topological indices, and many more, it offers a variety of built-in methods. The chemical and physical characteristics of molecules may be examined and described using these descriptors.
4. 2D and 3D Visualization: The GUI provides interactive and scalable 2D and 3D visualization of molecular structures. Users may apply numerous visual styles (coloring, shading, rendering) and examine molecules in various formats (stick, ball-and-stick, space-filling). Additionally, it enables interactive chemical structure study through panning, zooming, and rotation.
5. Database Integration: Integration with various tools and chemical databases is supported through the Open Babel GUI. Users may import and export data to and from public databases like PubChem and ChEMBL as well as search and retrieve chemical information from these databases. Through scripting or file interchange, it also offers smooth interaction with other computational chemistry or cheminformatics applications.
6. Scripting and Automation: Using the scripting features of the Open Babel GUI, users may automate routine processes or design unique workflows. Python and Tcl/Tk are only a couple of the programming languages it supports for scripting. Advanced users can expand Open Babel's capability and carry out intricate analysis or simulations thanks to this feature.

3.1.16 BIOVIA Discovery Studio

An extensive set of software tools and programmes called BIOVIA Discovery Studio (formerly known as Accelrys Discovery Studio) are created for drug discovery, molecular modeling, and computational chemistry. To help research and development in the life sciences sector, it offers a variety of cutting-edge functions and processes [65].

Here are some key features and functionalities of BIOVIA Discovery Studio:

1. Structure-based Drug Design: Users of the structure-based drug design tools provided by Discovery Studio may study protein-ligand interactions, carry out virtual screening, and forecast binding affinities. It has features including protein-ligand interaction analysis, homology modeling, pharmacophore modeling, and molecular docking.
2. Ligand-based Drug Design: Users of the programme may analyze and model ligand structures, carry out ligand similarity searches, and produce prediction models. The software offers tools for ligand-based drug discovery. It comprises methods like ligand-based virtual screening, 3D-QSAR, and quantitative structure-activity relationships (QSAR).
3. Molecular Dynamics Simulations: Users of Discovery Studio may examine the dynamic behavior of biomolecules, protein-ligand complexes, and lipid bilayers using molecular dynamics simulations supported by the software. Tools for system setup, simulation setup, trajectory analysis, and simulation result visualization are all included.
4. Pharmacophore Modeling: A series of active substances may be analyzed using the software's pharmacophore modeling capabilities to discover common chemical traits and patterns. Users may create pharmacophore models, search databases for possible matches, and rank substances according to how well they fit the pharmacophore.
5. Data Analysis and Visualization: Numerous tools for data analysis and visualization are available through Discovery Studio. Users may examine and view molecular structures, interactions between proteins and ligands, chemical characteristics, and other pertinent data. It has capability for data mining and has features like scatter plots, histograms, 2D and 3D visualization.
6. Workflow Automation and Integration: By designing unique protocols and procedures, the programme enables users to automate and simplify their activities. It facilitates the

integration of many Discovery Studio tools and modules as well as third-party tools and databases, enabling smooth data transmission and analysis.

Researchers and scientists in the pharmaceutical and biotechnology companies, academic institutions, and research organizations frequently utilize BIOVIA Discovery Studio. Users may speed up their research, improve drug candidates, and get insights into molecular interactions and characteristics thanks to the environment's robust and integrated capabilities for drug discovery, molecular modeling, and computational chemistry.

3.2 METHODOLOGY

3.2.1 Bioactive identification

The phytonutrients found in *T. terrestris'* fruits were discovered using data from the IMPPAT database [45]. For additional research, the IMPPAT IDs for these phytonutrients were noted. The features of ADME were examined in all the phytonutrients.

3.2.2 Potential gene target prediction

The Canonical SMILES of the phytonutrients contained in *T. terrestris* were obtained using the PUBCHEM database [66]. Then, an online application called Swiss Target Prediction was utilized to forecast the precise proteins or molecules with which these phytonutrients may interact [53].

3.2.3 Identification of differentially expressed genes (DEGs) of PAAD

From the GEPIA2 online tool, a list of DEGs for PAAD was retrieved [54] .

3.2.4 Identifying of intersection gene targets

The common gene targets of the active phytonutrients in *T. terrestris* and the gene targets linked to PAAD were examined for using the online Venny 2.1 tool. The genes that overlapped in both sets were considered as potential anti-PAAD gene targets.

3.2.5 Protein-protein interaction analysis

To undertake a protein-protein interaction study, the possible anti-PAAD gene targets were submitted to the STRING database. Tab-separated values (tsv) files containing the PPI analysis results from STRING were then imported into the Cytoscape programme to investigate possible anti-PAAD core targets [67].In the study, only targets unique to the *Homo sapiens* species and with a moderate confidence score > 0.4 were considered.

3.2.6 Prime targets identification using MCODE

The MCODE plug-in for Cytoscope was used to identify the essential elements of the PPI network of potential anti-PAAD main targets [68]. The MCODE analysis's settings for finding clusters throughout the whole network were degree cutoff = 2, node score cutoff = 0.2, k-core = 2, and maximum depth = 100.

3.2.7 Core targets identification using CytoHubba

The top 10 targets were filtered using "cytohubba" in Cytoscape version 3.9.1 [69]. Through the use of the four methods Degree, Maximum Neighbourhood Component (MNC), Maximal Clique Centrality (MCC), and Closeness, one may find the intersection of the goals reached. Consequently, the primary targets were found.

3.2.8 Network construction between the bioactive and the potential (prime and core) targets

Using the Cytoscape programme, a network connecting *T. terrestris*' active phytonutrients and the PAAD-related targets (prime and core) was developed [67].

3.2.9 GO and KEGG enrichment analysis

While maintaining the "Homo sapiens" species criterion, further study on potential core targets for anti-PAAD was conducted using GO functional enrichment techniques. Cellular component (CC), biological process (BP), and molecular function (MF) are the three groups of GO terms The top 30 KEGG pathways and the top 10 GO analysis data (BP, CC, and MF) were shown as an enrichment dot bubble by uploading the data to a bioinformatics platform. Statistical significance was established using the conventional hypergeometric test. The Benjamini-Hochberg approach was used to control the false discovery rate (FDR) for multiple hypothesis testing, and the adjusted $p < 0.05$ was used as the significant threshold in our research.

3.2.10 Molecular docking

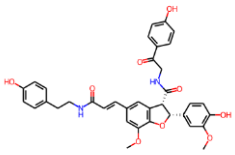
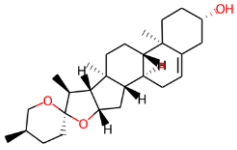
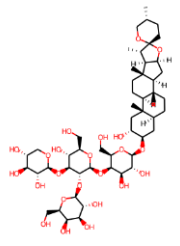
The current study links the active phytonutrients in *T. terrestris* with potential anti-PAAD core targets. The top ten probable anti-PAAD core targets' binding affinities with the top three active phytonutrients from *T. terrestris* were evaluated. All of the primary targets' crystal structures (PDB IDs: 4BQG, 5F1A, 6ESM, 7E73, 7R9V, 7OTE, 6S9X, 4OVV, and 3A4O), respectively, were obtained from the RCSB PDB in PDB format. Three of the active phytonutrients from fruit of *T. terrestris* (Diosgenin, Gitogenin, and Quercetin) were sourced from NCBI PubChem [66] . OPEN BABLE GUI was then used to convert their three-dimensional (3D) structures in PDBQT file format [70]. After uploading the PDB-formatted crystal structures of all the main targets to BIOVIA Discovery Studio Visualizer, the heteroatoms (water and other ligands) were removed and the polar hydrogens were then inserted. The resulting proteins were then loaded into AutoDock tools, stored in PDBQT format, and given the Kollman partial charges. After being loaded into AutoDock Vina [63] and tested for torsion, the formatted 3D structures of the active phytonutrients from *T. terrestris* were saved in pdbqt format. Proteins and phytonutrients that have been uploaded are chosen as ligands and macromolecules, respectively, and then stored in the PDBQT format. Then, using AutoDock Vina, a grid box was created for each protein in order to do blind docking. The molecular docking scripts were then created using the command prompt, and the obtained findings were shown as binding affinity. The BIOVIA Discovery Studio Visualizer was used to create 2D and 3D photos of the docked complexes.

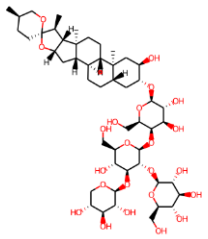
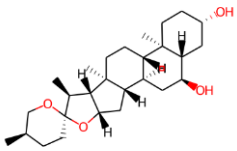
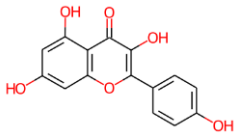
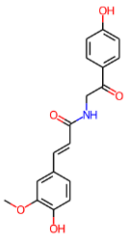
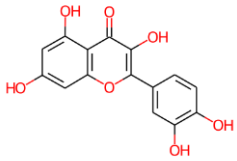
CHAPTER 4: RESULTS

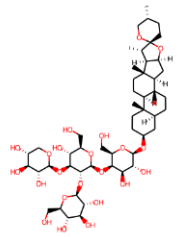
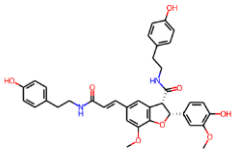
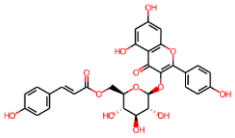
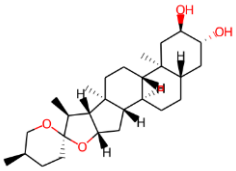
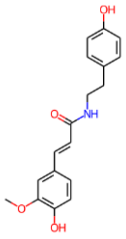
4.1 Screening of *T. terrestris's fruit* active phytonutrients.

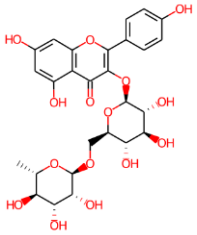
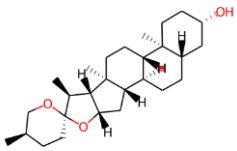
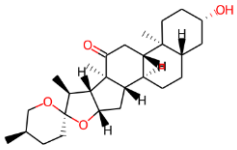
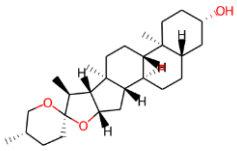
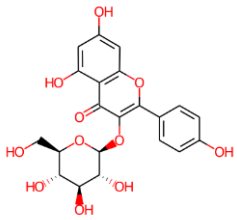
The *T. terrestris* plant produced 151 phytonutrients in total, according to the IMPPAT database. 20 of which, as stated in Table 4.1, are found in fruits. Additionally, active phytonutrient screening was done, and the fruit of *T. terrestris* contained 10 active phytonutrients based on OB of at least 50% and DL of at least 0.3.

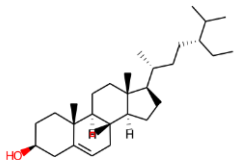
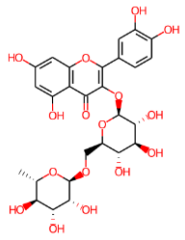
Table 4.1 The list of *T. terrestris* fruit's phytonutrients.

Phytochemical	**PubChem ID**	**IMPPAT ID**	**Oral Bioavailability**	**Drug Likeliness**	**Structure**
Tribulusamide B	10394345	IMPHY001098	0.78	0.11	
Diosgenin	99474	IMPHY003681	0.61	0.52	
Gitonin	441888	IMPHY004201	0.78	0.09	

F-Gitonin	44559009	IMPHY004202	0.78	0.09	
Chlorogenin	12303065	IMPHY004284	0.5	0.58	
Kaempferol	5280863	IMPHY004388	0.62	0.55	
Terrestriamide	5321824	IMPHY004424	0.52	0.56	
Quercetin	5280343	IMPHY004619	0.54	0.56	

Degalactotigonin	162401	IMPHY005415	0.75	0.43	
Tataramide B	5322012	IMPHY010322	0.8	0.1	
Tiliroside	5320686	IMPHY010580	0.78	0.14	
Gitogenin	441887	IMPHY011682	0.58	0.12	
Moupinamide	5280537	IMPHY011879	0.67	0.58	

Nicotiflorin	5318767	IMPHY011985	0.75	0.72	
Tigogenin	99516	IMPHY012273	0.54	0.16	
Hecogenin	91453	IMPHY012275	0.64	0.54	
Neotigogenin	12304433	IMPHY014759	0.54	0.59	
Astragalin	5282102	IMPHY014824	0.77	0.28	

Beta-sitosterol	222284	IMPHY014836	0.52	0.44	
Rutin	5280805	IMPHY015047	0.74	0.14	

4.2 Potential gene targets of *T. terrestris's* active phytonutrients

364 potential gene targets were found using the Swiss Target Prediction online database with a probability of "< 0". The 10 active phytonutrients were the main consideration while selecting these gene targets.

4.3 PAAD-related gene target

Using the Gepia2 online tool, 9290 gene targets associated with PAAD were found. In order to discriminate between over- and under-expressed genes on chromosomes with a q-value threshold of 0.01 and a log2FC cutoff of 1, these gene targets were discovered using the ANOVA differential approach.

4.4 Intersection gene targets analysis

According to Fig 4.1, VENNY 2.1 identified 180 gene targets that were shared by the 364 putative gene targets for the active phytonutrients in *T. terrestris*' fruit as well as the 9209 gene targets connected to PAAD. Candidates for anti-PAAD gene targets included these 180 gene targets.

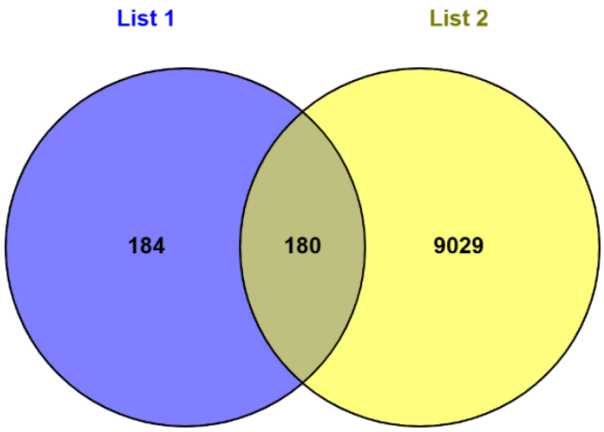
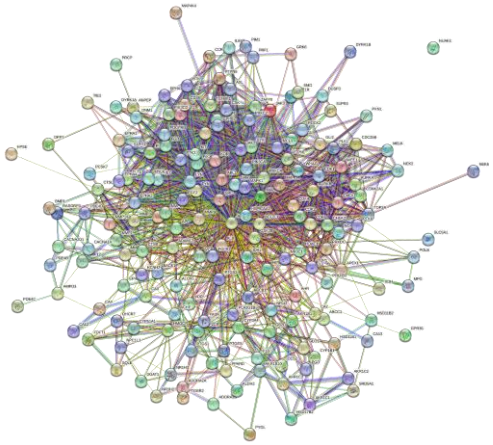


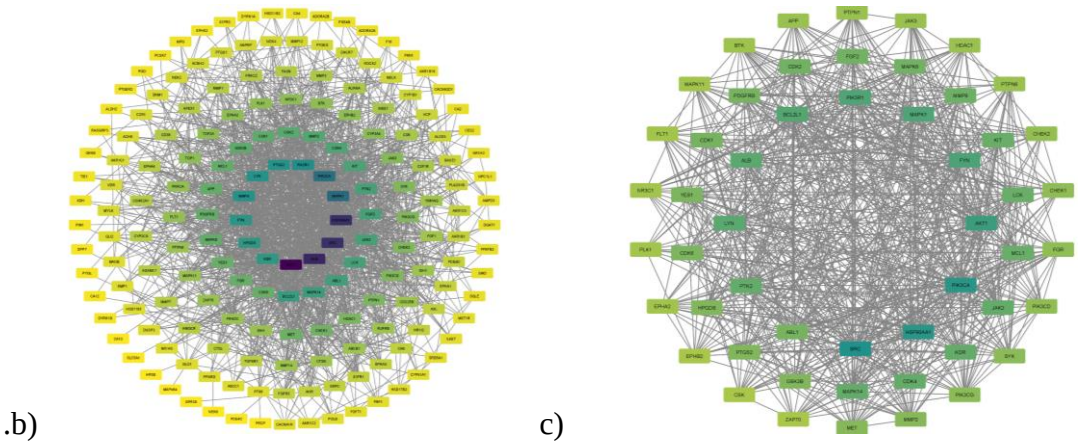
Fig 4.1 Venn diagram showing the commonness between predicted targets and DEGs.

4.5 PPI network analysis

A protein-protein interaction (PPI) network was examined using STRING, and it was discovered to have 180 nodes and 1546 edges, as shown in Fig 4.2 (a). The average node degree is 17.2 and the average local clustering coefficient is 0.534. There are 615 projected edges with an average PPI enrichment p-value of less than < 1.0e-16. The PPI network, however, has 179 nodes as seen in Fig 4.2 (b) based on Cytoscape analysis since there is 1 non-interacting node in the PPI network with 1546 edges and a typical route length of 2.206 between every pair of nodes. The network's density, diameter, average number of neighbors, clustering coefficient, and radius are all given as the following numerical values: 0.049, 7, 17.274, 0.246, and 1 accordingly.



a)



.b) c)

Fig 4.2 (a) STRING PPI interaction network. (b) PPI interaction network of 180 potential anti-PAAD prime targets and (c) 52 potential anti-PAAD core targets. The hue shifts from yellow to purple as the degree of each node increases.

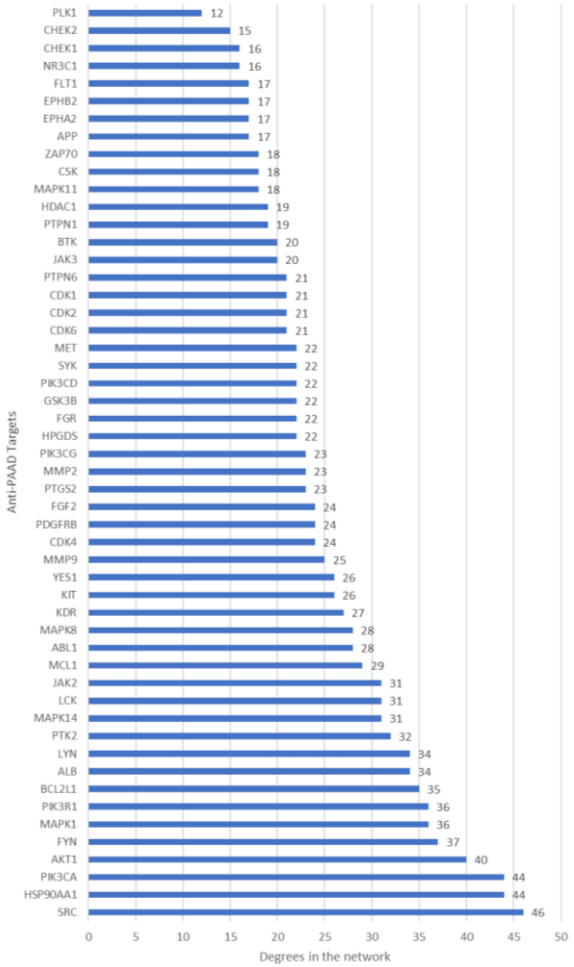


Fig 4.3 The 52 potential anti-PAAD core targets with their degree values.

52 nodes from the network were selected as the key anti-PAAD targets using the degree centrality (DC) ≥ average value (17.274) criterion, as shown in Fig 4.2 (c). These 52 nodes, which stand in for the key anti-PAAD gene targets, are sorted by DC and shown on a bar graph in Fig 4.3.

4.6 Cluster network analysis

The network of 179 potential targets that may be exploited to attack PAAD was examined using Cytoscape's MCODE plugin to identify clusters. According to the research, the PPI network of anti-PAAD prime targets had three cluster networks, as illustrated in Fig. 4.4.

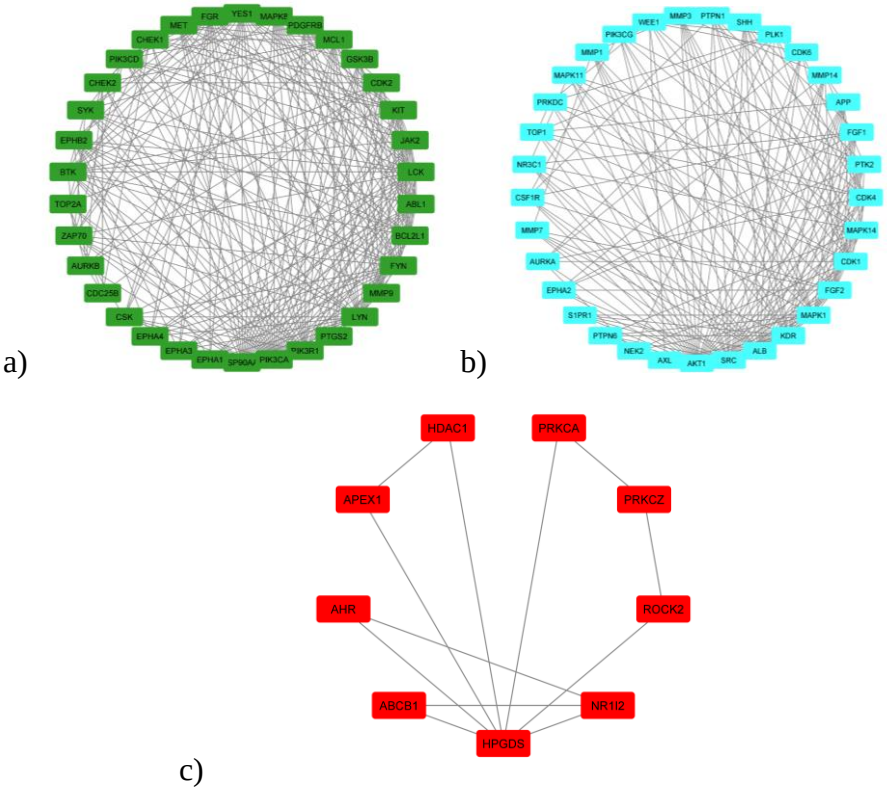


Fig 4.4 Network cluster analysis. (a) Cluster network 1, (b) Cluster network 2 and c) Cluster network 3.

The first cluster network in Fig. 4.4 (a) has 34 nodes and 250 edges, scoring 15.152. It displays several genes with several gene targets with a DC value ≥ 14.706, including FGR, YES1, MCL1, KIT, LCK, JAK2, ABL1, BCL2L1, FYN, LYN, PIK3R1, PIK3CA,

HSP90AA1, and BTK. With 33 nodes and 175 edges, the second cluster network, shown in Fig. 4.4 (b), scored 10.938. It demonstrates that a network with a DC value ≥ 10.606 has substantial interconnectivity between CDK1, CDK4, PTK2, FGF2, MAPK14, KDR, MAPK1, SRC, ALB, and AKT1. The third cluster network, represented by Fig. 4.4 (c), scored 3.000 and comprises 9 nodes and 12 edges. It demonstrates the strong connections between NR1/2 and HPGDS in a network with a DC value ≥ 2.667.

4.7 Core targets screening

The "cytohubba" software plug-in in Cytoscape 3.9.1 was used to identify the core targets, as shown in Fig. 4.5, using four different approaches: Degree, Maximum Neighbour Component (MNC), Maximal Clique Centrality (MCC), and Closeness. The top 10 core targets were filtered using each of these methods.

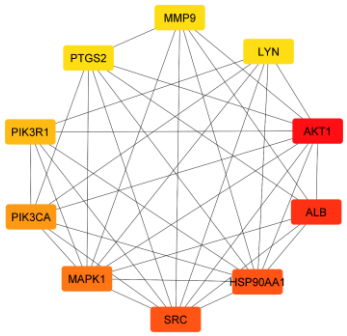
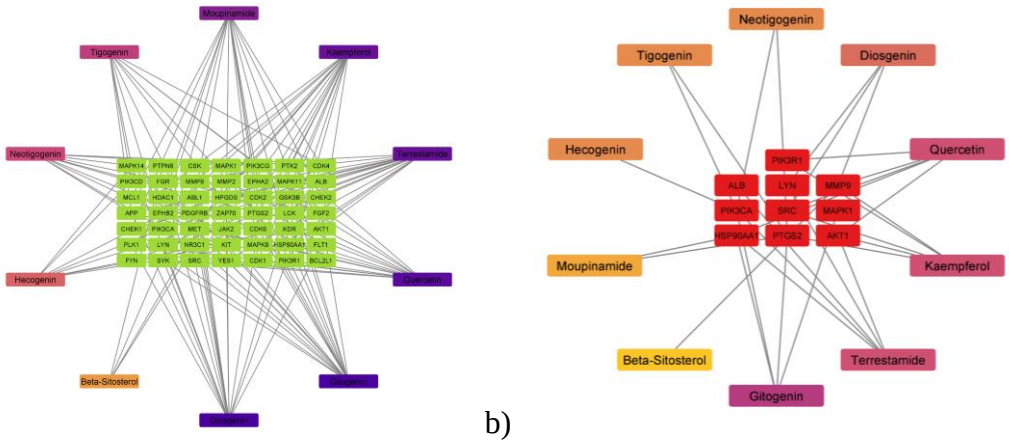


Fig 4.5 Hub genes prediction using CytoHubba

4.8 Network of *Tribulus terrestris* fruit's active phytonutrients and anti-PAAD targets

The association between the active phytonutrients present in *T. terrestris* and 49 key anti-PAAD targets is shown in Fig. 4.6 (a). There are 59 nodes and 138 edges in the network. Its radius and diameter are both 1, correspondingly. The network has a 0.039 density. The typical path length is 1.000 and the average number of neighbors is 4.542. 0.000 is the clustering coefficient. The edges in the network show how the active phytonutrients in *T. terrestris* interact with potential anti-PAAD targets. The degree of a node in a network describes how many edges link it to other nodes. Nodes get increasingly purple as their degree increases. The connection between the 10 primary anti-PAAD targets and the active phytonutrients present in *T. terrestris* is shown in Fig. 4.6 (b). There are 37 edges and 23 nodes in the network. Its radius and diameter are both 1, correspondingly. The network has

a 0.073 density. The typical path length is 1.000 and the average number of neighbors is 3.217. 0.000 is the clustering coefficient. The edges in the network show how the active phytonutrients in *T. terrestris* interact with potential anti-PAAD targets. The top 10 active phytonutrients found in *T. terrestris* fruit are shown in Fig 4.7, sorted according to their degree values in the hub network.



a) b)

Fig 4.6 (a) Network of 49 putative anti-PAAD primary targets and active phytonutrients of *T. terrestris* fruit. (b) The hub network of 10 potential anti-PAAD core targets and active phytonutrients of *T. terrestris* fruits. The gradient shift from yellow to purple as the node's degree increases.

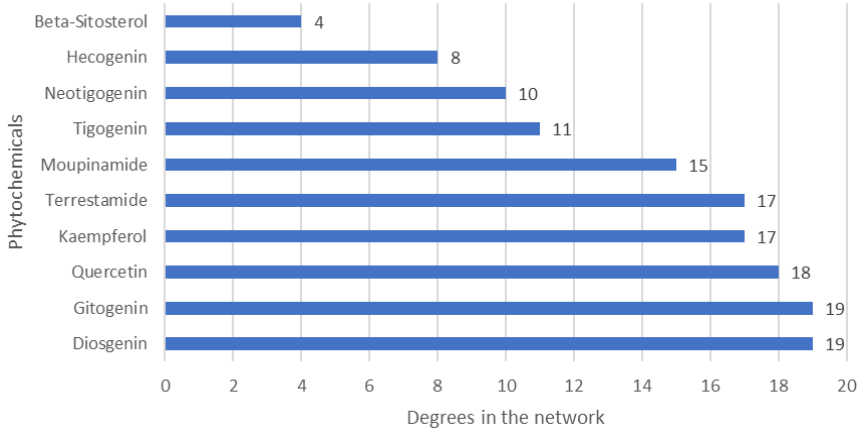
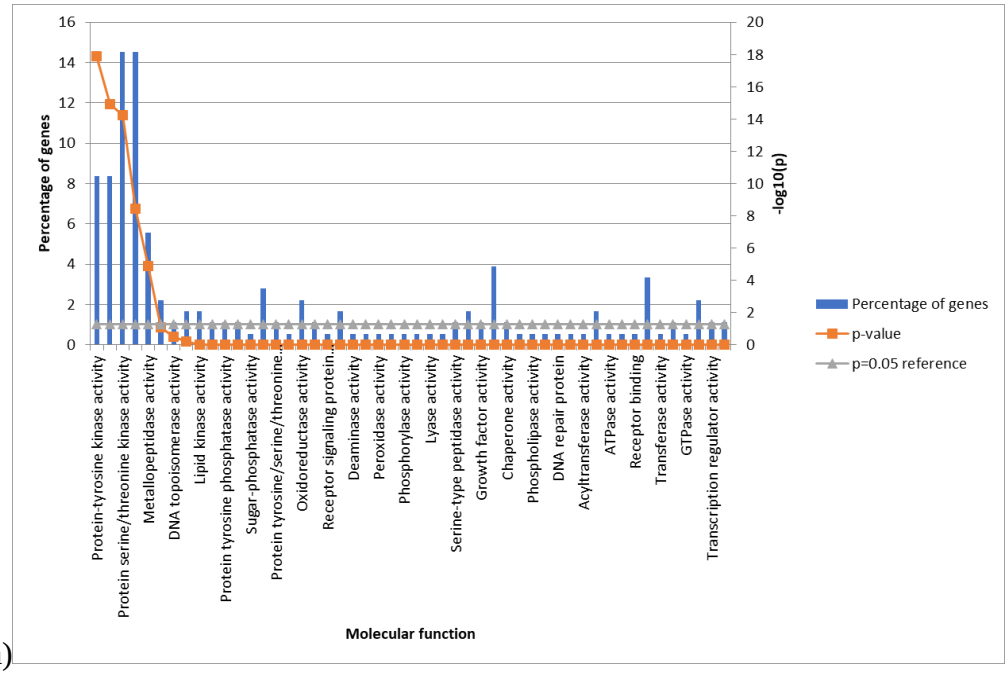


Fig 4.7 The hub network's top 10 *T. terrestris* fruit's most possible active phytochemicals with their degree values.

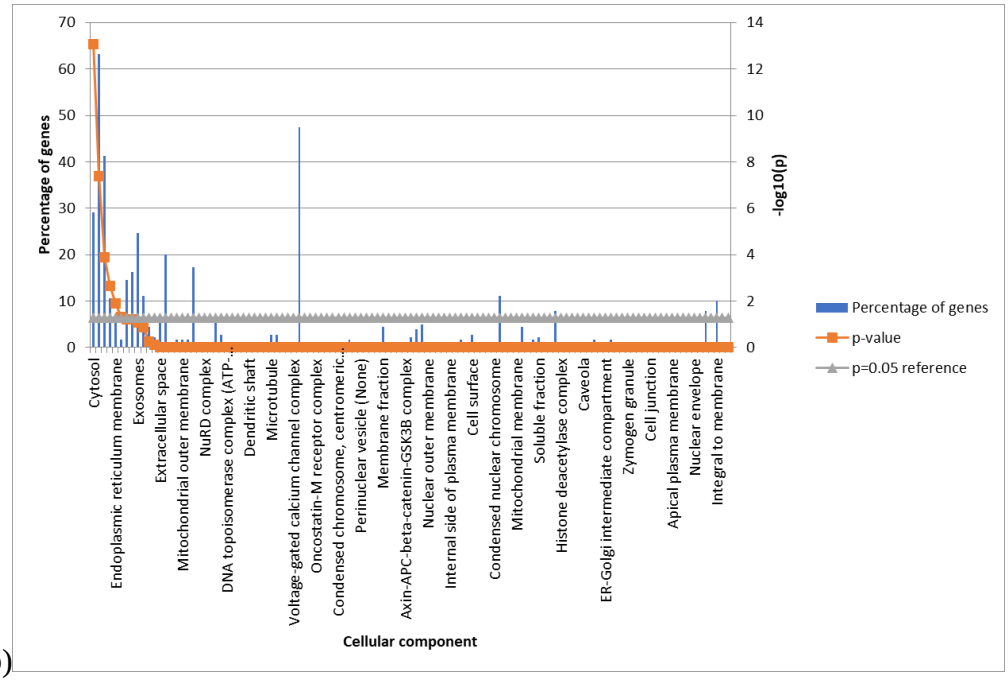
4.9 GO enrichment analysis

The top 10 enrichment words for BP, MF, and CC are presented in Fig 4.8 after further examining the 180 possible anti-PAAD targets for GO enrichment analysis using FUNRich software. The results of the investigation demonstrated the involvement of the gene targets in a number of biological processes, including signal transduction, cell-to-cell communication, metabolism, transport, etc. Gene targets were mostly discovered in the cytosol, exosomes, endoplasmic reticulum membrane, mitochondrial outer membrane, etc. in cellular components. Additionally, the enhanced molecular functions were heavily implicated in processes including protein tyrosine kinase, DNA topoisomerase, metallopeptidase, and protein serine/threonine kinase activities, etc.

a)



b)



c)

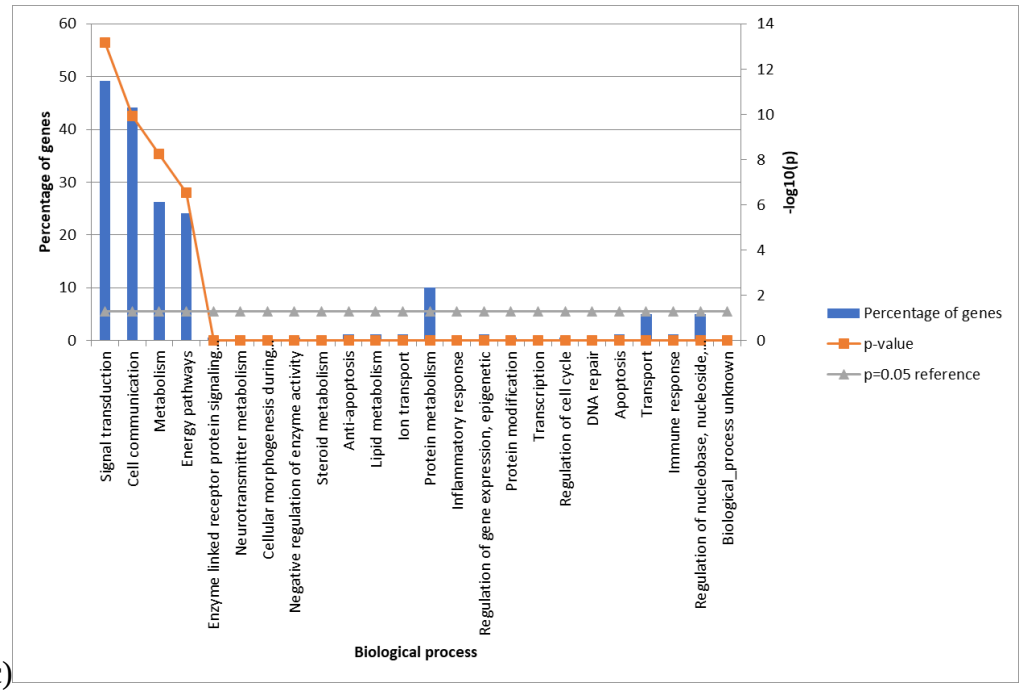


Fig 4.8 Analysis of 179 putative anti-PAAD prime targets using GO enrichment. the MF, CC, and BP are depicted on the X-axis, while the number of gene targets is displayed on the Y-axis.

4.10 KEGG analysis

To ascertain how *T. terrestris* may aid in reducing PAAD, KEGG pathway analysis was carried out. The top 30 KEGG pathways most related to the study with p 0.05 were found when we submitted 10 possible anti-PAAD targets to the ShinnyGo platform. The findings suggest that *T. terrestris* may be able to target pathways that are connected to processes like the VEGF signaling route, the TNF signaling system, the estrogen signaling pathway, etc. These pathways could be crucial to the molecular process by which *T. terrestris* treats PAAD.

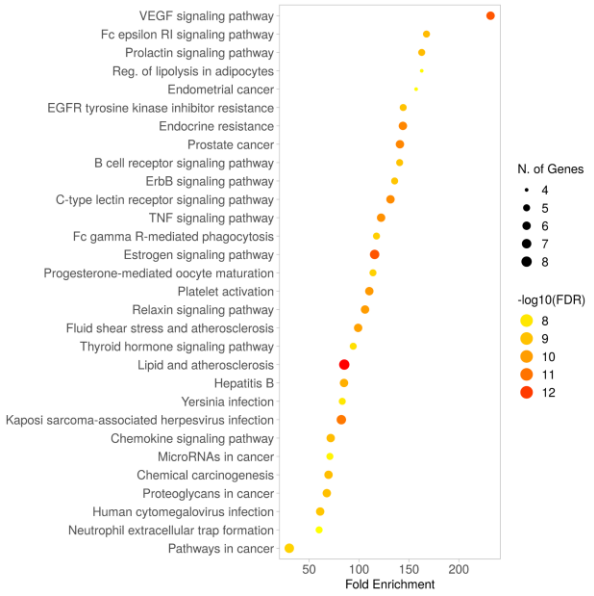


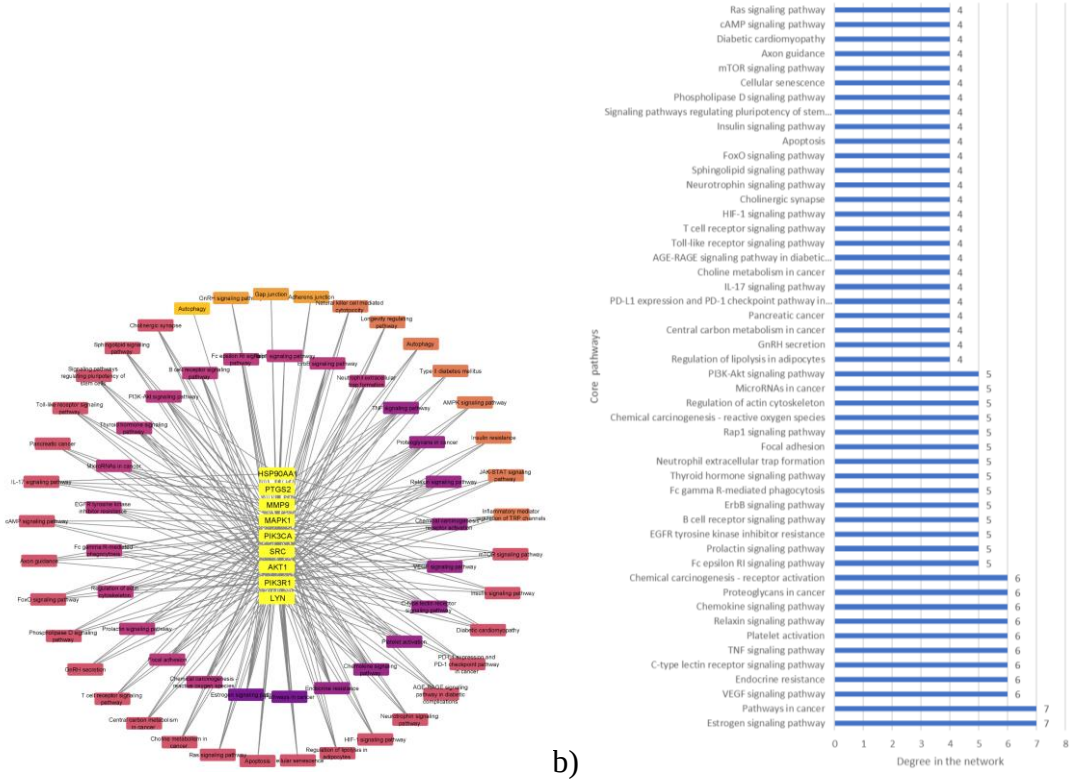
Fig 4.9 Top 30 KEGG pathways. Fold enrichment is indicated on the X -axis, numerous KEGG pathways are shown on the Y-axis, and the number of genes participating in each pathway is indicated by the size of the bubble.

4.11 Core pathways determination

The top 62 KEGG pathways and the top 9 anti-PAAD core targets were combined to create a network, which was then used to study the core pathways contributing to the anti-PAAD effects of the main active phytonutrients found in *T. terrestris* fruit (Fig. 4.10 (a)). The results show that the network contains 269 edges and 71 nodes. Additionally, the network's radius, density, and diameter were each 1, 1, and 0.054, respectively. According to the network's results, nine anti-PAAD core targets are interacted with by each of the sixty-two

routes in a distinct way. As the degree rises, the node's color shifts from yellow to purple (Fig. 4.10(a)).

In addition, the network's paths were sorted using the DC average value of (7.577), and fifty core pathways were found, as shown in Fig. 4.10 (b). The pathways used in cancer were followed by seven of the nine anti-PAAD core targets, while the VEGF signaling route was followed by the estrogen signaling pathway. These fifty basic pathways may thus have a role in the anti-PAAD actions of the main active phytonutrients in *T. terrestris* fruit.



a) b)

Fig 4.10. (a) Network of anti-PAAD core targets and pathways. The gradient shifts from yellow to purple with an increasing degree of a pathway. (b) 50 core pathways with associated degree values.

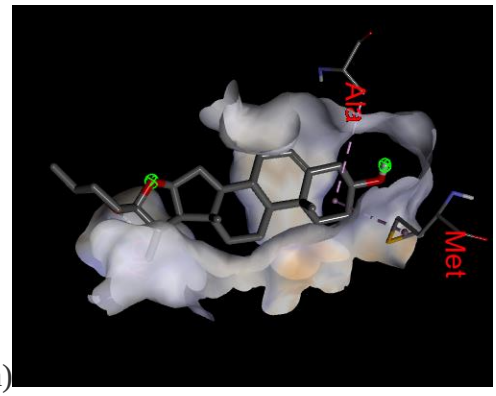
4.12 Molecular docking investigations

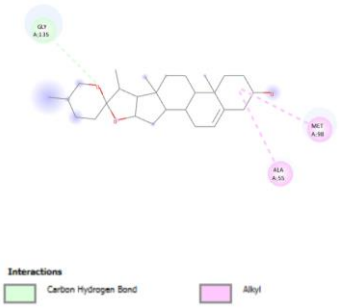
The top nine anti-PAAD core targets were molecularly docked with the top three active phytonutrients from *T. terrestris*, including HSP90AA1, PTGS2, MMP9, MAPK1, PIK3CA, SRC, AKT1, PIK3R1, LYN. As seen in Fig 4.11, the outcomes of the molecular

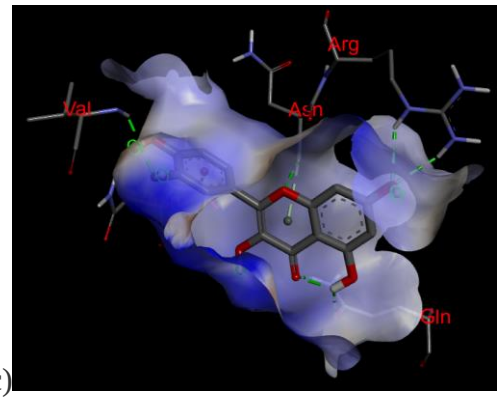
docking study in terms of binding affinity (kcal/mol) scores are displayed as a heatmap. Additionally, Fig 4.12 depicts their docked complexes in 2D and 3D formats. The higher binding capacity of the phytonutrients and targets is shown by the decreased binding affinity. The results demonstrated that all three of the main active phytonutrients in *T. terrestris* fruit had good to exceptional binding affinity scores with all anti-PAAD core targets, ranging from -6.4 to -11.5 kcal/mol, respectively. Gitogenin demonstrated excellent binding affinity scores with AKT1, LYN, MMP9, and PIK3CA (-11.5, -9.4, -9.2 and -9.0 kcal/mol, respectively). Diosgenin, on the other hand, showed superior binding affinity scores (-10, -9.4, -9.3 and -8.9 kcal/mol, respectively) for LYN, PIK3CA, MMP9, and SRC. PTGS2 and PIK3R1 showed the same binding affinities with diosgenin i.e. -8.7 kcal/mol. Despite having lower binding affinity scores than gitogenin and diosgenin, quercetin has shown high binding affinity against AKT1, PTGS2, MMP9 and PIK3CA (-9.7, -9.5, -9.0 and -8.9 respectively). Additionally, quercetin demonstrated favourable binding affinity scores against HSP90AA1, MAPK1, SRC, PIK3R1 and LYN of <-6.4 kcal/mol. Additionally, the binding affinity scores for diosgenin and gitogenin against SRC (-8.9 kcal/mol) and PTGS2 (-8.7 kcal/mol) were similar. The results of the molecular docking study therefore supported the idea that *T. terrestris* may reduce PAAD by modifying the activities or expressions of these targets.

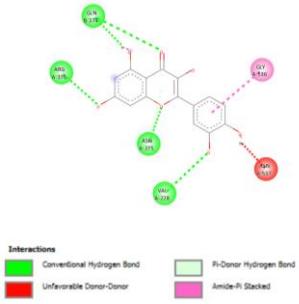
Targets	Diosgenin	Gitogenin	Quercetin
HSP90AA1	-7.7	-7.4	-6.4
PTGS2	-8.7	-8.7	-9.5
MMP9	-9.3	-9.2	-9
MAPK1	-8.4	-8.6	-7.6
PIK3CA	-9.4	-9	-8.9
SRC	-8.9	-8.9	-7.6
AKT1	-8.2	-11.5	-9.7
PIK3R1	-8.7	-8.2	-7.7
LYN	-10	-9.4	-6.5

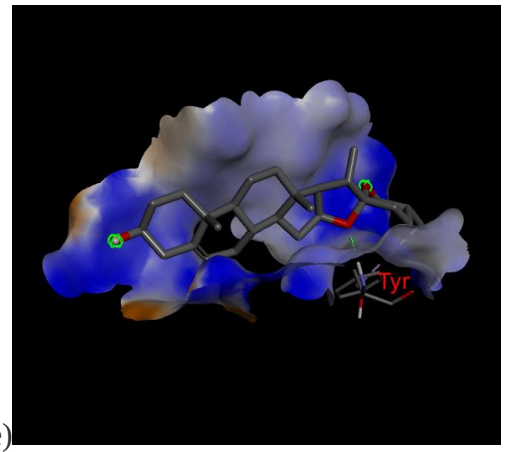
Fig 4.11 The heatmap of binding affinities (kcal/mol) for three of the most active phytonutrients found in *T. terrestris* fruit with the top nine anti-PAAD core targets. As the binding affinity scores drop, the color changes from yellow to green.

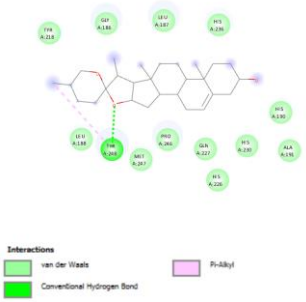
a) 
Arg
Met

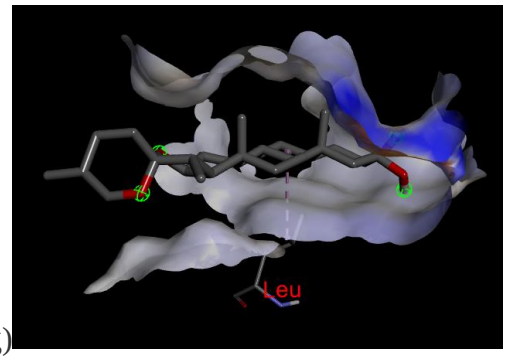
b) 
Interactions
Carbon Hydrogen Bond
Alkyl

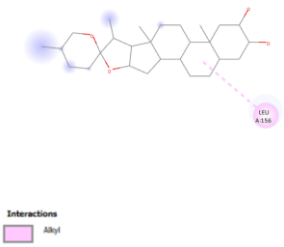
c) 
Arg
Val
Asn
Gln

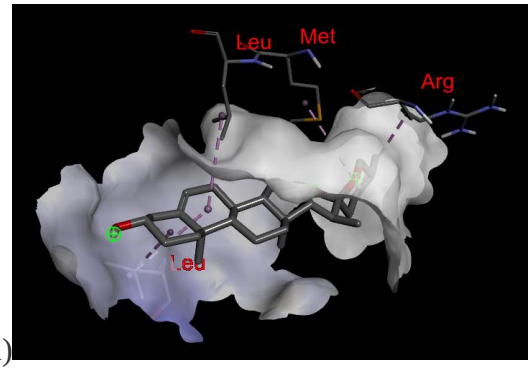
d) 
Interactions
Conventional Hydrogen Bond
Pi-Donor Hydrogen Bond
Unfavorable Donor-Donor
Amide-Pi Stacked

e) 
Tyr

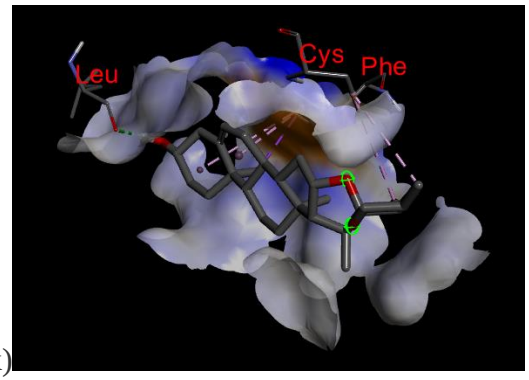
f) 
Interactions
van der Waals
Pi-Alkyl
Conventional Hydrogen Bond

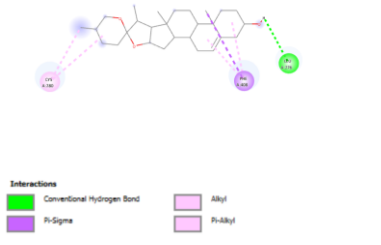
g) 
Leu

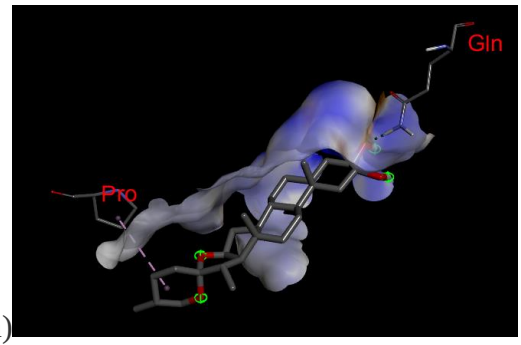
h) 
Interactions
Alkyl

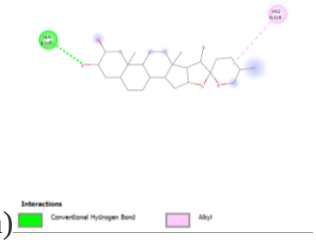
i) 
Leu
Met
Arg
Leu

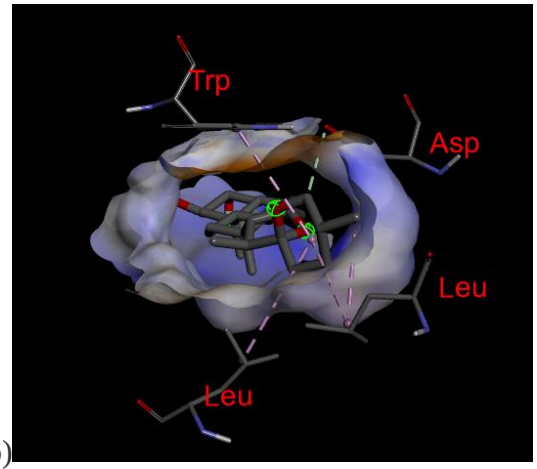
j) 
Interactions
Alkyl

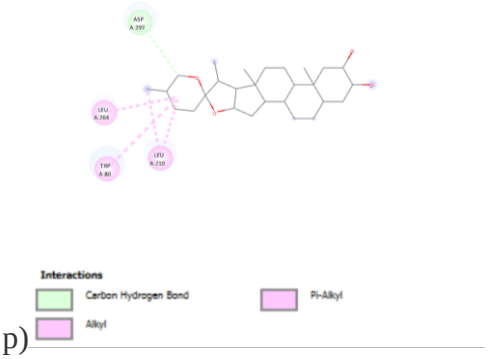
k) 
Leu
Cys
Phe

l) 
Interactions
Conventional Hydrogen Bond
Alkyl
Pi-Sigma
Pi-Alkyl

m) 
Gln
Pro

n) 
Interactions
Conventional Hydrogen Bond
Alkyl

o) 
Trp
Asp
Leu
Leu

p) 
Interactions
Carbon Hydrogen Bond
Pi-Alkyl
Alkyl

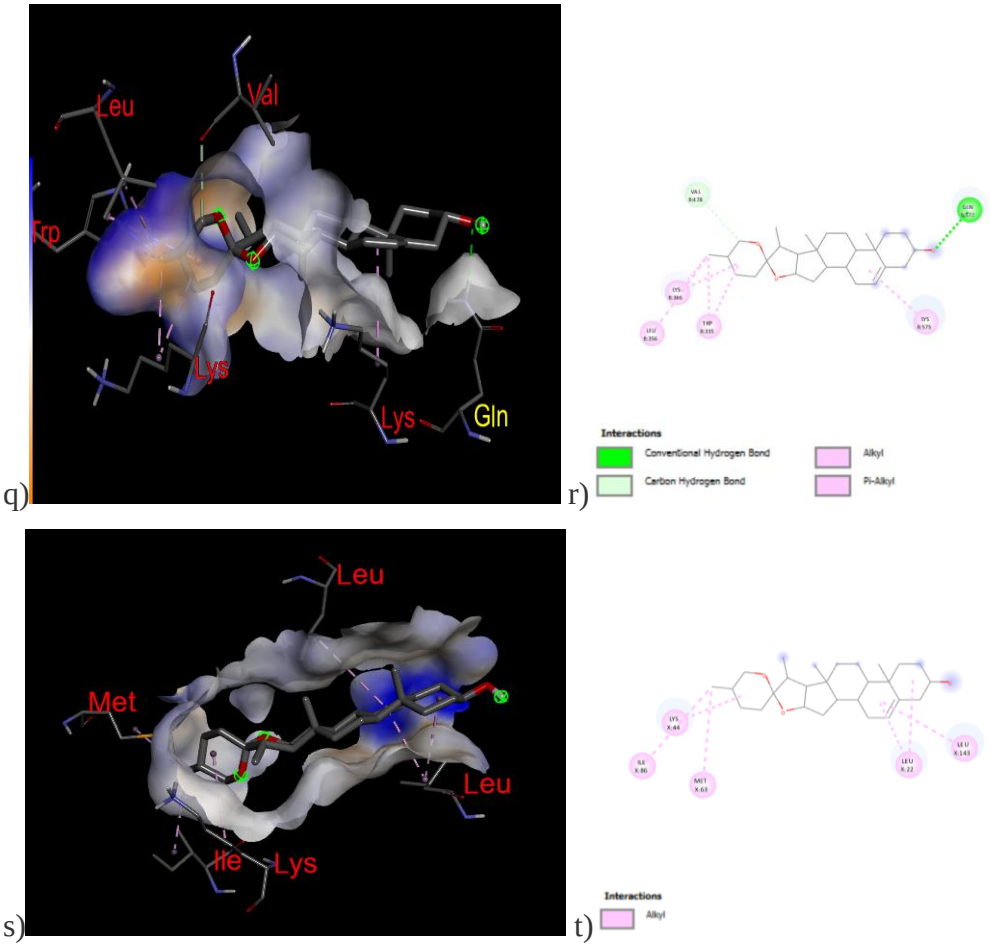


Fig 4.12 2D & 3D Molecular docking results. Diosgenin binds to (a, b) HSP90AA1, (e, f) MMP9, (i, j) PIK3CA, (k, l) SRC, (q, r) PIK3R1 and (s, t) LYN. Gitonin binds to the (g, h) MAPK1, (m, n) SRC and (o, p) AKT1. Binding of quercetin with (c, d) PTG.

DISCUSSION

In order to examine the potential of *T. terrestris* fruit, a medicinal plant, in the treatment of pancreatic adenocarcinoma (PAAD), the current study used a network pharmacological method. The study aims to discover the potential targets and pathways impacted by *T. terrestris* and understand its mechanisms of action in pancreatic cancer by integrating bioinformatics, computational biology, and pharmacological investigation. Ten phytonutrients in all were shown to be active phytonutrients in *T. terrestris* based on OB 50% and DL 0.3, as shown in Table 4.1. The network findings showed that several anti-PAAD core targets and numerous important active phytonutrients in the *T. terrestris* fruit worked together synergistically to reduce PAAD pathology.

Four distinct strategies were used in the CytoHubba study to reduce the targets to the core set. Based on degree, maximal clique centrality, maximum neighborhood component, and proximity, the top 10 core targets were determined. These core targets reflect the essential genes that may be extremely important in *T. terrestris*' anti-PAAD activities. In order to better understand their interactions, the network of active phytonutrients in *T. terrestris* and anti-PAAD targets was examined. The key anti-PAAD targets and the active phytonutrients may interact, according to the network study.

The selected gene targets' biological activities, molecular processes, and cellular components were revealed using GO enrichment analysis. According to the study, the gene targets were important for a number of biological functions, especially those involving signal transduction, cell-to-cell communication, metabolism, and transport. Cytosol, exosomes, the endoplasmic reticulum membrane, and the mitochondrial outer membrane were the main cellular components. Metallopeptidase, DNA topoisomerase, protein tyrosine kinase, and protein serine/threonine kinase activities were among the enriched molecular functions. Furthermore, KEGG pathway analysis found a number of pathways that could be pertinent to *T. terrestris*' molecular mode of action in treating PAAD. The top pathways linked to possible anti-PAAD targets were the VEGF signaling route, the TNF signaling pathway, and the estrogen signaling system.

The binding affinity of the active phytonutrients from *T. terrestris* with the main anti-PAAD targets was evaluated by molecular docking studies. The findings supported the theory that *T. terrestris* may exert its anti-PAAD effects by modulating the activities or expression of the targets (HSP90AA1, PTGS2, MMP9, MAPK1, PIK3CA, SRC, AKT1, PIK3R1, and LYN), showing favorable to excellent binding affinities between the active phytonutrients and the targets.

A molecular chaperone involved in the folding and stabilization of proteins is known as HSP90AA1 (Heat shock protein 90 alpha family class A member 1). HSP90AA1 has been discovered to interact with a number of oncogenic proteins, such as mutant KRAS, EGFR, and AKT [71]. Prostaglandin-endoperoxide synthase 2 (PTGS2), sometimes referred to as cyclooxygenase-2 (COX-2), is an enzyme that catalyzes the formation of prostaglandins from arachidonic acid. Tumor development, angiogenesis, and inflammation are all linked to increased PTGS2 expression [72]. A member of the family of matrix metalloproteinases, MMP9 is involved in remodeling the extracellular matrix. Overexpression of MMP9 promotes tumor invasion, metastasis, and angiogenesis [73]. MMP9 breaks down extracellular matrix elements, promoting tumor cell invasion and migration [74]. A component of the mitogen-activated protein kinase (MAPK) signaling cascade, MAPK1 (Mitogen-activated protein kinase 1) is sometimes referred to as ERK2. In cancer, activation of MAPK1 is commonly seen, and it is linked to tumor development, metastasis, and treatment resistance. Phosphatidylinositol 3-kinase (PI3K), a crucial regulator of the PI3K/AKT/mTOR signaling pathway, is encoded by PIK3CA (Phosphatidylinositol-4,5-bisphosphate 3-kinase Catalytic Subunit Alpha). The PI3K pathway is activated by PIK3CA mutations, which are frequent and improve cell survival, proliferation, and invasion. In cancer, PIK3CA mutations are linked to a poor prognosis. Non-receptor tyrosine kinase SRC, also known as the SRC proto-oncogene, participates in signal transduction pathways. In cancer, SRC is increased and activated, which promotes the growth and spread of tumors [75]. AKT1 (AKT serine/threonine kinase 1), commonly referred to as protein kinase B alpha, is a PI3K pathway downstream effector. Through genetic changes or pathway dysregulation, AKT1 is typically activated in cancer. The regulatory subunit of PI3K, which is important for stabilizing the catalytic subunit and

regulating its activity, is encoded by the gene PIK3R1. PAAD has been linked to PIK3R1 changes, such as mutations and deletions [76]. PIK3R1 dysregulation can result in increased PI3K/AKT signaling, which can support tumor survival and growth. The Src family of tyrosine kinases, which are important in cellular signaling, includes the LYN proto-oncogene. In cancer, LYN is overexpressed and activated, which aids in the survival, migration, and invasion of tumor cells. To encourage PAAD growth, LYN controls a number of downstream signaling pathways, including as MAPK and PI3K/AKT. The primary active phytonutrients in the *T. terrestris* fruit are thought to be responsible for the anti-PAAD actions, according to the results of recent studies.

CONCLUSIONS

The results of the study offer compelling evidence that *T. terrestris* may be useful as a treatment for pancreatic adenocarcinoma (PAAD). The study found active phytonutrients in *T. terrestris* fruit and clarified their interactions with key targets implicated in PAAD development by using a network pharmacological approach. The findings showed that *T. terrestris* significantly inhibits PAAD by modifying important molecular targets and pathways linked to the initiation and progression of cancer. This study's main finding is that *T. terrestris* may successfully inhibit the development and division of pancreatic cancer cells. *T. terrestris* promotes apoptosis and prevents the survival of cancer cells by downregulating important proteins including MMP9 and MAPK1. This shows that *T. terrestris* may be utilised as an adjuvant therapy to improve patient outcomes when combined with other pancreatic cancer therapies. The study also emphasises how crucial it is to examine natural chemicals like *T. terrestris* from a molecular standpoint while looking for non-conventional cancer therapies. Alternative remedies that are both safe and effective include natural supplements, which may help patients suffer less adverse effects. The use of a network pharmacological approach enables the development of tailored therapeutics by providing a thorough knowledge of the molecular processes behind the anti-PAAD activities of *T. terrestris*. It is essential to create suitable medication formulations and delivery mechanisms in order to effectively capitalise on *T. terrestris*' therapeutic advantages for people with pancreatic cancer. These developments may improve the pharmacokinetics and therapeutic effectiveness of *T. terrestris* drugs by increasing their bioavailability and tissue-specific targeting.

FUTURE PERSPECTIVES

On the basis of the findings of the investigation, the following future prospects can be taken into account:

1. Experimental Validation: In vitro and in vivo tests should be used to confirm the network pharmacological approach's findings. Preclinical and clinical research will show greater proof of *T. terrestris*' effectiveness and safety in treating pancreatic cancer.

2. Identification of Active compounds: Although the active phytonutrients in *T. terrestris* were found in this work, more investigation is required to identify and characterise the precise chemicals that have anti-PAAD actions. The creation of focused medicines and maybe a reduction in side effects will be made possible by better understanding of the active ingredients.

3. Combination Therapy: Due to the complexity of pancreatic adenocarcinoma, adding *T. terrestris* to other standard treatments such chemotherapy or targeted medicines may improve patient results. To create more efficient treatment regimens, synergistic effects between *T. terrestris* and current medications should be investigated.

4. Clinical studies: Well-planned clinical studies including a larger patient base will yield useful information on the effectiveness, safety, and optimum dose of *T. terrestris* for the treatment of pancreatic adenocarcinoma. Evidence-based medicine might benefit from randomised controlled studies contrasting *T. terrestris* with control groups receiving just routine care or a placebo.

5. Drug Formulation and Delivery Systems: The bioavailability and therapeutic effectiveness of *T. terrestris* compounds will be improved by the development of suitable drug formulations and delivery systems. The pharmacokinetics and tissue-specific targeting of *T. terrestris* drugs can be enhanced by using nanoparticles, liposomes, or targeted drug delivery systems.

APPENDIX: LIST OF PUBLICATIONS

- Bharmjeet, Asmita Das*. Racial disparities in cancer care, an eyeopener for developing better global cancer management strategies. Cancer Reports 2023; https://doi.org/10.1002/cnr2.1807.

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REVIEW

Cancer Reports | WILEY

Racial disparities in cancer care, an eyeopener for developing better global cancer management strategies

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Abstract

Background: In the last few decades, advancements in cancer research, both in the field of cancer diagnostics as well as treatment of the disease have been extensive and multidimensional. Increased availability of health care resources and growing awareness has resulted in the reduction of consumption of carcinogens such as tobacco; adopting various prophylactic measures; cancer testing on regular basis and improved targeted therapies have greatly reduced cancer mortality among populations, globally. However, this notable reduction in cancer mortality is discriminate and reflective of disparities between various ethnic populations and economic classes. Several factors contribute to this systemic inequity, at the level of diagnosis, cancer prognosis, therapeutics, and even point-of-care facilities.

Recent Findings: In this review, we have highlighted cancer health disparities among different populations around the globe. It encompasses social determinants such as status in society, poverty, education, diagnostic approaches including biomarkers and molecular testing, treatment as well as palliative care. Cancer treatment is an active area of constant progress and newer targeted treatments like immunotherapy, personalized treatment, and combinatorial therapies are emerging but these also show biases in their implementation in various sections of society. The involvement of populations in clinical trials and trial management is also a hotbed for racial discrimination. The immense progress in cancer management and its worldwide application needs a careful evaluation by identifying the biases in racial discrimination in healthcare facilities.

Conclusion: Our review gives a comprehensive evaluation of this global racial discrimination in cancer care and would be helpful in designing better strategies for cancer management and decreasing mortality.

KEYWORDS
cancer care, cancer health disparities, cancer management, cancer therapy, racial disparities

1 | INTRODUCTION

Despite coherent efforts that have led to a significant reduction in cancer mortality, it remains to be the second major cause of death, following cardiovascular diseases. With 215 deaths from cancer per 100000 individuals, the mortality rate peaked in 1991.[1] At the beginning of the present year, the American Cancer Society estimated a total of 1918030 collective cancer cases with 609360 deaths in

- 6th IEEE international conference on information systems and computer networks (ISCON 2023) held on 3rd - 4th march 2023 at GLA university Mathura, Anjali sinha , Bharmjeet and Asmita Das* corresponding author an insilico comparison of ginsenoside's anti cancer activity against the son of seven homolog one, https://doi.org/10.1109/ISCON57294.2023.10112037

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