Natural Alkaloids as a Source of Anticancer Agents

By

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A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (Hons.)

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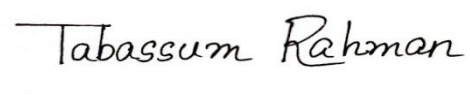

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Approval

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Ethics Statement

This study does not involve any human or animal trial.

Abstract

Cancer is a term used to describe disorders in which malignant cells divide uncontrollably and can infect neighboring tissues. Cancer cells have developed multidrug resistance to traditional anticancer treatments during the previous few decades, resulting in tumor recurrence. As a result, new and effective anticancer agents are urgently needed. Alkaloids have been used to treat several diseases, and they have shown great promise in causing cytotoxicity. *In-vitro* and *in-vivo* reports show that some alkaloids derived from natural herbs have antiproliferative and antimetastasis properties on a variety of malignancies. Thus, this review summarizes the mechanisms of action of natural alkaloids with potential anticancer characteristics, such as isoquinoline, vinca, steroidal, indole, vincamine, and other alkaloids, as well as marine, derived alkaloids. The data presented in this study will assist researchers in keeping up with current understanding in this field.

Keywords: Alkaloids, Anticancer, Cytotoxicity, Mechanism of action.

Dedication

Dedicated to my Parents

Acknowledgment

To begin, I would like to express my gratitude to Allah (SWT) Almighty for His unending blessings, mercy, and compassion. All glory to Him for providing me with immense patience, strength, courage, knowledge, and wisdom.

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List of Acronyms

ATF	Activated Transcription Factor
ADC	Antibody-Drug Conjugate
BBR	Berberine
CDC	Complement Dependent Cytotoxicity
CDK	Cyclin Dependent Kinase
CPT	Camptothecin
EPR	Enhanced Permeability and Retention
EMT	Epithelial-Mesenchymal Transition
FDA	Food and Drug Administration
HDACs	Histone deacetylases
MDR	Multi-drug Resistance
MTAs	Microtubule Targeting Agents
NPs	Nano-particles
NK	Natural Killer
ROS	Reactive Oxygen Species
RES	Reticulo-endothelial System
TNFR	Tumor Necrosis Factor Receptor

Chapter 1: Introduction

1.1 An overview of cancer and natural alkaloids

Cancer is a term that pertains to a collection of diseases marked by uncontrolled cell development and the capacity to infiltrate and spread throughout the body. External factors such as alcohol, radiations, infectious agents, tobacco, and chemicals, as well as internal considerations like hormones, immunological morbidities, hereditary abnormalities, and metabolic alterations, can induce DNA mutation in normal cells, leading to cancer development and progression (HUANG et al., 2017). In 2012, there have been 14.1 million cancer incidences globally. 8.2 million people passed away from cancer that year, with 32.6 million individuals surviving with cancer (within 5 years of diagnosis) over the globe. Less developed regions accounted for around 57 percent (8 million) of new cancer cases, 65 percent (5.3 million) of cancer deaths, and 48 percent (15.6 million) of 5-year prevalent cancer cases (DeBono et al., 2015).

Surgical intervention, chemotherapy, and radiation therapy, as well as a combination of these therapies, are currently available for cancer treatment. Chemotherapy is the most extensively used and acknowledged cancer treatment approach. But chemotherapy medications have limited clinical applicability due to their undesirable side reactions, such as nausea, vomiting, exhaustion, and hair loss. Natural products have been offered as an alternate treatment in this area due to their possible efficacy and safety (HUANG et al., 2017). Moreover, the majority of synthetic antineoplastic treatment methods on the market today are immunosuppressive and have a variety of adverse effects (Tomar et al., 2019).

Also, the issue with current therapies is multi-drug resistance (MDR) which is an obstacle to the therapeutic effectiveness of many unconnected cancer therapies due to its extensive prevalence within malignant cells. Two types of anticancer drug resistance exist, one that

occurs from the impeded delivery of drugs to the cancerous cells, and the other one that originates inside the malignant cell because of epigenetic and genetic abnormalities that affect the drug's sensitivity. Current chemotherapeutics have limited water solubility, which leads to higher drug elimination and metabolic activity, leading to reduced drug concentrations in the blood. As a result, drug diffusion from the bloodstream, through cellular membranes, and into the tumor mass decreases (DeBono et al., 2015). Chemotherapy contains many limitations that includes negative side effects, emergence of resistance to active ingredients, and the need for additional therapies to recover a patient in conjunction to chemotherapy. Chemotherapy typically works by interfering with DNA synthesis and mitosis, leading to the death of cancer cells that are rapidly multiplying. The chemicals are nonselective, meaning they can harm healthy normal tissues as well, resulting in significant unanticipated and unpleasant side effects. Indeed, the severe adverse impacts of chemotherapeutic drugs on normal tissues and organs are a key contributor to cancer patients' elevated fatality rates.

Plants have a vital role in the formulation of new anticancer medications. Plants and other natural origins, such as marine creatures and microbes, account for over 70% of currently accessible antineoplastic drugs (Tomar et al., 2019). Alkaloids are a complex category of substances found across the plant world, primarily in higher plants of the *Ranunculaceae*, *Leguminosae*, *Papaveraceae*, *Menispermaceae*, and *Loganiaceae* families. A lot of alkaloids have biological effects, like ephedrine's asthma-relieving properties, morphine's analgesic properties, and vinblastine's anticancer properties. Alkaloids are one of the most significant active ingredients found in natural plants. Several alkaloids, such as camptothecin (CPT), a well-known topoisomerase I inhibitor, and vinblastine, which interferes with tubulin, have already been successfully converted into chemotherapeutic drugs (Gupta et al., 2015). MTAs (microtubule-targeting agents) are highly effective therapeutic agents among the several alkaloids that work via various modes of action since they interrupt abnormal cancer cell

development by interfering with ongoing mitotic division. Vinca alkaloids like Vinflunine, Vinorelbine, Vincristine, Vindesine, and Vinblastine are the first MTAs to be developed and authorized for the management of hematologic and lymphatic malignant tumors in the clinic (Martino et al., 2018). Each anticancer drug has its unique mechanism for causing cancer cells to die. With numerous efficient anticancer treatments on the marketplace, such as paclitaxel and docetaxel, and additional molecules in clinical development, antimitotic therapies constitute a dominant class of cytotoxic drugs (Tomar et al., 2019).

1.2 Rationale of the study

Currently, chemotherapeutics are most effective for anticancer treatment. But regardless of this, cancer continues to be the biggest reason of death globally. As an instance, 589,430 fatalities and 1,658,370 new cancer cases were detected in the United States in 2015. Many people suffer from the drawbacks of low water solubility and substantial hazardous side effects of chemotherapy. For this reason, more effective anticancer agents and novel methods are urgently needed (Cragg John M Pezzuto Bethesda, 2016).

Natural compounds are the most consistent origin of innovative and effective antitumor drugs. Their efficacy can be due to the fact that natural products often contain built-in chirality, making them ideal for binding to complex proteins and other 3D biological receptors (Kingston, 2009).

There are a large number of naturally occurring alkaloids with anticancer effects that might be used as lead molecules in medication development. To create and construct anticancer medications, further study and testing into the relationship between natural alkaloids and cancer is required. The purpose of this study is to assemble information on some of the most significant and geographically diversified natural alkaloids with anticancer action. In this study, advances

on numerous common alkaloids with anticancer properties have been summarized and discussed some of their features so that anticancer medicines derived from alkaloids might be discovered faster. The chemical structures, anticancer characteristics, structure-activity connections, and mechanisms of action of new natural and synthesized - alkaloids with anticancer activity have been focused on in this literature.

1.3 Aim and objectives of the study

The aim of this review is to provide an overview of different natural alkaloids which can be used in cancer treatment.

The objectives of the study are:

- To present a detailed overview of the knowledge on alkaloid anticancer compounds and evaluate their potential for development as therapeutically useful anticancer drugs
- Compare the efficacy of natural alkaloids with that of conventional cancer treatments.
- To analyze the possibilities of modern drug delivery systems to develop more effective therapeutic approaches for the delivery of alkaloids for cancer therapy.

Chapter 2: Methodology

In this paper, the database was searched, and some naturally derived alkaloids were selected for reviewing such as steroidal alkaloids, isoquinoline alkaloids, vinca alkaloids, noscapine and its derivatives, vincamine alkaloids, carbazole alkaloids and coumarins, indole alkaloids, berberine derivatives, antofine analogues, curcumins, and various marine alkaloids with relatively more anticancer studies. This review is focused on latest and most relevant research articles from journals with a high impact factor. The information was gathered using reliable search engines such as Google Scholar, Researchgate, Sciencedirect, PubMed, Scopus, and Elsevier, among others. A thorough search of the journals was conducted, and the results were narrowed down to the most recent and relevant ones.

Chapter 3: Plant-derived alkaloids

3.1 An overview of different classes of plant derived alkaloids

Plants have been utilized as an origin of medication for the therapeutic purpose of numerous ailments for many years. Natural compounds are small-molecule secondary metabolites utilized by plants for survival, like alkaloids, flavonoids, and terpenoids. Secondary metabolites from plants are powerful weapons in the battle against pathogens including viruses, bacteria, and protozoans. They protect against herbivores such as insects, worms, and animals. They also serve as attractors to pollinators like insects, birds, and bats. Secondary plant metabolites are an important source of information for drug development. Secondary plant metabolites is an integral element in chemoprevention, or the prevention of carcinogenesis, in addition to cancer treatment (Fulda & Efferth, 2015). It's worth noting that 70% of currently available anticancer medications are made from natural ingredients or are derived from plants. According to reports, over 65 percent to 80 percent of the planet's population relies only on plants for their medical assistance. Furthermore, according to estimates, 35,000-70,000 plant species have been utilized for medical purposes across the world, accounting for 14 percent to 28 percent of the world's 250,000 plant species (Twilley & Lall, 2018). Alkaloids are amongst the most significant secondary metabolites derived from natural sources that are employed in anticancer therapy. An alkaloid's fundamental structure is a ring structure with a nitrogen atom. The nitrogen atom might be found in the molecule's ring structure or the side chain.

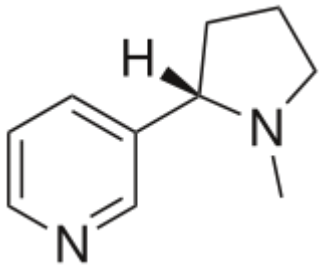

Figure 1. General structure of an alkaloid.

There are numerous more types of alkaloids and some of them are mentioned in the following table.

Table 1. Different classes of alkaloids, their sources with functions and mechanism of actions.

Classes of alkaloids	Examples and sources	Functions	Mechanism of anticancer action	Reference
Iso-quinoline	Berberine (*Berberis asiatica)*	Digestive stimulant, antimicrobial, immune stimulant, antitumor.	Cell death is induced through cell cycle inhibition, apoptosis, and autophagy.	(Yun et al., 2021)
	Colchicine (*Colchicum autumnale)*			
Indole	Ergotamine (*Ergot sclerotium, Claviceps purpurea)*	Vasoconstrictor used to treat migraines, used as psychedelic drugs, anticancer.	Tubulin polymerization inhibitors, which can connect with the colchicine binding domain and can target DNA topoisomerases, sirtuins, histone deacetylases (HDACs), PIM kinases, and receptors.	(Dadashpour & Emami, 2018)

Vinca	Vincristine, Vinblastine (*Vinca rosea)*	Antitumor, used to treat cancer.	Inhibits cell cycle progression by preventing mitosis. Microtubules are disrupted and tubulin polymerization is hampered.	(Martino et al., 2018)
Steroid	Solanine (*Solanum melongena)*	Anti-cholinesterase activity, anticancer, anti-inflammatory, antimicrobial, and analgesic	Triggers apoptosis by cell cycle disruption (at the G0/G1 and G2/M checkpoints), suppression of cell-signaling proteins (MMP-2/9 and AKT), and upregulation of transcriptional factors (p21WAF1/CIP1 and checkpoint kinase-2), induction of Bax expression, Bcl-2 suppression, and PARP-1 initiation.	(Dey et al., 2019)
Pyridine	Nicotine (*Nicotiana tobacum)*	CNS activity, antiasthmatic,	Topoisomerase I inhibitory activity.	(Thawabteh et al., 2019)

	Arecoline (*Areca catchu)*	anticonvulsant, antimuscarinic, anticancer, antidiabetic, and anti-inflammatory.		
Piperidine	Piperine (*Piper nigrum)*	Anticancer, antidepressant, herbicidal, anti-histaminic, CNS stimulant, insecticidal and fungicidal.	Causes cell growth arrest in the G2/M stage, resulting in apoptosis in 4T1 cells. NF-B, cAMP response element-binding (CREB), c-Fos, and activated transcription factor 2 (ATF-2) are all suppressed by this compound. P-glycoprotein (P-gp) and CYP3A4 are inhibited, which alters drug metabolism and re-sensitizes MDR cancer cells.	(Thawabteh et al., 2019)

Terpenoid indoles	Vincamine (*Rauwolfia serpentine)*	Antitumor.	It accelerates the death of A549 cells by activating a number of signaling pathways, including caspase-3-mediated apoptosis.	(Al-Rashed et al., 2021)
Quinoline	Quinine, Quinidine (*Cinchona officinalis)*	Antipyretic, antimalarial, and cardiotonic, anticancer.	Inhibits cell growth by Cell cycle stoppage, apoptosis, angiogenesis suppression, cell migration interruption, and modulation.	(Jain et al., 2019)
Tropane	Cocaine (*Erythroxylum coca)*	Antifungal, Digestive and urinary tract spastic disorders, used in anticolic and spasmolytic medicines.	Induce cell death through apoptosis, shows tyrosinase inhibition effect.	(Thawabteh et al., 2019)
	Scopolamine (*Datura stramonium)*			

The general anticancer mechanistic action of alkaloids are as follows (Twilley & Lall, 2018):

- Activates multiple cell cycle factors, including ERK1/2, p27, p21, death receptor 5 (DR-5), and phosphorylation of p53, along with cell cycle inhibition in the G1 or G2/M stages, to cause apoptosis.

- Inhibition of N-acetyltransferase, COX2, and telomerase, among other enzymes.
- Cyclin-dependent kinase proteins, B-cell lymphoma 2 (Bcl-2) proteins like Bcl-2, Bcl-xL, Bax, caspase-3, -8, -9 are all controlled.
- Causing the formation of reactive oxygen species (ROS) in neoplastic cells.
- They can block Nf-kb focal adhesion kinase, HIF-1, MMP-2, -3, -9, and VEGF which are all regulatory factors in metastasis and angiogenesis.
- Topoisomerase I, cAMP response element-binding, c-Fos, mitogen-activated protein kinase phosphatase 1, CYP3A4, STAT-3, activated transcription factor 2, P-glycoprotein (P-gp), and Wnt/b-catenin signaling, are all suppressed.
- They can lower the membrane potential of the mitochondrial membrane.
- TNF receptors (TNFRs) such as TNFR1 and 2, Fas-associated death domain, TNFR-1 associated death domain, TIMP-1, and Fas-receptor are upregulated.

3.2 Alkaloids with anticancer effects and their mechanism

3.2.1 Steroidal alkaloids

Steroidal alkaloids are secondary metabolites with a wide range of pharmacological effects, however, there are currently few thorough evaluations on their anticancer potential and mechanisms of action. SAs have a cyclopentanephenanthrene skeleton also known as a steroidal scaffold, with a nitrogen atom bonded to the molecule as a primary fragmentation in the ring, or side chain. The induction of apoptosis is among the most important carcinogenic actions of SAs. Apoptosis, or programmed cell death, is a cellular event that is well-coordinated and preserved, due to a well-organized matrix of inherent cellular suicide mechanisms. Cancer cell death morphometric markers on a microscopic scale, such as cell shrinkage, apoptotic bodies, membrane blebbing, chromatin condensation, and DNA breakage, were used to demonstrate that the death of cells was caused by apoptosis in the early research. Apoptosis-

inducing pathways are changed when the equilibrium between cell growth and death is disrupted, resulting in oncogenesis. Apoptosis can be triggered by receptor-mediated extensible networks or mitochondrion-mediated internal mechanisms. Other cellular organelles, such as the lysosomes, cytoskeleton, endoplasmic reticulum (ER), and nucleus, may also be involved in apoptotic transmission by recognizing or integrating pro-apoptotic impulses (Dey et al., 2019).

The anti-oncogenic impact of SA is mediated through the disruption of cell cycle progression. Mitogenic signaling allows cells to activate pre-programmed paths and proceed through the cell cycle. The master regulators of the cell cycle are CDKs. CDK disruption or change causes neoplasia. CDK hyperactivation is caused by mutations in CDK-regulated genes, and CDK-inhibitor genes have been related to a variety of cancers. As a result, the inhibitors or modulators have a lot of potential for new anticarcinogenic medicines. SAs such as solanidine, ritterazine b, and solamargine derivatives stop cancer cells from going through the G0/G1 and G2/M checkpoints. SAs also work by exerting anti-proliferative and anti-metastatic action. The suppression of different cell transduction cascades and proteins like MMP-2/9 and AKT that enable cancer cells to proliferate might be the biological mechanism underpinning SAs' antiproliferative actions. Solanine-induced suppression of MMP-2 and MMP-9 is linked to a reduction in A2058 human melanoma cell motility and intrusion, which adds to SAs' antimetastatic effect. α-tomatine inhibits Akt (protein kinase B) phosphorylation that controls several proteins associated with cancer cell growth and metastasis. Increased p21WAF1/CIP1 levels and checkpoint kinase-2 activation are also involved in the anti-proliferative effect of α-tomatine. SAs like solasodine and cephalostatin 1 can also cause DNA fragmentation and prevent protein synthesis (Dey et al., 2019).

Table 2. Anticancer mechanisms of some steroid alkaloids.

Name of SAs	Mode of action	Cancer types or cell lines	Reference
Tomatidine and α-Tomatine	• Tomatidine causes cytotoxicity by inhibiting the cell cycle's G0/G1 stage. • It also prevents A549 cells from invading and migrating where TIMP-1 and RECK are upregulated, while MMP-2 and 9 are downregulated. • Tomatidine effectively sensitizes cancer cells by decreasing the action of ABC transporters.	MCF-7 Breast Cancer Cells, Chang Normal Liver Cells, AGS Stomach cell line, HT-29 Colon Cancer Cell line, and HepG2 Liver Cancer Cell lines are all impacted by α-tomatine.	(Friedman et al., 2009)
Solamargine and solasonine	• Solamargine causes apoptosis by increasing expression of Tumor necrosis factor receptor I (TNFR-I), Fas-associated death domain (FADD), Fas receptor (Fas), and TNFR-I-associated death domain (TRADD) which are examples of extrinsic death receptors. • SM enhances the inherent ratio of Bax to Bcl-2 by increased expression of Bax and down regulation of Bcl-2 and Bcl-xL transcripts. The induction	**Solasonine:** Breast cancer (MCF7), Hepatocellular cancer (HepG2), Glioblastoma (M059J, U343, U251), Lung cancer (PC-12), Gastric cancer (MGC-803), Cervical cancer (HeLa). **Solamargine:** Lung cancer (H520, H69, H441, H661), breast	(Munari et al., 2014)

	of caspases-8, -9, and -3, as well as the discharge of mitochondrial cytochrome c, demonstrate that SM stimulated both external and internal apoptotic pathways in cancer cells. • SM lowers cell viability and promotes apoptosis by raising mRNA and protein expression of p53 and Bax. Bcl-2 (an anti-apoptotic protein) expression is also lowered.	cancer (MCF-7), colon cancer (HT-29, HCT116), osteosarcoma (Saos-2, U-2 OS, MG-63), Glioblastoma (M059J, U343, U251), Hepatocellular cancer (HepG2).	
Solasodine	• By increasing LAK (lymphokine-activated killer) and NK (natural killer) cell activity, encouraging lymphocyte proliferation, and enhancing IL-2 production. • 15mg/ml of solasodine hydrochloride and 20mg/ml cyclophosphamide reduced the formation of multidrug-resistant sarcoma tumors in mice by triggering apoptosis, which was accompanied by a decrease in topoisomerase II and P-gp expression.	Basal cell carcinoma, squamous cell carcinoma, prostate cancer, melanoma, colorectal cancer, osteosarcoma, bladder cancer, oral epidermoid carcinoma, Ovarian cancer, Breast cancer, myelogenous leukemia, liver cancer, lung cancer, pancreatic cancer, gastric carcinoma, glioblastoma, renal	(Dey et al., 2019)

		cancer, uterine cancer, mesothelioma, and endometrial cancer.	
α-Solanine and α-chaconine	• The downregulation of miR21 expression by α-solanine decreased tumor development and metastasis. • α-chaconine suppresses ERK1/2 phosphorylation and activates caspase-3 in the HT-29 cancer cell line, promoting death of cancer cells.	**α-Solanine:** Cervical cancer (HeLa), Colorectal cancer (HT-29), Hepatocellular cancer (HepG2), Prostate cancer (PC-3), Gastric cancer (AGS, KATO III), Pancreatic cancer (PANC1, SW1990, PaCa-2), Lymphoma (U937), Melanoma (A2058, A375). **α-chaconine:** Cervical cancer (HeLa), Colorectal cancer (HT-29), Hepatocellular cancer (HepG2), Lung cancer (A549), Gastric cancer (AGS, KATO	(Dey et al., 2019)

		III), Lymphoma (U937).	
Cyclopamine	• The cyclopamine Cnor-D homosteroidal alkaloid has been identified as an Hh signaling pathway antagonist and has been shown to be teratogenic in animals. • Cyclopamine is responsible for inducing apoptosis in PLC/PRF/5, Huh7, and SMMC-7721 cells by inhibiting the Shh signaling cascade and downregulating Bcl-2 expression. Modulation of the AKT and ERK pathways is part of the molecular mechanism by which cyclopamine induces apoptosis.	In diverse leukemia and lymphoma cells, cyclopamine preferentially attacks and lowers Gli1 expression, leading to cell growth arrest and apoptosis induction. Furthermore, cyclopamine has anticancer properties in GIT/gastric, lung, breast, pancreatic, biliary tract, and esophageal carcinomas, as well as oral squamous cell carcinoma and leukemia.	(Dey et al., 2019)

3.2.2 Isoquinoline alkaloids

One of the most diverse categories of natural compounds is alkaloids with an isoquinoline group. Isoquinoline is a heterocyclic molecule made up of a benzene ring fused to a pyridine

ring at C3/C4. Isoquinoline alkaloids are synthesized from tyrosine, which produces dopamine and p-hydroxyphenylacetaldehyde. The protoberberine class includes isoquinoline alkaloids such as berberine, palmatine, coralyne, and coptisine, whereas the benzo phenanthridine class includes sanguinarine, chelerythrine, and chelidonine. The benzylisoquinoline alkaloid class includes noscapine and scoulerine (Yun et al., 2021).

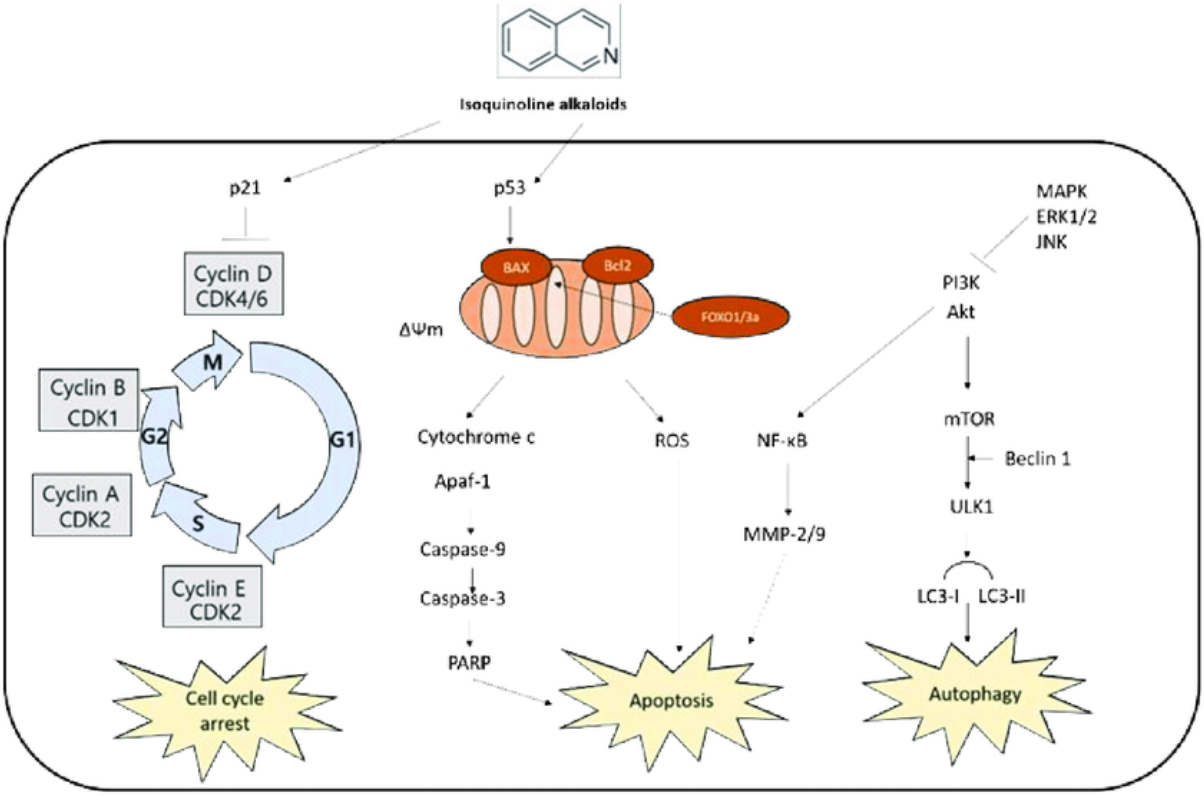

Figure 2. Molecular pathways involved in anticancer machinery of isoquinoline alkaloids.

Isoquinoline alkaloids have significant anti-cancer properties. As an option to complement chemotherapy, studies were done on plants rich in isoquinolines and isoquinoline alkaloids. In numerous cancer cell lines, they effectively trigger cell death. Isoquinoline alkaloids, according to research focused on *in-vitro and in-vivo* methods, shows strong anti-tumor actions by autophagy, cell cycle halt, and apoptosis, all of which end in the death of the cell (Yun et al., 2021).

Table 3. Mechanism of berberine and noscapine on different type of cancers.

Cancer Type	Mechanism	Effects	Compound	Reference
Colorectal cancer	Apoptosis	• Causes cell proliferation in the sub-G0 stage. • Increased expression of Bax.	Berberine	(Yun et al., 2021)
Breast cancer	Apoptosis	• Compressed chromatin with nuclei that are fractured. • Cell proliferation in the sub-G0 phase.	Noscapine	(Yun et al., 2021)
Lung cancer	Apoptosis	• Phosphorylation of the p38 MAPK protein increases. • An increase in FoxO3a transcriptional activity.	Berberine	(Jagetia & C., 2021)
Liver cancer	Apoptosis	• The PI3K/Akt/mTOR pathway becomes suppressed. • JNK phosphorylation elevates ROS (reactive oxygen species) production. • Expression of Bim and FoxO transcriptional action both increases.	Berberine	(Jagetia & C., 2021)

Oral cancer	Apoptosis	• Increased expression of FasL. • Bcl-2 and Bcl-xL expression both decreases. • Elevated Apaf-1, Bad, and Bax expression. • Caspase-3/8/9 and PARPI becomes activated. • The phosphorylation of p38 MAPK increases.	Berberine	(Jagetia & C., 2021)
Breast cancer	Cell cycle arrest	• Enhanced cyclin dependent kinase 1 (Cdk1)/cyclin B1 complex activity. • G2/M phase cell arrest.	Noscapine	(Yun et al., 2021)

3.2.2.1 Noscapine and its derivatives

One of the earliest alkaloids identified from *Papaver somniferum* was noscapine. Even though it is discovered in a plant with a high quantity of alkaloids, it chemically and pharmacologically has no similarities to the narcotic alkaloids found in *Papaver somniferum*. Early toxicity tests revealed that it was the least hazardous of the alkaloid opiates (DeBono et al., 2015). Noscapine has long been used as an anti-tussive medication. Its capacity to attach stoichiometrically to tubulin, halting division of cells at the metaphase step and ultimately to apoptosis, is credited with its function. Its safety and less toxicity have been proven, and a lot of techniques for manufacturing its physiologically active (-) - α isomer which is occurred naturally have been discovered (Tomar et al., 2018). Even at stoichiometric doses, noscapine did not depolymerize the microtubules.

Instead, it just reduced their dynamics. Although effective, some microtubule-interacting chemotherapeutics have adverse effects that are controlled by the therapy such as immunosuppression and peripheral neuropathies. Cancers that reoccur are frequently resistant to these treatments. Noscapine, on the other hand, is still effective in some cases, such as taxane-resistant ovarian cancer. Moreover, noscapine and its analogs have no neurotoxic or immunosuppressive effects (Mahmoudian & Rahimi-Moghaddam, 2009). While noscapine offers numerous benefits over current anti-cancer medications like toxicity is low, safety is excellent, bioavailability is high (particularly against drug-resistant cancer strains), and oral delivery, it also has the disadvantage of seeking a high Effective Dose (ED50) to exhibit substantial bioactivity. The activity of noscapine analogues is substantially more, making these molecules additionally appealing as anti-cancer medicines (Tomar et al., 2018).

In addition, the structure of 9-Bromo noscapine and its analogs was studied. In a rodent model of neurodegenerative illness and stroke victims, noscapine was found to be neuroprotective. Noscapine and its derivative 9-Br-Noscapine, like low dosages of colchicine, have anti-inflammatory properties. There is evidence that noscapine can be used to prevent ischemia-reperfusion damage and fibrosis. Within 401 kilobases of genomic DNA, the whole noscapine biosynthetic pathway is stored as a genes coding, enabling large-scale biotechnological manufacture of noscapine for medical purposes. As a result, noscapine and its derivatives (noscapinoids) may be inexpensive and safer anticancer agents. Because of its low toxicity, it might potentially be used as a preventative measure in high-risk scenarios (Mahmoudian & Rahimi-Moghaddam, 2009).

3.2.2.2 Berberine derivatives

Berberine (BBR) has been widely researched in both *in-vitro* and *in-vivo* investigations. BBR reduces cell growth via triggering apoptosis and regulating the cell cycle and autophagy. By hindering epithelial-mesenchymal transition (EMT) and downregulating the synthesis of

proteins and signaling cascades involved in metastasis, BBR also prevents cell invasion and metastasis. BBR also suppresses telomerase activity and reduces cell growth via interacting with microRNAs. BBR has anti-inflammatory and antioxidant characteristics, as well as the ability to modulate the tumor microenvironment.

Cancer develops as a result of changes in the cell cycle. BBR was shown to control the cell cycle and reduce cell growth in a variety of malignancies in studies. BBR inhibited the cyclin D1 and cyclin E1 expression in A549 lung cancer cells, causing them to enter the G1 stage of the cell cycle. In colorectal cancer cells, a combination of an Hsp90 inhibitor and BBR decreased cell development by inhibiting CDK4 expression and modulating cyclin D1. BBR decreased cyclin D1 expression in HepG2 human hepatoma cells *in-vivo* and *in-vitro*. Furthermore, in some neoplastic cells, BBR stopped the cell cycle in G1 by reducing cyclin B1 levels and suppressing CDC2 kinase indirectly (Wang et al., 2020).

By activating caspases, BBR has been demonstrated to cause apoptosis. BBR aided cell death in leukemia by raising the production of caspase-8 and caspase-9 and suppressing the expression of bcl-2 via caspase-3 actuation. BBR activated caspases via raising cytochrome C levels, activating AMPK (AMP-activated protein kinase), and increasing ROS (Reactive oxygen species) production. Mitochondria play a vital part in controlling apoptosis. External stimulation enhanced mitochondrial membrane susceptibility, which triggered the caspase pathway and caused apoptosis, according to research. In hepatoma cells, this signaling pathway was similarly engaged in BBR-induced apoptosis (Wang et al., 2020).

Autophagy is a type of programmed cell death that aids in the maintenance of cellular homeostasis. BBR activates autophagy in glioblastoma cells by attacking the AMPK/mTOR network. BBR caused autophagic death in breast cancer by regulating JNK (c-Jun N-terminal kinases) phosphorylation and causing the bcl-2/beclin-1 complex to dissociate.

BBR triggered autophagy in hepatoma cells by encouraging the discharge of beclin-1 from the bcl-2/beclin-1 complex. Autophagy-mediated drug resistance has been shown to play a crucial part in cancer progression, and tumor cells can dodge apoptosis by regulating autophagy. Drug resistance could be resolved with BBR via activating AMPK and controlling autophagy. Moreover, patients with cancer die as a result of unregulated cell growth, tumor cell invasion, and metastasis, which destroy tissues and organs. BBR decreased cell maturation and metastasis in breast cancer cells by attacking ephrin-B2 and lowering the formation of matrix metalloproteinases-2 (MMP-2) and MMP-9 (Wang et al., 2020).

Structures of significant anti-cancer isoquinolines including berberine and noscapine are given below:

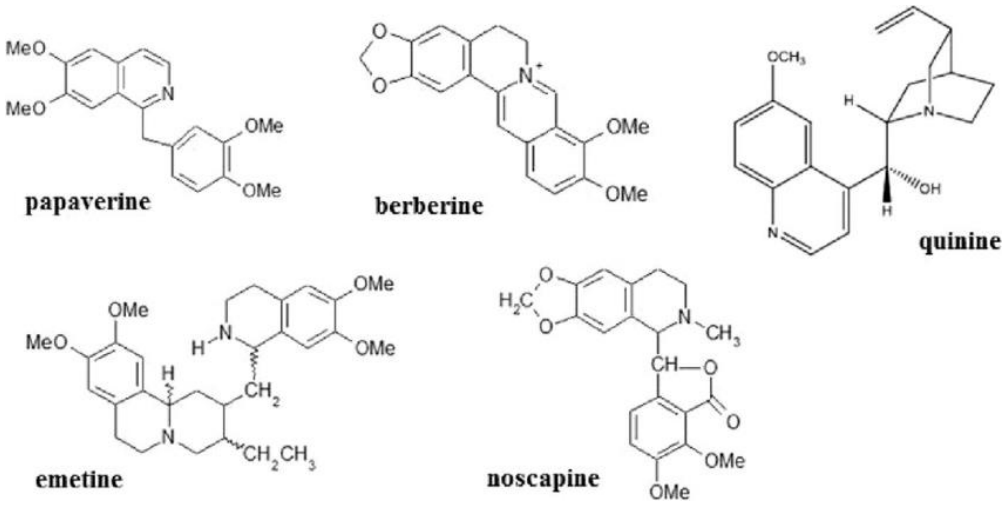

Figure 3. Structure of some anti-cancer isoquinoline alkaloids (Filipiak-Szok et al., 2018).

3.2.3. Vinca alkaloids

Vinca Alkaloids (VAs) were the only other antimitotic drugs to enter the pharmacological marketplace from the world of plants. Initially, VAs were thought to be hypoglycemic agents. This notion was quickly disproved when no drop in blood glucose levels was found in treated rabbits. The animals, on the other hand, died of septicemia as a result of leukopenia. This surprising outcome motivated researchers to dig deeper into this topic and look for a probable

link between VAs and cancer. As a result, phytochemical research guided the isolation and discovery of Vinblastine (VBL), the prototype of VAs, which can cause myelosuppression in leukemia xenograft mice models. This groundbreaking finding paved the way for a novel cancer treatment strategy. As a result, the FDA authorized VAs as a pharmacological approach against many tumor types (such as leukemia, Hodgkin's lymphoma, breast cancer, and lung cancer). Five vinca alkaloids are approved for clinical use, natural vinblastine (VBL) and vincristine (VCR), which have been officially approved in 1961 and 1963, respectively by FDA; semisynthetic derivatives vindesine (VDS), which is used clinically in a few countries; vinorelbine (VRL), which was approved by the FDA in 1994; and vinflunine (VFN), a bis-fluorinated VAs derivative obtained through superacid chemistry, which was approved by EMA (European Medicines Agency) in 2012 for second-line treatment of metastatic and advanced urothelial cancer. The presence of an indole nucleus (catharanthine) and a dihydroindole nucleus (vindoline) connected by a C-C bridge characterizes the structure of VAs (Martino et al., 2018).

Due to their way of interrelating with tubulins and restricting microtubule polymerization, microtubule-targeting agents (MTAs) are a beneficial family of anti-neoplastic drugs. This interaction inhibits the start of anaphase and allows the cells to stay in a state like the G1 phase or a protracted arrest state, as a result of which rapidly growing cells die. VAs are classified as MTAs. These molecules stop cells from dividing by attaching to tubulin proteins in the mitotic spindle, preventing them from polymerizing into microtubules. VAs have concentration-dependent effects on tubulin polymerization kinetics. VAs impede the production of microtubules at low concentrations by binding directly to the (+) microtubule structure, inhibiting GTP binding and preventing the cross-linking of MAP (Microtubule-associated proteins) indirectly. At elevated levels, VAs attach tubules in a specific shape by forming spirals and paracrystalline complexes, resulting in microtubule depolymerization. In general,

the final result is mitotic spindle disintegration, which causes chromosomes to accumulate in abnormal shapes (balls and stars), resulting in cell death via p53-dependent and/or independent apoptotic pathways (Martino et al., 2018).

Table 4. Characteristics of VAs in terms of pharmacokinetics and toxicology (Martino et al., 2018).

Parameters	**Vincristine (VCR)**	**Vinblastine (VBR)**	**Vindesine (VDS)**	**Vinorelbine (VRL)**
Plasma half lives	23-85	20-64	20-24	18-49
Metabolism	Hepatic	Hepatic	Hepatic	Hepatic
Clearance	Excretion via biliary route	Excretion via biliary route	Excretion via biliary route	Excretion via biliary route

The cytochrome P450 CYP3A enzyme in the liver is the key operator in the metabolism of these antimitotic medicines. In the case of VAs, P450 CYP3A4-mediated metabolism might be regarded as a detoxifying mechanism. Indeed, it encourages the conversion of VAs into compounds with lower toxicity profiles. Furthermore, the various metabolites produced by CYP3A4 may be linked to VA toxicity that is medically idiosyncratic. Patients with reduced hepatic activity may have abrupt and severe autonomic neurotoxicity at this stage of pharmacokinetics. Therefore dosage changes should be considered. Furthermore, VAs should not be used with erythromycin or other CYP3A4 inhibitors; otherwise, VA metabolism may be affected (Martino et al., 2018).

In terms of therapeutic relevance, VAs are frequently utilized as anticancer medications to treat acute lymphocytic leukemia, breast cancer, and osteosarcoma, either alone or in combination

with other treatments. They were first used to treat juvenile hematologic malignancies, later expanded to include solid and adult hematologic malignancies (Martino et al., 2018). Vincristine >vinblastine >vinorelbine are the tubulin affinities in sequence.

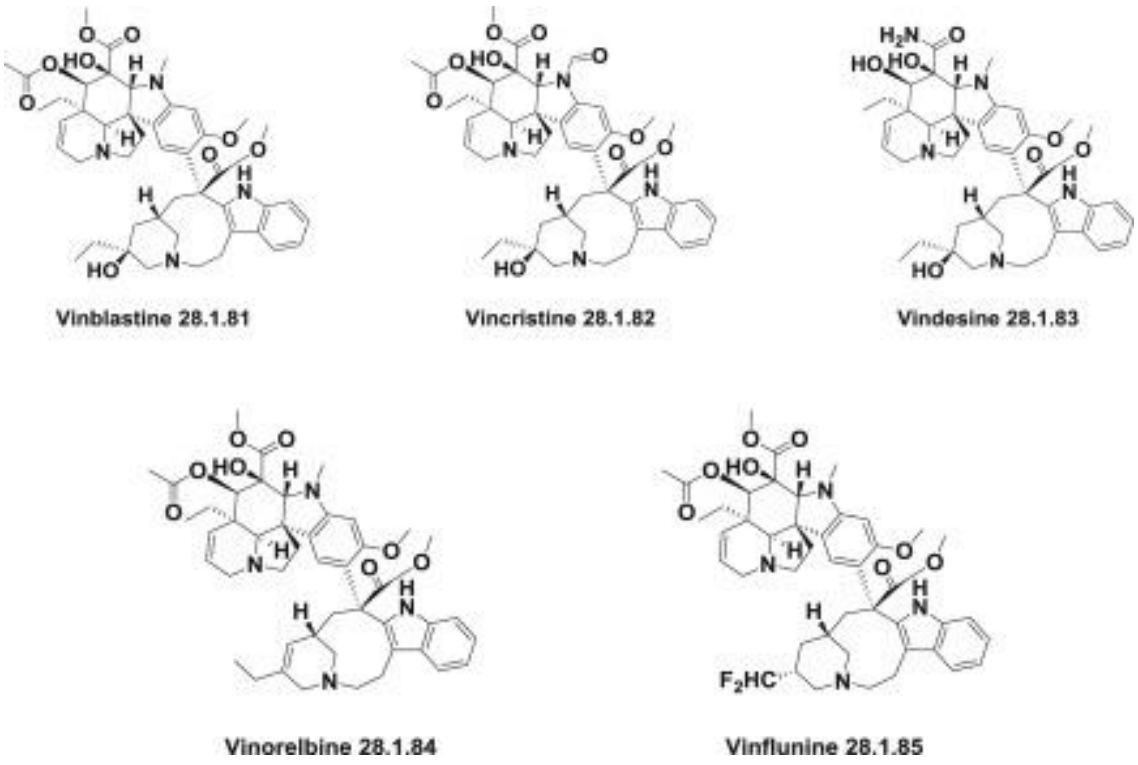

Figure 4. Structure of some vinca alkaloids (Vardanyan & Hruby, 2016).

Table 5. The use of vinca alkaloids (VAs) in various cancer therapies, their mechanisms and negative effects.

VAs	Treatments	Side effects	Mechanism	Reference
(VBL) Vinblastine	It treats leukemia, Ewing's sarcoma, Testicular carcinoma. Also, breast cancers, germ	It can be destructive to WBCs and can cause dyspnea, discomfort in	Vinblastine targets the cell cycle. The drug binds to tubulin selectively and prevents tubulin from polymerizing into	(Shah & Avashia-

	cell tumors, and Nephroblastoma.	chest, wheezing, nausea along with fever, vomiting, constipation. It can lead to antidiuretic hormone production and loss of weight.	microtubules, halting cell division in metaphase. Cell death is likely to occur if adequate chromosomal segregation is not achieved during mitosis. Melanoma cells were injected with vinblastine in amouse model, which caused apoptosis, maturation of tumor-infiltrating dendritic cells, and cytotoxic T-cell activity.	Khemka, 2021)
(VCR) Vincristine	Philadelphia chromosome-negative acute lymphoblastic leukemia, neuroblastoma, colorectal cancer, estrogen-receptor negative breast cancer, B-cell lymphoma, metastatic	Peripheral neuropathy, decrease in body weight, mouth sores, dizziness, stomach or abdominal pain or cramps, headache are all side effects. It can also	Their anticancer activity is thought to be mainly resulting due to its interaction with tubulin, which suppresses mitosis during metaphase. They can disturb the metabolism of cyclic AMP, amino acid, and glutathione as well as calmodulin-dependent Ca2+-transport ATPase action, cellular	(Martino et al., 2018)

	melanoma, and glioma are treated. It can also treat rhabdomyosarcoma, multiple myeloma, Wilm's tumor.	cause nausea, vomiting, diarrhea, bloating, Baldness, constipation, and a decreased appetite.	respiration, and nucleic acid and lipid biosynthetic pathway.	
(VDS) Vindesine	Utilized to treat pediatric solid tumors, esophageal, colon, breast, and renal cancers that have metastasized, acute lymphocytic leukemia, malignant melanoma, as well as blast crisis of chronic myeloid leukemia.	It can cause side reactions like leucopenia, thrombocytopenia, fatigue, paralytic ileus, constipation, decreased sensation, sore mouth, nerve pain, weight loss, diarrhea, convulsions, and depression.		(Martino et al., 2018)

(VFN) Vinflunine	After failure of platin-containing treatment, metastatic and advanced urothelial carcinoma develops which can be treated by using vinflunine.	Neutropenia, alopecia, leucopenia, anemia, myalgia, thrombocytopenia, fatigue, weight reduction, decreased appetite, vomiting or nausea, abdominal pain, diarrhea are all side effects.		(Martino et al., 2018)
(VRL) Vinorelbine	It can treat metastatic breast cancer, rhabdomyosarcoma, and Non-small cell lung cancer that has progressed or spread.	Undesirable reactions like nausea, anemia, vomiting, neuropathy, weight loss, constipation,	It suppresses cellular growth by attaching to tubulin, like other vinca alkaloids, although it varies from them in terms of anticancer action. *In-vitro* impacts on dynamic instability include a	(Townsend, 2007)

		diarrhea, muscular weakness can occur.	reduction in shortening duration, a rise in the duration of growth, and a slowdown in microtubule growth rate.	

3.2.4 Vincamine alkaloids

Plants have been demonstrated for being a natural origin for new compounds consisting of a variety of medicinal properties. The alkaloid vincamine is extracted from Vinca minor leaves, often called smaller ordwarf periwinkle. This alkaloid is popular for its anti-neoplastic and neuroprotective properties and also for its function in enhancing cerebral blood flow. Vincamine has indeed been demonstrated to improve brain metabolism by affecting ATP production and glucose and oxygen use, providing increased protection from ischemic and hypoxic attacks temporarily. Vincamine is an antioxidant, neuroprotective, peripheral vasodilator, and anti-apoptotic agent that helps to minimize oxidative stress. Vincamine is an alkaloid isolated from *Catharanthus roseus* and it was evaluated for efficacy as an antineoplastic agent using a variety of bioinformatics methodologies. It was tested in a lung carcinoma cell line and shown to enhance A549 cell apoptosis by triggering a variety of signaling cascades, including caspase-3-induced cell death (Al-Rashed et al., 2021).

Vincamine can pass the blood-brain barrier, and its hydrolysis to vincamic acid (a hydroxycarboxylic acid) induces a complex with Fe to develop in the urine. This procedure can lower the concentration of Fe in cancer cells, preventing them from multiplying. Since iron is required for the synthesis of DNA in rapidly developing cells, cancer cells demand elevated quantities of iron, a condition called iron addiction. To maintain this elevated iron

concentration, cancer cells regularly modify the expression of iron metabolizing genes and proteins (Al-Rashed et al., 2021).

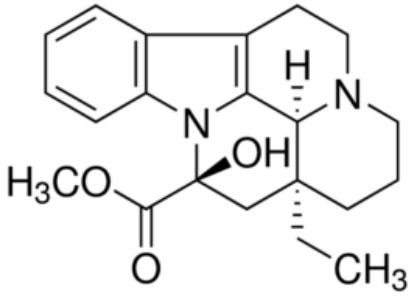

Figure 5. Structure of vincamine alkaloid.

Caspases-3 is responsible for intracellular mitochondrial-mediated apoptosis, which results in the cleavage of several cellular proteins. Vincamine binds tightly to caspase-3 and promotes caspase-3-mediated apoptosis, according to in-silico studies. Activation tests for caspase-3 produced similar results. Caspases-3 inhibition by Z-DEVD-FMK (a tetrapeptide), on the other hand, significantly lowered the anticancer potential of vincamine. As a result, it's reasonable to assume that caspase-3 activation is the primary cause of caspase-dependent apoptosis. Vincamine also lowered the potential of the mitochondrial membrane, which is thought to be an early step in the apoptotic cascade. Cytochrome C is discharged into the cytoplasm when the mitochondrial membrane is broken, resulting in caspase-3 activation and reduced cell survivability. Therefore, vincamine inhibits lung cancer by reducing intracellular iron levels, inducing programmed cell death by breaking the mitochondrial membrane, and activating caspase-3 directly. Vincamine is a benign substance that is often used as a dietary supplement, making it an excellent contender for anticancer clinical studies (Al-Rashed et al., 2021).

3.2.5 Carbazole alkaloids and coumarins

Carbazole alkaloids and coumarins have a significant antitumor effect in many cell types. They are mainly key components of Clausena plants. Carbazoles are tricyclic heteroaromatic

alkaloids with a core structure consisting of two benzene rings joined to a pyrrole ring. They are a crucial heterocyclic class of anticancer drugs, and their therapeutic functions have garnered growing interest because of their numerous biological actions. Clinical studies have been conducted on certain carbazoles with significant anticancer potential. Multidrug resistance has produced issues in patients recruited in the studies. As a result, only a few carbazole kinds have been authorized as anti-cancer agents. Aromatic, polycyclic, and planer carbazoles have anticancer properties through intercalating DNA or inhibiting DNA-dependent enzymes like telomerase and topoisomerase I/II. Clausena plants have been extensively studied as a source of natural carbazole alkaloids and several anticancer carbazole alkaloids have been found so far, which are included in the table below (HUANG et al., 2017).

Table 6. List of some carcinogenic carbazole alkaloids yielded from Clausena plants (HUANG et al., 2017).

3-Methylcarbazole	Clausine O
3-Formylcarbazole	7-Methoxymukonal
Methyl carbazole-3-carboxylate	Clauszoline J
Clauszoline K	Clausine Z
6-Methoxy-9H-carbazole-3-carboxylic acid	Clausine D
2-Hydroxy-3-methylcarbazole	Claulansine N
Mukonal	Clausamine D
O-Demethylmurrayanine	Heptaphylline
Clausines E	Clausine B
Murrayanine	Excavatine A
Murrayafoline A	Heptazoline
Clausine TY	7-Methoxyheptaphylline

Coumarins, on the other hand, are benzene and pyrone ring systems fused and have a variety of biological functions. Coumarins have a huge anticancer potential and just a few adverse effects, depending on the fundamental nucleus replacements. Coumarins with anticancer properties have been the subject of study for many years as significant components of Clausena plants (HUANG et al., 2017).

Table 7. List of some anticancer coumarins separated from Clausena plants (HUANG et al., 2017).

Xanthoxyletin	Clauslactone D
Nordentatin	Clauslactone E
Clausarin	Clauslactone F
Dentatin	Clauslactone H
Clauslactone A	Clauslactone I
Clauslactone B	Clauslactone J
Clauslactone C	Scopoletin

Su et al. discovered three chemicals from the medicinal plant *C. excavata* in 2009: nordentatin, clausarin, and clausenidin. These compounds were shown to have cytotoxic action versus 4 cancer cell lines from humans which are MCF-7, KB, A549, and KB-VIN, with EC50 values of 7.96, 1.61, 2.98, and 1.59 μg/ml respectively. Xanthoxyletin, nordentatin, dentatin, and clausenidin, all derived from the roots of *C. excavata*, were shown to exhibit cytotoxic action against the NCI-H187 cell line, with IC50 values of 35.54, 7.10, 15.92, and 8.63 μg/ml respectively. Furthermore, scopoletin was discovered to have cytotoxic action against the MCF-7 cell line. Dentatin, a substance derived from the roots of *C. excavata*, causes apoptosis

by activating mitochondrial signaling, nuclear factor κ-B (NF-κB) signaling, and arresting the G0/G1 cell cycle (HUANG et al., 2017).

3.2.6 Indole quinoline alkaloids

The indole skeleton is one of the most commonly used heterocycles in bioactive components, such as anticancer drugs. It has been a prominent concept for target-based design and production of anticancer therapies due to its diversity and adaptability. Several researchers have revealed many indole-based compounds showing the relevance of the indole motif in the development of antitumor drugs. The indole core is now being used in the research of novel anticancer medicines targeting DNA topoisomerases, sirtuins, PIM kinases, histone deacetylases (HDACs). Compounds generated from indoles that attack these cancer cell enzymes or receptors might be a promising anticancer therapeutic option. Among the natural indoles, vincristine and vinblastine, antimitotic drugs isolated from *Catharanthus roseus*, have a long history of usage in the treatment of many malignancies, including breast cancers, Hodgkin's disease, testicular cancers, non-lymphoma, and Hodgkin's Kaposi's sarcomas. Mitomycin C, which has an oxidized indole nucleus, is another example of anticancer indole alkaloids. *In-vivo* bioreductive activation of this antitumor antibiotic results in the formation of inter and intra-strand cross-links with DNA. Furthermore, bisindole alkaloids such as hyrtinadine A, dragmacidin, topsentin, nortopsentin D, and hyrtiosins B were discovered to be cytotoxic to cancer cell lines (Dadashpour & Emami, 2018).

Anticancer indoles target a variety of pathways in cancer cells, according to biological assessments and mechanistic studies. Many small compounds with an indole structure have been characterized as tubulin polymerization inhibitors with the ability to interact with the colchicine binding site. Anticancer indoles attacks DNA topoisomerases, histone deacetylases (HDACs), sirtuins, PIM kinases, and receptors. New anticancer drugs were discovered owing

to the design of new indole compounds based on targets and SAR analyses. Numerous HDAC inhibitors derived from indole were in various stages of clinical studies or were being commercialized as anticancer medicines. While there are various limitations to designing HDAC inhibitors that are specific for malignancy therapy, the indole-derived lead compounds indicated that both class-selective and isoform-specific HDAC inhibitors may be generated. A hydroxamic acid functionality as a metal-binding moiety connected to a capping group through an aliphatic or aromatic spacer is found in the majority of HDAC inhibitors. In the development of novel HDAC inhibitors, the indole skeleton has been employed as a capping group as well as an aromatic spacer. The interplay between each pharmacophoric part of the HDAC inhibitor for imposing isoform-specificity will be a significant topic of future research to create effective isoform-selective inhibitors based on the indole scaffold. In indole-based hydroxamic acids, individual modification of the capping group and linker can improve selectivity, simultaneous alteration of multiple of these areas is probably required to develop class-selective and isoform-selective HDAC inhibitors. The efficacy of indole-based HDAC inhibitors might vary depending on their physical characteristics and pharmacokinetic properties. Although the prerequisites for selectivity have not yet been clearly defined, a parallel study in this field is required to increase bioavailability orally and enhance HDAC inhibitor half-lives (Dadashpour & Emami, 2018).

3.2.7 Antofine analogues

Phenanthroindolizidine alkaloids are a small category of alkaloids extracted mostly from Cynanchum, Tylophora, Pergularia, and others. Antitumor, antiamoebic, antibacterial, and antifungal activity are among the biological effects of these pentacyclic natural compounds. The substantial cytotoxicity against a variety of cancer cell lines is the most fascinating trait among these intriguing biological activities. For example, against drug-sensitive KB-3-1 and multidrug-resistant KB-V1 cancer cell lines, antofine analogues have IC50 values in the low

nanomolar range, which are equivalent to those of clinically used cytotoxic medicines (Fu et al., 2007).

Phenanthroindolizidine alkaloids have gotten a lot of recognition as possible therapeutic leads because of their strong cytotoxic action and intriguing metabolic properties. This category of natural products, however, has yet to be established for therapeutic use. The strong central nervous adverse effects and low water solubility of this compound group are the key disadvantages to its possible therapeutic application. Furthermore, antofine has metabolic instability and so its pharmacokinetic profile is poor, according to some unpublished mouse findings. *In-vivo* metabolic instability was shown to be caused mostly by the methoxy moiety on the phenanthrene ring (Fu et al., 2007).

To summarize, a range of antofine analogues with various phenanthrene ring substituents were tested. The current study found that steric bulkiness is not tolerated at the 2-position of the antofine, but that minor changes at position 3 are likely to be accepted. The 6-position substituent appears to be associated with hydrogen bonding as a donor. These findings are important for developing the SAR of the phenanthrene ring in phenanthroindolizidine alkaloids and laying the groundwork for future research (Fu et al., 2007).

3.2.8 Curcumins

Curcumin's therapeutic efficacy has been observed in malignant cells or neoplasms *in-vitro* and ex vivo such as brain tumors, pancreatic cancer, lung cancer, breast cancer, leukemia, prostate cancer, skin cancers, and hepatocellular carcinoma, incorporating antimetastatic properties and cytotoxic activity on cancer stem cells (Seca & Pinto, 2018). Curcumin has also been shown to have potent anticancer properties, including tumor prevention and antiangiogenic action *in-vivo* and *in-vitro*, against variety of malignancies. Curcumin also has pleiotropic qualities that affect proteins including cyclooxygenase-2 (COX-2), thioredoxin reductase, protein kinase C

(PKC), tubulin, and 5-lipoxygenase, among others. Curcumin also influenced transcription factors, cytokines, growth regulators, and their receptors, and enzymes (Varsha et al., 2017).

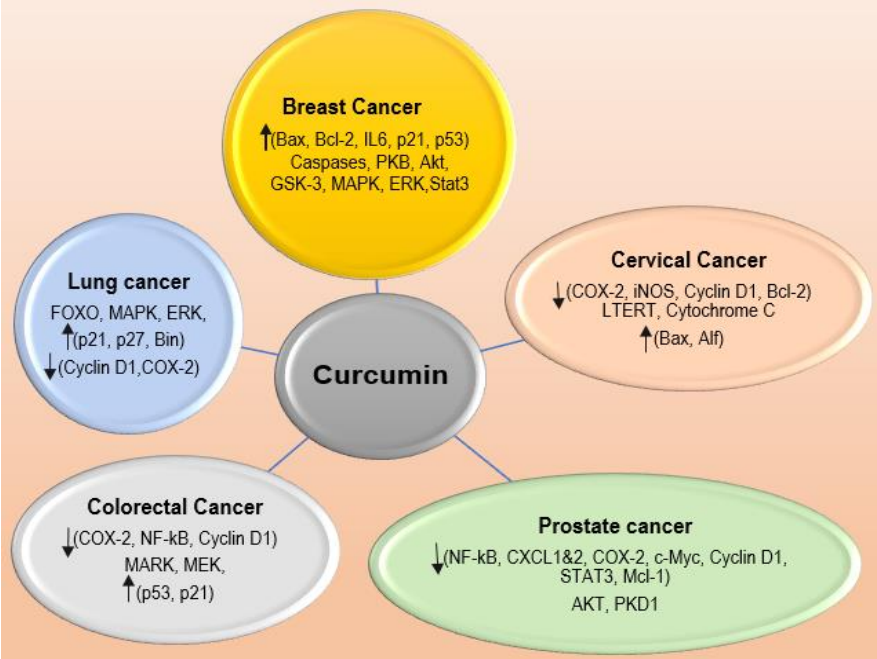

Figure 6. Molecular targets of curcumins in different cancer therapies (Bagla et al., 2015).

Dietary curcumin has poor bioavailability in phase I clinical study, as evidenced by quick metabolism, reduced plasma and tissue concentrations, and substantial quick elimination. Low aqueous solubility (higher solubility in alkaline solutions) and unpredictable absorption are two potential reasons that restrict curcumin's bioavailability. When curcumin is taken orally, it undergoes substantial hepatic first-pass metabolism in the liver. Furthermore, clinical research with human colorectal cancer volunteers revealed that the malignancy did not react to a daily large dosages of curcumin of 3.6 g given for four months. As a result, after concomitant administration of curcumin, there were no significant alterations in the volume of the tumor, the weight of the body, or tumor markers. Overall, studies have shown that curcumin has anticancer properties at a concentration of 5–30μM for 1–2 days. But attaining this therapeutic dosage at the tumor location in humans is challenging due to its poor solubility, low dissolution, inferior bioavailability, and greater metabolic activities. Curcumin must thus

be adjusted in nanomedicine to address these key physicochemical and pharmacokinetic constraints (Varsha et al., 2017).

Curcumin has also been proven to work as a chemosensitizer for several antitumor medications in clinical trials. (e.g., gemcitabine, 5-fluorouracil, paclitaxel, doxorubicin) and to have a synergic action when combined with other natural products (e.g., resveratrol, epigallocatechin-3-gallate honokiol, licochalcone, and omega-3) characteristics that might be employed as a viable method for overcoming tumor resistance and preventing recurrence. These findings imply that when curcumin is combined with other compounds, it can attain a higher therapeutic index, which could be beneficial in the treatment of various malignancies. In any case, further research is necessary to determine the actual mechanism of curcumin's synergistic impact. Curcumin's therapeutic applications has been hindered because of its decreased absorption, inadequate metabolism, and low systemic bioavailability, necessitating patients to take up to 8–10 grams of free curcumin orally every day to get quantifiable amount in the blood. As a result, a number of techniques have been offered to address curcumin bioavailability issues, including –(i) the utilization of adjuvants such as piperine that causes glucuronidation and disrupts curcumin metabolism, (ii) curcumin preparations using nanotechnologies, such as liposomes, micelles, and phospholipids, and (iii) the utilization of curcumin analogues. There are presently 17 open clinical trials employing curcumin, most investigations of combination curcumin therapy with other drugs for treating various forms of cancer, as a consequence of its anticancer potential and despite its clinical therapeutic limits (Seca & Pinto, 2018).

3.2.9 Taxane alkaloids

Taxane alkaloids are microtubule stabilizing compounds that is used to treat large solid tumors. Tubulin stabilizing drugs prevent microtubules from depolymerizing into tubules, resulting in an increment in the quantity of microtubule chains due to consistent polymerization. These

drugs primarily bind to the taxol binding site. Protofilaments are generated when GTP attaches to a tubulin dimer and is hydrolyzed. Such compounds attach to β-tubulin on the internal side of the microtubule lumens, causing the polymerization process to speed up (Mishra, 2011).

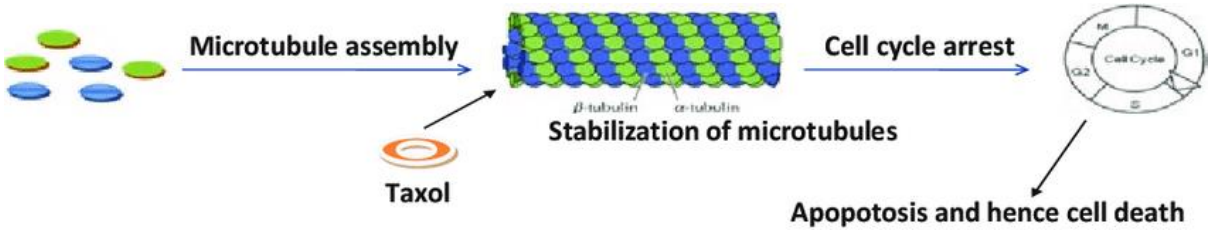

Figure 7. Mechanism of action of taxols (Ballout et al., 2019).

In 1971, Wall was the first to extract paclitaxel from *Taxus brevifolia* Nutt's stem bark. Ovarian, breast, prostate, nonsmall-cell lung cancers, and Kaposi's sarcoma are the most common malignancies treated with it. It is commercially manufactured from semi-synthetic alterations of Baccatin III, a natural substance derived from *Taxus baccata*, due to its limited abundance in the plant. Docetaxol, has been created to solve the current problem of significant adverse effects, resistance due to efflux pump upregulation, and poor bioavailability of paclitaxel (Bocci et al., 2013).

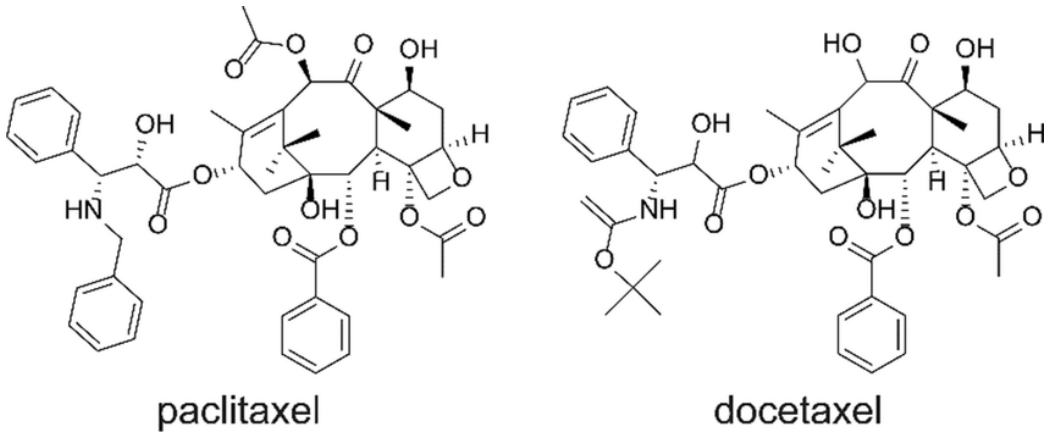

Figure 8. Molecular structure of paclitaxel and docetaxel (Tong et al., 2021).

Docetaxel is a drug that is offered under the label of Taxotere and is used to treat prostate cancer, stomach carcinoma, non-small cell lung cancer, head and neck cancer, and breast

cancer are all treated with it. Docetaxel and paclitaxel are taxanes that impede microtubule depolymerization and consequently cell progression through the M-phase of the cell cycle, but by a completely different action on microtubular function (Smith et al., 2022).

The emergence of resistance to paclitaxel and its similar molecules is a serious problem. There are a number of mechanisms, tubulin isotope amplification is among the most well-known. Paclitaxel attaches to tubulin through the β-isomer that is further divided into six isotypes. Due to paclitaxel's considerable sensitivity to Class III β-tubulin, resistance has developed. Several semi-synthetic compounds of taxane have been researched to combat the lack of tumor specificity and multidrug resistance. By modifying the taxane's side chains, semi-synthetic analogues were generated which have demonstrated to be quite effective in clinical trials (phase I and phase II) (Bocci et al., 2013). Cabazitaxel is a taxane analogue that is semi-synthetic. It kills the tumor cell lines P388/DOX, Calc18/TXT, KBV1, P388/TXT, HL60/TAX, and P388/VCR that are resistant to docetaxel. Another semi-synthetic analogue of taxane is Larotaxel that works in a similar way to docetaxel. It has been shown to have antineoplastic effect against a wide range of tumor cells that are resistant to taxol. Ortataxel, a semi-synthetic second-generation taxane derivative and has shown to be as effective as paclitaxel in tumor prototypes *in-vitro* and *in-vivo*. It showed promising outcomes in patients with breast cancer who were resistive to paclitaxel or docetaxel therapy in a phase I clinical trial. Tesetaxel is a taxane derivative that is semi-synthetic that may be taken orally. It showed greater anticancer activity compared to docetaxel and paclitaxel in several *in-vitro* and *in-vivo* investigations and also showed robust action in resistant cell lines up-regulating P-glycoprotein. In a phase II clinical study, it was utilized as a second-line treatment for metastatic colon cancer (Naaz et al., 2019).

Chapter 4: Marine-Derived Alkaloids

4.1 Overview of different classes of marine-derived alkaloids

Four chemotherapeutic medicines have so far been authorized, with eighteen more medication candidates in the queue, marine biodiversity has been extremely favorable in the zone of cancer. New bioactive marine natural compounds account for more than half of all new natural products which were separated between 1985 and 2012 that showed cytotoxicity towards experimental cancer models. 49 percent of anticancer medicines licensed before 2014 were classed as natural products or directly derived from marine sources, the establishment of these anticancer treatments confirmed the enormous effect of natural products on the existing chemotherapeutic regimen (Pereira et al., 2019).

A variety of bioactive alkaloids have been identified in cyanobacteria. The ability of cyanobacteria to build enormous colonies is due to their ability to photosynthesize and fix nitrogen. The large number of bioactive compounds identified from cyanobacteria made this group a promising subject for further research. 300 alkaloids have been identified from 800 compounds recovered from marine cyanobacteria, with a large number coming from the genera Lyngbya and Symploca. The majority of these marine alkaloids are generated as powerful predatory poisons. They are secondary metabolites with unique skeletons and a wide spectrum of anti-cancer properties. The structures, biosynthesis processes, and mechanisms of action of these alkaloids are being investigated in the hopes of producing novel cancer-fighting medications. Lyngbya majuscula, marine filamentous blue-green algae, produces around 30% of the bioactive compounds obtained from cyanobacteria. Lyngyabellins and hectochlorin attack the apratoxins which are cytotoxic and also target the polymerization of actin or tubulin. Hectochlorin and lyngyabellin are cyclic depsipeptides that address the actin filaments of cells. In human Burkitt lymphoma CA46 cells, hectochlorin, which causes actin

hyperpolymerization, causes G2/M phase arrest. This metabolite's anti-neoplastic actions on colorectal, squamous cell carcinoma, ovarian and renal malignancies were demonstrated in cytotoxic tests against 60 cancerous cell lines. Lyngyabellins, like hectochlorin, exhibited anti-proliferative properties in a variety of cell types. Lyngyabellins E, D, F, and H were shown to be highly toxic to the human lung cancer cell line NCIH460, whereas lyngyabellin I was found to be cytotoxic to mouse neuroblastoma neuro2-a cells. The most thoroughly researched molecule, Lyngyabellin A, was demonstrated to impair the microfilament framework. As a result, cytokinesis occurs in colon cancer cells, causing the generation of apoptotic cells. Lyngyabellin B is thought to behave similarly to lyngyabellin A, and its biological action is currently being researched. Above all, the selectivity of these lyngyabellins for malignant cells makes them promising candidates for cancer therapies (Tohme et al., 2011).

Marine fungi have brought new alkaloids. The diverse range of secondary metabolites generated by marine fungus is largely attributable to the harsh environments in which they develop, as well as various symbiotic connections with other marine animals. Marine fungi face various pressures, temperatures, nutrient scarcity and saltiness than terrestrial fungi, resulting in the formation of new biochemical pathways and the synthesis of new compounds that help them adapt to severe environments. When marine fungus metabolites were tested for anti-cancer potential, it was discovered that several of them displayed promising anti-tumorigenic capabilities as opposed to a number of different human cancer cell lines. Bioassay-guided fractionation was used to extract the most researched alkaloids from species of *Actinomycetes*, *Penicillium*, and *Aspergillus*. Alkaloids from *Penicillium janthinellum* and *Actinomycetes Z2039-2* have been discovered to trigger apoptosis in cancerous cells. Shearinine A, D, and E, three indole alkaloids extracted from *Penicillium janthinellum*, were found to trigger apoptosis in HL-60 human pro-myelocytic leukemia cells. Two indolocarbazole alkaloids (K252C and arcyriaflavin) were separated from *Actinomycete*

Z2039-2 and demonstrated to trigger apoptotic cell death in a cell line with myeloid leukemia using bioassay-guided fractionation (K562) (Tohme et al., 2011).

Marine sponges, which live in salty water, have been around for a long period and are one of the ancient living species on the planet. Although the amounts of secondary metabolites generated by these marine creatures are minimal, they aid in predator avoidance and competition with sessile species. In addition, sponges have symbiotic interactions with some microbes, such as bacteria and fungus that are thought to supply the bioactive molecules. A few of the found compounds and analogs have gone to phase I and II cancer clinical trials, such as eribulin mesylate, an analog of the macrocyclic polyether halichondrin B, which is being evaluated for breast cancer treatment that has progressed to other sections of the body. Alkaloids produced from sponges have been found to show activity comparable to Paclitaxel and camptothecins which are chemotherapy medicines produced from plants that are being used in clinical practice. These marine-derived alkaloids suppress cell growth by modulating the equilibrium of anti-apoptotic and pro-apoptotic proteins and targeting topoisomerases and tubulin polymerization. Some can even stop cells from migrating and invading, preventing metastasis (Tohme et al., 2011).

4.2 Anticancer activity of marine-derived alkaloids

Antibacterial, anti-inflammatory, antifungal, antimalarial, antiplasmodial, anti-HIV, carcinogenic, larvicidal, glucose uptake stimulatory, trypanocidal, anti-cholinesterase, and vasodilatory actions are among the bioactivities of marine-derived alkaloids. Furthermore, alkaloids derived from marine creatures often have unique scaffolds not seen in terrestrially related species (Wibowo et al., 2021).

About 2,000 alkaloids were discovered in tropical plants, but just a few in marine species. Trabectedin is a tetrahydroisoquinoline alkaloid that was isolated from the Caribbean sea squirt

Ecteinascidia turbinata at the time of the National Cancer Institute's (NCI) extensive extraction and testing of natural products in the 1960s. Data from many tumor models confirm trabectedin's anticancer efficacy, and its acting mechanism also included an effect on the tumor microenvironment, primarily macrophages. Since 2007, the EMEA has authorized trabectedin for the management of progressive soft tissue sarcoma cases that have failed to respond to anthracyclines and ifosfamide or who are otherwise unsuitable for it. The FDA authorized trabectedin in October 2015 for the therapy of metastatic liposarcoma and leiomyosarcoma individuals, who had been treated before with an anthracycline-based regimen. This novel authorization was developed after a phase III trial revealed a 45 percent decrease in the possibility of cancer progressing or mortality in comparison to dacarbazine, making trabectedin the first medicine to be approved by the FDA for liposarcoma therapy. Lately, trabectedin is being tested in clinical trials for different cancers such as breast, bone, prostate, and ovarian cancer, either alone or in combination with other drugs (Barreca et al., 2020).

Lurbinectedin was authorized by the FDA for adult individuals with metastatic small cell lung cancer and it progressed after or while receiving platinum-based chemotherapy. This was found on the basis of the findings of phase II research that showed an ORR of 35% and a median response time of 5.3 months (Barreca et al., 2020).

Table 8. Apoptosis-inducing mechanisms of some marine alkaloids (Tohme et al., 2011).

Alkaloid	**Organism**	**Mechanism of activity**
Apratoxin	Cyanobacteria	Suppression of FGFR and G1 cell cycle halting.
Largazole	Cyanobacteria	HDAC inhibitor
Hectochlorin	Cyanobacteria	Actin filament hyperpolymerization

Shearinine E	Fungi	Suppression of EGF
Lyngyabellin	Cyanobacteria	Actin filament hyperpolymerization
Trabectedin	Tunicates	Alkylation of DNA, Disintegration of RNA pol II, cell cycle halt in the S phase, Increment in CCL2, Decrease in VEGF, Drecrease in IL-6.
Hemiasterlin	Sponges	Depolymerization of tubulin
Variolin B	Sponges	Cell cycle is halted in the G1 and G2 phase.
Granulatimide	Tunicates	Inhibition of G2 checkpoint
Polycarpines	Tunicates	↑p53
Kuanoniamine A	Sponges	Cell cycle halt in the G1 stage.

Various anti-cancer medications developed from marine substances have been licensed for using clinically, and marine-based pharmaceuticals have begun to have an influence on modern pharmacology such as cytarabine, enfortumab vedotin, vidarabine, belantamab mafodotin, nelarabine (prodrug of ara-G), plitidepsin, trabectedin, eribulin mesylate, fludarabine phosphate (pro-drug of ara-A), brentuximab vedotin, lurbinectedin, and polatuzumab vedotin (Barreca et al., 2020).

4.3 Chemotherapeutic line of marine alkaloids

4.3.1 Licensed drugs and agents in clinical trials

Looking back, it's worth noting that there were just four medications originated from marine sources that were licensed for cancer and melanoma therapy at the start of 2018. These included cytarabine (Cytosar-U®, Pfizer's first marine-derived drug licensed in 1969), trabectedin (Yondelis®, PharmaMar), eribulin mesylate (Halaven®, EisaiInc.), and the antibody-drug conjugate (ADC) brentuximab vedotin (Adcetris®, SeattleGenetics). In 3 years since 2018, 5 new medications were authorized worldwide for the cure of different malignancies; two of these were only recently approved in 2020. The following drugs were added to the five marine pharmaceuticals described above (Dyshlovoy & Honecker, 2020):

- Plitidepsin (Aplidin®, manufactured by PharmaMar), which causes oxidative stress in cancer cells, was licensed for the first time in Australia in 2018 for the cure of leukemia, multiple myeloma, and lymphoma (Dyshlovoy & Honecker, 2020).
- Polatuzumab vedotin (PolivyTM, developed by Genentech, Roche) is an ADC (antibody-drug conjugate). By triggering ADC cleavage and MMAE discharge, the antibody delivers MMAE (Monomethyl auristatin E) to cancer cells in a targeted manner. After that, tubulin polymerization is disrupted, causing the demise of cancer cells. In 2019, the FDA authorized the medicine to treat B-cell lymphomas, non-Hodgkin lymphomas, and chronic lymphocytic leukemia (Dyshlovoy & Honecker, 2020).
- Enfortumab vedotin (PADCEVTM, Astellas Pharma, and Seattle Genetics) is another ADC that contains MMAE and a nectin-4-specific antibody. In 2019, it got FDA authorization for the therapy of metastatic urothelial carcinoma (Dyshlovoy & Honecker, 2020).

- Belantamab mafodotin (BlenrepTM, GlaxoSmithKline) is another ADC that uses MMAF (monomethyl auristatin F, one of the MMAE derivatives) as the warhead and is linked to a BCMA (B-cell maturation antigen) targeting antibody). MMAF, like MMAE, is a tubulin polymerization blocker. In 2020, the medicine will be licensed for the management of patients with relapsed or refractory multiple myeloma (Dyshlovoy & Honecker, 2020).
- Lurbinectedin (ZepzelcaTM, PharmaMar) is a synthetic trabectedin analogue that adheres to DNA's minor groove and inhibits and degrades RNA polymerase II, which has anticancer properties. In 2020, the medication received FDA approval for treating small cell lung carcinoma that has spread metastatically (Dyshlovoy & Honecker, 2020).

The unique skeletons and structural complexity of Marine macrocyclic natural products (MMNPs) have aided in the identification of anticancer medications like trabectedin, a tetrahydroisoquinoline alkaloidal derivative that has been authorized as an anticancer treatment by the FDA and the European Agency for the Evaluation of Medicinal Products (EMEA) (Althagbi et al., n.d.). Despite the fact that the mechanism of action is unclear, trabectedin is proven to attach to the DNA minor groove via a covalent reversible link and associates with several binding proteins of the Nucleotide Excision Repair (NER) pathway. Trabectedin requires a competent NER system to function. In tumor cells, trabectedin causes a cell cycle inhibition at G2/M, and the apoptotic action is unaffected by the presence of p53. Trabectedin was developed and authorized for STS and ovarian cancer according to *in-vitro* and *in-vivo* outcomes. The product is now being tested in Phase II studies for breast, lung, prostate, and pediatric cancer, as well as Phase III trials for STS first-line treatment. In terms of its safety profile, reversible neutropenia and transaminase increases, which are similarly temporary,

appear to be the most common adverse events. There were no signs of mucositis, alopecia, neurotoxicity, cardio toxicity, or cumulative toxicity (Mayer et al., 2010).

Batzellines are pyrroloiminoquinones alkaloids derived from the deep-water Caribbean sponge *Batzella sp*, which have antifungal and carcinogenic actions against murine leukemia cells. They have a structural resemblance to other marine chemicals such makaluvamines and discorhabdins, which have potent cytotoxic activity against different cancer cell types. Furthermore, the batzellines, like other marine pyrroloiminoquinones, appear to have many mechanisms of action. The key mechanisms appear to be topoisomerase II suppression and their capability to disrupt DNA synthesis by intercalating with DNA. Surprisingly, batzellines that operate primarily through DNA intercalation appear to be most efficient in combating pancreatic cancer cell lines (Guzmán et al., 2009).

There are now 23 "marine" compounds at various phases of clinical development in various cancer types, according to Prof. Alejandro M. S. Mayer and colleagues'. The great majority of these drug candidates are being evaluated as anticancer therapies (i.e., 19 out of 23 (83 percent)). It should be emphasized, however, that the majority of the compounds (70%) are either ADC analogues of MMAE or MMAF or are currently licensed medications in the process of being approved in new entities (e.g., lurbinectedin in ovarian, breast, and small cell lung cancer) (Dyshlovoy & Honecker, 2020).

4.3.2 Overcoming the challenges and limitations in the approval of marine drugs

There are numerous important problems in obtaining pharmaceuticals from marine sources. Variable environmental components may have a part in the emergence of distinct metabolites from the same organism at different times. The bioactive substances are produced by microorganisms residing in the aquatic organisms, not by the invertebrate marine hosts, which presents considerable difficulty. Microorganisms cannot be grown in a pure colony by

themselves in a symbiotic interaction. Their growth is influenced by the actions of their hosts. Some biosynthetic genes go unnoticed and are not expressed *in-vitro*. Attempting to exploit these animals' entire metabolism potential is a huge task. In this context, the analysis of genetic data from whole populations, known as metagenome research, is becoming increasingly important. It permits genomes to be directly exposed to uncultivable microorganisms and microbial populations. Furthermore, maintaining a steady supply of extracted and known lead compounds might be difficult. The lead compound is only present in small amounts, and isolating it can be difficult. The quantity sought for any anticipated use of the molecule (drug, cosmetics, etc.) might be as little as a few grams for preclinical medication manufacturing and tolerability studies in a variety of forms, from kilos for clinical studies in various stages, to several tons for cosmetics. So, the number of lead compounds might be a major worry (Saeed et al., 2021).

The development and manufacture of some very strong marine new compounds have been hampered by a lack of sustainable availability of the target molecule. This problem has been addressed by increasing the synthetic or hemisynthetic analog derivatives manufacture with desired and customized features, or by developing a lower-complexity pharmacophore with a simpler manufacturing technique. The chemical formula of the isolated compound, the exact composition (planar connectivity), the intramolecular bond arrangement, the core design, and the improper allocation of one or more stereocenters could all be affected by the isolated compound's structural intricacy and the low yield typical of marine compounds. To solve the problem of daily supply, the use of natural resources should be regulated, and the growth of aquatic species in their natural environment should be favored via farming, often known as mariculture. Another option is to produce marine species in artificial environments using aquaculture technology. Furthermore, the entry requirements for marine-derived drugs are the same as for terrestrial medicines. This means that acceptance takes a long time (typically 8 to

15 years) and a lot of money (on average US$ 900 million) from exploration to market. Complications, toxic consequences, accessibility that isn't durable, as well as other indications that resulted in making several appealing marine medications to have trouble making it through the development phase. Similarly, changing culture conditions using epigenetic processes, or co-cultivation are ways that may be used to overcome these limitations and enhance chemical diversity. A better understanding of the structure's key components (pharmacophore) and advances in chemical synthesis might aid in the design of less intricate structures and the development of commercially feasible synthesis processes. The continual supply of suitable amount of organisms and compounds without destroying the marine ecosystem is a significant stage in the process of medicine manufacturing from marine creatures and also it is an obstacle. Marine medications will only be allowed to enter the market if supply can be managed in an economically and environmentally sound manner. If natural selection cannot be regulated sustainably, chemical synthesis/semi-synthesis/modification or marine biotechnology (aquaculture/mariculture/fermenter cultivating; genetic engineering; enzymatic biosynthesis or modification) can be used to alleviate the supply issue (Saeed et al., 2021).

Natural marine compounds have unique chemical, physical, and biological characteristics that cannot be detected on the surface of the earth, but the features of previously characterized compounds can be used to refine anticancer activity through developing a novel method for the synthesis and molecular modeling of new compounds. A crucial aspect is the concept of environmental conservation as well as the importance to protect the marine environment to conserve and protect biodiversity and avoid the loss of ecosystem functions. Marine biotechnologies are highly focused on establishing new methodologies depending on the assessment of the long-term viability of organisms sampled for later use in conjunction with novel selection criteria and the establishment of marine biobanks (Conte et al., 2020).

Chapter 5: Drug delivery of alkaloids in Oncology

5.1 New molecular targets, routes, and therapy

Female breast cancer (523,000 cases), colon cancer (500,000), lung cancer (470,000), and prostate cancer (450,000) were the most prevalent cancer sites. These four types of carcinomas account for 50 percent of the total cancer load in Europe. Lung cancer (388,000 deaths), colon carcinoma (243,000), breast cancer (138,000), and pancreatic cancer (128,000) were the major reasons for death. The primary reasons for greater mortality among cancer patients are late diagnosis and non-responsive treatment. Better knowledge about the principles behind tumor biology has resulted in substantial advancements in cancer prevention, diagnosis, and therapy in recent years. Surgery, chemotherapy, radiation therapy, targeted hormone therapy, and immunotherapy are all examples of traditional cancer treatments. Unfortunately, due to their low specificity, these techniques are sometimes restricted, since they might also harm non-cancerous cells or harm the healthy immune system, resulting in undesirable reactions. Furthermore, all cancer treatments excluding surgery can cause tumor cells to develop a drug-resistance response. As a result, anticancer therapy research is always focused on finding more effective therapeutic techniques (Colone et al., 2020).

Combination treatment is a potential technique for decreasing the negative consequences of vinca alkaloids that are used in conjunction with additional chemotherapeutic medications to boost anticancer activity. To enhance their therapeutic impact, drugs are frequently given in a combination or in sequential order. CVP is an amalgam of vincristine, cyclophosphamide, and prednisone that is used as a first-choice treatment for follicular B-cell lymphoma. CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) is a combination of cyclophosphamide, doxorubicin, vincristine, and prednisone that is used in first-choice treatment for people with follicular or diffused large B-cell lymphoma. For patients with

diffused large B-cell lymphoma and follicular lymphoma, rituximab coupled with CVP or CHOP offers a new first-line therapy. When combined with other chemotherapeutic medicines, vinblastine can be used in the cure of a wide range of carcinomas. Non-small-cell lung cancer stages IIIA and IIIB are treated with vinblastine, cisplatin, and radiation treatment (VCRT). In people with disseminated non-seminomatous germ-cell tumors, CISCA/VB (cisplatin, doxorubicin, cyclophosphamide, vinblastine, and bleomycin) is utilized. Conventional chemotherapy treatment for Hodgkin's lymphoma includes doxorubicin, bleomycin, vinblastine, and dacarbazine (Lee et al., 2015).

Table 9. Vinca alkaloids in combination therapy for cancer treatment (Lee et al., 2015).

Formula	Applications
CVP (cyclophosphamide, vincristine, and prednisone)	First-line therapy for follicular B-cell lymphoma.
CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone)	Front line therapy for follicular or diffuse large B-cell lymphoma.
Rituximab combined with CVP or CHOP	First-line therapy for diffused large B-cell lymphoma and follicular lymphoma.
VCRT (vinblastine, cisplatin, and radiation therapy)	Treat IIIA and IIIB non-small cell lung cancer.
CISCA/VB (cisplatin, doxorubicin, cyclophosphamide, vinblastine, and bleomycin)	Non-seminomatous germ-cell tumors.
ABVD (doxorubicin, bleomycin, vinblastine, and dacarbazine)	Standard chemotherapy for Hodgkin lymphoma.

Vinorelbine and cisplatin	Adjuvant chemotherapy for non-small cell lung cancer.
A thoracic radiation scheme, vinorelbine, and cisplatin	Stage III A and stage III B non-small cell lung cancer.

In case of new molecular routes, trabectedin, and lurbinectedin, two structurally similar minor-groove alkylators, possess a complicated pleiotropic anticancer mechanism that affects both tumor cells and the tumor microenvironment. Unlike traditional alkylators, both molecules attach to the exocyclic amino group of guanines, with a choice for the DNA minor groove's guanine-cytosine-rich triplets, generating abnormal bending in the direction of the major groove. As a result, a series of synergistic processes are triggered, halting cell growth, differentiation, and death. In conjunction with the binding domain (subunits A and B), a subunit (subunit C) extends from the DNA backbone that interacts actively as well as passively with other DNA-binding proteins such as transcription factors and DNA repair proteins. Due to this unique molecular property which is not shared by other anticancer agents, trabectedin has shown to be particularly effective against soft-tissue sarcomas in both experimental and clinical tests. The relocation of oncogenic fusion proteins that is important for cancer metamorphosis and tumor growth, appears to be linked to the exceptional sensitivity of translocation-related sarcomas (Pereira et al., 2019).

5.2 Drug Delivery systems

5.2.1 Nanotechnology

The nano-biotechnological industry is seeing increased growth in the application of nanomaterials for establishing antineoplastic treatments. Nanoformulations are linked to a

broad range of nanoparticles, and their polymerized structures are becoming a popular method for generating anticancer medicines (Colone et al., 2020). Nanomedicine is gaining as a technique of increasing cancer therapeutic efficacy, decreasing adverse effects and combating drug resistance. In medicine, nanoparticles (NPs) or nanocarriers (NCs) are increasingly being examined as a prospective choice for safely delivering drug substances into specified compartments in an organ, tissue, or cell. To encapsulate the medication in carriers, many nano-micro delivery methods have been developed (liposomes, polymeric nanocapsules, and dendrimers), masking the molecule's negative biopharmaceutical characteristics and substituting these with the attributes of the materials of the nano-delivery system. Nanomedicines are also being utilized to deliver medication to specified locations. In this setting, it is necessary to integrate nanoparticle delivery system research in the preformulation stage for the establishment of novel drug formulations. To investigate this strategy, biomaterial science has progressed towards the development of smart materials and nanoscale drug delivery systems. Moreover, Doxil®/Caelix®, Lipocurc^{TM}, Marqibo®, Abraxane®, Myocet®, DaunoXome®, ThermoDox®, are FDA-approved nanodevices (Colone et al., 2020).

Table 10. Summary of the properties of various nanomaterials, active and passive targeting, as well as their benefits and drawbacks.

Nano-materials	Passive targeting	Active targeting	Benefits	Drawbacks	Reference
NPs made of lipids	By using the EPR effect, this method accumulates.	Chances of using a specialized ligand to design	Decreased toxicity, biocompatible, disintegrating	High level of clearance by RES (Reticuloendothelial system)	(Szwed et al., 2020)

Polymeric NPs	Long periods of circulation	In tumor areas, drug concentrations are higher.	Design is simple, and there are a lot of different shapes to choose from.	Inflammatory responses are induced.	(Colone et al., 2020)
Carbon-based NPs	Increasing the amount of accumulation in tumor regions	Toxicity is lower, and effectiveness is higher.	Biocompatible	The immunogenic response may occur as well as the possibility of blood clotting.	(Fabbro et al., 2012)
Metallic and Magnetic NPs	Diagnosis and therapy in combination	Related with multimodal cancer therapy to improve drug uptake	There is no specialized distribution of drugs.	High toxicity, limited stability, and biocompatibility are all concerns.	(Colone et al., 2020)

Chitosan is a chemical polymeric biomaterial found in the exoskeletons of shellfish like shrimp and crabs. Furthermore, chitosan is defined as a big biopolymer based on polycationic that is non-toxic, decomposable, and biocompatible that are commonly used in the fields of agriculture and biotechnology. Similarly, due to its decomposable qualities, chitosan has a decent chance of being employed as a drug transporter with a longer storage time and higher

bioavailability of pharmaceuticals. Chitosan transparently acts on neoplastic cells, affecting digestion of cells and inhibiting cell proliferation. A previous study found that chitosan had antineoplastic actions both *in-vivo* and *in-vitro*, allowing it to be a suitable candidate for use as an antitumor aid agent and carrier. Furthermore, research has revealed how chitosan nanoparticles are constructed for tumor cells. Pharmaceutical uses that are best, such as immunization transmission, mucosal processing, antioxidants, genes are all emphasized in colorectal or cancer cures which have all been captured by chitosan nanoparticles (CNPs). Chitosan is coupled with copper, iron, graphene oxide, silicon, and silver nanoparticles and can be ionotropically gelled, polyelectrolyted, microemulated, micellar-reversed, and emulsified, among other forms (Saeed et al., 2021).

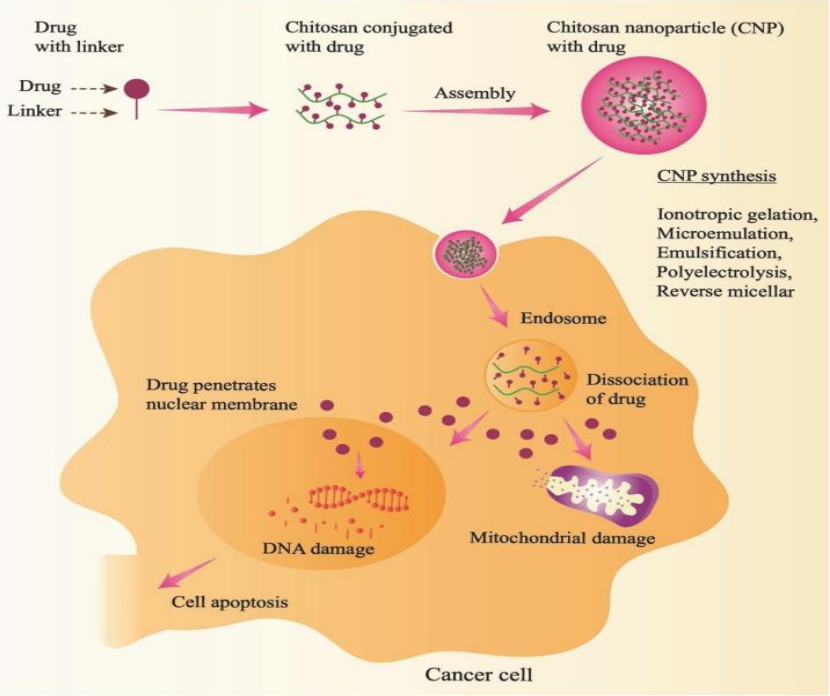

Figure 9. Illustration of anticancer drugs using CNPs.

Anticancer therapeutics are made with chitosan-conjugated NPs such as gold, silver, and silicon. The formulation is triggered when it enters the cell through the endosomal membrane,

reaches the mitochondria or nucleus, and disrupts apoptosis and DNA replication (Saeed et al., 2021).

Nanomaterials are divided into four classes: Nanomaterials that are inorganic-based, carbon-based, organic-based, or composite-based. Nanomaterials based on carbon come in different geometry and sizes, including spheres, ellipsoids, tubes, and horns. These nanomaterials may be divided into graphene quantum dots (0-D), carbon nanotubes (1-D), and graphene (2-D) depending on their dimensions, with 0-D denoting no dimension, 1-D denoting one dimension, and 2-D denoting two dimensions at the nanoscale. Metal-based and metal oxide-related nanoparticles are examples of inorganic-based nanomaterials. Pure metal nanoparticles make up metal-based nanoparticles (i.e. nanoparticles of iron, zinc, magnesium, titanium, platinum, gold, copper, silver, and alginate). Metal oxide nanomaterials (zinc oxide, silver oxide, and so on) can be formed when metal-based nanoparticles are bonded to oxygen. Organic-based nanomaterials, unlike inorganic and carbon-based nanomaterials, are largely made of organic matter. Through noncovalent interactions, these organic-based nanomaterials may be converted into liposomes, micelles, dendrimers, and polymer nanoparticles, which are highly effective in drug administration. Composite-based nanomaterials are made up of many phases, one of which is on the nanoscale and combines distinct nanoparticles with large or sophisticated materials, such as hybrid nanofibers and metal-organic structures. These nanomaterials have the potential to change the way illnesses like cancer are detected and treated (Loh et al., 2021).

5.2.2 Magnetic microspheres

Chemotherapy, either individually or in conjunction with the help of surgery and radiotherapy, photodynamic therapy, and immunological therapy are all used to treat cancer. The non-specificity of chemotherapeutic drugs causes cytotoxicity, which is a key drawback of cancer treatment. It has an easy approach and simplicity of command over site-specific dispatch in

exterior conditions. As a result, according to a considerable amount of published papers on site-selective and targeted drug release, magnetically targeted drug carrier techniques have piqued attention. The loading of medications with magnetic microspheres (MMS) has been the topic of study and development. A magnetic core and a polymeric covering are usually found on MMS. Targeted drug administration, cell dissociation, enzyme and protein immobilization, immunoassay, nucleic acid refinement, magnetic resonance imaging (MRI), and tumor heating are just a few of the uses for MMS in tissue engineering and other biomedical sciences. A variety of approaches for the manufacture of magnetized polymeric microspheres with inorganic nanoparticles and polymeric core–shells have been developed to date. MIONs (microspheres with inorganic nanoparticles) are produced and subsequently crushed with polymer particles or adsorbed onto polymer microspheres in these procedures. MIONs on core shells have been shown to inhibit magnetic hyperthermia when cross-linked to create polymer clusters. MIONs covalently linked to the surface of MMS interacted directly with neighboring cells, speeding up healing and medication delivery. Magnetic microspheres have been studied as a carrier for anticancer drugs, employing both recyclable and non-recyclable polymers. The sustained release properties of the poly (lactic-co-glycolic acid)-embedded drug carriers have been impressive. PLGA, a popular environmentally sustainable polymer, has been extensively explored in the last two decades because it decomposes hydrolytically and produces safe and biodegradable by-products. As multifunctional biological drug delivery carriers, non-recyclable polymers are preferred and so PLGA can be used in a variety of controlled medicine delivery applications. Optimizing the kinetics of drug release from PLGA-based drug carriers is therefore critical for maximizing their chemotherapeutic efficacy. Anti-metabolites, such as 5-FU, are a kind of drug. It destroys cells that are very active in mitosis. 5-Fluorouracil is a pyrimidine-analog antimetabolite with strong action against solid neoplasms (including the

gastrointestinal system, pancreatic, breast, ovary, brain, and liver), as well as solo and combination theranostic therapy for cancer (Ayyanaar et al., 2022).

5.2.3 Liposome based nanoparticle drug delivery systems

Liposomes and lipid nanocapsules (LNCs) are two types of lipid-based nano delivery systems. Liposomes are built of a hydrophilic core enclosed by lipid bilayers and it can include hydrophobic or hydrophilic drugs in the lipid bilayer or the aqueous core. Because the lipid bilayer's geometry is comparable to that of the cell membrane, the liposomal nanoparticle can either combine with the cell membrane or lyse, delivering the drug once attached to intracellular organelles. Furthermore, liposomes' capacity of encapsulation of both therapeutic and diagnostic molecules brings up the possibility of using liposomal delivery systems in theranostic platforms. Liposome distribution to cancer cells is frequently dependent on targeting passively and the increased permeability and retention action. Furthermore, the inclusion of polyethylene glycol (PEG, also called stealth liposomes) can extend the duration in which they circulate. Clinical studies for liposome-based systems with various targeting ligands are now underway, including an anti-HER2 mAb with Paclitaxel and a mAb 2C5 with Doxorubicin (Doxil®). An external stimulus, such as heat, was used to induce the release of the medication absorbed into liposomes with ThermoDox®, which was lately licensed by the US FDA. Lipid nanocapsules (LNCs), which are enclosed by a membrane made of PEGylated surfactants, are another form of the lipid-mediated delivery mechanism. Some antineoplastic medications, such as doxorubicin and paclitaxel, have been encapsulated within LNCs and have shown adequate efficacy *in-vivo* and *in-vitro*, as evidenced by the increased intracellular drug administration and decreased tumor size noticed when LNC formulations were delivered (Allen & Cullis, 2013).

Because tumor angiogenesis is poorly controlled, a tumor is frequently linked with a faulty, leaky vascular structure. Furthermore, a tumor's interstitial fluid is frequently insufficiently evacuated by a imperfectly constructed lymphatic system. that is why submicron-sized particulates may so significantly extravasate and be maintained within the tumor. The "enhanced permeability and retention" (EPR) action is a term used to describe this phenomenon. A well-developed nanoparticle technique, such as SLN (Solid Lipid Nanoparticle), can take use of this EPR phenomenon to accomplish passive tumor targeting. The aforementioned problem of inadequate tissue specificity can be partially overcome this way. Furthermore, with advancements in surface-engineering technologies, the biodistribution of SLN might be additionally adjusted by enhancing the surface physical and chemical features of SLN to direct it to the desired tissue. This enhances the quantity of drugs that goes to the targeted tumor sites while lowering systemic toxicity. Physical stability, preservation of labile medicines from deterioration, controlled delivery, and simplicity of manufacturing are all benefits of SLN, as are other forms of drug carriers utilized for cytotoxic drug transfer, such as polymeric systems and liposomes. SLN (Solid Lipid nanoparticles), on the other hand, avoids some of the problems that other drug delivery methods have. SLN does not have the comparatively elevated costs linked with bulk-scale liposomal formulation manufacture. When compared to systems like liposomes, they have reduced storing and drug leaking issues. SLN lacks the considerable toxicity and acidity linked with a variety of biodegradable polymeric compounds (Wong et al., 2007).

Because of their unique, attractive qualities for drug administration, lipid-based nanoparticles, notable liposomes, have been researched and used in a wide range of pharmacological applications. Lipid-based nanoparticles are expected to largely influence health of the general public, specifically with the recent victory of the COVID-19 mRNA vaccines from BioNTech/Pfizer and Moderna. Through analysis, evidence on the effectiveness and toxicity

of anticancer alkaloids incorporated in lipid-based nanoparticles *in-vitro and in-vivo* is obtained (Loh et al., 2021).

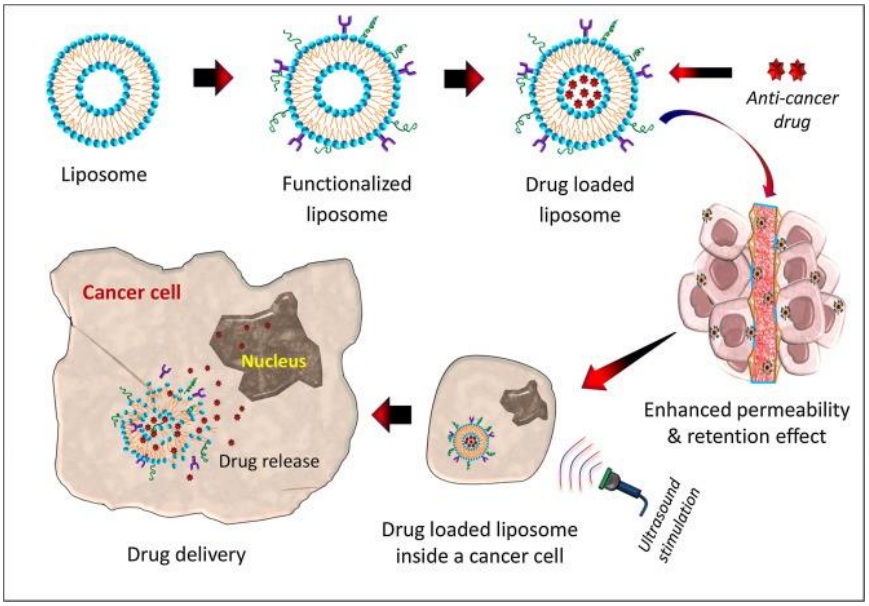

Figure 10. Illustration of liposome-based smart drug delivery system for cancer therapy (Hossen et al., 2019).

In different malignancies, encapsulated alkaloids have better *in-vitro* cytotoxicity and a better *in-vivo* effectiveness and toxic potential than unbound alkaloids. This is due to lipid-based nanoparticles' better physicochemical qualities as well as pharmacokinetic, pharmacodynamics, and biodistribution profiles. Encapsulated alkaloids have also been shown in studies to be able to conquer the long-feared MDR that is the leading reason for non-successful cancer therapies and mortality. Thus, lipid-based nanoparticle encapsulation has shown to be an effective method for increasing anticancer alkaloids' clinical translation. Onivyde, Marqibo, and Lipusu, three liposomal nanoformulations of alkaloids, have been authorized for clinical usage, with additional lipid-based nanoparticles now encountering various stages of clinical testing. To improve efficacy and acceptability, most of these lipid-based nanoparticles have been engineered to take advantage of EPR actions (Loh et al., 2021).

Chapter 6: Discussion

In this study, recent advances on numerous common alkaloids with anticancer properties and discussed some of their features has been summarized. Anticancer alkaloids have several properties or difficulties that need to be addressed (Lu et al., 2012). Methodological issues such as the absence of a control or placebo group, limited sample sizes, and insufficient trial durations have been noted in the majority of relevant clinical studies. As a result, it is too early for many alkaloids to infer that they have anticancer properties, and large-scale and well-controlled clinical trials are required to evaluate their efficacies, side effects, and safety before they are used to treat cancer. However, incorporating the benefits of both traditional and modern medication has previously been recommended as a potential strategy for discovering and commercializing novel plant-derived medicines (Choudhari et al., 2020.).

The majority of the alkaloids have been shown in both *in-vivo and in-vitro* trials to have synergistic or enhancing effects in cancer cells, as well as lower side effects, when taken with chemotherapeutic medications. So, combination treatment is likely to be the best arena for practical use of these substances. Furthermore, maximum of the alkaloids have limited dispersibility in water and bioavailability, making them difficult to get to the tumor location. Aside from structural changes, another method may be to change the drug delivery mechanism. The advancement of nanotechnology may provide promise for resolving these issues, and there have already been some successful examples (Lu et al., 2012). Finally, the toxicity of these substances must be considered like anaphylaxis, constipation, and skin reactions are among the most prevalent berberine side effects. Berberine can induce kernicterus, jaundice, and brain damage in babies by displacing bilirubin from serum binding proteins (Tang et al., 2009). As a result, alkaloids extracted from natural plants are not always considered harmless. Factors like dose, drug administration routes and therapeutic methods, among other things, are critical.

The use of innovative medication delivery technologies and the alteration of chemical structures may help to lessen the toxicity of these substances (Lu et al., 2012).

MDR (Multi-Drug Resistance) is a significant barrier to the clinical effectiveness of several oncogenic therapies due to its ubiquitous prevalence within malignant cells. Two types of anticancer drug resistance exits, one that emerge within the malignant cell due to genetic and epigenetic modifications that influences the drug's sensitivity and the other type occurs from the impeded transfer of a drug(s) to the cancer cells (DeBono et al., 2015). Resistance to medication therapies and the likelihood of relapses are two downsides of anticancer therapy, with one of the key goals being to uncover and characterize new medicines (Conte et al., 2020). One of the well-researched pathways for resistance of drug is the upregulation of drug efflux pumps like P-glycoprotein and multidrug resistance-associated protein 1 (MRP1). Although unrestrained cell proliferation in cancer encourages clonal expansion, the existence of chemotherapeutics leads to the natural selection of tumor cells that can survive and multiply, contributing to the development of resistance. Current chemotherapeutics have limited water solubility, which contributes to higher drug metabolism and excretion, leading to reduced drug concentrations in the blood and, as a result, drug diffusion from the blood decreases across cellular membranes, and into the tumor mass (DeBono et al., 2015). New anticancer drugs are generally developed using a multidisciplinary approach that commences with the discovery and extraction of novel bioactive molecules from natural origin that are then subjected to preliminary biological activity assessments, toxicological examinations, and chemical/biotechnological formulation (Conte et al., 2020).

Poor water solubility and limited bioavailability, which restrict oral delivery, are two important problems in alkaloid research. If we can produce compounds that boost water solubility via semisynthetic and biochemical transformation approaches, we can increase bioavailability. Similar procedures have been used to improve the solubility of different alkaloids as well as

their potency in water (Mondal et al., 2019). Regarding marine-derived alkaloids, the difficulties in locating natural alkaloids of marine sources and gathering adequate amounts for clinical and preclinical testing frequently prevents the isolation of natural biomolecules, delaying the creation of hopeful medicines. Even though the epigenetic signature of natural chemicals has previously been studied, factors associated with the formulation of novel marine-derived anticancer bio-compounds that emphasizes the diversity that distinguishes organisms and their environment have not been thoroughly explained (Conte et al., 2020).

Alkaloids, like other secondary metabolites, are extracted and identified using chromatographic methods. Paper chromatography and thin-layer chromatography were popular in the past. However, for the isolation of alkaloids, the high-performance liquid chromatography (HPLC) approach is currently commonly used. It is extremely precise and capable of detecting extremely small amounts of a substance. Another popular approach for analyzing alkaloids is gas chromatography. Although not as well-known as HPLC, this approach has been used to analyze alkaloids qualitatively and quantitatively. The easy method and spectacular outcomes, even at low efficiency, were the key reasons for its adoption. Ultrasound-based techniques, microwave-based methods, supercritical carbon dioxide extraction methods, and techniques that combine ultrasound and surfactants are all relevant ways of extracting alkaloids. The spectroscopic instruments that include high-resolution mass spectrometry and nuclear magnetic resonance are the latest methodologies for the identification and characterisation of structural features of alkaloids. These spectroscopic approaches can be utilized individually or in conjunction with chromatographic procedures (Mondal et al., 2019).

Apoptosis-mediated programmed cell death is the utmost studied *in-vivo* cell death pathway. In the past few years, there seems to be a greater focus on understanding alternative types of cell death, including necrosis, autophagy-dependent cell death, necroptosis, and senescence, because these mechanisms might contribute more to *in-vivo* programmed cell death.

Understanding the cancer pathogenesis and the molecular mechanisms of anticancer treatments have never been more important. Although the underlying mode of action has been extensively studied in a variety of cellular models, the *in-vivo* mechanisms are frequently unknown. Another significant problem is the anticancer medicines' negative effects and dose considerations. Comprehensive knowledge of the toxicity profile of novel chemical structures produced as anticancer drugs is required for proper interpretation of the toxicological profile. To ensure optimal impact, most developed medications are dosed to the highest degree of tolerance. The toxicities attributed to anticancer drugs might be due to how they work. As a result, future studies should concentrate on determining the precise anticancer mechanisms of alkaloids, investigating impactful combinational treatments that may work in unison with natural alkaloids to kill cancer cells more efficiently, developing effective drug delivery devices, and enhancing cancer cell selectivity (Mondal et al., 2019). Therefore, (1) Using novel therapeutic tools, the specific anticancer mechanisms of alkaloids must be elucidated; (2) By utilizing pharmaceutical chemistry the chemical structures of these lead molecules must be modified to reduce toxicity; (3) Strategies of successful combinational treatment should be investigated; (4) Efficient drug delivery systems should be established; and (5) Further clinical trials for these alkaloids should be conducted for their anticancer activity (Lu et al., 2012).

Chapter 7: Conclusion and Future Recommendations

Cancer has become a prominent disease in both developed and developing nations. But the anticancer drugs discovered and used in chemotherapy have downsides, mostly because of their detrimental effects on non-targeted tissues, which creates issues in the human body. As a result, alternative therapies are in high demand, with natural-source anticancer drugs being the best option. In this study, many natural alkaloids have been highlighted for their antiproliferative effectiveness on cancer cell lines, which includes factors like cell proliferation, growth, metastasis, angiogenesis, and invasion. However, further study into the safety and effectiveness of most of these promising natural alkaloids, as well as their pharmacokinetic profiles, is required. Moreover, the current data reviewed and analyzed in this study clearly show that nanotechnology-based innovative biotechnological techniques provide new possibilities for cancer therapy and it is crucial for the successful administration of alkaloids in cancer therapy. Therefore, anticancer natural alkaloids research is important not just for introducing them into clinics. The knowledge acquired from studying these molecules might be used to create derivatives with superior pharmacological properties for cancer therapy, such as increased bioavailability and pharmacokinetics, reduced resistance, and fewer adverse effects etc.

Future Recommendations:

- Plant cell culture can be researched in the future since it can be utilized as an alternative strategy for producing the low-yield compounds.
- Simple synthetic techniques for bioassays might be researched, and advanced combinatorial chemistry can be used to design new analogues of the parent molecules.
- Future research should concentrate on determining the precise antiproliferative mechanism of alkaloids, investigating effective combination treatments that can work

in conjunction with natural alkaloids to speed cancer cell death, developing functional drug delivery techniques, and improving cancer cell specificity.

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