Bioactivity of Herbal Extracts use as Antidiabetic and Anticancer drugs

MS Thesis

by

Rabia Nawaz

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Rabia Nawaz

CIIT/FA22-R06-016/LHR

Department of Chemistry

Faculty of Sciences

COMSATS University Islamabad

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Name	Registration Number
Rabia Nawaz	CIIT/FA22-R06-016/LHR

Supervisory Committee

Supervisor

Dr. Muhammad Javid Iqbal
Associate Professor
Department of Chemistry
COMSATS University Islamabad
(CUI) Lahore

Co-Supervisor

Dr. Muhammad Ihtisham Umar
Assistant Professor
Department of Pharmacy
COMSATS University Islamabad
(CUI) Lahore

Member

Dr. Mazhar Amjad Gilani
Associate Professor
Department of Chemistry
COMSATS University Islamabad
(CUI) Lahore

Member

Dr. Sara Riaz
Associate Professor
Department of Chemistry
COMSATS University Islamabad
(CUI) Lahore

Certificate of Approval

Bioactivity of Herbal Extract use as Antidiabetic and Anticancer drugs

This thesis titled

By

Rabia Nawaz

CIIT/FA22-R06-016/LHR

has been approved

for the degree MS Chemistry

at COMSATS University Islamabad,

Lahore Campus

External Examiner: _____

External Examiner's Name>

University Name

Supervisor: _____

Dr. Muhammad Javid Iqbal

Associate Professor

Department of Chemistry

(CUI) Lahore

Head of Department: _____

Prof. Dr. Zulfiqar Ali

Department of Chemistry

(CUI) Lahore

Declaration

I, Rabia Nawaz CIIT/FA22-R06-016/LHR, hereby declare that I have produced the work presented in this thesis, during the scheduled period of study. I also declare that I have not taken any material from any source except referred to wherever due to that amount of plagiarism is within an acceptable range. If a violation of HEC rules on research has occurred in this thesis, I shall be liable to punishable action under the plagiarism rules of HEC.

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It is certified that Rabia Nawaz, CIIT/FA22-R06-016/LHR has carried out all the work related to this thesis under my supervision at the Department of Chemistry and Department of Pharmacy, COMSATS University Islamabad, Lahore Campus and the work fulfills the requirements for award of MS degree.

Date: _____

Supervisor:

_____

Dr. Muhammad Javid Iqbal
Associate Professor
Department of Chemistry
COMSATS University Islamabad
Lahore Campus

Head of Department_____

Prof. Dr. Zulfiqar Ali
HOD, Department of Chemistry
CUI, Lahore Campus.

Dedication

I would like to dedicate my thesis with the utmost respect, love, and appreciation to my beloved (late) father, mother and my sisters who are always with me through thick and thin and kind teachers who have been a constant source of knowledge & inspiration.

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Rabia Nawaz

CIIT/FA22-R06-016/LHR

Abstract

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Plants are essential for human well-being, providing vital resources and medicinal benefits. This study focused on a medicinal plant *Salvadora oleoides*, investigating its potential as antidiabetic and anticancer agents. Synthetic drugs have limitations, while herbal medicines offer safer alternative research. Current study aimed to find effective and safe phytoconstituents to replace synthetic drugs. Different extracts were obtained through sequential extraction using methanol, acetonitrile and 2-propanol. Phytochemical screening confirmed the presence of key chemical constituents. A multimodal approach was used, including in vivo CAM assay on fertilized eggs, albino rat experiments. The study evaluated *Salvadora oleoides'* ability to stop cancer cell proliferation and trigger apoptosis in a physiological setting, providing new insights into its anticancer potential. Additionally, tests were conducted on blood glucose regulation in albino rats. The 500mg/ml of 2-propanol extract showed significant antidiabetic activity, with inhibition rates of 83%. The antiangiogenic activity of extracts was evaluated using the CAM assay. The methanol extracts at 1000µg/ml showed significant potential with a 57.91% inhibition rate. The positive control, Sorafenib, exhibited a significant inhibition rate of 58.89%. The 500mg/ml of 2-propanol extract showed significant antidiabetic activity, with inhibition rates of 83%. The results suggest *Salvadora oleoides's* potential as a natural treatment for cancer and diabetes. The results showed that extracts of *Salvadora oleoides* extract with 2-propanol has highest antidiabetic activity. Overall, the results highlighted the superior synergistic anti-diabetic and anti-angiogenic potential of the methanol and 2-propanol extracts in comparison to the acetonitrile extract, suggesting their suitability for the development of natural antidiabetic and antiangiogenic agents.

Keywords: CAM Assay, *Salvadora oleoides*, Antiangiogenic, Antidiabetic

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COX	Cyclooxygenase
TNF-alpha	Tumor necrosis factor alpha
NSAIDs	Non-steroidal anti-inflammatory drugs
HIF	Hypoxia-derived growth factor
FGF	Fibroblast growth factor
CAM	Chorioallantoic membrane
PDGF	Platelet-derived growth factor
NO	Nitric oxide
DMSO	Dimethyl sulfoxide
HPLC	High performance liquid chromatography
GC-MS	Gas chromatography mass spectrometry
BV	Blood vessels
IL-I	Interleukin-I
VEGF	Vascular endothelial growth factor

Chapter 1
Introduction

1.1 Importance of Medicinal Plants

Plants are of immense significance to human life as they provide oxygen, food, medicine, and ecological services. They are vital for sustaining life on Earth and maintaining a healthy ecosystem [1]. There is a lot of evidence that medicinal plants are used for healing purposes, written evidence and plant evidence. The search of drugs from plants is an old practice. Plant parts such as seeds, fruits are used against illness. In modern pharmacology, a lot of drugs are from plant origin that have been used by old civilization. The awareness about the use of plants as medicines leads the physicians towards the development of new drugs from medicinal plants and face the difficulties related to the development of drug discovery [2].

Nature has bestowed upon us an immense bounty of medical cures. In contemporary treatments, about half of all medications are composed of natural compounds or their derivatives. Conventional drug research and development is extremely expensive and challenging due to its poor success rate and large capital investment requirement. Researchers have spent the last several decades concentrating on the development of new drugs derived from botanical or herbal sources, which constitute a significant category of complementary and alternative medicine (CAM) treatments [3].

These days, plants provide around 80% of the medications used to treat cancer, cardiovascular disease, immunosuppression, and antibiotics. It is well acknowledged that over 80% of medicinal ingredients are created from natural compounds or directly obtained from natural items. Indeed, around half of all medications come from chemicals that were initially extracted or recognized as active components from plants, animals, and other species [4].

Herbs generated from plants are an important component of traditional and folk medicine, and they may be divided into two main categories:

1. Herbs found in databases that offer a thorough and cohesive explanation of their history and theories of usage, such as those used in Ayurvedic and traditional Chinese medicine (TCM; Zhong-Yi in Chinese)
2. Herbs used in traditional medicine don't have enough literature on their background or intended application.

Traditional medicines and treatments' clinical experiences continue to inform the evolution of modern medicine[5]. At least 130–140 novel medications, either as isolated chemicals taken from herbal remedies or as artificially altered molecules, are presently being used in clinical settings in China. Anisodamine, induribin, huperzine, and bicyclol are a few examples.

- The proportion of naturally occurring plants used in conventional and folk treatments is rather small. Thanks to developments in biological science and analytical technologies, several bioactive chemical entities present in plants or food products have been discovered through plant chemicals and pharmacological study. For instance,

- One important anticancer drug, Taxol (paclitaxel), comes from the Pacific Yew tree. It is commonly known that lutein, which is derived from marigold, enhances vision and may prevent the formation of cataracts.

- Lycopene from tomatoes is thought to offer protection against certain types of cancer. Even though humans have improved drug synthesis, plants remain an invaluable resource for medication research.

Thus, pharmaceutical companies used plant material to generate a number of well-known drugs, including podophyllotoxin, paclitaxel, camptothecin, artemisinin, vincristine [6].

The use of medicinal products to treat a range of medical conditions is still growing quickly. Both in established and emerging nations, there is a huge increase in the public's acceptance of and interest in natural medicines; these herbal cures may be found in grocery shops as well as pharmacies[7]. Herbs are commonly used in medicinal practices like Ayurveda, Unani, Homeopathy, and Sidha. They are also used in contemporary medications and nutritional supplements for food and drink.

Traditional medicines have thousands of years of use, ensuring their safety and efficacy [8]. The use of natural goods as nutritional supplements, Phyto cosmetics, and other herbal products is becoming increasingly popular, providing novel chemical entities for modern medications [9].

A botanical substance that may be utilized as an extract or diluted form is herbal medicine. When traditional medication proves to be unsuccessful due to increasing drug resistance, the usage rises. The Indian herbal business uses a wide variety of plant species. These medications are the result of generations of doctors in practice synthesizing their therapeutic expertise. The naturally occurring mixtures of substances have antioxidant, antiviral, antibacterial, and antiprotozoal properties[10]. People continue to utilize traditional herbal remedies for good healthcare since they are more concerned about utilizing contemporary treatments due to the developing drug resistance [11]. Several hundred plants are utilized globally in traditional medicine to treat bacterial infections, serving as the foundation for the creation of antibacterial chemicals. While some of these have also undergone in vitro screening, few controlled clinical trials have thoroughly examined the effectiveness of these herbal remedies [12].

Because medicinal plants are a rich source of phytoconstituents and are utilized worldwide for the prevention of illnesses and disorders, the usage of herbal medicine has expanded recently [13]. This global movement replaces synthetic medications with natural ones. Many historical societies continue to treat their illnesses using natural treatments. In this regard, India is home to several internationally renowned indigenous medical systems, including, yoga, homeopathy and naturopathy. India's rural and urban communities are beginning to embrace and utilize herbal treatments more since they are inexpensive, readily available, and safe [14]. Aspirin (derived from willow bark), morphine (derived from opium poppy), digoxin (derived from foxglove), and quinine (derived from cinchona bark) are only a few examples of common drugs that have natural origins. Because herbal medicine is nontoxic and has no negative effects, it is used as primary healthcare around the world. There are now a number of regulatory models available for herbal medicines, including those for prescription medications, over-the-counter pharmaceuticals, traditional remedies, and nutritional supplements [15].

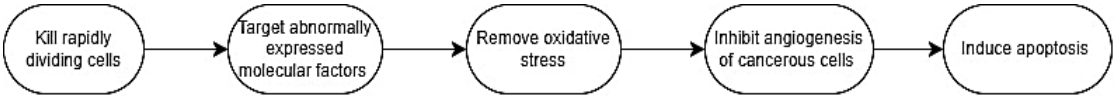
1.2 Phytochemicals in Plants

Phytochemicals found in plants have long been utilized as flavoring, aromatic, and coloring agents in addition to being used medically. These substances have a variety of biological functions and are crucial to both plant and human physiology[16]. They exhibit a diverse array of biological activities, encompassing anti diabetic, antibacterial, antioxidant, antimicrobial, and anticancer effects. There are a range of phytochemicals that have the hypo glycemic property some of these phytoconstituents are flavonoids, amino acids, peptidoglycans and polysaccharides [17] .

Many pharmacological functions are performed in the human body due to many phytochemicals and secondary metabolites present in plants. The phytochemical that are involved in this process are phenolics, alkaloids, resins and glucosides[18]. The phytochemicals that have antitumor properties are following:

- Lycobetaine
- Curcumol
- Tetrandrine
- Curidione
- 10-Hydroxycamptothecin
- Vinblastine
- Taxol
- Etoposide
- Ipomeanol
- Monocrotaline
- Indirubin
- Gossypol
- Homoharrington

Different phytochemicals follow different mechanisms for antitumor activity. Some of them use antioxidant mechanisms and some use apoptosis mechanism. The basic mechanism involved in this process is as follows:



Some phytochemicals use antioxidant mechanisms for this purpose for example gingerol, coumarin, rutin, thymol, epigallocatechin gallate and curcumin. Some phytochemicals follow the apoptosis mechanism for antitumor process for example flavonoids (methoxy isoflavone), polyphenols (Gallo catechins) [19].

1.3 Diabetes

One of the serious ailments related to glucose metabolism is diabetes mellitus. A lot of complications are linked with diabetes mellitus including microvascular and macrovascular problems.

- Macrovascular (stroke, vascular diseases, heart diseases)
- Microvascular (neuropathy, retinopathy, nephropathy)

The ratio of diabetes is increasing worldwide because of the global rise in obesity. Obesity and diabetes are interlinked [20]. The classification of diabetes, its mechanism and diagnosis are complex, and it has been under discussion for last many years. Around 500 genes are involved in diabetes. Diabetes is a serious public health condition due to serious health conditions linked with it like reduced life expectancy, morbidity and other financial conditions [21] .

Table 1.1: Some of the most common signs and damages caused by diabetes[22]

Signs	**Damages caused by Diabetes**
Diabetic retinopathy	Diabetes cause damage to eyes is diabetic retinopathy. Blood vessels of retina are affected by this, which can also bring about the gradual loss in vision leading to blindness.
Diabetic neuropathy	Diabetic neuropathy is damage caused by the nerves in diabetes. Symptoms linked with diabetic neuropathy are pain sensation, paresthesia, numbness and skin damage.
Diabetic foot ulcer	Diabetes induce foot problem like diabetic foot ulcers may occur likely, difficult to treat, in some cases requiring amputation.
Cardiovascular diseases	Diseases that are linked with damage to blood vessels are cardiovascular diseases. The risk of cardiovascular diseases is increased in diabetes. Coronary artery diseases are the major cause of death linked with diabetes, almost 75%. This may lead to stroke.
Diabetic	Diabetic ketoacidosis is the problem linked with metabolic

Ketoacidosis	process. The complications are nausea, vomiting, reduced consciousness, abdominal pain. Patients with diabetes type 1 usually suffer this problem.
Diabetic dermadromes	Diabetic dermadromes is a collective term for skin rashes caused by diabetes. Diabetes also causes skin issue like skin itching.
Hyperosmolar nonketotic	A severe but rare condition in diabetes type 2 patients mostly caused by dehydration.

1.3.1 Background of Diabetes

The problems linked with diabetic mellitus are metabolic disorders involving protein metabolism, carbohydrate metabolism and lipid metabolism. This is due to hyperglycemia it results from problem in insulin action and insulin secretion. From ancient times, diabetes mellitus has been known[23].

Its description has been provided in Chinese, Indian, Arab and Greek medical literature as well as in Egyptian papyri.

- An accurate explanation of diabetes was first provided by the Aretaeus of Cappadocia in the 2nd century.
- In 17th century, the term mellitus was also added to diabetes by Thomas Willis, described urine as sweet taste in case of diabetes.
- In 19th century, this disease was further studied by Claude Bernard which is a French physiologist. He studied the glycogenic action of liver.
- In 1889, two scientists performed an experiment in which they remove the pancreas of a dog which results in severe diabetes that ultimately became fatal. These scientists are Oskar Minkowski and Joseph von Mering.
- In 1921, two scientists extend the experiment of Minkowski's and Mering's experiment, insulin was extracted from the islets of pancreas, and it was provided to the patients. This was performed by the Fredrick Banting and Charles Best, which proved a huge hallmark in the discovery of treatment of diabetes [24] .

1.3.2 Origin of Diabetes

The metabolic disorder that is linked with problem in insulin secretion and insulin action or in some cases may be both is Diabetes mellitus[25]

Insulin is an anabolic hormone, and it is involved in the metabolism of carbohydrates,

lipids and proteins. The abnormality in the metabolism of carbs, proteins and lipids in diabetes indicates the importance of insulin hormone. The abnormalities in metabolic functions are due to multiple reasons.

- low levels of insulin
- Insulin resistance

Insulin resistance effect target tissues at the receptor levels. It affects the normal signal transduction system, receptors and effector enzymes at the level of liver. The major tissues that are effected by insulin resistance are adipose tissues and skeletal muscles[26]. The symptoms and the severity of diabetes depend on the type of diabetes and the duration. In diabetes type 2, in early years of diabetes the patient is asymptomatic sometimes. While the children with diabetes experience many complications like hyper glycemia, polydipsia, weight loss, polyuria and blurred vision. If diabetes remains untreated or uncontrolled it may lead to coma, stupor or even death due to ketoacidosis. And it may lead to a rare syndrome nonketotic hyperosmolar syndrome [27] .

Insulin resistance, insufficiency, or both, are the most common etiological elements of diabetes mellitus, a diverse primary disease of carbohydrate metabolism. Diabetes eventually results in hyperglycemia, the hallmark of this illness condition, regardless of the source[28]. Triglyceride and low-density cholesterol levels have been linked to an increased risk of early arteriosclerosis in NIDDM. Vascular disease is the cause of between 70 and 80 percent of diabetes patient fatalities. A medication that not only regulates blood sugar levels but also stops arteriosclerosis and other diabetic problems from developing would be the perfect therapy for diabetes[29].

Native American cures for diabetes mellitus and high cholesterol levels were in use long before insulin was widely employed. Patients' requests for the usage of herbal remedies with antihyperlipidemic and antidiabetic properties have been growing. This is mostly due to the fact that insulin cannot be taken orally and that administering insulin via injection has a risk of hypoglycemia as well as harm to other bodily processes including the liver[30]. The unfavorable effects and limitations of synthetic medications, together with their unsuitability for use in pregnancy, have led researchers to explore hypoglycemic medicines derived from plants. Numerous plant-based products and herbs have been demonstrated to have antihyperlipidemic and antihyperglycemic properties[31].

Diabetes mellitus represents a long-term condition brought on by a partial or whole lack of insulin, which results in poor regulation of blood sugar levels and both acute and long-term consequences. The primary consequence of diabetes mellitus is still premature and widespread arteriosclerosis affecting the renal, lateral, and cardiovascular arteries[3]. Diabetes is known to cause changes in the blood lipid profile, which may raise the risk of coronary heart disease. For these patients' long-term prognosis, a decrease in blood lipid levels—especially in the VLDL and LDL fraction and triglycerides—should be taken into consideration. Reduced risk of cardiovascular diseases appears to be linked to lowering blood glucose and cholesterol levels in the blood by medication and dietary changes [32].

1.3.3 Types of Diabetes

Table 1.2: Brief overview of various Diabetes types[33]

Diabetes Types	Brief overview of Diabetes
Insulin dependent diabetes mellitus (IDDM)	Destruction of beta cells in insulin dependent diabetes mellitus (IDDM) that leads to insulin deficiency.
Non-Insulin Dependent Diabetes Mellitus (NIDDM)	Loss in insulin secretion in non-insulin dependent diabetes mellitus (NIIDM) leads to insulin resistance.
Gestational diabetes	Diabetes is diagnosed during pregnancy, usually in the second trimester or third trimester.
Monogenic diabetes	Monogenic diabetes is a relatively rare form of diabetes. Monogenic diabetes is of two types: neonatal diabetes and MODY
Exocrine pancreas disease	Exocrine pancreas disease is linked with disorder of pancreas, cystic fibrosis and pancreatitis.
Chemicals/drug induced diabetes	Chemical/drug diabetes usually caused by organ transplantation drugs, HIV drugs and glucocorticoids.

1.3.5 Disease caused by Diabetes

The problems that are linked with diabetes are categorized as:

- Macrovascular Complications
- Cardiovascular diseases and stroke

- Perivascular disease (leads to injuries, gangrene and eventually amputation)
- Microvascular Complications
- Nephropathy (renal system damage)
- Retinopathy (eye damage)
- Neuropathy (nervous system damage) [34]
-

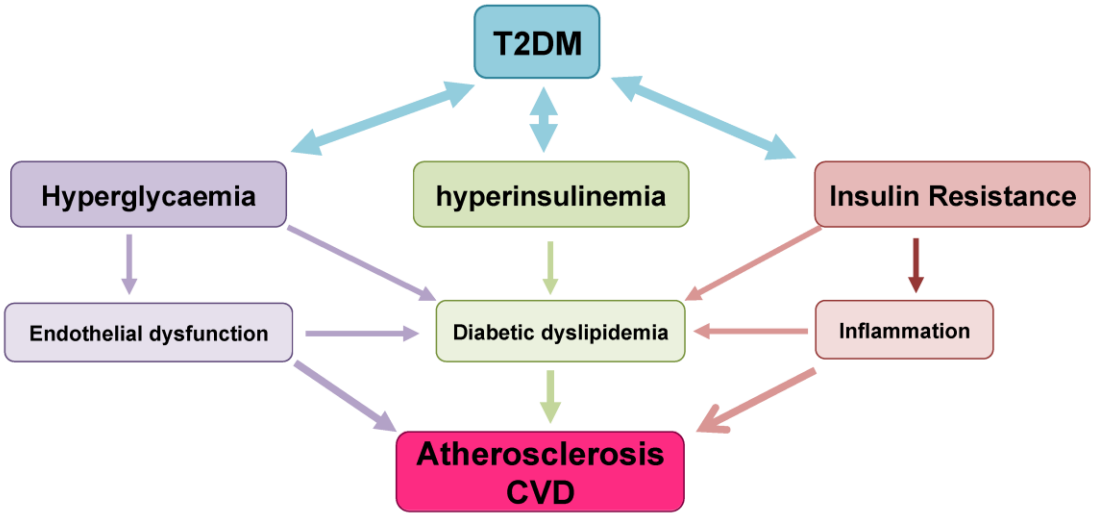


Fig 1.1: Pathophysiology of Type-2 Diabetes

1.4 Cancer

The uncontrolled and abnormal growth of cells is cancer. The small cells grow uncontrollably that originate from body organ. In some cases, cancer is detected at an early stage occasionally and in some cases through some laboratories. The mass of cells that grow uncontrollably usually becomes detectable when it reaches 1cm in size or almost a million cells. At this stage, it is called lump, tumor or lesion. Leukemia and lymphoma are exception to this, because no mass is produced in this type of cancer, but they can be identified through other body tests [35].

The process of progression of normal cells into cancer cells is due to the inability of immune system to distinguish cancer cells at the early stage and remove them from the body. The immune system further get affected in those patients that have weak immunity due to age factor, in case of previous history of chemotherapy, or it may be due to use of some sort of medications for example, corticosteroids and analgesics [36].

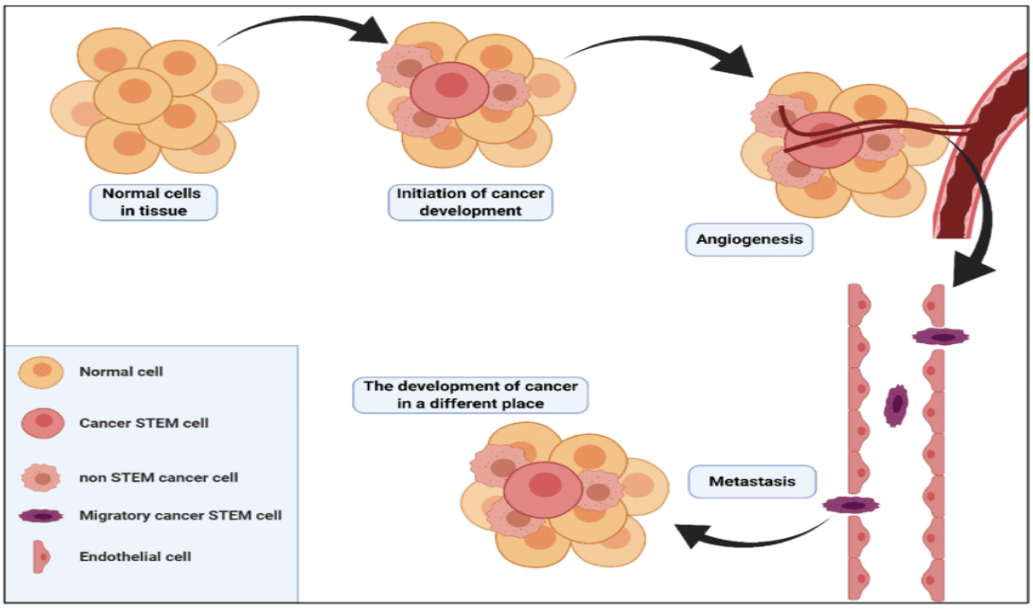


Figure 1.2: Process of formation of cancer cells from normal cells

Background of Cancer

The term cancer stems from the Greek word for carb, karkinoma, which the physician Hippocrates used to describe the radiating appendage-like projections spreading from breast tumors. Cancer could be a s solid tumor or in case of leukemia it is non solid tumor[37]. A tumor is defined as a lump or a mass that could be malignant or benign. Malignant tumor cells penetrate the surrounding normal tissues, whereas benign tumor cells remain grouped in a single mass and are commonly encapsulated. Benign tumors are normally not lethal, however at specific anatomic places, such as in the skull, they can impose pressure on the other organs and lead to considerable morbidity and, if ignored, death [38]. There are several factors that can measure the malignancy of tumor, along with degree of differentiation and metastatic potential. In most cases, the cause of death of cancer patients is metastasis, that is the most lethal form of cancer and highest degree of aggressiveness.

Table 1.3: Difference between malignant and benign tumor [39]

	Malignant tumor	**Benign tumor**
Differentiation	Undifferentiated	Differentiated, resembles tissue of origin
Invasiveness	Invasive	Noninvasive
Metastasis	Often metastatic	Never
Rate of growth	Rapid	Slow, sometimes static

1.4.1 Cancer Progression

Cancer development in distinct phases, frequently over a long period, a prospective cancer cell acquires abnormalities that results in growing tumor aggressiveness. The early mutagenesis events of cancer start bypass growth growth-restrictive processes, thereby offering a selective advantage to aberrant cells, allowing them to outgrow and displace their neighbors[40]. In addition, the accumulation of genetic abberations found in cancer may come from a mutator phenotype. Mutator phenotypes are induced by mutagenesis events early in cancer development that subsequently endanger genomic stability and DNA integrity, hence predisposing tumor cells to the accumulation of future mutations. Given the known mutation rate of normal cells, it is improbable that cells will acquire sufficient mutations to become malignant [41]. In other words, the enormous number of genetic mutations seen in many malignancies cannot be accounted for by random mutagenesis events. Therefore, the mutator character of premalignant cells allows them to be more sensitive to mutations than normal cells [42].

Most solid tumors develop from areas of hyperplasia, defined by increased local tissue size due to aberrant cellular proliferation. Cells from hyperplastic tissue keep their natural architecture and orientation[43]. When normal cellular architecture and orientation are lost, the abnormal tissue is described as cancer. Genetic abnormalities can continue to accumulate in malignant cells and may influence the growth rate, the level of invasiveness, and the metastatic potential.

Progressive genetic changes may also influence the efficacy of:

- Chemotherapy
- Surgeries
- Radiation therapy (radiotherapy)

1.4.2 Cancer genes

Some genes are involved in cancer progression and initiation, and some genes are involved in the normal growth of cells. They can be recognized as 3 types of genes:

1. Tumor-suppressor genes
2. DNA repair genes
3. Proto-oncogenes

Mutation in these genes causes cancer. These types of mutations can be acquired by

germline transmission or by external osmotic damage [44]. During normal process of replication, proto-oncogenes are involved are involved in maintenance and growth. Some genes involved in the creation and maintenance of normal cells and tissues may, in some situations, be involved in cancer initiation and advancement[45]. These genes fall into three categories: proto oncogenes, tumor-suppressor genes, and DNA repair genes[46]. Mutations in proto-oncogenes, tumor-suppressor genes, and DNA repair genes cause cancer. These mutations can be acquired by germline transmission or by external somatic damage. Proto-oncogenes survive mutations that impair their normal regulation, they become hyperactivated and uncontrollably accelerate cellular proliferation. Hyperactivated mutant alleles of proto-oncogenes are dubbed oncogenes [47].

Table 1.4. Some of most common forms of cancer [48]

Types of cancer	**A brief overview of cancer**
Carcinoma	Cancer lines the internal organs and also starts from skin. There are many types of carcinomas, it can me squamous cell carcinoma, adenocarcinoma, transitional cell carcinoma.
Sarcoma	The cancer that grows in the connective tissue is sarcoma, it may be blood vessels, cartilage and bone.
Lymphoma and myeloma	Lymphoma and myeloma begin in the immune system. Cancer that starts in the plasma cells is myeloma. Cancer that develops in the lymphatic system is called lymphoma.
Leukemia	The cancer of white blood cells is leukemia. Leukemia starts in the bone marrow, that make red blood cells.
Spinal cord and brain cancers	Cancer of the central nervous system is cancer of brain and spinal cord. Cancers that develop in spinal cord and brain sometimes are benign that develop slowly. And some tumors of the central nervous system are cancerous. The cancer of glial cells is the most common type of cancer, called glioma.
Hodgkin's lymphoma	A rare form of cancer is Hodgkin's lymphoma. This is cancer of the lymphatic system.

1.5 Angiogenesis and Cancer

A natural process that is linked with biomolecules is angiogenesis. Process of development of new blood vessels[49]. The complicated and natural process of

angiogenesis is regulated by a number of different molecules that the body produces. The cells in endothelial cells and cells of smooth muscle work together to repair damaged blood vessels through endogenous chemical signals that can be localized or systemic[50]. The vascular tree grows as a result of endothelial cells "sprouting" from previously present blood cells to produce new blood vessels. Angiogenesis involves the manufacturing of proteases, migration and proliferation of endothelial cells, the construction of vascular tubes, the anatomical joining of newly formed tubes, , inclusion of smooth muscles as well as pericytes [51] .

Cancer is a deadly disease that can spread to distant organs. Tumor cells can enter lymphatic or blood arteries, enter intravascular flow, and metastasize. The formation of a vascular network is crucial for cancerous tissue's metastatic spread[49]. Angiogenesis and lymph angiogenesis are processes that create new blood and lymphatic vessels, which are essential for developing a circulatory network that supplies nutrients, oxygen, and immune cells while eliminating waste [52]

Angiogenesis is a crucial process in cancer progression, involving a four-step process: local tissue injury, angiogenic stimuli, endothelial cell movement, cell multiplication, and ongoing impact from angiogenic factors [53]. This process is accelerated when tumor tissues seek nutrition and oxygen and is regulated by both activator and inhibitor molecules. However, up-regulation of angiogenic factors alone is not sufficient for neoplasmic angiogenesis [54].

1.5.1 Angiogenesis and the Prognosis of Cancer

Numerous investigations have demonstrated the important role angiogenic activators play in the development and metastasis of cancer. Approximately 50% of the human cancers examined had a VEGF protein and their receptors expressed, according to immunohistochemistry testing [55]. It is established that these variables impact the prognosis of carcinomas that have spread to the stomach, ovary, and cervix of the uterus. Furthermore, it has been shown that there is a significant correlation between the level of expression of VEGF and the prognosis in cases of malignant mesothelioma, head and neck squamous cell carcinoma, lung cancer, colorectal cancer. These studies also shown that tissue levels of angiogenic substances indicate the rate at which tumor cells proliferate, and as a result, they may be used to forecast whether high-risk individuals would develop [54].

1.5.2 Angiogenesis as a therapeutic agent

Surgery may be curative for limited solid malignant tumors, but radiation plus cytotoxic chemotherapy is a better treatment option for those that cannot be surgically removed. Despite advancements in both therapies, treatment outcomes for advanced illness remain poor. A new therapeutic approach is needed to address the bleak treatment outcomes [56]. Angiogenesis is controlled by both activator and inhibitor chemicals, and a shift in the local balance between these regulators is necessary for the transition to the angiogenic phenotype. The discovery of angiogenic inhibitors could reduce cancer mortality and morbidity.

Five classes of angiogenic antagonists are currently under clinical trials:

- Protease inhibitors,
- Endothelial cell migration,
- Proliferation,
- Matrix proteins on cell surfaces like copper and integrins, and
- Inhibitors with distinct mechanisms [54].

1.5.3 Signals for Angiogenesis

Angiogenesis is a complex biological process involving various signals and mechanisms, including TGF, PDGF, VEGF, fibroblast growth factor, HIF, and angiopoietins[54]. These signals aid in the formation of new blood vessels, crucial for tissue growth and repair. Understanding these mechanisms is vital for developing new treatments for diseases like cancer and cardiovascular disease. These signals, produced by various body cells, can function alone or in combination, making it essential to comprehend the signals involved in angiogenesis [57].

1.5.4 Cancer and Angiogenesis Activators and Inhibitors

Table 1.5: List of activators and inhibitors of cancer and angiogenesis [58]

Activators	Inhibitors
Vascular endothelial growth factor	Interleukin-10
TGF-β (Transforming growth factor)	Interleukin-12
Epidermal growth factor	Sorafenib
Fibroblast growth factor	Semaxanib
PDGF (Platelet derived growth factor)	Erlotinib

Angiopoietin	Activator inhibitor I
EGF	Angiopoietin-2
Erythropoietin-I	Angiostatin II(AT2 receptor)
Angiostatin	Caveolin-I , 2
Angiogenin	Endostatin (collagen XIII fragment)
Prostaglandin E	Interferon-alpha
Gelatinase A,B	Thioredoxin Inhibitor
Nitric oxide synthase	IFN-α
HIF-1α	TNP-470
TGF-α (Tumor growth factor-α)	Bevacizumab
HB-EGF	Aflibercept

1.5.5 Potential of herbs in Angiogenesis Inhibition

Therapeutic method for treatment of many diseases including diabetic retinopathy, cancer, and cardiovascular disease is angiogenesis inhibition. Growing attention has been exhibited in the ability of herbs to decrease angiogenesis in recent years [59].

Many herbs have been shown to possess substances with anti-angiogenic qualities, which can assist in suppressing the production of new blood pots and so prevent the growth and spread of cancer and other diseases. Plants contain numerous chemical components at a time, and unlike chemical medications, which implies that they influence several elements of disease pathology concurrently [60]. Many herbs have been shown to possess substances with anti-angiogenic qualities, which can assist in suppressing the production of new blood pots and so prevent the growth and spread of cancer and other diseases. Plants contain numerous chemical components at a time, contrary to chemical medications, they can have synergistic effects, which implies that they influence several elements of disease pathology concurrently [61].

1.6 Traditional Herbal Medicine

Around the world, more than 50,000 plants have therapeutic characteristics. For the treatment of cancer, 80% of the population depend on natural plant sources [20]. There are 6000 types of wild plants in Pakistan, of which 400-600 are estimated to have medical potential [21]. In Pakistan, 84% of people apply traditional treatments to manage their disease [22]. In the distant and rural mountainous regions of Pakistan, between 50,00 and 60,000 hakims (local healers) and a comparable number of

unregistered practitioners employ more than 200 plants as ordinary therapies for treating a wide range of ailments [23]. Plants that are utilized as medication are significant because they are widely available, have no bad effects, and are inexpensive. Traditional herbal remedies are a form of complementary medicine that have been used for many years to cure diseases and enhance health and wellbeing [24]. Because herbal medicines have fewer negative effects in the treatment of angiogenesis-related disorders, research on plants to identify and create such compounds could be a therapeutic technique. As a result of the lengthy legacy of employing herbs as cancer treatments, they have traditionally functioned as the key element in traditional medicines used to treat a variety of disorders [25]. Plants are nevertheless recognized as significant resource for the development of innovative therapeutic components by researchers even though the actual compounds created from them aren't generally used as medications [62].

Along with providing the body with the vitamins and minerals it needs, herbs have roughly 25,000 different chemical components, the bulk of which have biological functions and properties. These treatments are from plant extracts and are typically prepared into teas, powders, tinctures, or capsules [20]. For the treatment of many illnesses, herbal medicines are used, such as reproductive illnesses, and digestive troubles [21]. They are widely used in conjunction with conventional medicine and, when used appropriately, and are generally regarded as safe [22]. Additionally demonstrating their therapeutic potential, some of the active components of conventional herbal treatments have been isolated and turned into current medications [23].

Anti diabetic and anti-angiogenic activity rich medicinal herbs Medicinal herbs with anti-diabetic and anti-angiogenic characteristics offer intriguing medicinal promise. These plants have bioactive chemicals that can inhibit angiogenesis and diabetes, two processes that are associated with the development of numerous disorders, including diabetes and cancer. By exploiting the potential of these plants, new medicines, and techniques for treating diabetes and angiogenesis-related disorders may be established [52].

Table 1.6: List of plants those inhibit Diabetes and Cancer [63]

Plant	Phytochemicals
Aloe barbadensis	Triterpenoid
Andrographis paniculata	β-sitosterol
Annona squamosa	Eugenol
Argemone Mexicana	Mycotoxin
Anacardium occidentale	Epicatechin gallate
Asparagus racemosu	Diosgenin
Azadirachta indica	Catechins
Amaranthus tricolor	Amaranthine
Boerhaavia diffusa	Punarnavine
Bryophyllum pinnatum	Bryophyllin B and A
Costus speciosus	α-tocopherolquinone
Cocculus hirsutus Linn.	Anthocyanin
Moringa oleifera	Niazinin A, b
Murraya koenigii	Coumarin
Piper betle Leaves	Caryophyllene
Terminalia catappa	Ellagitannins
Terminalia chebula	Quinic acid
Wedelia chinensis	Apigenin
Salvadora persica	m-anisic acid
Salvadora oleoides	Terpenoids
Syzygium cumini	Myrecetin
Mikania cordata	Saponins
Kalanchoe pinnata	Triterpines

1.7 *Salvadora oleoides*

Salvadora oleoides decne is a naturally occurring tiny tree or shrub is found in alkaline and dry parts of Pakistan and western India. It's a member of the salvadoraceae family and locally called as "jal," "pilu," or "wan". It has various therapeutic applications in traditional treatments. S. oleoides' mature fruits are ingested because they are regarded as nutritious [64]. This plant's aerial branches are used as miswaks to brush teeth, maintain gums healthy, and reduce gum inflammation. Fever, rheumatism, and an enlarged spleen are all treated with fruits and leaves. Additionally, leaves are utilized

to heal ulcers, boils, and coughing. Leg discomfort is treated with the leaf paste, which is also used directly on open wounds. Its stem bark is excellent for treating tumors, piles, pneumonia, and rheumatic discomfort. In addition to these customary applications, *S. oleoides* is believed to have anti-oxidant, anti-inflammatory, anti-hyperlipidemic, hypoglycemic, and antibacterial effects [65]. The chemical composition of *S. oleoides* has not been extensively studied, but what is known includes an alkaloid in the stem, a few fatty-acids and lipidic compound in the upper parts, a few flavonoids in the leaves, glucosinolate in the shoot parts, stem, and root, iso-coumarin in the whole plant [21].

Salvadora oleoides Decne (Salvadoraceae) is a major tree species in the wide region of Gujarat, growing spontaneously by seed germination. Gujarat is home to just two species of Salvadora, Salvadora oleoides. This little plant grows well in arid highland regions of Gujarat and among rocks. Typically, the plant grows from June to September, which is the wet season [66]. The natives of Gujarat's Kachchh area have long employed the whole plant to cure a variety of uterine and skin conditions. When rheumatism strikes, the oil derived from seeds is administered. Unripe fruit decoction is used to treat rheumatic fever and enlarged spleen. Fruits are beneficial for stomach issues and asthma, while leaves are utilized for dry coughs [67].

The Salvadora oleoides leaf extract has dry extract/ml and has anti plasmodial action. *Anopheles stephensi* was 100% poisonous to Salvadora oleoides seed oil at 0.01%. Horses are fed leaves as a purgative and to ease coughs. One uses root bark as a vesicant [68]. Fever, rheumatism, and enlarged spleen are all treated with fruits. The fat from seeds is used to make suppositories, cure rheumatic aches, and serve as a foundation for ointments. Unidentified component used in India to cure domestic animals' sore throats. People utilize the herb, which has high medicinal potential, to cure a variety of illnesses. An open wound was treated with the leaf paste [69].

The *Salvadora oleoides* tree, member of the Salvadoraceae family, is a multifunctional plant with high therapeutic oil. It is extensively suited to India's arid and dry regions. Many of the medications used today were developed from plant components, and this method was vital to the pharmaceutical industry's efforts to create new pharmaceuticals. India is referred to be the "Botanical Garden of the World" due to its abundant biodiversity, which is a natural blessing. There are over 45,000 different plant species found in India; 7,500 of those are known to exist. A variety of serious illnesses can be effectively treated with extracts or portions of medicinal plants. The medical

significance of these plants may lie in their potential to yield novel sources or molecules with potent antimicrobial, antiviral, and antifungal agent activity. Terpenoids, phenolic chemicals, alkaloids, glycosides, and flavonoids are present in *Salvadora oleoides* extracts and are commonly utilized against a variety of microbial activity. The plant extract has tumors, spleen illness, antihyperlipidemic, brochities, hypoglycemic, rheumatic pain, bronchitis, appetizer, laxative, carminative (leaves), and stimulant (alexipharmic) properties from stem bark. It is also commonly used as an antibiotic [70].

Figure 1.2 Salvadora oleoides Plant

1.7.1 Taxonomic classification of Salvadora oleoides

- Kingdom: Plantae
- Family: Magnoliophyta
- Class: Magnoliopsida
- Subclass: Dillieniidae
- Order: Capparales
- Family: Salvadoraceace
- Genus: Salvadora
- Species: Oleoides
- Local name: Pilu
- Part used: Stems and leaves.

1.7.2 Traditional use of Salvadora oleoides

The Salvadoraceace family includes the well-known plant Salvadora oleoides, which thrives in Pakistan's Thar desert. The plant is the most effective component in many

therapeutic treatments since it can make oil. Additionally, indigenous people already use the herb as a treatment for a range of ailments. The usefulness of leaf decoction as a cough suppressor, spleen tonic, and fever treatment is established [71]. Similarly, the blooms of S. oleoides work as a blood purifier and nerve tonic. It is believed that the anti-ulcer, analgesic, and anti-inflammatory effects of the leaf paste are useful. The stem exhibits diuretic and anthelmintic effects. The current research picked the plant for assessment depending on its conventional use as well as membrane stabilizing and antioxidant properties [64]. Moreover, the research was organized to comprehensively examine the phytochemicals present in Salvadora oleoides. Assessments were subsequently expanded to screen for antioxidant and antibacterial abilities of such phytochemicals in opposition to *Aspergillus terreus*, *E. coli, Aspergillus niger* and *Shigella spp* [21].

A naturally occurring tiny tree or shrub, the *Salvadora oleoides* found in dry of western Pakistan. Known locally as peelu, it belongs to the Salvadoraceace family. In traditional remedies, it has a number of therapeutic uses. Fruits that have grown on *S. oleoides* are eaten because they are thought to be healthy. The aerial branches of *S. oleoides* plant are used for miswaks to avoid gum inflammation, maintain healthy gums, and brush teeth. Fruits and leaves are used in the treatment of fever, rheumatism, and enlarged spleen. Furthermore, leaves are utilized to treat coughs, and ulcers [72] .Leaf paste may be applied directly to open injuries as well as utilized to relieve leg soreness. Its stem bark helps with rheumatic pain, piles, pneumonia, and malignancies. Apart from its traditional use, S. oleoides is believed to possess antibacterial, anti-inflammatory, anti-hyperlipidemic, antioxidant, and hypoglycemic qualities. Though S. oleoides' chemical makeup has not been thoroughly investigated, what is known about it includes an alkaloid found in the stem, a small amount of fatty acids and lipidic compounds in the upper sections, a small number of flavonoids in the leaves, glucosinolate in the stem, root, and shoot sections, iso-coumarin throughout the plant [73].

The genus Salvadora has three genera and ten species. The natural habitats are found in grassy savannahs, salty soils, marshes, thorny plants, and seasonal wet places. In desert climates, they may also be found along sewage lines [74]. The large distribution of Salvadora species may be attributed to its ability to thrive in a wide range of conditions, including water, soil. Certain varieties of Salvadora can also be found along riverbanks in areas where groundwater levels are higher. Only Salvadora, a solitary genus including two species (*S. oleoides* and *S. persica L.*), is present in Pakistan [75].

Salvadora species are perennial shrubs or trees with axillary terminal inflorescences that are densely fascicled or laxly panicled, and simple, opposite, petiolate leaves. The tiny, sessile or subsessile, bisexual, tetramerous flowers are pale green in color. Fruits are globose drupes with a solitary seed and a prolonged calyx. In addition, S. persica L. has mature fruits that are red or white, as opposed to *S. oleoides* Decne, which has reddish-brown fruits. In comparison to *S. oleoides*, *S. persica* L. has leaves that are both smaller and more numerous. Both Salvadora species are facultative halophytes with great salt tolerance and deep-rooted mesomorphic xerophytes [76].

S. persica L. is commonly referred to as a toothbrush tree that is frequently used for tooth cleaning. The tree is more widely recognized globally under the commercial name Miswak. In terms of important religions and regional knowledge systems, *S. persica* L. is likewise more significant culturally. It is one from the seventeen families of plants that have been identified as plants that are referenced in the Holy Quran [77] . *Salvadora oleoides,* is a member of the Salvadoraceace family. This plant species is advantageous from an ecological, social, and economic standpoint [70]. It is cultivated as a plant with several uses. It is a well-known plant species that is often found in arid parts of Pakistan, India, and Kutch. In the semiarid middle area of Saurashtra, extending southward to Kutch in Gujrat, India, *Salvadora oleoides* is also found. Its edible, medicinal qualities usually make it significant. Additionally, its wood is utilized as fuel [78]. *Salvadora oleoides* is a small shrub and plant that may reach a height of between six and nine meters in ideal circumstances. Its branches are many, stiff, drooping, and occasionally swollen at the tips. It has gray or whitish-gray bark. The leaves are leathery, rather meaty, linear with dark greenish-yellow juvenile leaves and gray adult leaves [79]. Flowering occurs from January to March in the western parts of India. Sessile, greenish-white, minuscule, with four rounded, blunt lobes, the calyx is cup-like and sometimes seen in groups. It is also covered in tiny spikes. The month of May is fruiting. When ripe, the globose, greenish-yellow fruits—drupes—become reddish-brown. Trees can be toppled, or fruit removed with a strong shake of the trees. A mature tree yields between 10 kg of fresh fruit and 2-3 kg of dried fruit. Fruits taste sweet, pleasant, aromatic, peppery, and somewhat spicy [74].

1.7.3 Biological Active compounds in Salvadora oleoides

The Salvadoraceace family, primarily found in dry regions, includes two underutilized species*: Salvadora oleoides (S. oleoides*) *Salvadora persica (S. persica)*. *S. persica*, a

small tree or shrub, has numerous pharmacological properties, including astringency, diuretic, aphrodisiac, anti-plaque, bitter stomachic, alexiteric, analgesic, anti-inflammatory, anti-microbial, and anti-plaque. It is used in folk literature, particularly by Muslims, and mentioned in the Holy Quran. *S. oleoides*, an oil-producing, multifunctional plant, is believed to have antihyperglycemic, hypolipidemic, analgesic, anti-inflammatory, and antibacterial properties[73].*Salvadora oleoides* leaves, known for their antibacterial, analgesic, antihyperlipidemic, anti-inflammatory and antioxidant properties, were analyzed for their antioxidant and membrane-stabilizing properties based on historical uses [21].

Significant amounts of hydrocarbon (41.3%) and phenolic chemicals (25.7% and 25.7%, correspondingly) are present in the stems and leaves of Salvadora oleoides. The essential oil of Salvadora oleoides was shown to share 23 chemical components between its leaves and stems. The main constituents of the essential oil of leaves include:

- 25.4% Methoxy-4-vinylphenol,
- 16.8% (Z)-cis-3-Hexenyl benzoate,
- 13.9% Phytol,
- 6.9% n-hexadecenoic acid,
- 2.1% trans-ß-damascenone

These are the major components of the essential oil of leaves, instead stems have an elevated concentrations of 2-methoxy-4-vinylphenol (21.6%), phytol (12.9%).

Additionally, this species includes salvadourea, alkaloids, salvadorine, salts, salvadorine, primary chlorides. Methanolic extract of upper parts possesses:

- β - amyrin
- Ursolic acid
- 3β- erythrodiol
- Trimethylamine
- Tannins
- Proteins
- Carbohydrates
- Tetracosone
- Noctacosonal
- β-sisterol

- Flavonoids
- Mucilage

Table 1.7: Types of fatty acids along with their composition **[80]**

Type of fatty acid	Myristic acid	Lauric acid	Palmitic acid	Linoleic acid	Oleic acid
Percentage	28.4%	47.2%	28.4%	1.3%	12%

Because of their high quality and few side effects, natural products are becoming more and more popular. As a result, it is necessary to investigate underused species that have a strong historic usage against diseases. Since no prior studies have been published in the literature, the purpose of this study is to examine the anti-inflammatory and antioxidant qualities of polyphenol-rich fraction of S. oleoides and the S. persica fruits and aerial parts [72].

Plants have long been used medicinally, and in developing nations, their contribution to the system of disease prevention is crucial. Most of the therapeutic herbs utilized in conventional medicine are harvested from the wild. The doctor would gather, give, and inspect the medications on their own using their sense of smell, color, texture, taste, and sound, according to the ancestral idea [72]. Certain plants were utilized to treat distinct ailments based on their form and environment. Eventually, information on medicinal plants was included in herbal pharmacopoeias. Many natural compounds that are found in plants have been a valuable resource for preserving health for a long time. Currently, the first screening of bioactive compounds is part of the quality assessment process, which depends on phytochemical evaluation [81].

In addition, proximate elemental analysis, fluorescence analysis, and chemo-profiling are employed to guarantee the efficacy and safety of the herbal medication. It takes deliberate work to accurately identify and assess the medicinal plants that should be used in medicine administration. Primary and secondary compounds found in plants are extremely important in treating human illnesses [65]. They possess pharmacogenetic qualities such antibacterial, anticancer, antioxidant, anti-inflammatory, and anti-diarrheal effects. In the biotechnological industries, lipids, carbohydrates, amino acids, nucleotides, and other primary metabolites have defined functions and can be used as raw materials. Secondary metabolites of carbohydrates are often found in one species of plant [82].

1.7.4 Medicinal use of Salvadora oleoides

The medical properties of plants have been known for many decades, and their position in the disease prevention system is crucial in developing countries. As per archaic theory, physician used to gather, administer, and check the drugs by themselves with the help of odor, color, texture, taste and sound. Based on habitat and form, distinct plants were employed to cure ailments. Herbal pharmacopoeias eventually contained information about therapeutic herbs[70]. Plants possess many kinds of natural products, which have been a vital source for keeping a healthy existence. In the current age, phytochemical evaluation is vital for quality assessment, which involves the early screening of bioactive metabolites. Along with that, chemo-profiling, fluorescence analysis, and proximate elemental analysis are employed to ensure the safety and efficacy of the herbal medication. Conscious efforts need to be made to correctly discover and analyze the medicinal plants to implement for medicine administration [83]. In plants, primary and secondary metabolites play a very major role in healing human illnesses. They have pharmacogenetic qualities such as anti-inflammatory, antidiarrheal, antioxidant, anticancer, antibacterial, etc. Chlorophyll, amino acids, nucleotides, carbohydrates, lipids, and other primary metabolites have established roles in the biotechnological industries and can be used as raw materials[84]. While the essential primary metabolites are prevalent across the plant kingdom, secondary metabolites (amino acids, sugars, and acyl lipids) are typically found in a single plant species or a taxonomically related group of species. That indicates that forms of secondary metabolites are usually isolated from a particular plant species, while all types of secondary metabolites are not found in all plant species [21].

1.7.4.1 Medicinal uses of Leaves

• Cannabis sativa L. leaves are combined to make a paste that is administered to the anus to treat piles; the paste is also used to treat dry cough, asthma, and digestive issues.

• Also used as a popular purgative and to treat rheumatism, mild fever, coughs, snake bites, and enlarged spleen.

• To cure corneal opacity, leaf paste combined with water is sprayed into the eyes.

• The leaves' decoction is used to treat placenta retention and cough.

• A paste is applied topically to treat conjunctivitis; it is also used to treat fever, enlarged spleen, and cough [85].

1.7.4.2 Medicinal Uses of Fruits

- Rheumatic fever and enlarged spleen are treated with this mixture. both as an aphrodisiac and to cool the body.
- Fruit is chewed and suggested for rheumatism & purgative. Cooling effect; used to cure calculi, constipation, indigestion, and stomatitis. Opens the body's pores and acts as an appetizer, laxative, carminative, alexipharmic, and is beneficial for piles, tumors, bronchitis, spleen illnesses, ascites resolvent, expectorant, and diuretic [86].

1.7.4.3 Medicinal Uses of Roots and stem bark

- Boiled root ash was used to cure blisters and rheumatism, as well as to fight mange and remove hair.
- The infusion is used as a febrifuge and to control menstrual cycles.
- Young stems and rootlets are used to make miswak, a tooth-clearing agent.
- The bark of the roots is used as a vesicant [87].

1.7.4.4 Medicinal Uses of seed and seed oil

• To test for camel bite, seeds are combined with jaggery and administered.
• Topically, seed oil is administered to relieve rheumatic discomfort [22].

Chapter 2
Literature Review

2.1 Literature review of selected Medicinal Plant

2.1.1 Reported Pharmacological study of *Salvadora oleoides*

Akash Garg et al. (2013) found that *Salvadora oleoides* had hypoglycemic action in diabetic albino rats, contrasting with conventional drugs. Blood samples were collected from rats with albinism and blood sugar levels were measured at various points. Different solvents were used to administer the *Salvadora oleoides* doses. The ethanol extract-treated rats showed the highest hypoglycemic action, while the ethanolic extract-treated euglycemic and alloxan-induced rats showed the greatest drop in blood glucose level [35].

Yadav et al., (2008) reported hypo glycemic activity of Salvadora oleoides (leaves) in albino rats. The extracts of *Salvadora oleoides* were produced using different solvents and fed to diabetic rats induced by drug alloxan and euglycemic albino rat group. The results were highly noteworthy using ethanolic extracts of *Salvadora oleoides* and blood glucose level drops down is substantial at 21st day that is the end of treatment period, with p value ($P < 0.001$). The p value is likewise pretty excellent for lipid profile with value of ($p< 0.001$) in both alloxan induced rat group and euglycemic rat group. Blood glucose level showed maximum reduction in blood glucose levels in rat group administered the usual medication tolbutamide. Reduction in blood glucose level was steadily increased moving from day 7, 14 and maximum reduction were recorded on 21st day. The drop in glucose level is 10% on day 7, 20% on day 14 and 26% on day 21. But this is less than the typical medicine used for *Salvadora oleoides* that is 36%. Ethanolic extracts of *Salvadora oleoides* were effective in reducing blood glucose level [36].

Kanam et al., 2022 reveals radical scavenging assay of methanolic extract value 46.90 ± 0.34 % discovered in leaves of *Salvadora oleoides* L. The present investigation determined the DPPH radical scavenging value 20.17 % in leaves extract. Kumari and Parida, 2016 author observed ABTS activity value 4.5µg/ml and the present investigation of *Salvadora oleoides* L. plant ABTS value is 90%. So, this plant has stronger antioxidant potential. Some plants have compounds that have antioxidant

effects. A study on the Salvadora plant was carried out utilizing several antioxidant approaches. Methanolic extracts have the highest antioxidant action. The greatest radical scavenging activity in ABTS assay is 90% shown at 200 µg/ml concentration of methanolic extract of plant [37].

S. oleoides, had effects on blood glucose levels when combined with *Coccinia indica* Wight & Arn. When compared to the standard medication Glipizide the methanol extracts reduced the blood glucose level of diabetic rats at a dose of 150mg/kg with ($p<0.01$). They showed a good overall antidiabetic potential by demonstrating a substantial ($p<0.01$) effect on blood urea level and creatinine, lipid profile, and ALS/AST activity [38].

The study found that one of the most common phenolic acids in *S. oleoides* and *S. persica* fruit extracts and aerial parts is chlorogenic acid. The highest levels of chlorogenic acids were found in the extracts of *S. oleoides* and *S. persica*. The highest concentrations of gallic acid were found in the aerial component extract of *S. oleoides* and the extracts of *S. persica*. The hydroxyl benzoic acid content was highest in the fruit extracts of *S. persica*. The aerial components of *S. oleoides* and *S. persica* contained elevated levels of cinnamic acid. The aerial component extracted from *Salvadora persica* showed high levels of myricetin, rutin, and catechin. Fruit extracts had similar levels. In aerial part of *S. oleoides and S. persica* catechin was the most important compound. The antioxidant activity was correlated with flavonoid concentrations and phenolic concentration. The most important antioxidant compounds were chlorogenic acid and gallic acid. The antioxidant activity was found to be favorable with these compounds.

Phenolic molecules are well-connected and have a significant role in antioxidant activity. When compared to other solvents, methanol, a polar solvent, may extract a greater amount of phenolics. Saleem et al. [31] reported that the amounts of total flavonoid (0.21 mg QE/g) and whole phenolic in the methanol extract of *S. oleoides* aerial parts were lower than our findings. The total estimated phenolics and flavonoids in the fruit's methanol extract of S. persica were measured by Kumari et al. [39] to be 120.38 mg/100 g DW and 77.59 mg/100 g DW, respectively; no data regarding TFC and TPC of fruit of *S. oleoides* have been reported.

Kaneria et al. [40] reported that total phenol levels were higher in *S. persica* than in *S. oleoides* had a higher total phenolic content of 253.10 mg/g and total flavonoid content of 43.65 mg/g in leaf methanol extracts, while *S. persica* had total phenolic content of

252.770 mg/g. Variations in TFC and TPC results may have been due to changes in agro-climatic, regional, and seasonal variables [41] .There is proof that the leaf extract of this particular tree has analgesic, anti-inflammatory, and anti-ulcer qualities.

Seema Dhankhar et al, 2013 reported the effects of different extract fractions (methanol, acetone, and aqueous) extracted from the mycelia of 17 endophytic fungi Salvadora oleoides, on glucose-loaded fasting and alloxan-induced diabetic albino rats, as well as their antidiabetic and hypolipidemic properties. A glucose tolerance test revealed that only four extracts significantly reduced blood glucose levels: the unknown fungus (water-soluble), *Aspergillus sp*. JPY1 (methanol), and *Phoma sp.* (acetone). *Aspergillus sp*. JPY2(methanol).

The study found that rats with diabetes caused by alloxan experienced the most significant decrease in blood glucose levels five hours into acute treatment trial and on the 14th day of the subacute treatment. In the long-term treatment, blood glucose levels were reduced by 11.3% to 28.04%, while the usual medication tolbutamide reduced them by up to 40%. The aqueous extract of an unknown fungus significantly improved the rats' body weight and lipid profile. The primary components were phenol and 2, 6-di-tert-butyl-p-cresol and 2, 6-bis (1, 1-dimethylethyl)-4-methyl, respectively, according to GCMS (gas chromatography mass spectrometer) analysis of the aqueous bioactive fraction of the unidentified fungus and the methanolic extract fraction of *Aspergillus sp.* JPY1. The findings also showed that the four bioactive fractions stated above had a strong margin of safety and were not responsible for any fatal side effects in animals up to dosages of 1000 mg/kg by weight. in addition to being safe to use on human erythrocytes at levels of up to 500 µg /ml [42].

Numerous pharmacological activities, including hypolipidemic and hypoglycemic activity, anti-ulcer, antibacterial activity, anti-inflammatory, analgesic and antifungal action, have been reported from *S. oleoides*. The extract of methanol was separated using activity direction, and the results showed that the fraction consisting of n-butanol had the highest level of hyperlipidemic activity. Sub-fractions were also isolated as a result of the n-BuOH fraction. In keeping with our efforts to extract the active ingredient from S. oleoides, a flavonoid glycoside was recovered from the n-BuOH fraction [43].

Chronic and degenerative illnesses including diabetes mellitus, atherosclerosis, and cancer are mostly caused by free radicals [31]. The study analyzed antioxidant activity of various extracts, with aerial, stem, and root extracts showing the highest scavenging in the DPPH assay. Aerial methanol extract was the most active in the reducing power

experiment, followed by stem methanol and root methanol. DCM extracts showed decreased capacity for scavenging and decreasing. Aerial components had more antioxidants than stem and root, and the high flavonoid concentration of the leaf extract may be responsible for its greatest efficacy.

Researchers have found a linear relationship between the amount of bioactive ingredients and their capacity to scavenge radicals. However, when compared to methanol extracts, the aerial, stem, and root DCM extracts showed the best overall antioxidant activity for the phosphomolybdenum assay. This is due to the presence of non-phenolic antioxidants in DCM extracts, such as tocopherol or vitamin C. This finding is consistent with previous studies (Albayrak et al., 2010; Liorent-Martınez et al., 2017), which showed the higher overall antioxidant capacities of DCM solvent [44].

2.1.2 Reported Phytoconstituents of *Salvadora oleoides*

Withania somnifera and *Salvadora oleoides* are two common plants in Pakistan that are known to have a variety of medicinal uses. The goal of the current study was to subjectively and quantitatively assess the presence of various phytochemicals in these plants. Examining these phytochemicals' antioxidant and antibacterial capabilities towards *E. coli, Aspergillus terreus*, and *Aspergillus niger* and *Shigella Spp* marked the conclusion of this work [69].

Furthermore, a quantitative analysis of reducing sugars, sugars, phenolics, total proteins, flavonoids was conducted in addition to a qualitative assessment of the phytochemical contents, including glycosides, steroids, flavonoids, alkaloids, tannins, and terpenoids. Antioxidant and antibacterial properties of extracts from plants were also investigated. The findings showed that both plants had a broad variety of phytoconstituents, such as flavonoids, saponins, steroids, glycosides, alkaloids, flavonoids, tannins, and terpenoids[88]. The varied reaction was quantified for a small number of phytoconstituents that are pharmaceutically relevant. Antioxidants were detected in S. oleoides in extracts made with methanol of leaves and in *W. somnifera* in water leaves extract. The medicinal plants' leaves, bark, and roots all showed inhibitory action against the tested bacterial and fungal strains when extracted in methanol. Our research provides strong proof that these therapeutic plants include phytoconstituents with pharmacological significance, suggesting that they might be a useful substitute for conventional medication [45].

The fact that phenolic compounds make up one of the biggest and most prevalent groups

of plant metabolites helps to explain the current study's findings. They have biological properties that include anti-carcinogen, anti-atherosclerosis, cardiovascular protection, anti-aging, anti-inflammatory, and enhancement of endothelial function. They also decrease cell proliferation and angiogenesis [34]. However, the current study's results are very promising and strongly suggest that additional research be done on *W. somnifera* and *Salvadora oleoides* to determine other potential therapeutic benefits [46]. Nidhi Varshney et al. found that Cr in S. oleoides leaves and stems is within the permitted limit, as per the WHO/FAO. Cr is a micronutrient essential for human metabolism of proteins, fats, and carbohydrates. It is also a component of the glucose tolerance factor and is considered the primary trace element in treating and preventing hyperlipidemia and hyperglycemia in type II diabetes mellitus. The permitted Cr level in medicinal plants is 2 ppm [34].

Plants use metabolic processes to manufacture a variety of organic compounds. They are used as artificial medicine substitutes because of their antimicrobial and antioxidant properties [52]. The findings, indicate that successive extracts of both plant parts included main (carbohydrates, proteins, lipids and amino acids) secondary (alkaloids, flavonoids, phenols, mucilage etc.) metabolites [47]. Alkaloids were detected in trace amounts in the ethyl acetate extracts of both plant sections and identified in non-polar to relatively more-polar solvent extracts of leaves. Alkaloids are thought to be a source of waste products and nitrogen because of their noticeable physiological effects [56]. Because it has a higher proportion, it was discovered that a chloroform-based extract of *S. oleoides's* leaf and stem may be utilized to extract glycosidic compounds. Strong pharmacological properties are found in glycosides, and their phenolic group content increases the likelihood that they will be used in the manufacturing of herbal medications [57]. Both the chloroform and ethyl acetate extract of the stem and the ethanolic leaf extract have an excess of flavonoid components. One of the main advantages of consuming *S. oleoides* appears to be its high flavonoid content, which is essential for blocking the formation, progression, and promotion of cancer through a variety of mechanisms [58]. Compared to other non-polar solvent extracts, phenolic compounds were found in very small amounts in polar solvent extracts of the leaf and stem [48].

The study examined the hypolipidemic and antidiabetic properties of extract fractions from seventeen endophytic fungi. Four endophytic fungi showed significant antihyperglycemic effects in glucose-loaded normal rats and alloxan-induced diabetic

rats. The extract fractions improved blood glucose tolerance nearly as much as tolbutamide [82]. Fungal extracts showed marginal reduction in blood glucose levels in rats with alloxan-induced diabetes after acute and subacute treatment. The fungus's aqueous extract fraction showed stronger antidiabetic efficacy due to tannin and terpenoids, and significantly decreased blood sugar levels over extended periods [67]. A strong antidiabetic compound with minimal damage to human cells is produced by an unidentified endophytic fungus, according to the study. Plant extracts from S. oleoides have been demonstrated to have antidiabetic effects, lowering blood sugar levels in rats and mice. After three hours, the ethanolic extract brought the mice's blood glucose levels down to 17.00%, however the diabetic rats' blood glucose levels did not decrease with immediate therapy [67]. Because S. oleoides contains organic sulfur compounds, diabetic rats administered alloxan showed a considerable reduction in blood glucose levels on day 14. These substances are thought to have anti-diabetic and hypoglycemic effects. The phytoconstituents of the host plant may have reduced blood glucose because they may have passed genetic data from the plant to fungus or vice versa [42].

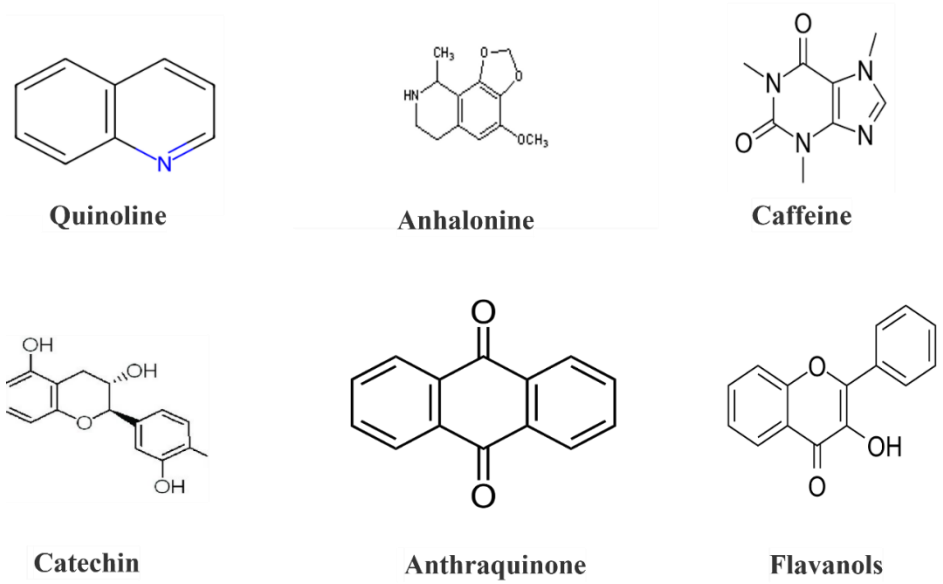


Figure 2.1 Structures of some isolated compounds from *Salvadora oleoides*

Chapter 3
Materials and Methods

3.1 Chemicals and Equipment's

Soxhlet apparatus, Buchi rotavapor (Labortechnik AG9230 Flawil/Switzerland). Chemicals were methanol, acetonitrile, 2-propanol, DMSO, ethanol were purchased from Sigma Aldrich, RCI LABBSCAN LIMITED, Unichem and Ducksin.

3.2 Selection Collection and Identification of Plant

The selected plant Salvadora oleoides was selected based on its use as antidiabetic and antioxidant plant. The plant was collected on 14 august 2023 from Sahiwal, Punjab, Pakistan. The plant was authenticated with the help of plant taxonomist, Dr. Abdul Rehman Niazi, University of the Punjab, Lahore. The plant samples were submitted in herbarium section of the said institute and details of plant specimen are mentioned in Table 3.1: Collected part of plants, composition and voucher number are present

Plant name	Part collected	Voucher number
Salvadora oleoides	Stem and leaves	LAH#095024

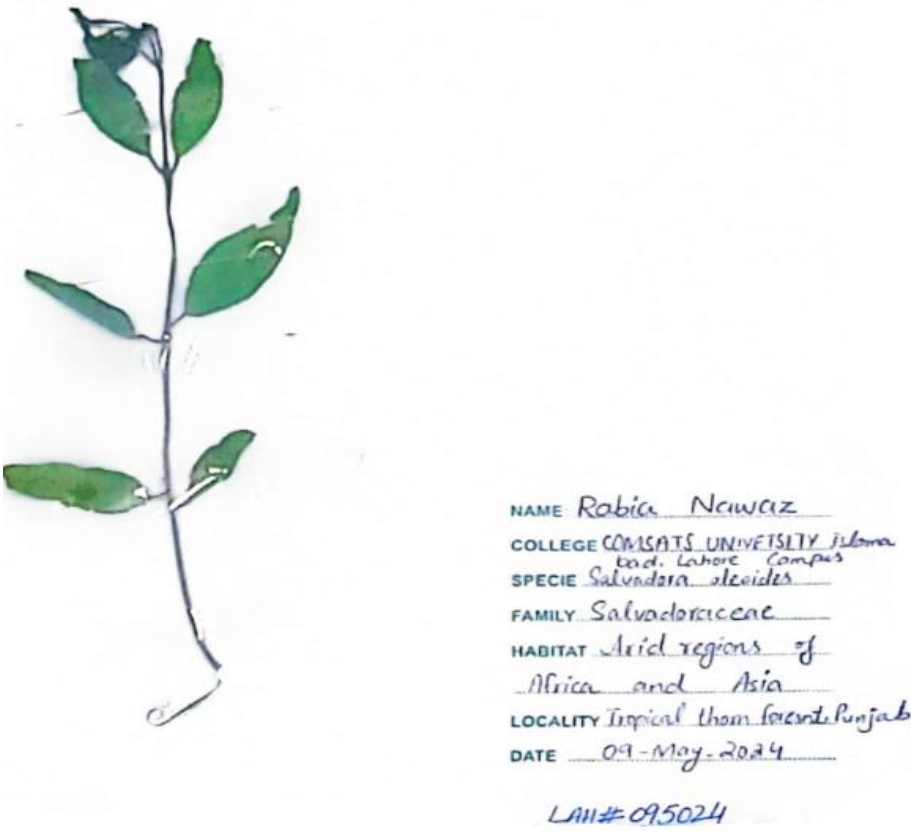


Figure 3.1 Authentication of *Salvadora oleoides*

3.3 Processing and extraction of Plants

In a recent study, the leaves of Salvadora oleoides were carefully washed and subsequently dried at 45℃ temperature in oven. This process ensured that the leaves were in optimal condition for further analysis. Plant samples were undergoing a grinding process to produce a fine powder. This was achieved by a grinding mill, which effectively reduced the plants materials to a powdered form. The powdered material weighing 1.5kg underwent a sequential extraction process using methanol (2.5L), acetonitrile (2.5L) and 2, propanol (2.5L) respectively. The purpose of this extraction was to isolate specific compounds present in the material. The process involved the use of solvents with varying polarities to extract different compounds. A rotavapor (Labortechnik AG 9230 Flawil/ Switzerland) was used to concentrate extracts obtained from a filtration process using filter paper. The resulting yields were 84.5 g (5. 63%), 41g (2.73%), and 140g (9.33%) of methanol, acetonitrile and 2, propanol, respectively. After the extraction process, the resulting extracts were carefully stored in a refrigerator at a temperature of4℃ ,until they were ready to be utilized.

3.4 Preliminary Phytochemical Tests

The selected plant extracts were analyzed using a conventional method to identify various compounds such as carbohydrates, alkaloids, flavonoids, phenolic compounds, tannins, amino acid, proteins, steroids, terpenes, and saponins using standard procedure with the slight modification.

3.4.1 Carbohydrate Identification Test

1 gram of methanol extract and 1 gram of acetonitrile extract were separately diluted in 5ml of distilled water and filtered. The resulting filtrates were used in experiments to identify carbohydrates.

3.4.1.1. Benedict's test

The emergence of an orange or red tint suggested the presence of reducing sugars. 1 ml of Benedict's reagent and 1 ml of the stock solution's plant extract filtrate were combined in a test tube. After that the final mixture spent five minutes in boiling water. Reducing sugars are indicated by the appearance of red or orange.

3.4.1.2. Molisch's Test

One milliliter of filtrate from the stock solution was taken, and two drops of the naphthol solution were added. The development of a violet ring at the test tube's bottom proved that there were carbs present.

3.4.2 Test for Alkaloids

To make the stock solution, each methanol extract and acetonitrile extract (50mg) were dissolved in 5ml HCl (1%) and the test tube was gently heated for 1 minute on a water bath.

3.4.2.1 Dragendorff's Test

A test combination of 0.2ml of weal HCl and 1ml of extract solution was prepared in a test tube using a stock solution. One milliliter of Dragendorff's reagent was added to this blend. The emergence of a characteristic crimson or orange precipitate indicated the presence of alkaloids.

3.4.2.2 Mayer's Test

The experimental procedure, a test tube was employed to carefully mix 1ml of a filtered stock solution with 0.5ml of Mayer's reagent, specifically Potassium Mercuric Iodide. The purpose of this combination was to determine the presence of alkaloids in the solution. The formation of a white or yellow precipitate served as an indicator for the appearance of alkaloids.

3.4.3 Tests for Flavonoids

3.4.3.1 Lead Acetate Test

A test tube was utilized to carry out the following experimental procedure. Initially, 0.5 ml of a 10% lead acetate solution was carefully introduced into the test tube. The same test tube was then filled with 2 milliliters of the sample solution. The purpose of this process was to investigate the presence of flavonoids in the sample solution. The indication of flavonoid presence was demonstrated by the occurrence of a yellow precipitate in the test tube.

3.4.4 Test for Phenolic Compounds

3.4.4.1 Ferric Chloride Test

To begin the experimental procedure, 2 grams of extract obtained from specific plant extract was diluted in 10ml distilled water. The resulting solution was filtered using test

tube. Next, 1ml of a 5% w/v solution of ferric chloride was added to filtrate. The objective of this step was to detect the presence of phenols in the solution. A visual indication of the presence of phenols was observed through the formation of a green or bluish-black precipitate.

3.4.5 Test for Tannins

3.4.5.1 Gelatin Test

To detect tannins, a test tube was used to mix 2ml of the test tube solution from the stock solution, 1 ml of gelatin solution (1%), and 1ml of NaCl solution (10%). The detection of tannins was accomplished by a straightforward visual observation. When a sample containing tannins was treated with gelatin, a white precipitate was formed, indicating the presence of tannins.

3.4.6 Test for Amino Acids

3.4.6.1 Ninhydrin Test

To identify the presence of amino acids, a test tube was employed for the following experimental procedure. First, 1 gram of the sample was mixed in 5ml of distilled water and created a test solution. Then, 2ml of this test solution was combined with 1ml of a 0.2% ninhydrin solution in the test tube. The resulting mixture was subsequently heated in a water bath for 2-3 minutes. If amino acids were present in the sample, a distinct blue or violet color would manifest, indicating their presence.

3.4.7 Test for Proteins

3.4.7.1 Xanthoproteic Acid Test

The material was combined with 0.5 milliliter of strong nitric acid in testing tube and shaken for about five minutes. The emergence of a unique yellow tint indicated the presence of proteins.

3.4.8 Test for Steroids

3.4.8.1 Salkowski’s Test

Five milligrams of selective herbal extracts were mixed in one milliliter of chloroform to find steroids. After that, 0.5 cc of sulfuric acid solution was added very carefully. The test tube's bottom had a golden yellow tint, which suggested the presence of steroids.

3.4.9 Terpenoid

3.4.9.1 Copper Acetate Test

The following stages were part of the experimental approach to confirm the existence of diterpenes. In test tubes, 1g of carefully chosen plant extracts was first combined with 2ml distilled water. Next, the mixture was mixed with 2.5 milliliters of copper acetate solution. The emergence of an emerald, green tint indicated the existence of diterpenes.

3.4.10 Test for Saponins

3.4.10.1 Foam Test

In the process of testing for the presence of saponins, 0.5g extract was combined 2ml distilled water in a test tube. Mixture was vigorously mixed several minutes. Presence of saponins was determined by the formation of a persistent, continuous froth that approximately 10 minutes. The presence of this froth served as an indicator for the presence of saponins in the extracts.

3.5 Anti angiogenic Activity

We evaluated anti-angiogenic potential of samples using CAM assay in fertilized chicken eggs as mentioned by [89] with minor modifications.

3.5.1 Fertilized Chicken Eggs

We obtained 2-3 days old, fertilized chicken eggs from a nearby poultry farm.

3.5.2 Materials and Reagents

The materials used in the experiment were Whatman Filter paper, sterile surgical tape (Nichophore surgical tape No.21), sorafenib (Sonib, Pharmasol Private Limited), DMSO (Sigma-Aldrich, Germany), cotton swab, and sterile distilled water.

3.5.3 Equipment's

The equipment used in the experiment included an HDD-Egg Incubator (China), an egg candler, push pin, needle nose forceps, sterile forceps, dissection scissors, 8-number feeding tube, 10 ml syringes, and 2-20 µl pipette.

3.5.4 Preparation and Administration of Test Samples

The DMSO and apparatus were autoclaved, while were sterilized using UV. The plant extracts (10mg) were dissolved in 300 µl of DMSO using a vertex mixer, and distilled water was gradually added to achieve volume of 10ml. Table 3.2 outlines the dilutions made from the stock solutions. The positive control (Sorafenib, 100 µg/ml) was

prepared in a similar way, and the vehicle used was considered the negative control. On the fifth day of incubation, 25µl of each sample were applied dropwise on a 3 mm filter paper and placed on the CAM with the highest density of blood vessels.

Table 3.1: Group and doses of all extracts and positive control

Sr. #	Groups	Drug/ Extract used
1	Group A	125 µg/ml of Methanol extract
2	Group B	250 µg/ml of Methanol extract
3	Group C	500 µg/ml of Methanol extract
4	Group D	1000 µg/ml of Methanol extract
5	Group E	125µg/ml of Acetonitrile extract
6	Group F	250µg/ml of Acetonitrile extract
7	Group G	500µg/ml of Acetonitrile extract
8	Group H	1000µg/ml of Acetonitrile extract
9	Group I	125µg/ml of 2-propanol extract
10	Group J	250µg/ml of 2-propanol extract
11	Group K	500µg/ml of 2-propanol extract
12	Group L	1000µg/ml of 2-propanol extract
13	Group M	100µg/ml of standard drug- sorafenib
14	Group N	Negative control (DMSO)

3.5.5 Experimental design

The eggs were sterilized with a cotton swab and incubated facing the narrow pole downward at 60/70% humidity and 37-38℃. In an automatic egg incubator, the temperature is set. The eggs were candled in a candling light after 48 hours of incubation by shining the light at the blunt ends of the eggs where the air sack is located to distinguish the fertilized eggs from the unfertilized eggs. On the fifth day of incubation, a small hole was carefully cut in narrow pole egg with the scissors, and at least 2ml of egg albumin was sucked out with a syringe. To minimize the contamination and albumin leakage, and shell-puncture was closed with sterile surgical tape. The eggs were then incubated for another 2,3 hours to allow the chorioallantoic membrane to separate from the eggshell. As shown in Table 3.2, different quantities of materials were produced in DMSO and distilled water. Whatman filter paper was used to make 3 mm filter paper discs using a paper punch machine. Several extract concentrations were

applied to the CAM using 3 mm filter paper discs. The negative er control was the vehicle, while 100µg/ml sorafenib sterilize the broader pole of the eggshell. Prior to creating the window, the surgical tape was adhered to the shell to avoid creating significant cracks in the eggshell and to make the cutting process smoother. A small opening was carefully made using sterilized scissors and fine forceps to expose the CAM. The filter paper disc loaded with the extract was sample was tested in at least six replicates. After applying the various doses, the opening was sealed with sterile surgical tape, and the eggs were incubated for an additional 24 hours. Then, the filter paper discs were delicately removed, and images of the vascular bed were captured using a digital camera.

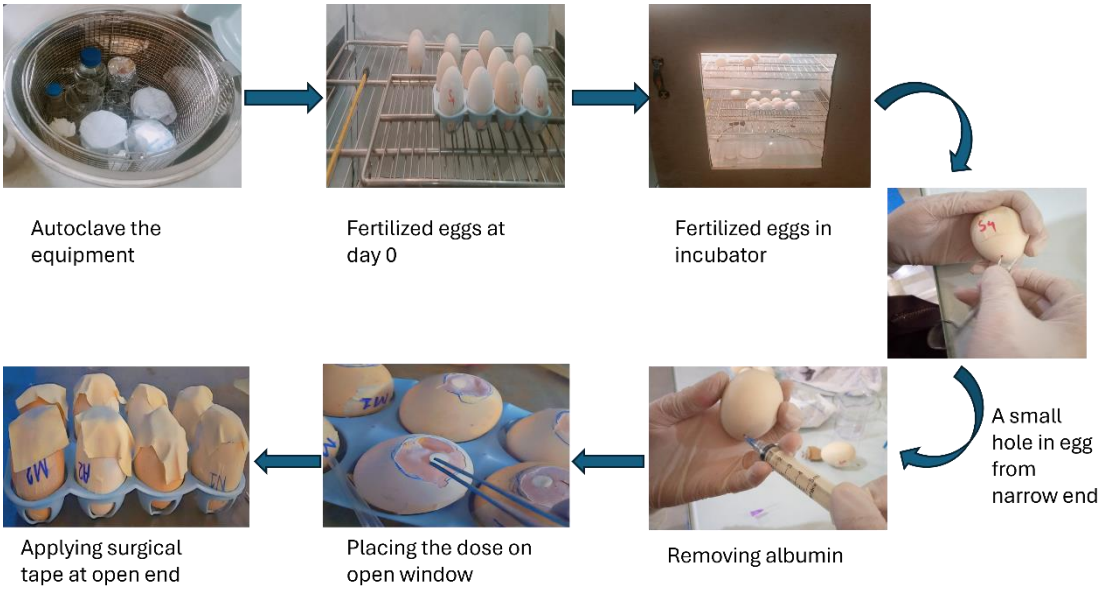


Figure 3.2: Steps involved in CAM Assay

3.5.6 Measurement of Antiangiogenic Response

The photographic analysis was used to observe and determine the antiangiogenic response of each dose.

3.5.7 Photographic Analysis

Images of the CAMs were captured using a digital camera after 24 hours of treatment with each sample. The number of blood vessels and their branches were manually counted, and their mean values were compared with those of the positive and negative control groups. % inhibition of blood vessel growth was calculated using following formula:

$$\% \text{ Inhibition} = \left(1 - \frac{\text{Number of Vessels in Treatment group}}{\text{Number of Vessels in Control group}}\right) \times 100$$

3.5.8 Statistical Analysis

ANOVA test (one way analysis of variance) and Tukey's post hoc test were used in the statistical study to compare the treatment and control groups. The significance threshold was established at $p < 0.01$(**), $p <$ (***), $p < 0.05$(*). The average of the data was displayed together with the standard error of the means, or SEM.

3.6 Hypoglycemic Activity on Albino Rats

We used a slightly modified approach to evaluate hypoglycemic effect of *Salvadora oleoides* leaf extracts.

3.6.1 Animal Collection

Male albino rats weighing 100-200 grams were procured from animal research and service center department of Pharmacy COMSATS university Lahore and were housed in the animal transit room where all the experimental procedures were conducted. The rats were given free access to food and water, except during the experimental procedures, for which the food was withdrawn for 12 hours prior to the commencement of the procedure.

3.6.2 Preparation and Administration of Test Samples

Doses of varying concentrations, namely 125mg/ml, 250mg/ml, and 500mg/ml, were prepared for all groups using each extract. To facilitate appropriate binding, 1% Tween 80 was added to the preparations. The total volume of each dose was adjusted to 1ml by incorporating distilled water.

Table 3.2: Drug doses of different concentrations

Groups	**Drug/ Extract used**
Group A	125mg/ ml of Methanol extracts
Group B	250mg /ml of Methanol extracts
Group C	500mg/ ml of Methanol extracts
Group D	125mg/ ml of Acetonitrile extracts
Group E	250mg/ ml of Acetonitrile extracts
Group F	500mg/ ml of Acetonitrile extracts
Group G	125mg/ ml of 2-propanol extracts
Group H	250mg/ ml of 2-propanol extracts
Group I	500mg/ml of 2-propanol extracts
Group J	5mg/ml of standard drug-
Group K	Negative control (Distilled water+ Tween 80)

3.6.3 Experimental Design

Eleven groups made up of three replicates each were created by randomly assigning the rats to each group. The herbal extract was given orally to rats in group 1 through 9 after an overnight fasting interval. The doses given for methanol, acetonitrile, 2-propanol extracts were 125mg/ml, 250mg/ml, and 500mg/ml respectively. The extracts were reconstituted in distilled water with 1% Tween 80. A single limit dosage of positive control, 5mg/kg, reconstituted in 1% Tween 80 in distilled water, was administrated to group 10 rats. Rats in group 11 acted as control group and distilled water containing 1% Tween 80. After 30 minutes, each rat orally received a dose of herbal extracts with methanol, acetonitrile and 2-propanol. The blood glucose level was measured in each rat with digital glucometer before and after the dosage. A small puncture was introduced in the tail of each albino rat for blood. The strips were used to check the blood glucose level, a drop of blood was applied onto the strip and blood sugar levels were noted. The mean differences between control groups and treated groups were compared.

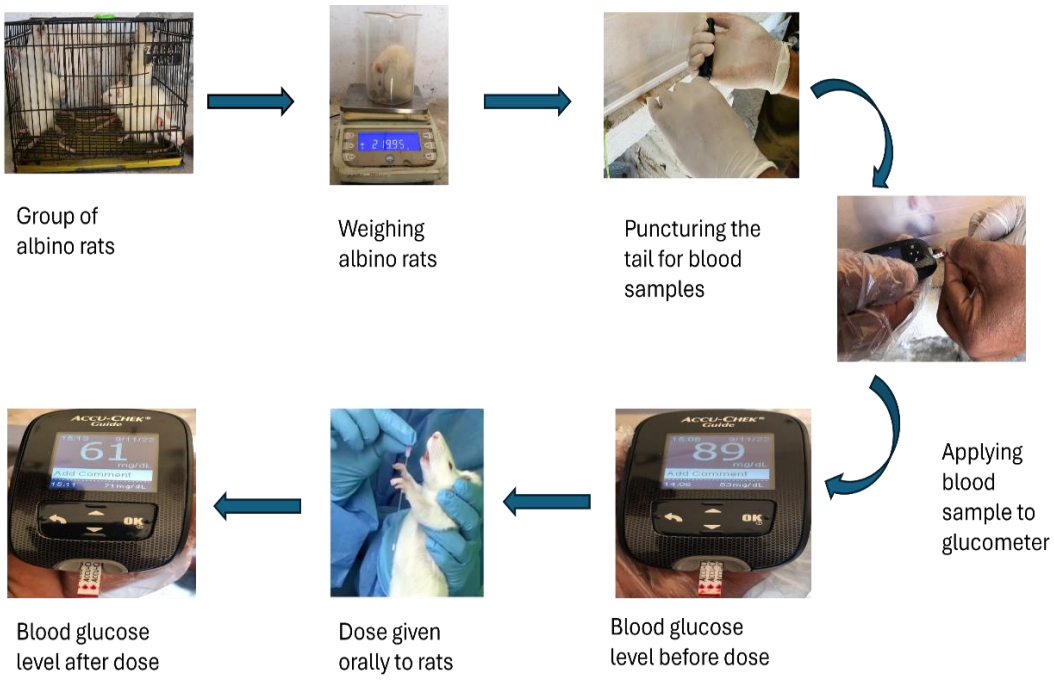


Figure 3.4.13: Hypoglycemic activity on albino rats

3.6.4 Data Collection

Recorded the blood sugar levels of each rat before and after the dose given with the help of digital glucometer.

3.6.5 Measurement of Anti-Diabetic response

The antidiabetic response was measured utilizing the following formula:

$$\% \text{ inhibition} = \frac{b-a}{b} \times 100$$

A= After dose value & B= Before dose value

% inhibition was calculated by using this formula:

$$\% \text{ inhibition} = \left(1 - \frac{\%\text{Inhibition in treatment group}}{\%\text{ inhibition in Control group}}\right) \times 100$$

3.6.6 Statistical Analysis

ANOVA test, two-way analysis of variance (ANOVA) and Tukey's post hoc test were used in the statistical study to compare the treatment and control groups. The significance threshold was established at $p<0.05$ (*), $p<0.01$ (**), and $p<0.001$ (***). The standard error of means, or mean plus SEM, was used to present the data.

Chapter 4
Results and Discussions

4.1 Preliminary Phytochemical test of Methanol extract

Various tests were conducted in the initial phytochemical examination of extract from methanol to determine the makeup of its compounds. Significant phytochemical groups, such as steroids, flavonoids, carbohydrates, alkaloids, tannins, phenols and terpenoids, were found in the data. But the methanol extract did not include any proteins, amino acids, or saponins. Results are mentioned in table 4.1.

Table 4.1: Preliminary Phytochemical test of Methanol extract

Phytochemical Constituents	**Chemical Test**	**Presence**
Carbohydrates	Benedict's test	+
	Molisch's test	+
Alkaloids	Dragendorff's tests	-
	Mayer's test	-
Amino acids	Ninhydrin test	-
Tannins	Gela tin test	+
Flavonoids	Lead acetate test	+
Proteins	Xanthoproteic Acid test	+
Steroids	Salkowski's test	+
Terpenoids	Copper Acetate test	+
Saponins	Foam test	+
Phenols	Ferric chloride test	+

(Present: + Absent: -)

4.2 Preliminary Phytochemical test of Acetonitrile extract

A preliminary investigation was conducted on a acetonitrile extract to determine the primary groups of phytochemical components. Multiple phytochemical tests were performed for this purpose. The results unveiled the presence of significant groups of phytochemical components like carbohydrates, terpenoids, phenols, saponins, flavonoids, tannins alkaloids. However, the analysis of the acetonitrile extract did not indicate the presence of amino acids, proteins, and steroids.

Table 4.2:Preliminary Phytochemical test of Acetonitrile extract

Phytochemical constituents	**Chemical test**	**Presence**
Carbohydrates	Benedict's test	+
	Molisch's test	-
Alkaloids	Dragendorff's test	+
	Mayer's test	+
Tannins	Gelatin test	-
Flavonoids	Lead acetate test	+
Proteins	Xanthoproteic Acid test	+
Steroids	Salkowski's test	-
Terpenoids	Copper acetate test	-
Saponins	Foam test	+
Phenols	Ferric chloride test	+

(Present: + Absent: -)

4.3 Preliminary Phytochemical test of 2-Propanol extract

A preliminary investigation was conducted on 2-propanol extract to determine the primary groups of phytochemical components. Multiple phytochemical tests were performed for this purpose. The results unveiled the presence of significant groups of phytochemical components such as carbohydrates, alkaloids, flavonoids, phenols, tannins, terpenoids, and saponins. However, the analysis of the 2-propanol extract did not indicate the presence of amino acids, proteins, and steroids.

Table 4.3: Preliminary Phytochemical test of 2-Propanol extract

Phytochemical constituents	**Chemical test**	**Presence**
Carbohydrates	Benedict's test	+
	Molisch's test	-
Alkaloids	Dragendorff's test	+
	Mayer's test	+
Tannins	Gelatin test	-
Flavonoids	Lead acetate test	+
Proteins	Xanthoproteic Acid test	-
Steroids	Salkowski's test	+
Terpenoids	Copper acetate test	+
Saponins	Foam test	-
Phenols	Ferric chloride test	+

(Present: + Absent: -)

4.4 CAM ASSAY

The antiangiogenic effects of Herbal extracts were examined using CAM assay. Sorafenib with concentration of 100µg/ml was used as positive control. DMSO and distilled water were used as negative control. For the evaluation of antiangiogenic activity of sequential extract of petroleum ether, chloroform, methanol, photographic analysis, and manual counting of the number of blood vessels and branches method were used. Doses of different concentrations (125, 250, 500,100 µg/ml) of sample were used and the obtained results of treatment groups were compared with positive and negative control groups as shown in fig.4.1. The mean values of number of BV and capillaries per CAM per group were counted and the graph in which both number of BV and % inhibition of each group was compared with the mean values of negative and positive control groups as shown in fig. All the results are summarized in fig and the values are compared in the graph shown in figure 4.2.

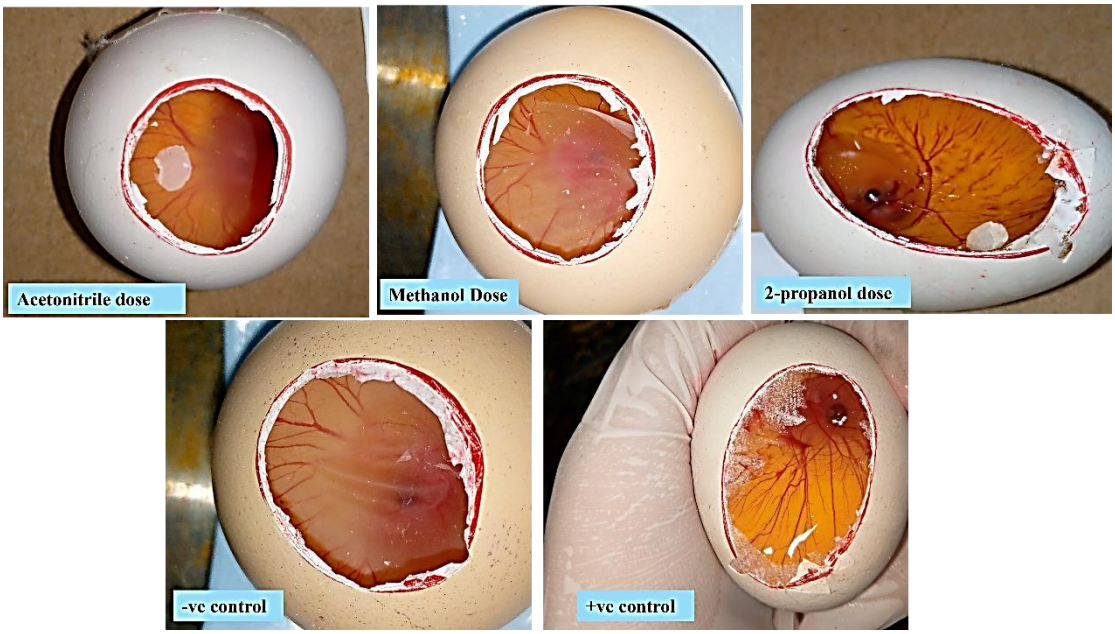


Figure 3.4.1: CAM assay, blood vessels with different extracts along with positive and negative control

4.5 Antiangiogenic activity

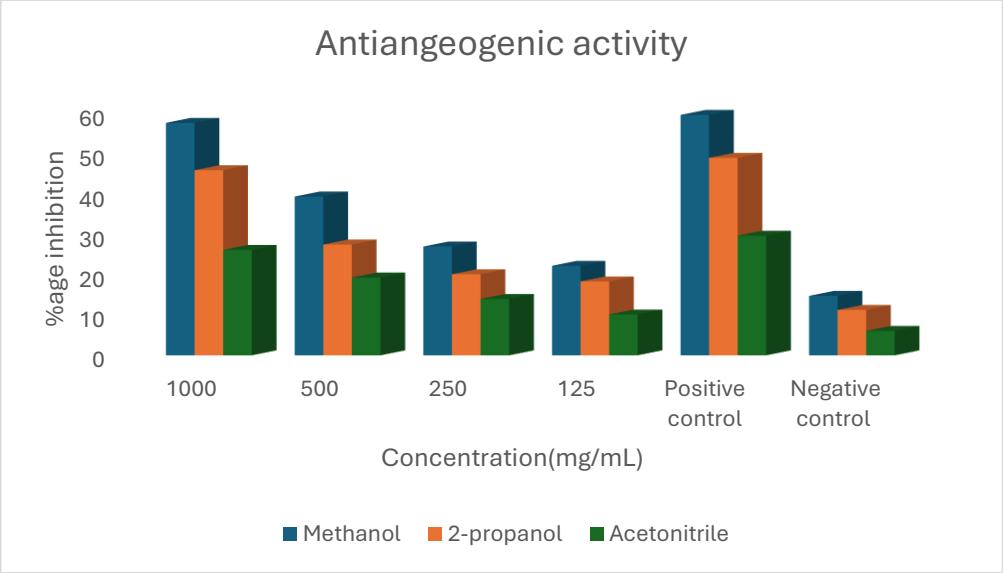


Figure 4.2: Antiangiogenic activity with extracts, positive and negative control

4.5.1 Antiangiogenic potential of Methanol extract

In comparison to the negative and positive control groups, where the average value of the number of BVs and branches was 148.5 and 65.5, respectively, the highest dosages of 1000 µg/ml concentrations of the methanol extract applied on CAMs demonstrated significant ($p<0.001$***) antiangiogenic potential. After administering a 1000 µg/ml dosage of methanol extract, % inhibition in BVs were 57.91%. In contrast, the substantial positive controls (sorafenib, 100 µg/ml) showed 59.89% suppression of BVs. When compared to methanol extract and the positive control, the inhibition of methanol extracts was less successful. Additionally, there were less BV and branches beneath the discs containing 1000 µg/ml of methanol extract.

Images taken after the 24-hour treatment showed a capillary-free region underneath the discs and a noticeable reduction in the large pre-existing BV as well as branches. With a mean value that was 148.5 per CAM, the group with a negative control had significantly more BVs and branches.

When CAMs were treated with a 500 µg/ml concentration of methanol extract from certain plants, they likewise shown substantial ($p<0.05$*) antiangiogenic action, with a mean value of 107.5 branches and BVs, in contrast to the negative control, which had a mean value of 148.5 branches and BVs. After receiving a 500 µg/ml dosage, the

proportion of the blood vessels inside CAMs that were inhibited was 39.56%, whereas both the negative and positive control groups showed a percentage of blood vessel inhibition of 58.89%. The number of larger already present blood vessels was moderately affected in relation to the effects of the positive control while 2-propanol extract, but the small capillaries under the disk were severely impacted and nearly absent after treatment at 500 µg/ml of the extract.

Following treatment with a sample concentration of 250 µg/ml for methanol extracts, the blood vessels as well as branching patterns of CAMs were not significantly altered; however, the region beneath the disc was. Insignificantly, there were 118.25 fewer blood vessels as well as branches on average than in the negative control group. Only 27.1% of blood vessels were inhibited, compared to 58.89% in the positive control group. With 129.5 average values of the number of BVs as well as branches, the concentration of 125 µg/ml of the plant extract demonstrated a very little antiangiogenic impact on the blood vessels, which is less than the value of the group with a negative control (148.5).

4.5.2 Antiangiogenic potential of 2-propanol extract

In comparison to the negative and positive control groups, where the average value of the number of BVs and branches was 148.5 and 65.5, respectively, the highest dosages of 1000 µg/ml concentrations of the 2-propanol extract applied on CAMs demonstrated significant ($p<0.01$**) antiangiogenic potential. After administering a 1000 µg/ml dosage of 2-propanol extract, % inhibition in BVs were 46.12%. In contrast, the substantial positive controls (sorafenib, 100 µg/ml) showed 49.2% suppression of BVs. When compared to methanol extract and the positive control, the inhibition of 2-propanol extracts was less successful. Additionally, there were less BV and branches beneath the discs containing 1000 µg/ml of 2-propanol extract.

Images taken after the 24-hour treatment showed a capillary-free region underneath the discs and a noticeable reduction in the large pre-existing BV as well as branches. With a mean value that was 148.5 per CAM, the group with a negative control had significantly more BVs and branches.

When CAMs were treated with a 500 µg/ml concentration of methanol extract from certain plants, they likewise shown substantial ($p<0.05$*) antiangiogenic action, with a mean value of 107.5 branches and BVs, in contrast to the negative control, which had a mean value of 148.5 branches and BVs. After receiving a 500 µg/ml dosage, the

proportion of the blood vessels inside CAMs that were inhibited was 27.6%, whereas both the negative and positive control groups showed a percentage of blood vessel inhibition of 11.3% and 49.2% respectively. The number of larger already present blood vessels was moderately affected in relation to the effects of the positive control while 2-propanol extract, but the small capillaries under the disk were severely impacted and nearly absent after treatment at 500 µg/ml of the extract.

Following treatment with a sample concentration of 250 µg/ml for methanol extracts, the blood vessels as well as branching patterns of CAMs were not significantly altered; however, the region beneath the disc was. Insignificantly, there were 118.25 fewer blood vessels as well as branches on average than in the negative control group. Only 20.2% of blood vessels were inhibited, compared to 49.2% in the positive control group. With 129.5 average values of the number of BVs as well as branches, the concentration of 125 µg/ml of the plant extract demonstrated a very little antiangiogenic impact on the blood vessels, which is less than the value of the group with a negative control (148.5).

4.5.3 Antiangiogenic potential of Acetonitrile Extract

A high dose of 1000 µg/ml of acetonitrile with a mean value of number of BV (113) and an inhibition of just 26.26% was observed in the CAM treated with selected plant acetonitrile extract; other doses of acetonitrile extract, 500 µg/ml, 250 µg/ml, and 125 µg/ml showed inhibition of 19.38%, 14.04%, and 10.16%, respectively, were compared to the positive control with an inhibition of 29.8%.

4.6 Antidiabetic activity of Salvadora oleoides

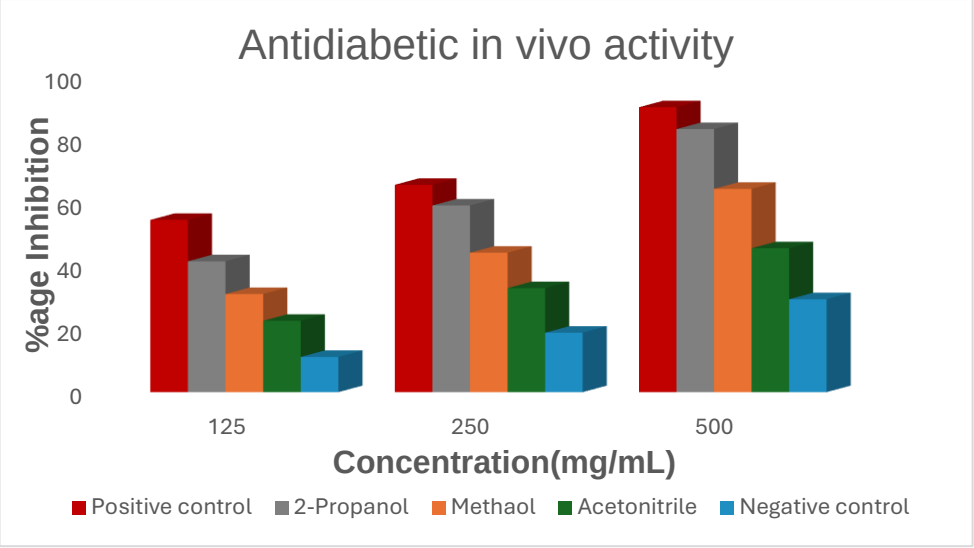


Figure 4.3: Antidiabetic in vivo activity of *Salvadora oleoides*

Antidiabetic activity in vivo of Salvadora oleoides extract is studied against different solvents which are 2-propanol, methanol, acetonitrile and negative control at different concentrations of 125mg/mL, 250mg/mL, and 500mg/mL. 2-Propanol gave percentage of inhibition at 500mg/mL, 250mg/mL and 125mg/mL were 83%, 58% and 41%, respectively. While methanol gave percentage inhibition at 500mg/mL, 250mg/ml and 125mg/ml were 64%, 44% and 30% respectively. Additionally, acetonitrile exhibited percentage inhibition at 500mg/ml, 250mg/ml and 125mg/ml were 45%, 32% and 22% respectively. Negative control depicted the percentage inhibition at 500mg/ml, 250mg/ml and 125mg/ml were 29%,18% and11% respectively. Positive control tolbutamide depicted the percentage inhibition at 500mg/ml, 250mg/ml and 125mg/ml were 89.9%, 65.42% and 53.34%. These results depicted that Salvadora oleoides extract in 2-propanol gives highest inhibition in 500mg/mL concentration**.**

4.6.1 Anti-diabetic potential of 2-propanol extract

Comparing the various extract concentrations to both positive and negative controls, it was discovered that the 500 mg/ml concentrations exhibited considerable antidiabetic effect. Following dosage inoculation, 500 mg/ml quantities of 2-propanol extract exhibited considerable ($p<0.001$***) antidiabetic efficacy. Thirty minutes after dosage inoculation, the methanol extract showed a maximal inhibition of 83% at the concentration of 500 mg/ml. When compared to the conventional drug's 89.9% inhibition, the reduction was statistically significant. Following dosage injection, the negative control exhibited a 29% inhibition rate. The 2-propanol extract at a dosage of 250 mg/ml showed a significant ($p<0.001$***) hypoglycemic effect. Thirty minutes after dosage inoculation, the 2-propanol extract showed an inhibition rate of 58.15% at a concentration of 250 mg/ml, which was very similar to the conventional drug's inhibition of 65.42%. Following dosage inoculation, the negative control showed an 18% inhibition rate. Thirty minutes after the dosage inoculation, the 2-propanol extract at 125 mg/ml showed a strong 41% inhibition, like the 53.34% inhibition of the reference medication. The percentage inhibition of the negative control was 11%. The 2-propanol extract at 125 mg/ml concentration showed a significant ($p<0.01$*) hypoglycemic action.

4.6.2 Anti-diabetic activity of Methanol extract

Comparing the various extract concentrations to both positive and negative controls, it was discovered that the 500 mg/ml concentrations exhibited considerable antidiabetic

effect. Following dosage inoculation, methanol extract concentrations of 500 mg/ml exhibited considerable ($p<0.001$***) antidiabetic efficacy. Thirty minutes after dosage inoculation, the methanol extract showed a maximal inhibition of 64% with the concentration of 500 mg/ml. When compared to the conventional drug tolbutamide 89.9% inhibition, this inhibition was of statistical significance. Following dosage injection, the negative control exhibited a 29% inhibition rate. The 2-propanol extract at a dosage of 250 mg/ml showed a significant ($p<0.001$***) hypoglycemic effect.

Thirty minutes after dosage inoculation, the 2-propanol extract showed a reduction in activity of 44% at a concentration of 250 mg/ml, which was substantially like the conventional drug's inhibition of 65.42%. Following dosage inoculation, the negative control showed an 18% inhibition rate. Thirty minutes after dosage inoculation, the 2-propanol extract at 125 mg/ml showed a 30% inhibition, which was significant like the conventional drug tolbutamide 53.34% inhibition. The percentage inhibition of the negative control was 11%. The 2-propanol extract at 125 mg/ml concentration showed a significant ($p<0.01$*) hypoglycemic action.

4.6.3 Anti-diabetic activity of Acetonitrile extract

Comparing the various extract concentrations to both positive and negative controls, it was discovered that the 500 mg/ml concentrations exhibited considerable antidiabetic effect. Following dosage inoculation, acetonitrile extract concentrations of 500 mg/ml shown considerable ($p<0.001$***) antidiabetic efficacy. Thirty minutes after dosage inoculation, the acetonitrile extract showed a maximal inhibition of 45% at a concentration of 500 mg/ml. When compared to the conventional drug tolbutamide, 89.9% inhibition, this inhibition was significantly higher. Following dosage injection, the negative control exhibited a 29% inhibition rate. The 2-propanol extract at a dosage of 250 mg/ml showed a statistically important ($p<0.001$***) hypoglycemic effect.

Thirty minutes after dosage inoculation, the 2-propanol extract showed a reduction in inhibition of 32 percent at a concentration of 250 mg/ml, which was substantially like the conventional drug tolbutamide inhibition of 65.42%. Following dosage inoculation, the negative control showed an 18% inhibition rate. Thirty minutes after the dosage inoculation, the 2-propanol extract at 125 mg/ml showed a strong 22% inhibition, like the 53.34% inhibition of the reference medication, tolbutamide. The percentage

inhibition of the negative control was 11%. The 2-propanol extract at 125 mg/ml concentration showed a significant ($p<0.01$*) hypoglycemic action.

Discussion

Diabetes mellitus (DM) is a condition characterized by high blood sugar levels due to insufficient insulin production or poor cell response. Symptoms include frequent urine, increased thirst, and increased appetite. Diabetes is classified into three categories: Insulin-dependent diabetes mellitus (IDDM), also known as type 1 diabetes, where the body cannot produce insulin, requiring insulin pumps or injections, and insulin resistance, also known as non-insulin-dependent diabetic mellitus (NIDDM), where cells cannot utilize insulin. Diabetes is often classified into three categories. Gestational diabetes, the third type, is caused by elevated blood glucose levels in pregnant women who have never had diabetes before. Currently, insulin and oral hypoglycemic medications are used to treat diabetes mellitus, but they do not maintain normal glucose homeostasis for extended periods and have lifelong side effects like hypoglycemia, renal disease, GIT issues, hepatotoxicity, heart risk issues, and insulinoma. Several herbal medications have shown efficacy due to their natural properties [90].

Angiogenesis is a complex process that involves the coordinated action of various vascular components, including endothelial cell migration, vascular basement membrane breakdown, and endothelial cell proliferation. It is essential for physiological activities like reproduction, embryogenesis, organ differentiation, and tissue repair. Angiogenesis is triggered by external stressors like mechanical stress, hypoxia, and hypoglycemia. Hypoxia stimulates vasculature development during embryogenesis by upregulating HIF-1, endoplasmic reticulum kinases, soluble guanylate cyclase, and mTOR. Angiogenesis also contributes to maintaining the vasculature in adults [51].

Diabetes mellitus is a prevalent metabolic disease affecting 2.8% of the global population and is expected to reach 5.4% by 2025. Despite the use of biguanides and sulfonylureas in oral hypoglycemic medication, herbal therapies are increasingly being explored due to their potential to treat the disease. Herbal remedies have been a respected source of medicine throughout history and their widespread use in contemporary medicine indicates a growing prevalence of their use. The use of herbal therapies, primarily derived from plants, has gained popularity due to the negative effects of oral hypoglycemic drugs for treating diabetes mellitus [91].Over a thousand plant species are used in traditional medicine to treat diabetes, with the chemical

makeup of these plants affecting their biological function. Herbal medications have become increasingly popular as a source of hypoglycemic drugs in recent years. Herbal items, rich in components like phenolic chemicals, terpenoids and coumarins, have been found to lower blood glucose levels. These remedies are recommended due to their perceived efficacy, less adverse effects in clinical practice, and affordable price. For centuries, medicinal plant items have been used to treat diabetes mellitus [92].
Diabetes mellitus and cancer are two prevalent life-threatening illnesses that share risk factors. The development of therapeutic interventions for diabetes and cancer has long attracted attention. However, side effects, financial difficulties, and patient compliance are among the issues with traditional therapies that are being contested. To address these problems, safe and effective therapy approaches must be found. The antioxidant and pro-oxidant qualities of phytochemicals derived from edible plants have emerged as a substitute technique. Naturally occurring substances known as phytochemicals are thought to be promising agents having positive effects that can be used to treat a variety of ailments. Numerous phytochemicals have been identified to have either pro- or antioxidative activities that regulate cell division, proliferation, apoptosis, and cell cycle arrest. These actions can be therapeutic and preventative against diabetes and cancer [93].

Nowadays, there is a lot of interest in phytochemicals' ability to fight cancer. It's interesting to note that depending on their quantities, and structures, phytochemicals can have both pro- and antioxidant qualities. Phytochemicals often have antioxidant qualities when present in low concentrations, but at excessive amounts, they can cause oxidative stress. Phytochemicals with antioxidant activity reduce intracellular ROS levels, which hinders signal transmission for cell proliferation and helps prevent carcinogenesis caused by oxidative DNA damage.

It was also shown that flavonoids, which have antioxidant qualities, may change from pro-oxidants to pro-oxidants at low doses, which causes cancer cells to die. The presence of metal ions, such as copper ions, which are known to be more prevalent in cancer cells compared to normal cells, mediates this process. ROS are produced when a flavonoid undergoes the redox process and is converted into semiquinone in presence of copper ions. According to several research, phytochemicals have demonstrated the ability to prevent cancer and have therapeutic benefits via modulating ROS. Many

clinical investigations on phytochemicals' ability to prevent cancer are now underway [94].

Antioxidant chemicals including diterpenes, tannins, monoterpenes, flavonoids, phenylpropanoids, lignans, cinnamic acid and triterpenes may be found in all parts of plants such wood, bark, stems, pods, leaves, fruit, roots, flowers, pollen and seeds in increasing amounts. According to studies, these phytochemicals offer preventive properties against illnesses like diabetes that are caused by oxidative stress [95]. To validate the presence of various phytochemical constituents, the phytochemical analysis of the selected plant extracts was conducted. The preliminary phytochemical study of methanol extract confirmed the existence of carbohydrates, alkaloids, flavonoids, phenols, tannins, steroids, and terpenoids. However, amino acids were not detected in the extract. The results are consistent with the previously reported findings in the existing literature [65]. To confirm the presence of various phytochemical constituents, a preliminary phytochemical investigation was performed on 2-propanol extract. The analysis revealed the presence of carbohydrates, alkaloids, flavonoids, phenols, tannins, terpenoids, and saponins in the extract. However, tannins, proteins and saponins were not detected in the extract. These findings are consistent with the previously reported results in the existing literature [96]. To confirm the presence of various phytochemical constituents, a preliminary phytochemical investigation was performed on acetonitrile extract. The analysis revealed the presence of carbohydrates, alkaloids, flavonoids, phenols, tannins, terpenoids, and saponins in the extract. These findings are consistent with the previously reported results in the existing literature [82].

The various concentrations of methanol, 2-propanol, and acetonitrile were tested for their anti-diabetic effects in vivo on groups of albino rats. Blood glucose levels were measured during fasting and 30 minutes after the dose was administered using a glucometer. Among these, methanol and propanol at 500 mg/ml concentrations both showed notable hypoglycemia effects. The 2-propanol extract had an 83% inhibition rate. In a similar vein, the methanol extract displayed a 68% inhibition rate. These findings suggest that methanol and 2-propanol extracts have strong antidiabetic properties. Both outcomes were in line with the drug's typical inhibitory rate. Moreover, both the methanol and 2-propanol extracts at 250 mg/ml had strong antidiabetic effects. The percentage of inhibition for methanol and 2-propanol at a dose of 250 mg/ml was 58% and 44% respectively.

However, only at 500 mg/ml concentration did the acetonitrile extract exhibit mild antidiabetic action. Thirty minutes after dosage inoculation, at this level of concentration, it showed inhibition rates that were 32% and 22%, respectively. Following dosage inoculation, the 2-propanol extract at the concentration of 500 mg/ml demonstrated an impressive 83% inhibition rate. Comparing this inhibition to the conventional medication, there was statistical significance.

The findings together imply that the extracts of methanol, 2-propanol, and acetonitrile have differing degrees of anti-diabetic along with antiangiogenic qualities. When it comes to antidiabetic efficacy, 2-propanol outperforms methanol and acetonitrile among these extracts. In addition, the antiangiogenic activity of the methanol extract is superior to that of the acetonitrile. As a result, it may be deduced that the polar phytochemicals in the herbal combination are more effective than the less polar ones. These results provide credence to the prospective application of the plant extracts in the creation of organic compounds that are antiangiogenic and antidiabetic.

Chapter 5
Conclusion

This research was mainly focused on the antidiabetic and anticancer activity of *Salvadora oleoides*, a plant known for its antidiabetic and anticancer properties, has been thoroughly investigated using various methods. Activity assessments on albino rats revealed its ability to control blood glucose levels. The CAM assay on fertilized eggs also showed its strong anticancer effects, and inhibiting cancer cell proliferation. These findings provided valuable insights into the plant's potential as a potential treatment for diabetes and cancer.

The study has been carried out in well-established labs. The analysis of phytochemicals exhibiting antidiabetic and anticancer effects provides valuable information. The findings of this thesis highlight *Salvadora oleoides's* potential as a complementary medicine for cancer and diabetes. A thorough grasp of its pharmacological characteristics and possible therapeutic uses has been made possible by the collaborative use of model animals, in vitro tests, and analytical methods. In the end, the study adds to the increasing amount of data that encourages the investigation of Salvadora oleoides, for the creation of innovative treatments to alleviate the worldwide burden of cancer and diabetes. However, further investigation is needed to understand the specific processes behind these effects and maximize their therapeutic use.

Future Findings:

- Identifying the specific mechanisms of action that underlie Salvadora oleoides' impacts on the suppression of cancer cells and blood glucose control.
- Examination of Salvadora oleoides' impact on metastasis & angiogenesis in cancer models—two important parameters affecting the development and dissemination of tumors.
- Exploration of Salvadora oleoides' ability to mitigate oxidative stress and DNA damage, key factors implicated in both diabetes and cancer pathogenesis.

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