CHAPTER 2

LITERATURE REVIEW

Many animal secretions have been exploited to generate medications for ailments such as heart attacks and cancer. Snake venom has been used to cure a variety of diseases. Snakes are notorious for their lethal bites. Nonetheless, venom is what makes a snakebite so lethal. Snake venom is made up of hundreds of peptides, poisons, and enzymes, and it is produced by each snake. Toxins of two categories predominate: hemotoxins and neurotoxins. Hemotoxins target the circulatory system and prevent coagulation, producing uncontrollable bleeding, whereas neurotoxins target the central nervous system and stop working with the muscles, perhaps leading to choking. Neurotoxin toxins are hazardous because their proteins can disrupt channels that enable ions to pass through neuronal membranes. Interrupting the communication channel may cause the entire body to crash and instantly die. Hemotoxins-derived medicines are used to treat heart and blood crises. The incidences of stroke, illness, and diabetes decrease with these medications. Neurotoxin medications are used to treat brain injuries and diseases such as Alzheimer's and Parkinson's. Snakebites have become a public health concern in India. In India, about 250,000 snakebites are documented on average in a single year. In terms of length and body weight, snakes in India have a wide range of species. On tough terrain, snakes covered deserts, woods, marshy places, streams, and rivers **(Saini *et al.*, 1984).**

2.1. Snake Venom

Snake venoms are toxic snake fluids that are stored and generated in their venomous glands. Glands release zootoxins, altering and encapsulating the parotid gland on each side of the skull, near the eye. Poisons are stored in the glands of the big alveoli and injected into the body during the bite via tubular fangs. Snake venom contains proteins, enzymes, and peptides that can be toxic if consumed. Phospholipase A2 (Pla2), mycotoxins, hemorrhagic metalloproteinase, and other proteolytic enzymes are all present in serpentine venom **(Leon *et al.,* 2011).**

2.2. Composition of snake venom

Snake venom contains enzymes, proteins, neurotoxins, coagulants, anticoagulants, and several cytotoxic chemicals. They are water-soluble and have acidic pH values. Inorganic cations, including sodium, potassium, magnesium, and tiny quantities of zinc, nickel, cobalt, and iron, are also present in snake venom. The enzymes in snake venom primarily hydrolyze proteins and membranes, which contribute to blood coagulation and necrosis**(Jin and Varner *et al.,*2011)**. Snake venom contains a mixture of enzymatic and non-enzymatic proteins that are highly effective in immobilizing and digesting prey. It is incorrect to state that all snakes are poisonous. The modes of action of the snake venom enzymes are shown in **Table 2.1**. Venomous snakes were divided into two groups.

1. ELAPINES: It includes cobra, mamba, and coral snakes, and venom is Neurotoxic as it paralyzes the airways.

2. PIT VIPERS AND TRUE VIPERS: The venom is Hemotoxic, anticosteroid, and necrotic. It is a member of the viperine family. Examples are copperheads, rattlesnakes, vipers, and adder puffs.

Currently, almost 20 deadly enzymes are found in snake venoms across the world. Not all snake venoms have 20 enzymes, although most contain 6-12 of those enzymes. The roles of all toxic enzymes are distinct. Among them are:

1. Phospholipase A2 (PLA 2)
2. ATPase
3. Hyalourinidase
4. Collagenase
5. L-amino acid oxidase (L – aao)
6. Lipooxygenase
7. Serine protease
8. Acetylcholinesterase
9. Protenase
10. Metalloproteinase
11. 5'Nucleotidase

2.3. Anti-venom

Currently, the only available treatment for snakebites is anti-venom. Calmette developed the first antidote against the Indian cobra. Activities can be performed by immunizing mammals such as horses, goats, and rabbits with particular snake venom and then isolating blood from specific immunoglobins. Antibodies are collected and utilized to combat poisoning as a component of the immune

response to active poisonous molecules. Among the two types of anti-poisons, monovalent is species-specific, whereas polyvalent is effective against several species **(Lake *et al*., 2004).**

2.4. Side effects of Anti-venom

The side effects of anti-venom therapy are anaphylactic reactions. E.g.

1. Swallowing and breathing difficulty
2. Swelling of eyes and face
3. Serum sickness
4. Rashes and itching
5. Joint inflammation

(*Maya Devi *et al.,* 2002*)

2.5. Anti venom issues

Currently, ASV (anti-snake venom) is the most effective antidote to snake venom and consists of Fab fragments of purified IgG from horse or sheep plasma or serum immunized with snake venom. It can be monovalent like CROFAB, which works against the poison of rattlesnakes, copper heads, and cottonmouths. If polyvalent, ASV can be used against species such as Russell viper, common cobra, common krait, and saw-scaled viper**(Whitaker and Whitaker, 2012).**

The use of ASV is not as daily as the availability and less, and the specific activity of the species and storage problems contribute to the lower use of this anti-poison. The main problem with immunotherapy is its specificity, as there is a great deal of variation between species and geographic areas that restricts the use of certain ASVs. In addition, it cannot be raised against all species and subspecies because

of a lack of knowledge of the geographical diversity of poisonous snakes; aside from the above issues, marketing, production, and delivery are also issues. Because ASV is time-consuming to manufacture, ideal storage facilities are required. These can be made in lyophilized form, but the process is too costly, and physicochemical changes are likely higher, so monovalent ASV is not made in India **(Simpson and Jacobsen, 2009)**. Other drawbacks include fatal anaphylaxis, serum sickness, and sometimes, offspring reactions.

2.6. Herbal as a choice

Plants can be a source of various plant flavonoids, triterpenoids, and terpenoids, such as gedunin, which are effective against snake venom **(Gupta and Peshin, 2012)** shown in **Table 2.2 and Table 2.3**. There are various plants that traditional healer‟s use, which can be obtained from plant sap, roots, and leaves-moreover, given in the form of powders, pills, and pastes. Plant-based compounds can act as inhibitors (**Table 2.4. and Table 2.5.).** This can be useful in remote areas of India and other parts of the world.

Table 2.1 Snake venom enzymes with their mode of action

S.NO	ENZYME	ACTIVITY	SOURCE SNAKE
1.	Phospholipase A2	Neurotoxic	
2.	ATPase	Cardiotoxin	All species
3.	Hyalourinidase	Cardiotoxin	All species
4.	Collagenase	Hemotoxins Myotoxic	Viperidae
5.	L-aao	Hemotoxins	All species
6.	Lipooxygenase	Neurotoxic	Viperidae
7.	Serine protease	Pro coagulant	Bothrops pirajai

8.	Acetylcholinesterase	Neurotoxic	Elapidae
9.	Protenase	Enzyme inhibition	Viper Pit viper
0.	Metalloproteinase	Proteolytic Pro coagulant	c.d.terrificus B. jararaca
1.	5‟ Nucleotidase	Hemotoxins	All species

Table 2.2 List of plants with their bio-active medicinal compounds

PLANT	ACTIVE PRINCIPLE	REFERENCE
Achyranthes Aspera	Glycosides, oleanolic acid	*Toxicol Int. 2012* *Dwivedi et al., 2009*
Allium cepa	sulfurous volatile oils, Quercitin, protocatechuic acid, and oleanolic acid.	*Toxicol Int. 2012* *Dwivedi et al., 2009*
Amaranthus spinosus	Oleanolic acid, a-spinosterol, saponoside	*Toxicol Int. 2012* *Dwivedi et al., 2009* Sikdar and Dutta , 2008
Argemone Mexicana	Alkaloids, tannins, terpenoids, flavonoids	*Toxicol Int. 2012* *Dwivedi et al.,2009* Sikdar and Dutta ,2008
Bryophyllum pinnatum	Alkaloids, triterpenes, glycosides	*Toxicol Int. 2012* *Dwivedi et al.,2009*
Ehretia buxifolia	Ehretianone, a-amyrin	*Selvanayagam et al. 1996* *Dwivedi et al.,2009*
Enicostemma axillare	Tannins	*Toxicol Int. 2012* *Dwivedi et al.,2009*
Glorosia superb	Esters	*Toxicol Int. 2012*

Ipomoea digitata	Triterpenoids, flavonoids	*Toxicol Int. 2012* *Dwivedi et al.,2009*
Pimpinella anisum	Anisic acid	*Dwivedi et al.,2009*
Rauwolfia serpentine	Alkaloids	*Toxicol Int. 2012* *Dwivedi et al.,2009*
Salix alba	Salicylic acid	*Dwivedi et al.,2009*
Tephrosia purpurea	Alkaloids, flavonoids, saponins, tannins, triterpenoids.	*Toxicol Int. 2012* *Dwivedi et al.,2009*
Trichosanthes tricuspidata	Trichotetral, cucurbitasne glycosides, cucurbitacins	*Toxicol Int. 2012* *Dwivedi et al.,2009* *Bhandari et al.,2008*
Eclipta prostrata	Wedelolactone	*Toxicol Int. 2012* *Sikdar and Dutta , 2008*
Casearia sylvestris	Ellagic acid	*Toxicol Int. 2012*
Harpalyce brasillana	Eudunol	*Toxicol Int. 2012*
Curcuma longa	Turmurin, turmerone	*Panghal et al.,2010*
Piper sps	4-nerolidylacatechol	*Hiremath and aranath, 2010*
Strychnosnux vomica	Caffeic acid	*Hiremath and aranath,2010*
Cordial Verbenaceae	Rosmarinic acid	*Hiremath and aranath,2010*
Emblica Officinalis	Triterpenoid phthalates	*Panghal et al.,2010*
Hemidesmus indices	Lupeol acetate	*Hiremath and aranath,2010*
Guiera senegalensis	Tannic acid	*Hiremath and aranath,2010*

Table 2.3 Reported activities of plant‟s bio-active compounds

Active principle	Enzyme inhibitory activity	Anti hemorr-hagic activity	Anti-inflam-matory activity	Anti-coagulant Activity	Anti-bacterial activity	Anti myotoxic activity	References
Terpenois	+	-	+	-	-	-	*Gupta & Peshin, 2014.*
Polyphenol Quercitin, Curcumin, Tannic acid	+	+	-	-	-	-	*Gupta & Peshin, 2014.*
Aristolochic acid	+	-	-	-	-	-	*Gupta & Peshin, 2014.*
Wedelolactone, D-mannitol, Sitosterol, Stigmasterol	-	+	-	-	-	-	*Gupta & Peshin, 2014.*
Phthalate Triterpenoids	+	+	+	-	-	+	*Gupta & Peshin, 2014.*
Triterpenoid Glycoside	+	-	-	-	-	-	*Gupta & Peshin,2014*
2-hydroxy-4methoxy benzoic acid Lupeol Acetate	+	+	+	+	-	-	*Gupta & Peshin, 2014.*

Table 2.4 Snake venom Enzymes and their inhibitors

ENZYME	INHIBITOR COMPOUND
Phospholipase A	Turmerin Eudunol Wedelolactone 4-Nerolidylcatechol Ellagic acid Chlorogenic Caffeic acid Rosmarinic Betulinic acid Tannic acid Lupeol acetate Quercitin
ATPase	Triterpenoid saponin
Hyalourinidase	Tannic acid, glycoprotein wsg
Collagenase	Aristolochic acid
L -aao	Aristolochic acid
Lipooxygenase	Triterpenoids
Serine protease	Cucurbitacins , Tannic acid
Ache	Tumerone
Protenase	Aristolochic acid
Metalloproteinase	Tannic acid Tumerone
5‟Nucleotidase	Tannic acid

Table 2.5 Natural bio-active inhibitor compounds against snake venom enzyme activity

SR. NO	VENOM ACTIVITY	ENZYMES INVOLVED	ANTI-VENOM COMPOUNDS	REFERENCES
1.	Proteolytic	Metalloproteinase	Neoclerodane Eudunol Tannic acid Tumerone	*Borges et al., 2005* *Ferreira et al., 1992* *Mahadeswaraswamy et al , 2009* *Silva et al., 2004* *Castro et al., 2003* *Mukherjee et al., 2008* *Machiah et al.,2006* *Nunez et al.,2005* *Chethankumar and Srinivas , 2008*
2.	Pro - coagulant	Serine protease Metalloproteinase	Triterpenoids Tannic acid Tumerone	*Borges et al., 2005* *Assafim et al , 2006* *Alam and Gomes , 2003* *Ferreira LA, et al , 1992*
3.	Neurotoxic	Ache Phospholipase A 2	Betulinic acid Quercitin Lupeol acetate	*Chatterjee et al., 2006* *Alam and Auddy, 1994* *Pithayanukul et al.,2010* *Girish et al., 2004* *Soares et al., 2005*
4.	Haemotoxic	Collagenase Phospholipase A 2	Quercitin Tannic acid Curcumin Wedelolactone Caffeic acid Triterpenoids Phthalate triterpenoid Neoclerodane Tumerone	*Borges et al., 2005* *Chatterjee et al., 2006* *Alam and Auddy 1994* *Pithayanukul et al.,2010* *Girish et al., 2004* *Chatterjee et al., 2004* *Assafim et al., 2006* *Alam and Gomes, 2003* *Ferreira et al., 1992* *Mukherje et al., 2008*

			Lupeol acetate	*Machiah et al.,2006* *Nunez et al.,2005* *Skaria et al., 2005* *Chethankumar and Srinivas , 2008*
5.	Myotoxic	Phospholipase A 2	Eudunol Ellagic acid Wedelolactone Phthalate triterpenoid	*Nunez et al.,2004* *Silva et al., 2008* *Ushanandini et al., 2009* *Mendes et al., 2008* *Assafim et al., 2006* *Alam and Gomes, 2003* *Mahadeswaraswamy et a. , 2009* *Silva et al., 2004* *Castro et al., 2003*

2.7. Azadirachta Indica

It is usually referred to as neem and has been renowned for its therapeutic qualities in India for over 2000 years. It belongs to the Meliaceae family. Neem extracts are abundant in more than 300 active ingredients, including phenols, alkaloids, triterpenoids, tetraterpenoids, and alkaloids. Moreover, gedunin, the most common alkaloid, and terpene have shown anti-PLA activity against snake venom **(Mukherjee *et al*., 2008).**

2.8. Cancer

Cancer is one of the world‟s leading causes of death, and most malignancies exhibit distinct symptoms. However, we need adequate diagnostic methods and the skills of experienced healthcare professionals to identify and interpret cancer symptoms. Cancer is a collection of more than 100 illnesses that arise from diverse initial causes (National Cancer Institute).

First stated in the 1721 universal etymological dictionary of Nathan Bailey, but with its source, diagnosis, and many phases, cancer avoided precise revelation and challenged scholars and medical professionals to define and discriminate between "genuine" and fake cancer. Of interest, several common names were taken from the same Greek term of karkinos, which signifies "canker," "cancer," "chancre," or "kanker." Briefly, cancer formation comes from a normal cellular process abnormality involving the growth and division of cells in a properly maintained and accurate calculation system, which is the fundamental human body's structural and functional and new cell production. Old, damaged, and senescent cells usually die to replace youthful ones. Cell death is often planned, with apoptosis being the most famous and discussed**(Elmore *et al.,* 2007).** Aberrant genetic alterations produce cancers with unregulated cell proliferation, dispersion, and life in cells, which may hinder their systemic cellular structure. Daughter cells have these abnormalities **(Fouad and Aanei, 2017)**. Therefore, cells have begun to overgrow, most of which are visibly changed or expanded by a mass known as a tumour. However, suppose it grows and reaches other unspecified body parts. In that case, the tumour can either be benign (specified non-spreading neoplastic body) or malignant. Despite certain hereditary genetic defects (e.g., BRCA1 and BRCA2), environmental factors (pollution), infections, carcinogenic chemical exposure, etc., cancer cells are produced mainly by unfavourable mutations in DNA. Conceivable mutations and increased cancer risk (e.g., dichloro-diphenyl-trichloroethane (DDT), poor lifestyle options, and addiction) are also possible (e.g., alcohol abuse and smoking) **(Parsa *et al.*,2012).** Although mutant cells with intrinsic cellular machinery are usually a defensive approach, DNA damage can be detected and repaired, and when not able to do so, the prospective cancer cell is

generally removed from the system via apoptosis; if this is not possible, cancer will develop abnormally by growth, split, and spread.

The four primary types of cancer are typical. (1) Carcinoma: carcinoma, a malignant tissue epithelium that includes both the surface and skin of the internal organs and glands. Neoplastic entities associated with carcinoma are frequently solid tumors. Carcinomas, such as breast, colorectal, lung, and prostate cancers, are among the most common diseases. (2) Sarcoma is a body-supporting and connecting connective tissue cancer. Sarcoma can have a varied, complicated origin space, which covers fats, muscles, nervous tissues, tendons, joints, arteries of the blood, lymph vessels, cartilage, or bone. (3) Leukemia: Leukemia often occurs when blood cells become malignant and uncontrollably proliferate, and the four major leukemia types include chronic lymphoid leukemia, acute leukemia, chronic myeloid leukemia, and acute myeloid leukemia. There are two main types:(4) Lymphoma is a lymph system cancer that includes Hodgkin's lymphoma and non-Hodgkin lymphoma**(Niladri Mukherjee *et al.*, 2002).**

2.8.1. Liver cancer

Liver cancer is the leading cause of cancer-related death worldwide; its aetiologies are infection with hepatitis C and B, alcohol intake, smoking, obesity, hepatic illness, diabetes, and iron surpluses. Many pathways for cellular signalling are implicated in hepatocarcinogenesis, such as YAP-HIPPO, Wnt-β-catenin, and Nuclear Factor-Group B, exploring possible treatment goals.

2.8.2. Prostate cancer

The most common genetic anomalies include TMPRSS2 fusion with ETS family genes, MYC oncogene amplification, PTEN, TP53 deletion or mutation, in late

illness, and androgen receptor amplification and mutation (AR). Blood tests and digital rectal examinations are commonly used to identify prostate cancer.

2.8.3. Ovarian cancer

Due to differences in access to diagnostic and therapeutic services, Africans have the highest death rate. By eliminating or reducing risk factors, one-third to two-fifths of all cancer cases can be averted. Understanding the incidence, mortality, and geographic distribution of ovarian cancer is critical for planning and avoiding difficulties.

2.8.4 Herbal choice as an anticancer agent

Cancer has been a continuous challenge, with several curative and preventative therapies. The disease is marked by the continual proliferation of cells that are neither controlled nor stopped in the human body. The tumour of malignant cells with metastatic potential is therefore formed. Current therapies include chemotherapy and radiation therapy. Patients might experience great hardship and severely harm their health with such treatments as chemotherapy. Therefore, it is essential to use alternative treatments and cancer medicines.

Herbal remedies **(Table 2.6)** have been utilized and continue to be a significant source of medical care in underdeveloped nations for many years; for their inherent antibacterial qualities, plants have been utilized in medicine. Therefore, research has been conducted on terrestrial plant extracts' possible features and uses to produce nano-substance medications, including cancer. Many plant species are already utilized for cancer treatment or prevention. Multiple studies have found plant species with anticancer effects firmly focused on those utilized in traditional medicine in impoverished nations. Compounds that are typical of the

plants and required for the organism's plant survival and "household" are examined for their potential to suppress growth and start cancer cell death **(Greenwell *et al.*, 2015).**

A. Polyphenols

Polyphenolic compounds include flavonoids, tannins, curcumin, and resveratrol. Resveratrol is present in foods such as peanuts, grapes, and red wine. Gallacatechins are also present in green tea. Polyphenols are thought to enhance health and reduce cancer risk through the dietary use of natural antioxidants. Polyphenols are believed to undergo apoptosis, which produces characteristics that can be used to indicate anticancer capabilities. The mechanism for initiating polyphenols is to regulate the mobility of copper ions that are linked to the chromatin-induced dizziness of DNA. Resveratrol can destroy DNA (II) in the presence of Cu.

B. Flavinoids

Polyphenoidal flavonoids are a comprehensive class of 10,000 known secondary plant metabolites. They are physically active in plants and are interested in their health advantages **(Greenwell *et al*., 2015).**

Several plants, including ferns and plants for traditional Chinese medicines like litchi leaf, have been investigated regarding their flavonoids and their impact on cancer cells. Many flavonoid chemicals, such as anthocyanins, flavones and flavonols, Chalcones, and more seeds, are present in one plant structure. Inhibited the NF-β expression needed for survival, angiogenesis, and cancer cell growth. Flavonoids exhibit cytotoxicity and high free radical activity in cancer cells. Anticancer effects have also been identified in several human diseases, including

hepatoma (Hep-G2), cervical carcinoma (Hela), and breast cancer by purified flavonoids (MCF-7).

C. Brassinosteroids

Brassinosteroids (BRs) naturally exist in plants with hormone-related signalling to govern cell development and differentiation, elongation of the stem and root cells, and other functions such as illness, stress, and tolerance also used for the control of plant senescence **(Whiting *et al.,* 2011).** They are also necessary for plant growth. BRs are another natural substance with therapeutic value in cancer etiology. BRs may elicit responses that inhibit growth and promote apoptosis by interacting with the cell cycle.

Table 2.6 Herbal compounds act as anticancer agents.

COMPOUND	ISOLATED FROM	ACTIVITY	REFERENCES
Taxol	Taxane	Block mitosis Induce apoptosis	Jordan *et al.,* 2004
Vinblastine	C. roseus	Anti-mitotic Anti-tumor	Ocker *et al*., 2009
Roscovitine	Brassicaceae	Cyclin-dependent kinase Inhibition	Unnati *et al.,* 2013
Flavopiridol	D.binectariferum	Anti-inflammatory	Unnati *et al*., 2013

2.8.5 Gedunin as an anticancer agent

One of the primary chemical components of the neem tree is gedunin (a tetranortriterpenoid). Recent research has demonstrated that gedunin can suppress cancer cells, including prostate, breast, pancreatic, ovarian, and colon growth. The Hsp90 (Heat Shock Protein 90) inhibitor is also reported. Moreover, the pharmacological similarity of gedunin for β-catenin chain A in cancer stem cells

has been found in recent silica research**(Kamath *et al*., 2009) (Uddin *et al*., 2007).** Many photochemical have been investigated for their ability to treat and prevent carcinogenesis and counteract the severe adverse effects of chemotherapy. The tree *Azadirachta indica*, sometimes known as neem, is primarily found in Asian and African nations, where it is widely utilized for several medical benefits. Isolated neem extracts have been investigated for their anti-cancer properties in several malignancies, including breast, colon, and cervical cancers**(Uddin *et al.,* 2007)**. We previously showed that ethanolic neem leaf extracts could prevent mammary carcinogenesis by affecting proliferation, apoptosis, and angiogenesis. The active component gedunin (tetranortriterpenoid) was extracted from the neem extracts. Gedunin has been found to exert anticancer activity by decreasing breast cancer cell growth by modulating specific heat shock proteins. Additionally, gedunin therapy has been shown to exert anti-proliferative effects in ovarian cancer cells by regulating critical signalling pathways.

Cancer is a term used to describe a group of disorders characterized by abnormal cell proliferation that can infiltrate or spread to other body regions. In contrast, the benign tumour does not spread to other body areas. A lump, unusual bleeding, persistent cough, unexplained weight loss, and change in bowel motion are all possible indications and symptoms. Although these symptoms might suggest cancer, they could signal something else **(Uddin *et al*., 2007)**. Approximately 100 different forms of cancer affect humans. Cancer is asymptomatic when it first appears. Signs and symptoms arise as the tumour develops or ulcerates. The type and location of malignancy determine outcomes. There are a few unique symptoms. Many of these conditions are common among people with other health problems. Cancer is a "great imitator," as the saying goes. Therefore, it is typical

for cancer patients to have been treated for other diseases that are thought to cause their symptoms.

NAD+ kinase phosphorylates nicotinamide adenine dinucleotide (NAD+) coenzyme to convert it to NADP+.NADP+ is a coenzyme that is reduced to NADPH via the pentose phosphate pathway to supply reducing power for biosynthetic activities, including fatty acid and nucleotide synthesis. The structure of NADK from Archaeoglobus fulgidus, archaea, has been previously discovered **(Anand *et al.*,2008)**.NAD+ kinases are encoded by the NADK and MNADK genes found in the cytosol and mitochondria. Similarly, yeast has cytosolic and mitochondrial isoforms, with the latter accepting NAD+ and NADH as phosphorylation substrates. It is used to operate as a reducing agent for the reductive synthesis of nucleic acids, proteins, and lipids; highly growing cancer cells require adequate levels of NADH and NADPH **(Jayasekara *et al.*, 2016).** A shortage of these precursors can cause the cells to stop growing and eventually die. NADPH is also involved in neutralizing the reactive oxygen species (ROS) associated with fast growth, which is important for maintaining a healthy redox state in cells. Cancer cells can alter the expression and regulation of metabolic genes to meet the metabolic and ROS-mediating needs associated with fast growth. Oncogenes and tumour suppressor proteins are involved in the regulation of numerous genes**(Khushi *et al.*, 2012).**

KRAS mutations are observed in more than 90% of pancreatic cancer patients, and GLI1, a downstream mediator, is responsible for KRAS-induced pancreatic development/transformation. According to other findings, GLI1 transcription factors function synergistically with activated KRAS to generate metastatic

pancreatic cancer. Abnormal activity of the Hedgehog/GLI signalling pathway has been linked to cancer development and progression in various cancers, including pancreatic cancer. Sonic hedgehog (Shh) is abnormally expressed in 70 % of pancreatic cancer tissues. Pancreatic intraepithelial neoplasia (PanIN) is induced by Shh overexpression alone. Shh signaling stimulates GLI transcription factors, which have oncogenic properties and are dysregulated in malignancies, resulting in invasion and metastasis, chemoresistance, and epithelial-mesenchymal transition **(Anguiano *et al.,* 2012).** Studies have shown that blocking the upstream target sonic hedgehog (Shh) causes a reduction in GLI1 expression, which inhibits tumor development in xenograft models. This emphasizes the importance of the hedgehog/GLI signalling pathway in chemotherapy development in K-RAS-driven pancreatic cancer.

Gedunin caused high ROS production, resulting in DNA damage and cell cycle arrest in the G2/M phase, halting cell growth. Following cytochrome C release, caspase 9 and 3 activations, and PARP cleavage, ROS increase resulted in mitochondrial stress and membrane depolarisation, resulting in mitochondria-mediated death. Transmission electron imaging (TEM) of gedunin-treated cells revealed apoptosis-like subcellular characteristics. Furthermore, stress kinases, such as phospho-ERK 1/2, phospho-p38, and phospho-JNK, were upregulated in gedunin-treated cells. N-acetyl-L-cysteine (NAC), a free radical scavenger, reversed all these effects, resulting in enhanced cell survival, cell cycle arrest abrogation, mitochondrial membrane potential restoration, and suppression of apoptotic markers. Intriguingly, gedunin inhibits the evolutionarily conserved molecular chaperone Heat Shock Protein 90 (hsp90), which is involved in cellular homeostasis **(Magni *et al.,* 2006).** Because many oncogenic proteins are also

hsp90 client proteins, targeting this chaperone might be an appealing technique for developing cancer therapies. These findings shed light on the molecular mode of action of gedunin, which might benefit the development of medications against ovarian cancer.

2.9 Gedunin ($C_{28}H_{34}O_7$)as anti diabetic agent

Diabetes mellitus (DM) is a metabolic condition characterized by persistent hyperglycemia due to a deficiency in insulin production, insulin action, or both. It is frequently accompanied by carbohydrate, lipid, and protein metabolism problems and severe consequences of diabetes, such as retinopathy, neuropathy, nephropathy, cardiovascular issues, and ulceration. By 2030, the WHO Health Organization estimates that diabetes will be the seventh leading cause of death, affecting up to 366 million people worldwide **(Beverley *et al.*,2003).** Lowering hyperglycemia by delaying and reducing the digestion of ingested carbohydrates is a successful treatment method for diabetes and obesity control. By delaying starch breakdown, inhibiting carbohydrate-degrading enzymes lowers the postprandial rise in blood glucose levels after a meal.

This reduction in postprandial hyperglycemia slows down the course of DM-related vascular problems. Human pancreatic amylase (HPA, 1,4-glucan-4-glucanohydrolase, E.C. 3.2.1.1) is one such enzyme that plays a crucial role in DM. It catalyzes the first step in the hydrolysis of starch to maltose, which is then degraded to glucose by -glucosidases. HPA inhibition slows starch digestion, which is vital for controlling postprandial hyperglycemia in type II diabetes **(Beverley *et al.*,2003)**. The rate of starch hydrolysis is slowed when HPA is inhibited in the small intestine, thereby delaying digestion. One of the most

effective ways to minimize postprandial hyperglycemia is to extend the digestive process. That minimizes the quantity of glucose created and released into the blood. Because existing medications include adverse effects such as hypoglycemia and weight gain, novel anti-diabetic targets and strategies for glycaemic control are needed. The inadequacy of existing medications to manage hyperglycemia without causing adverse effects, as well as their high cost and limited availability, has driven researchers to look for alternative treatments, such as traditional herbal remedies, which may provide valuable leads and therapeutic solutions. HPA inhibitors have also been reported to have no adverse effects **(Bastaki *et al.*,2005)**. Natural plant products are increasingly being used as supplements for DM therapy, with over 1200 plants claimed to have anti-diabetic properties. The absence of sufficient documentation, thorough quality control, and identification of critical bioactive components and their mechanisms of action has hampered the use of alternative medicines. Furthermore, there are only a few thorough investigations on the scientific validation of traditional anti-diabetic medicinal herbs, making them a promising source of HPA inhibitors. For more than 2000 years, the 'wonder tree' neem (*Azadirachta indiciaA. Juss*.; Meliaceae), native to the Indian subcontinent but planted throughout the tropics, has been renowned for its several medical properties **(Bastaki *et al.*, 2005).**

Previous research has demonstrated that the aqueous leaf extract of neem causes hypoglycemia in normal rats and lowers blood sugar levels in streptozotocin-induced diabetic rats. It is one of the most abundant producers of secondary metabolites, particularly tetranortriterpenoids (limonoids). Over the last five decades, more than 150 skeletally varied and oxygenated triterpenoids have been isolated and described from various portions of the neem plant and have been

found to exhibit a wide range of pharmacological actions and insecticidal effectiveness. The skeleton of limonoids is 4, 4, 8-trimethyl-17-furanylsteroidal and can be replaced with different functional groups. Basic limonoids (4, 4, 8-trimethyl-17-furanylsteroidal skeletons, such as azadirone, azadiradione, and gedunin) and C-seco limonoids (with modified and rearranged C-rings, such as azadirachtin, salannin, and Nimbin) are the two types of neem limonoids **(Wild *et al.,* 2004).** There have been limited investigations on the effects of tetranortriterpenoids on -amylase. In streptozotocin-induced diabetes in mice, the tetranortriterpenoids meliacinolin and azadirachtolide isolated from A. indica leaves and swietenine extracted from Swietenia macrophylla have recently been found to inhibit -amylase **(Whiting *et al*., 2011).**