CAPITAL UNIVERSITY OF SCIENCE AND TECHNOLOGY, ISLAMABAD

In Silico Evaluation of Insecticidal Potential of *Rhazya stricta* against *Anopheles gambiae* and *Apis mellifera*

by

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CERTIFICATE OF APPROVAL

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Abstract

Insect pests pose a huge problem for agriculture, causing big yield losses and economic harm globally. They are also becoming resistant to standard synthetic insecticides, which also bring environmental and health issues. So, there's a great need for safer, more effective options. Because of this, plant-based bioactive compounds are being focused on due to their structural variety and biological effects. This study focuses on the insecticidal potential of *Rhazya stricta* phytochemicals, a plant rich in different alkaloids, targeting key parts of insect nervous systems. The aim is to discover natural alternatives to traditional chemical insecticides. Aspidospermidine, serpentine, dihydrocorynantheol, tabersonine, vincadifformine, tetrahydroalstonine, eburnamine, eburnamonine, and quebrachamine were among the metabolites whose interactions with important target proteins, including acetylcholinesterase and GABA receptor, were assessed. In order to evaluate binding affinities and interaction kinetics, molecular docking studies were performed using the three-dimensional structures of these target proteins and metabolites. Notably, tetrahydroalstonine, a crucial metabolite of *Rhazya stricta*, had an excellent protein-ligand interaction profile, a potential ADMET profile, and the best docking score of -9.4 against protein acetylcholinesterase, suggesting its usefulness as an insecticidal agent. Tetrahydroalstonine was tested against the widely available pesticide malathion to determine its continued efficacy. Tetrahydroalstonine and malathion are equal in ADMET analysis, according to a comparison of all insecticide-like features; however, the molecular docking values and interactions for tetrahydroalstonine are far better than that for malathion. It suggests that tetrahydroalstonine is a viable insecticidal option for use in future treatments.

Keywords: Insecticides, *Rhazya stricta*, Malathion, Phytochemicals, Acetylcholinesterase, ADMET profile, Tetrahydroalstonine.

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Abbreviations

AChE	acetylcholinesterase
BBB	barrier of blood and brain
CADD	computer-Aided Drug Designing
CNS	Central Nervous System
CYP2D6	cytochrome P450 2D6
GRAVY	grand average of hydropathicity
HBA	Acceptor for hydrogen bond
HBD	Donors for hydrogen bond
II	Instability Index
IPM	integrated pest management
MW	molecular weight
NR	negatively residues
PDB	protein data bank

Chapter 1

Introduction

Insect pests continue to be a major problem for agriculture, food security, and public health worldwide. Beyond just damaging crops, these pests affect yield stability, nutrition, and the financial health of farming communities everywhere, whether in rich or poor regions [1]. Annually, we lose a big chunk of our global crop output because of pests, roughly 20% to 40% of the total yield, costing us billions of dollars globally. This is especially worse in developing countries that might not have easy access to the latest technology in pest control and often rely on older farming methods. The continuing nature of this problem speaks about the weaknesses in our current global food systems. When a significant portion of the crop yield is lost as a result of insect infestations, the consequences go far beyond the fields and include rising market prices and starvation in impacted homes. This can be particularly difficult in places where small-scale farming is the primary economic activity and crop failure is not only a sign of a bad year but also a difficult-to-break cycle of debt and poverty [2].

In such cases, crop vulnerability to bugs really upset farmers while boosting food insecurity and squeezing already weak rural economies. And it gets more complicated than just agriculture; these pests are major public health hazards too. They spread a range of serious diseases in both humans and animals. Diseases like malaria, dengue fever, and Zika are all tightly linked to insects, especially mosquitoes. This shows that managing pests is not just vital for farming, it is

also key to protecting everyone's health. Over the past few decades, synthetic chemical insecticides have been the main tool for keeping pest insects away because of how well they work and how quickly they act. Though there is no doubt in the success of synthesized insecticides in medicine and farming, we now have to face the true costs of our dependence on them. Synthetic medicines have definitely helped increase crop yields and reduce illnesses spread by bugs. Still, their overuse and lack of targeted application have caused major problems for our environment, health, and ecosystems. A huge issue is that insects are evolving to resist these chemicals [3].

We need more of the spray or something stronger as insects adapt to it, which increases farmer expenses and environmental dangers. These same chemicals also seriously contaminate the environment, making them harmful beyond just the pests they target. Pesticide residues stick around in soil, water, and air, causing lasting harm to ecosystems. Aquatic life is especially at risk from pesticide runoff, which hurts fish, amphibians, and other creatures. Additionally, soil contamination messes up vital microbial communities needed for soil health. Soil deterioration could be considered one of the problems that we have completely overlooked in relation to pest management. Soil is an ecosystem that contains a lot of microorganisms that contribute to a healthy environment for plant development and nutrient circulation. When we pour this system with toxins that do not decompose quickly, we basically create a dead zone where all organisms that are crucial for the development of plants cannot survive. This leads to increased vulnerability of plants to disease and need for more pesticides, creating a vicious circle. Fixing this problem requires moving on from harmful substances and using products that dissolve harmlessly in nature [4].

Toxic residues also build up in organisms, moving up the food chain and posing serious risks to both wildlife and humans. Exposure to these toxins over time has been linked to things like neurological problems, hormone issues, and cancer. Also, useful pollinators like bees and butterflies suffer from these pesticides, threatening biodiversity and the reliability of our food supply. Facing these issues, there is a big move toward safer and greener pest control. Plant-based insecticides are

stepping up as great substitutes because they are eco-friendly, they break down faster and are less toxic to harmless critters and plants. The main disadvantage of traditional synthetic pesticides is that they usually act through a single mechanism on the insect, by attacking only one enzyme or protein in the organism. Despite this feature being regarded as revolutionary at some point, it actually facilitated the process of evolution. Since the insect population is constantly under the influence of the same chemical stressor, the insects with a little bit different gene structure and able to avoid or neutralize the chemical influence will survive and transfer their characteristics to the following generation. Such a process of development is called target site resistance and makes the majority of previously effective chemicals useless, forcing farmers to apply higher doses, thereby speeding up the development of resistance even more [5].

The systematic investigation of plant defenses opens a door toward the convergence of ethnomedicine and agricultural biotechnology. The majority of plants utilized for decades in indigenous medicine contain molecules which are naturally biologically tuned, meaning that these compounds have been developed to function with living things while avoiding the formation of non-degradable residues which result from artificial petrochemical pesticides. Yet the lack of organized information about these plants has kept them from being employed in agriculture. The utilization of modern scientific and computational techniques to analyze these traditional medicinal plants will make it possible to develop a safer and scalable alternative. Unlike manmade substances, which are relatively simple, plants have undergone an extremely complex and ongoing process of development to protect themselves from insect attacks. After thousands upon thousands of years of evolutionary development, they managed to develop very complicated secondary metabolites that do not use only one type of action [6].

Secondary metabolites work through synergic processes, some compound prevents the insect from proper digestion, another one prevents its hormones from doing what they should be doing, etc. Insects have to deal with various physiological problems at once, which makes it virtually impossible for any mutation to protect them from the plant completely. In essence, we are utilizing the highly developed

understanding of evolution itself when creating our own phytochemical combinations. Plant insecticides use a variety of natural compounds, whereas standard insect sprays typically focus on one process inside bugs. These compounds hit several systems in insects, making them more effective and less likely to cause resistance. The move towards using plant-based alternatives is an evolution that is required for us to take an environmentally sustainable stance. Over millions of years, nature has been using its skills at developing complicated chemicals that protect plants from external sources, and in most cases, the chemicals found in plants are more intricate than what we can create in the laboratory. Since these chemicals co-existed with the insects, they are well designed in such a way that they interact with biological processes with the least effect on any other form of life [7].

Medicinal plants contain a variety of different chemicals called secondary metabolites like alkaloids, flavonoids, terpenoids, phenolics, tannins, and saponins, which have powerful effects. These compounds can repel insects, slow their growth, or act like poisons, seriously messing with insect biology. So, scientists are seeing these plants as a great resource for finding new insect fighters that are not only powerful but good for the environment too [8]. Among many medicinal plants being studied, *Rhazya stricta* stands out because of its interesting chemistry and useful properties. This perennial shrub, part of the Apocynaceae family, grows naturally in dry spots across South Asia and the Middle East. People there use it in traditional remedies for ailments like infections, inflammation, and metabolic issues. The remarkable range of compounds found in plants like *Rhazya stricta* is another interesting feature of using them. A combination derived from medicinal plants is a blend of many compounds, as opposed to an artificial pesticide, which is composed of a single, unchangeable molecule. Because insects must simultaneously adjust to the attack of many biochemical compounds, it greatly complicates the development of pesticide resistance [9].

Scientists found that *Rhazya stricta* is full of bioactive compounds, mainly alkaloids, which can really affect living things. These alkaloids show promise against microbes, as antioxidants, and even against cancer. So, the plant could be super

helpful for finding natural medicines. So *Rhazya stricta* not only fight diseases, but it might also keep pests away. It fights bugs due to its secondary metabolites, which interfere with insects' key functions, i.e., nervous signaling, enzyme activities, hormone controls, and growth tracks [10].

These compounds show potential, but research on using them as natural pest killers is still sparse, focusing more on their medicinal benefits. The broadening of the scope of usage of the biological properties of *R. stricta* in the area of agriculture beyond medicine constitutes a chance for cross-field scientific research. Although the effect of the plant on human pathogenic bacteria has been thoroughly studied, the use of the biological effects on the pest organisms has not yet been explored. Through the analysis of how the alkaloids affect the physiological functions of the agricultural pests, we can open the door to the next level of biopesticides development [11].

Advancements in bioinformatics have transformed insecticide discovery through efficient computer-based methods. Techniques like molecular docking allow researchers to explore interactions between small molecules and target proteins, revealing critical bonding interactions without the high costs of traditional experiments.

Virtual screening enables the rapid evaluation of numerous compounds, while ADMET profiling assesses the properties and safety of these candidates, ensuring they are effective yet environmentally safe. Collectively, these methods streamline the identification of lead compounds, resulting in significant time and resource savings [12].

This study uses advanced computational methods like molecular docking and ADMET analysis to explore *Rhazya stricta*'s phytochemicals for their insecticidal properties. It integrates phytochemical profiling with computer evaluations to find potential lead compound. This could be ecofriendly alternative to synthetic insecticides. The results will offer a solid scientific basis for future experiments and help develop sustainable farming practices that keep the environment safe while controlling pests effectively.

1.1 Problem Statement

The increasing reliance on synthetic insecticides has led to problems such as insect resistance, environmental pollution, and negative impact on non-target organisms. This creates an urgent demand for safer and more sustainable pest control options. *Rhazya stricta*, known for its rich content of biologically active phytochemicals, has not been thoroughly investigated for its insecticidal properties.

1.2 Hypothesis

Bioactive metabolites of *Rhazya stricta* may have an active role in relation to insecticides.

1.3 Aim and Objectives

This research is designed to investigate potential bioactive compounds from *Rhazya stricta* that have properties which can be used to produce an insecticide. The goals of this research are stated below:

i. To screen *Rhazya stricta* metabolites with insecticidal properties.

ii. To examine how particular Rhazya stricta metabolites interact with the intended target proteins.

iii. To determine which docking metabolite is most effective and less toxic at inhibiting target proteins.

Chapter 2

Literature Review

2.1 Insect Pests and their Effect on Global Food Supplies

The structure of global agricultural networks, thermodynamics of local ecological systems, and macroeconomics of global food supply chains face continual threats from populations of invasive insect pests. The role of these herbivores as biological stressors is significant since they directly feed on plant leaves, bore through plant vessels, damage root structures, and directly degrade the quality and quantity of the agricultural products. Research shows that infestations and feeding activities of insect vectors contribute to approximately 20 to 40 percent decrease in global crop production annually. Financial losses resulting from insect pest populations pose serious threat to socio-economic survival of millions of farming rural communities around the globe. Developing countries with resource constraints are particularly vulnerable to these biological stressors due to poor technological advancements in agriculture [13].

The devastating effects of phytophagous insects are seen from the time of the most vulnerable period of the plant, the seedling stage until the vegetative differentiation, reproductive processes, and even to the storage of the harvested grain as shown in figure 2.1. In addition to tissue destruction caused by feeding activities,

phytophagous insects reduce defense mechanisms of the host plants, consequently, there arise opportunities for the development of secondary pathogens, which can be represented by bacteria, fungi, and phytopathogenic viruses. Particularly important is the fact that many insect orders serve as extremely effective vectors for infectious plant diseases [14].

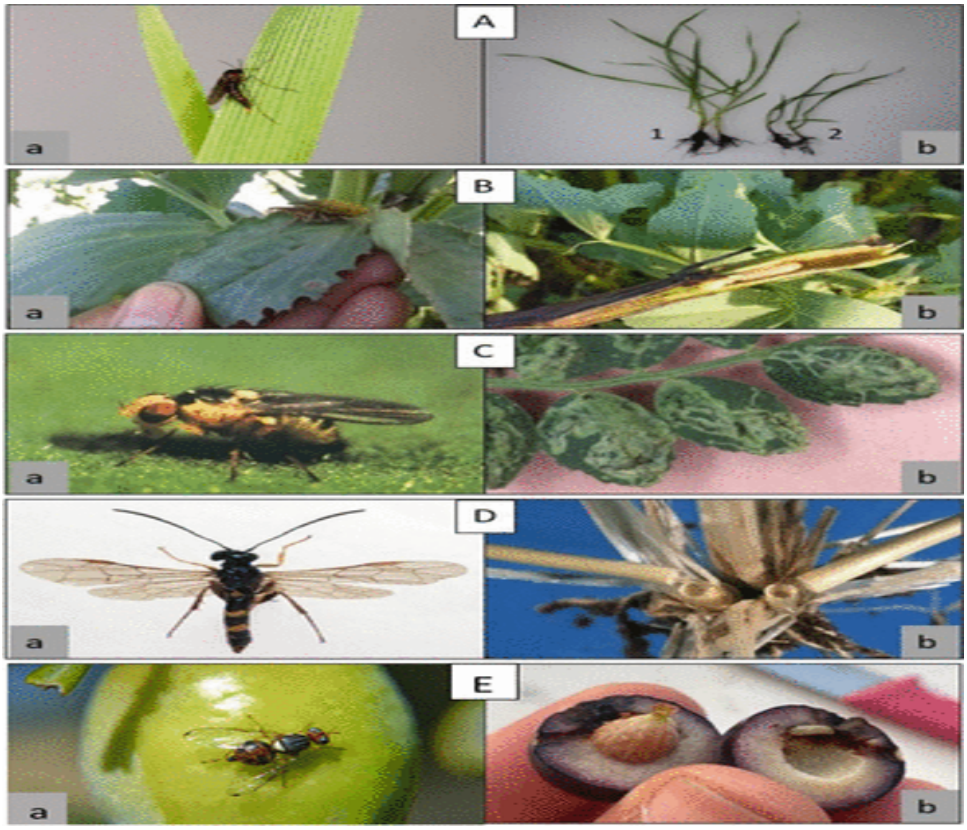


FIGURE 2.1: Different insect pests. This picture demonstrates that multiple insect species. The "a" shows the insect vectors or pests responsible for the infestation. At the same time, "b" illustrates the resulting plant pathology, such as stunted growth, stem boring, leaf mining, and fruit/seed destruction [15].

Beyond agriculture, insect pests pose serious threats to public health and veterinary science. As an instance, the vectors in the genus Culicidae can cause diseases like malaria, dengue, chikungunya, and Zika viruses among millions of people every year. Such a danger not only to agriculture but also to the public health emphasizes the importance of pest control measures targeting multiple purposes. Economic consequences of uncontrolled pest outbreaks affect the entire structure of regional and international market systems. In addition to increased production

costs caused by synthetic pesticides, farmers have to invest in specialized sprayers and manual labor [15].

In situations where heavy infestations cause complete losses of yields, food chains within the affected areas will be disturbed leading to high volatility in market prices and ultimately regional food insecurity. In the midst of all this, anthropogenic climate change has greatly changed environmental factors to the extent that there is now an environment highly conducive for rapid pest life cycles and distribution into new farming regions which were not previously affected. Ecologically, this will disrupt natural predator prey relationship and lead to reduced biodiversity as well as disruption of the ecosystem services offered by the beneficial insects [16].

Insects are responsible for a considerable amount of damage to agriculture through infestation of important crops, affecting the growth of the plants negatively and exhibiting symptoms that reduce crop production. The Hessian fly is responsible for attacking wheat plants, where larvae feed on stem tissues, resulting in stunted seedlings and no tiller elongation. Stem-boring insects, including sawfly larvae and stem caterpillars, attack brassica and canola plants by burrowing into their stems, resulting in longitudinal splits, weakening the structure and causing lodging/breakage of plants.

Leafminer flies damage crops of chickpea and legumes by forming numerous serpentine mines on the foliage, which decreases the photosynthetic surface area, accelerates leaf senescence, and ultimately leads to weak plant health. The wheat stem sawfly results in stem base girdling and internal destruction of tillers in wheat crops due to obstruction of nutrient flow, resulting in mature stems breaking and falling down before harvesting time. Similarly, the olive fruit fly damages olives by laying eggs under the skin of immature fruits; the larvae then eat up the inner flesh of the fruit, thereby ruining the interior [16].

Though usually harmless to the human population, animals, and the environment, honey bees (*Apis mellifera*) may also present themselves as pests. For instance, the stings of the insects can cause pain, inflammation, redness, and irritation to the

affected area. Some people can get allergic reactions that can lead to anaphylaxis due to honey bee stings. Honey bees may also attack farm animals, house pets, and wild animals with stings in cases where their hives have been disturbed, causing damage to or even killing them. While honey bees are essential pollinators, their presence in huge numbers in artificial colonies can become a threat to native bees and other pollinating insects since they compete with them when flower sources are few. In addition, honey bees may be carriers of some diseases and parasites to other bee communities. As such, in insecticidal research, honey bees are considered to be beneficial organisms, and insecticides should be selective against target pests and less toxic to honey bees [17]. The *Anopheles gambiae* are dangerous as the female of this species is capable of transmitting malaria parasites, known as Plasmodium, to human beings. The bite of this mosquito may make one feel itchy and irritated, but malaria results in symptoms like fever and chills, as well as anemia.

2.2 Synthetic Insecticides and the Crisis of Resistance Selection

While synthetic chemical insecticides have long served as a primary tool for rapid pest suppression and crop protection, their continuous, intensive application has driven a severe biological challenge, i.e., the widespread development of insecticide resistance. This phenomenon operates through intense evolutionary selection pressure. When a genetically diverse pest population is repeatedly exposed to a single chemical input, susceptible individuals are eliminated, while rare individuals possessing resistant traits survive to reproduce and pass these advantages to subsequent generations, as shown in figure 2.2. This rapid selection cycle quickly reduces the field efficacy of standard synthetic chemistry. As chemical treatments lose effectiveness, growers often respond by increasing application frequencies, raising chemical concentrations, or mixing stronger synthetic formulations. While this may provide temporary control, it increases production costs, creates a cycle of chemical dependency, and accelerates the development of resistance within pest populations [17].

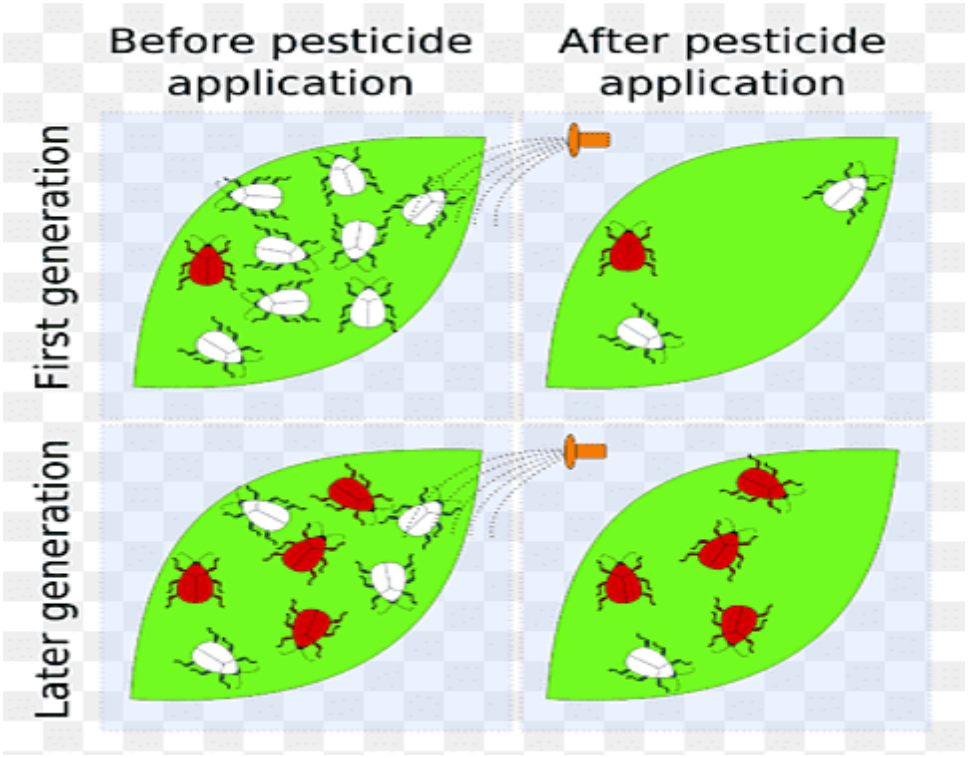


FIGURE 2.2: Pesticide resistance. This diagram illustrates the evolutionary process of pesticide resistance in an insect population over time [18].

The physiological mechanisms driving this resistance are complex and can be categorized into four primary pathways. First, metabolic detoxification occurs when pests upregulate endogenous enzyme systems, such as cytochrome P450 monooxygenases, glutathione S-transferases, and esterases, to neutralize toxic compounds before they can reach their intended physiological targets.

Second, target-site resistance involves structural mutations that alter the specific proteins or receptors targeted by the chemical, which prevents the pesticide from binding effectively. Third, pests can employ penetration resistance by modifying their cuticular wax layers to slow down the absorption of contact insecticides. Finally, behavioral avoidance allows pests to adapt their feeding and movement patterns to completely avoid treated plant surfaces [19].

To address these resistance challenges, agriculture must shift toward multi - targeted, bio-based alternatives, such as the phytochemical complexes found in *Rhazya stricta*, which can disrupt multiple physiological targets simultaneously and delay the onset of pest resistance.

This resistance crisis is worsened by cross-resistance, where a single mutation or metabolic adaptation protects an insect population against multiple distinct classes of synthetic pesticides simultaneously. This occurs because many synthetic options share similar chemical backbones or target identical single receptor sites within the pest's physiology.

When an insect evolves resistance to one synthetic chemical, it frequently gains immediate resistance to related compounds, rendering whole classes of pest control chemistry ineffective. This biological vulnerability highlights the urgent need for complex, multi-component botanical alternatives that attack multiple physiological targets at once, making it nearly impossible for pests to adapt successfully [18].

2.3 Environmental and Public Health Consequences of Conventional Agrochemicals

Although synthetic chemical pesticides offer short-term crop protection, their widespread, heavy application has caused significant damage to global ecosystems and public health. A primary concern is extensive soil pollution. During application, a large percentage of the chemical spray misses the target pest and accumulates in the surrounding soil matrix. These persistent chemical residues disrupt beneficial soil microbial communities, inhibit essential nutrient cycling, and degrade overall soil fertility, threatening long-term agricultural sustainability. Water contamination represents another critical environmental hazard [20].

Rainwater runoff and irrigation leaching carry pesticide residues from agricultural fields into nearby surface waters and underground aquifers. Once inside these aquatic ecosystems, the chemicals cause widespread damage to non-target organisms, including fish, amphibians, and vulnerable aquatic invertebrates. Because water systems can transport these contaminants over vast distances, the ecological damage often extends far beyond the original application site [21]. Uncontrolled synthetic residues accumulate across ecological trophic levels, causing severe reproductive and systemic damage to apex consumers and farm workers, as detailed in table 2.1.

TABLE 2.1: Systemic hazards of synthetic inputs [21]

Environmental Recipient	**Disruption Modality**	**Long-Term Biological Outcome**
Soil Microcosm	Microbial biomass reduction, breakdown of nitrogen fixers.	Suppressed fertility, failure of vital nutrient cycles.
Hydrosphere Matrix	Non-target accumulation via chemical leaching and surface runoff.	Massive aquatic mortality, collapse of food webs.
Human Consumption	Intake of crop chemical residues, occupational exposure.	Chronic illnesses, systemic toxicity hazards.

Furthermore, the indiscriminate nature of synthetic pesticides causes severe damage to beneficial non-target insects, such as honeybees, wild pollinators, and natural pest predators. Disruption of these beneficial species weakens natural pollination and biological pest control, creating ecological imbalances that reinforce the need for biodegradable, eco-friendly botanical alternatives like *Rhazya stricta*. The chronic public health risks associated with long-term pesticide exposure present a severe global challenge [22]. Farmworkers and consumers are regularly exposed to trace chemical residues on harvested food products, leading to chronic health issues, neurological disorders, hormone disruption, and increased cancer risks, as shown in figure 2.3.

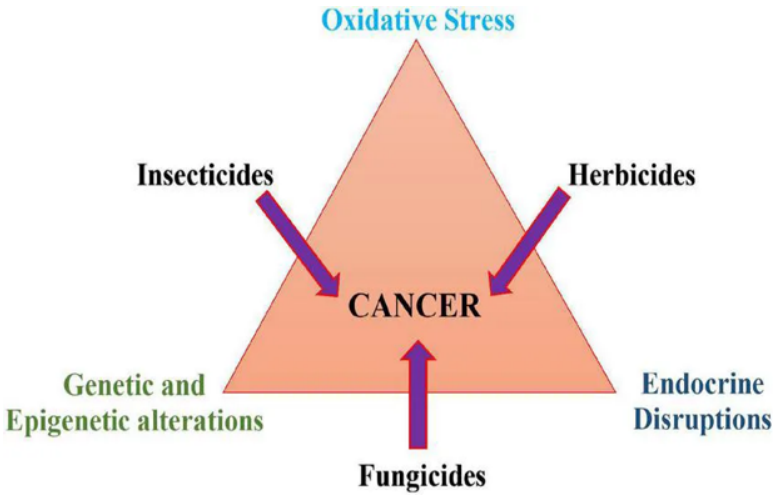


FIGURE 2.3: Health damage caused by insecticides. Synthetic herbicides, insecticides and fungicides are causing cancer, genetic and epigenetic alterations and endocrine disruptions [23].

These severe impacts on human health and ecosystems highlight the unsustainability of our current reliance on synthetic chemistry [23]. Transitioning to biodegradable plant extracts like *R. stricta* is essential to safeguard global ecosystems while supporting long-term food safety.

2.4 Botanical Insecticides as a Core Component of Sustainable Pest Management

Botanical insecticides serve as a core component of sustainable agriculture, offering an eco-friendly alternative to hazardous synthetic chemical inputs. Derived from natural plant secondary metabolic pathways, these biopesticides are highly compatible with ecological farming practices and Integrated Pest Management (IPM) strategies. Unlike synthetic chemicals that persist in ecosystems, botanical compounds break down rapidly through photolytic, thermal, and microbial processes, minimizing environmental accumulation and protecting vital soil and water resources. A major advantage of botanical biopesticides is their low toxicity toward non-target organisms [24]. While conventional synthetic chemical options often cause widespread mortality among beneficial insects, plant-derived extracts typically feature selective biological activity. This target selectivity helps preserve vital pollinator populations and native predatory species, allowing natural pest regulation and nutrient cycling to continue uninterrupted.

Additionally, the complex chemical matrix of compounds that combines alkaloids, flavonoids, terpenoids, phenolics, and saponins disrupts multiple physiological systems in pests simultaneously. This multi-targeted approach reduces the likelihood of insects developing rapid metabolic or target-site resistance, making phytocompounds an effective option for long-term pest management. When integrated into IPM frameworks alongside cultural rotations and biological controls, botanicals provide an effective, sustainable method for managing pest surges. Furthermore, because these medicinal plants can often be grown and processed locally, they offer

a cost-effective, accessible solution for smallholder farmers in developing regions, supporting agricultural self-reliance and environmental health [25].

The use of plants within IPM programs supports natural biological control by protecting indigenous predators and parasitic wasps. In conventional systems, broad-spectrum chemicals often eliminate these beneficial predators, leading to secondary pest resurgences where minor pests multiply rapidly due to a lack of natural predators [26]. Botanical extracts target pest surges selectively while leaving beneficial predator populations intact, allowing natural ecological balances to help regulate pest populations over the long term as shown in figure 2.4.

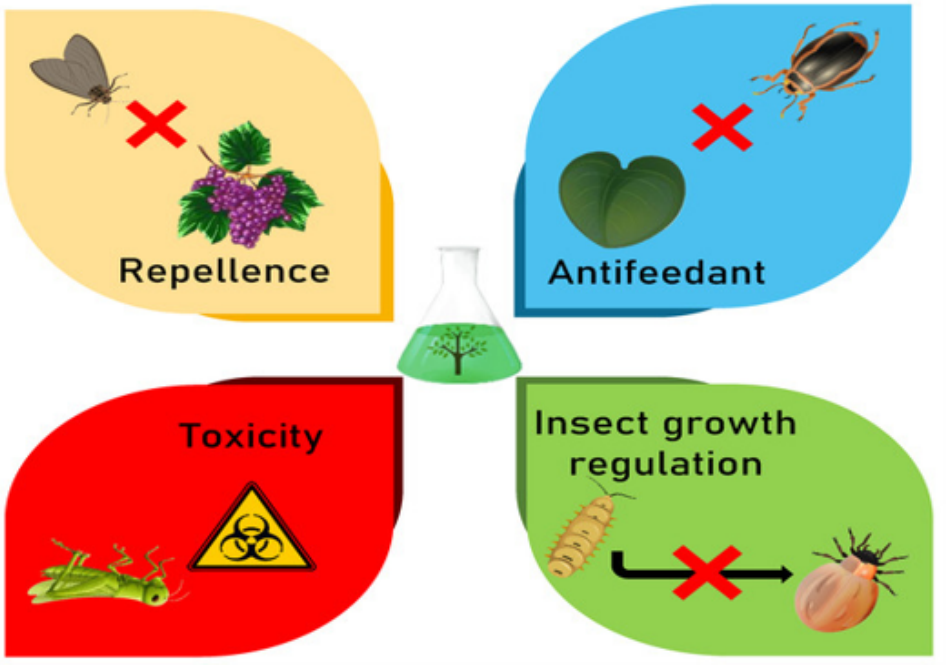


FIGURE 2.4: The process of botanical insecticides. Botanical insecticides exert their effect by repellence, insect growth regulation, toxicity and as antifeedant [26].

2.5 The Shift from Chemical to Plant-Based Pesticides

Driven by rising regulatory bans and a growing awareness of the hazards associated with synthetic chemical compounds, agricultural science is shifting toward safer,

bio-based alternatives. While conventional synthetic pesticides offer rapid, short-term suppression of target populations, their prolonged, indiscriminate application has triggered severe ecological crises. These include accelerated selection pressure leading to widespread insecticide resistance, persistent environmental pollution across physical terrestrial and aquatic ecosystems, and chronic and acute health toxicities in humans and non-target organisms [27]. Consequently, finding biopesticides that combine high target efficacy with rapid environmental degradation has become an agricultural priority as shown in figure 2.5.

Botanical insecticides, derived from the secondary metabolic pathways of diverse flora, represent a promising frontier in sustainable pest management. These naturally synthesized compounds feature a diverse matrix of complex molecules that exhibit insecticidal, repellent, antifeedant, and growth-regulating activities.

A key ecological advantage of botanical insecticides is their excellent biodegradability. Unlike synthetic organochlorines or organophosphates, plant-derived compounds break down rapidly when exposed to ambient UV radiation, soil moisture, and microbial activity, preventing long-term bioaccumulation in soil and water systems [28].

Additionally, botanical extracts generally exhibit low toxicity toward non-target organisms, such as insect pollinators like Apis mellifera, beneficial predatory mites, and parasitic wasps.

This target selectivity stems from specific biochemical modes of action that differ markedly from the broad-spectrum neurotoxicity of synthetic chemicals, making botanicals highly compatible with Integrated Pest Management (IPM) systems. Phytochemical matrices typically consist of complex blends of alkaloids, flavonoids, terpenoids, and phenolic compounds that target multiple physiological systems in an insect simultaneously.

This multi-site mechanism makes it exceptionally difficult for pest populations to evolve resistance, bypassing a major limitation of single-target synthetic chemistry.

Furthermore, because medicinal plants are widely distributed and can often be cultivated locally at low cost, they offer an accessible, self-reliant pest control solution for resource-limited smallholder farmers in developing economies [29].

FIGURE 2.5: A shift towards natural pesticides. This figure is showing a chemical pesticide sprayer on one side and natural spray being used on crops on the other. That image clearly depicts the move from synthetic control methods to eco-friendly plant-based alternatives [27].

2.6 The Role of Bioactive Compounds as Modern Biopesticides

Bioactive secondary metabolites offer a highly viable paradigm for developing modern, environmentally safe biopesticides as shown in figure 2.6. Developed over millions of years of evolutionary pressure, these molecules serve as a plant's natural defense network against herbivores and pathogens. Their structural diversity allows them to interact with a wide range of biological targets in target pests, causing severe physiological disruption through multiple modes of action. The diverse chemical structures found in botanical extracts enable them to function as multi-targeted management agents. For example, an insect population exposed to a crude extract of *Rhazya stricta* must simultaneously cope with neurotoxic alkaloids, growth-disrupting terpenoids, and digestive-inhibiting phenolics. This complex physiological pressure makes it exceptionally difficult for pests to develop metabolic or target-site resistance, extending the useful lifespan of these bio-based tools [30].

In addition to their efficacy, botanical pesticides offer excellent environmental safety profiles. They are highly biodegradable and break down rapidly when exposed to environmental elements, minimizing bioaccumulation in food webs and safeguarding vulnerable aquatic and terrestrial ecosystems. This rapid degradation makes them highly compatible with Integrated Pest Management (IPM) strategies, as they can be applied to control pest surges while minimizing harm to beneficial predators and native pollinators.

Furthermore, by utilizing locally sourced medicinal plants, rural agricultural communities can reduce their reliance on costly, imported synthetic inputs, supporting economic self-sufficiency and sustainable farming practices [31]. The shifting regulatory landscape further highlights the critical relevance of biopesticides in modern agriculture. As international regulatory bodies enforce stricter limits on synthetic residues in food products and ban legacy chemicals due to environmental damage, farmers urgently require compliant alternatives. Botanical biopesticides fit this need perfectly, matching modern sustainability standards while offering a reliable tool to protect global food production from changing pest pressures [32].

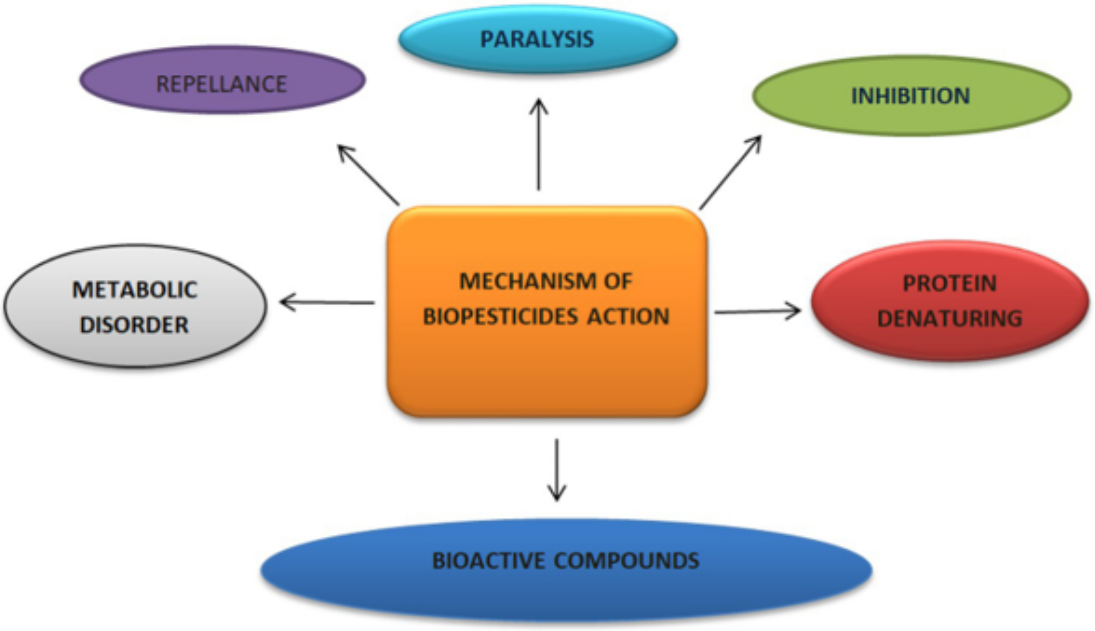


FIGURE 2.6: Bioactive compounds and their pesticidal actions. Instead of killing pests with toxic chemicals bioactive compounds repel them, stop them from mating or growing and damage their cells. They are modern because they're eco-friendly, safe for humans, and pests don't easily become resistant to them [30].

2.7 *Rhazya stricta*, A Potential Medicinal Plant

Rhazya stricta also known as rangobul, is a plant classified as an evergreen, perennial shrub that falls under the Apocynaceae family. The species is broadly spread within the harsh arid and semi-arid environments of South Asia and the Middle East, including documented instances within the regions of Pakistan, India, Saudi Arabia, and Iran. Adapted to endure extremely harsh conditions, the plant exhibits an excellent capacity for growth in the scorching desert plains, gravel screes, and harsh environments characterized by heat stresses, drought, and nutrient-poor soil conditions [33].

In traditional ethnomedicine, indigenous communities have long utilized crude preparations of *R. stricta* leaves, stems, and roots to treat a wide array of ailments. Local healers frequently prescribe decoctions and poultices of the plant to manage inflammatory disorders, persistent fevers, cutaneous infections, and diabetes mellitus. Modern pharmacological screenings have validated many of these traditional uses, demonstrating clear antimicrobial, anti-inflammatory, antidiabetic, antioxidant, and antineoplastic activities. These therapeutic properties are largely driven by the plant's exceptionally diverse secondary metabolic profile, which is particularly rich in specialized indole alkaloids. Alongside these alkaloids, a dense array of flavonoids, condensed tannins, and terpenoids enhances its biological activity [34].

While the pharmacological benefits of *R. stricta* are well documented, its potential application as an agricultural biopesticide remains largely understudied. Most historical literature focuses primarily on its clinical and therapeutic uses, leaving a significant gap regarding its utility in crop protection and sustainable pest management. Given the urgent demand for bio-based pest control options, the plant's abundant, highly active phytochemical constituents warrant systematic evaluation. Fortunately, recent advances in bioinformatics and computational biology provide powerful tools to address this gap. Through advanced *in silico* screening, molecular docking, and structural modeling, researchers can efficiently map and predict the precise molecular interactions between *R. stricta* ligands and

critical insect receptor targets, streamlining the discovery of effective, eco-friendly biopesticides [35].

FIGURE 2.7: *Rhazya stricta*. This image is showing the whole plant in its natural habitat, ideally showing the upright shrub form, narrow leaves, and flowering/fruiting branches [33].

2.8 Morphological Features of *Rhazya stricta*

Structurally, *Rhazya stricta* grows as a compact, sub-shrubby perennial that typically reaches heights of 1 to 2 meters as shown in figure 2.7. It features an upright, highly branched growth form adapted to survive severe desert environments. Its mature stems are heavily lignified, erect, and grow in dense clusters, providing robust structural integrity against abrasive, wind-blown desert sands. The outer bark is smooth and exhibits a distinct grayish-green coloration, which helps reduce water loss in hyper-arid conditions. The leaves are simple, entire, and assume a narrow, linear-to-lanceolate morphology, typically measuring between 3 and 10 cm in length. Arranged in an opposite decussate pattern along the stem axis, the leaves possess a thick, leathery, coriaceous texture. This structural adaptation minimizes transpiration rates and maximizes water retention within the internal parenchymal tissues. The leaf surface is glabrous and features a prominent, raised central midrib, allowing for efficient photosynthesis under intense solar radiation [36].

The plant produces small, white to pale pink, hypocrateriform, tubular flowers arranged in dense terminal or axillary cymes. These entomophilous flowers feature a deeply five-lobed corolla designed to attract native desert pollinators as shown in figure 2.8. Following successful fertilization, *R. stricta* develops elongated, paired, erect, cylindrical follicles filled with numerous compressed, winged seeds. This anemochorous seed structure ensures broad geographic dispersal across open arid terrains. At the microscopic level, the foliar and cauline histologies reveal specialized secretory structures and laticiferous cells dedicated to synthesizing and storing dense concentrations of secondary metabolites, particularly indole alkaloids. These profiles are systematically distributed throughout the foliar, cauline, and radicular systems to serve as a robust chemical defense network against severe herbivorous pressure [37].

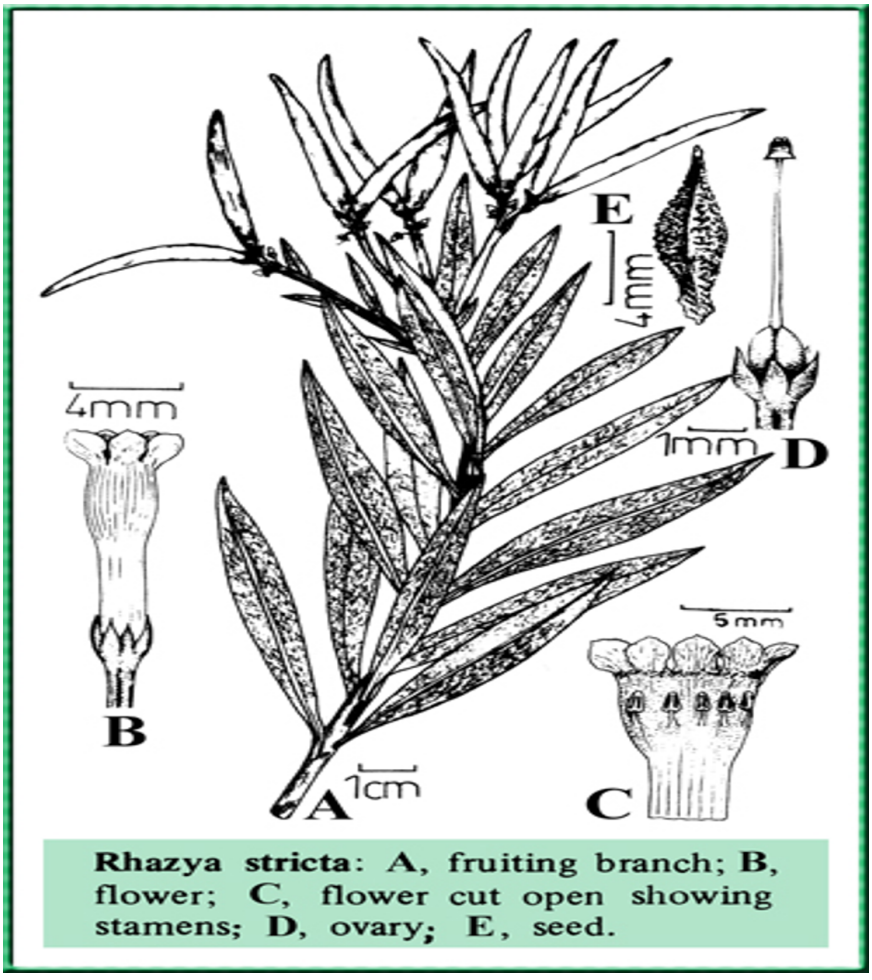


FIGURE 2.8: *Rhazya stricta* and its parts. This picture depicts morphological features of *Rhazya stricta* i.e., fruiting branch, flower, stamens, ovary and seed [36].

Furthermore, the physical structure of the cuticular layer acts as an initial line of defense, preventing structural breaching by macroscopic chewers and microscopic pathogens alike. The internal cellular arrangement includes specialized vacuoles within the palisade parenchyma where these highly reactive chemical compounds remain sequestered until tissue damage occurs. This spatial compartmentalization protects the plant's own primary metabolic machinery from self-toxicity while optimizing the immediate release of insecticidal secondary compounds during a herbivore attack. Consequently, this intricate macroscopic and microscopic architecture makes *R. stricta* a highly resilient species and a rich source of bioactive compounds [38].

2.9 Taxonomic Classification of *Rhazya stricta*

Properly positioning *Rhazya stricta* within the plant kingdom provides an essential framework for understanding its evolutionary relationships, biological traits, and phytochemical characteristics. Its taxonomy is outlined below in table 2.2. This taxonomic designation highlights *R. stricta*'s close evolutionary links to other pharmacologically important genera within the Apocynaceae family, such as Catharanthus and Rauvolfia. These genera are renowned for producing potent, neuro-active alkaloid complexes, supporting the hypothesis that *R. stricta* serves as a valuable source of bioactive molecules for both medicine and agriculture [39].

TABLE 2.2: Taxonomic position of *Rhazya stricta* [34]

Taxonomic Rank	**Systematic Nomenclature**
Kingdom	Plantae
Clade	Tracheophytes
Division	Angiosperms
Class	Magnoliopsida
Order	Gentianales
Family	Apocynaceae
Genus	Rhazya
Species	*Rhazya stricta* Decne.

The systematic classification under the order Gentianales and family Apocynaceae is particularly apparent of the plant's genetic predisposition for complex biochemical synthesis. Members of this lineage share highly conserved enzymatic pathways responsible for generating intricate structural scaffolding, such as the monoterpenoid indole alkaloid pathway. This evolutionary background confirms that *R. stricta* is not merely an isolated desert anomaly, but part of a well-documented group of chemically armed plants whose defensive strategies can be repurposed for modern agronomic protection [40].

2.10 The Secondary Phytochemical Matrix of *Rhazya stricta*

The diverse biological and insecticidal properties of *Rhazya stricta* are directly attributable to its rich phytochemical composition as shown in figure 2.9. As a specialized desert survival strategist, the plant dedicates significant metabolic resources to synthesizing secondary metabolites. Rather than supporting primary growth, these complex molecules function as defensive agents against environmental stress and herbivore pressure, providing an excellent source of natural bioactive compounds. Alkaloids represent the most abundant and extensively documented class of secondary metabolites in *R. stricta*, with over 100 distinct indole alkaloids identified across its leaves, stems, and roots. These nitrogenous bases exhibit broad cytotoxic and neuroactive properties. In an agricultural context, they act as potent neurotoxins by disrupting insect ion channels, neurotransmitter receptors, and vital synaptic enzymes, leading to rapid paralysis or death [41].

Complementing this alkaloid profile is a dense array of polyphenolic flavonoids, including prominent compounds like quercetin, kaempferol, and rutin. While these molecules primarily protect the plant from oxidative stress caused by intense UV radiation, they function as powerful feeding deterrents and growth inhibitors when ingested by insects, severely disrupting pest development and reproduction. The plant's volatile and non-volatile terpenoid fractions also play an important role in

its chemical defense system. These lipophilic compounds act as natural repellents and larvicides, penetrating insect cuticles to disrupt hormonal balance, interfere with molting, and prevent successful metamorphosis [42].

Additionally, *R. stricta* synthesizes significant quantities of complex phenolics and condensed tannins. When consumed, these molecules bind non-specifically to dietary proteins and digestive enzymes within the alkaline environment of the insect midgut, reducing nutrient absorption and weakening the pest. Finally, the presence of amphiphilic saponins adds a membrane-disrupting component to the plant's defenses, causing cell lysis in target tissues and enhancing the overall insecticidal efficacy of *R. stricta* extracts [43].

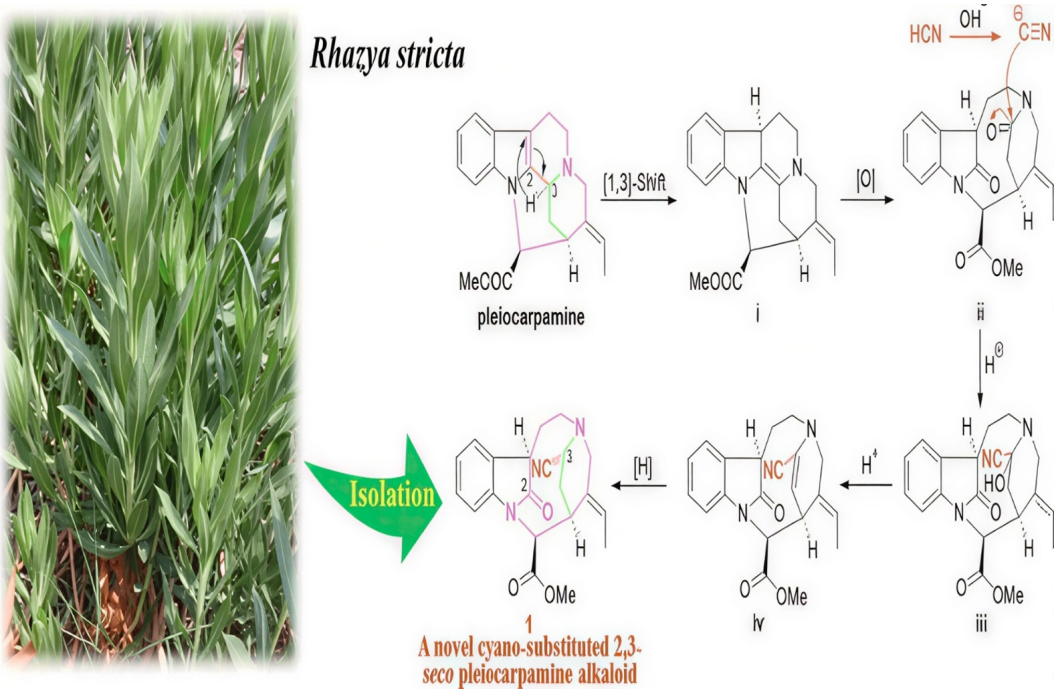


FIGURE 2.9: Screening of *Rhazya stricta* metabolites. Secondary phytochemical matrix of *Rhazya stricta* distribution of alkaloids, flavonoids, terpenoids, phenolics, and other metabolites across plant parts [42].

2.11 Characterization and Identification of Specialized Metabolites

To evaluate the biopesticidal potential of *Rhazya stricta*, its metabolic outputs can be categorized into primary and secondary pathways. While primary metabolites

maintain fundamental cellular function, the secondary metabolic pool drives ecological interactions and pest defense [44]. The major phytochemical groups, along with representative reference compounds and their primary modes of insecticidal action, are summarized in table 2.3.

TABLE 2.3: Phytochemical Profile and Target Biopesticidal Activities [44]

Phytochemical Class	**Key Molecular Markers**	**Target Mechanism within Pest Physiology**
Alkaloids	Rhazine, Stricticine, Vallesiachotamine	Synaptic block, competitive inhibition of AChE, paralysis.
Flavonoids	Quercetin, Kaempferol, Isoflavonoid glycosides	Midgut oxidation, taste receptor inhibition, antifeedant action.
Terpenoids	Monoterpenes, Sesquiterpenes, Phytol structures	Juvenile hormone interference, cuticular entry, larvicide.
Phenolics	Gallic acid, Ellagic polymers, Condensed tannins	Non-specific cross-linking of luminal proteins, starvation.
Saponins	Triterpenoid and Steroidal core structures	Sterol complexation, epithelial cell lysis, toxic synergy.

This multi-component chemical profile forms the basis for advanced *in silico* virtual screening, allowing researchers to evaluate how these diverse molecules can be used to develop multi-target botanical pesticides. The structural characterization of these specialized metabolites has been significantly enhanced by advanced spectroscopic and chromatographic techniques. By identifying individual chemical structures within the crude matrix, researchers can pinpoint exactly which compounds drive specific toxic outcomes. Rather than viewing the plant extract as a single entity, modern biochemistry treats it as an optimized, multi-component chemical cocktail where each molecular group plays a distinct role in weakening the target pest's defenses [45].

Furthermore, identifying these key molecular markers allows for standardization across batches of botanical extracts. Because environmental factors like temperature, soil quality, and water availability can alter a plant's metabolic output,

establishing a clear profile of these reference compounds ensures consistent quality and efficacy in field applications, establishing a reliable transition from raw desert shrub to a standardized agricultural input.

2.12 Biochemical Mechanisms of Phyto chemicals Activity

The insecticidal activity of plant-derived compounds relies on complex, multi-targeted biochemical and physiological pathways that disrupt essential life processes in target insects. Unlike conventional synthetic chemical pesticides, which typically target a single receptor site, botanical extracts leverage a complex mixture of phytochemicals to attack multiple biological systems simultaneously. This multi-site mode of action significantly enhances overall efficacy while reducing the likelihood of pest populations evolving resistance, as shown in figure 2.10.

Disrupting the insect nervous system is one of the fastest and most effective mechanisms of botanical toxicity. Many plant alkaloids and terpenoids interfere with normal neurotransmission by interacting with critical ion channels, neurotransmitter receptors, and regulatory enzymes [46].

A primary target is acetylcholinesterase (AChE), an essential enzyme responsible for breaking down the neurotransmitter acetylcholine within synaptic clefts. When plant molecules inhibit AChE, acetylcholine accumulates continuously, leading to prolonged postsynaptic stimulation, muscular spasms, paralysis, and ultimate death [47].

Beyond the AChE pathway, specific phytochemicals disrupt alternative neurological targets, including the GABA (gamma aminobutyric acid) gated chloride channels and octopamine receptor pathways, which coordinates vital physiological processes in insects. In addition to neurotoxicity, plant compounds disrupt essential metabolic and digestive enzymes. Complex tannins and phenolic compounds

form strong covalent and hydrogen bonds with dietary proteins and digestive enzymes within the alkaline environment of the insect midgut. This structural binding deactivates vital enzymes, impairing nutrient absorption and leading to severe weight loss, stunted growth, and reduced reproductive capacity [48].

Botanical compounds also disrupt endocrine regulation and development. Pests rely on precise balances of juvenile hormones and ecdysteroids to coordinate molting, pupation, and metamorphosis. Certain plant terpenoids mimic or antagonize these endocrine signals, causing incomplete molting, structural deformities, and adult sterility.

Furthermore, plant compounds can act as potent antifeedants and repellents, altering taste receptor perception to prevent crop feeding, or leverage amphiphilic saponins to disrupt cellular membrane integrity and cause cell lysis. This multilayered defense makes botanicals a valuable tool for sustainable pest management [49].

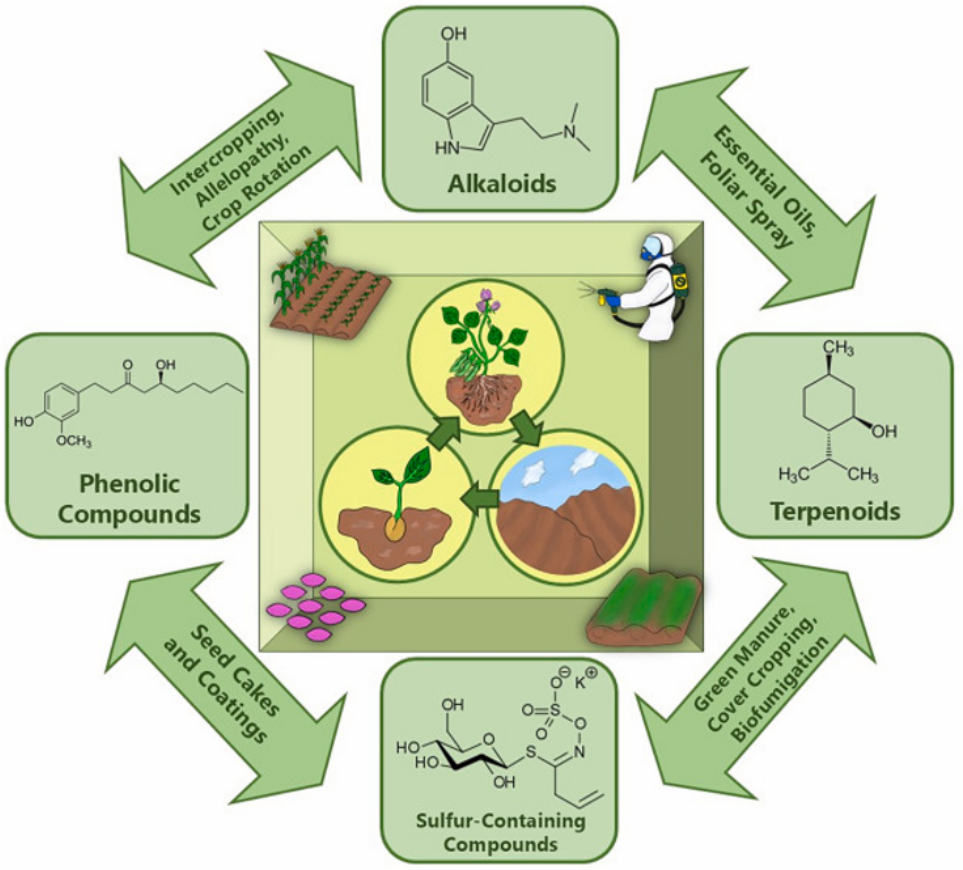


FIGURE 2.10: Therapeutic role of plant-derived metabolites. Phytochemicals and oils extracted from various plant tissues exert targeted activities [46].

2.13 Previous *in silico* Research on Plant Insecticides

The use of computer-aided discovery workflows to evaluate plant-derived insecticidal compounds has expanded significantly in recent years. Computational tools such as molecular docking, virtual screening, and molecular dynamics (MD) simulations allow researchers to identify and analyze potential biopesticides with high efficiency and lower costs, supporting the development of safer pest management solutions. Several recent studies demonstrate the effectiveness of *in silico* methods for identifying plant-derived enzyme inhibitors. For example, virtual screenings of Nerium oleander extracts revealed that key constituents, such as phytol and neophytadiene, exhibit exceptionally strong binding affinities with insect cytochrome P450 detoxification enzymes [50].

By blocking these metabolic pathways, the plant compounds prevent insects from neutralizing toxic inputs, increasing pest mortality and highlighting the potential for synergistic botanical formulations as shown in figure 2.11. Similarly, *in silico* evaluations of plant-derived flavonoids have shown high efficacy in inhibiting insect acetylcholinesterase. Specific compounds, such as methoxypelargonidin, demonstrated superior binding energies and active-site stability compared to conventional synthetic formulations like DTT. Additionally, computational modeling of limonoid compounds from Melia volkensii against the destructive Fall Armyworm Spodoptera frugiperda showed binding affinities equivalent to commercial chemical inputs [51].

Interestingly, these botanical ligands interacted with novel allosteric sites on the insect receptor, suggesting they could help bypass existing target-site resistance to synthetic chemicals and provide a valuable starting point for screening the phytochemicals of *Rhazya stricta*. These foundational computational studies demonstrate that *in silico* modeling can accurately predict true biological activity in field and laboratory settings. By showing that natural structures match or exceed the binding efficiency of synthetic agrochemicals, this literature confirms that complex

plant metabolites are well-suited for targeting critical insect proteins, justifying the systematic computational evaluation of *R. stricta*'s unique indole alkaloid profile [52].

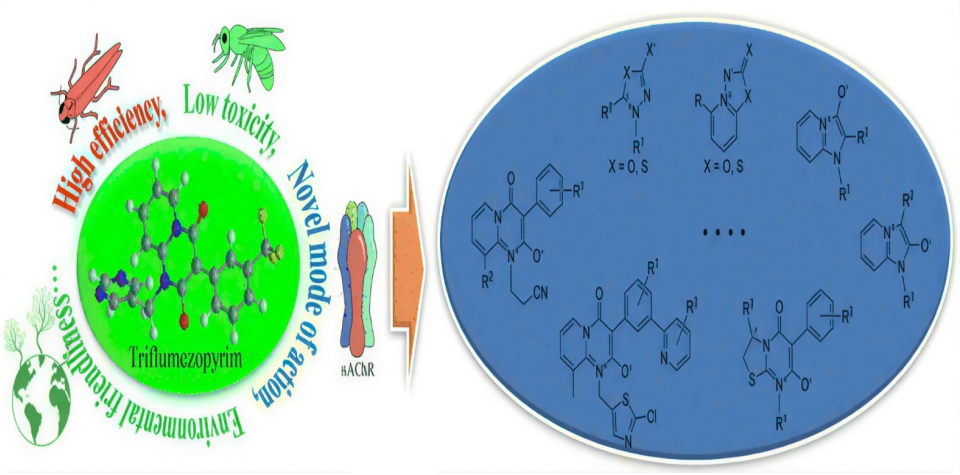


FIGURE 2.11: Insecticidal activity of phytocompounds. Phytocompounds have natural mode of action, environmental friendliness, high efficiency and low toxicity [51].

2.14 Molecular Docking and ADMET Profiling Frameworks in Biopesticide Discovery

The docking has become an essential tool in *in silico* drug development, allowing researchers to evaluate the spatial and energetic interactions between bioactive ligands and target proteins as shown in figure 2.12. By simulating these molecular dynamics, this computational approach can predict the binding affinity, structural stability, and mechanism of action of candidate compounds, significantly accelerating the discovery and development of plant-based pesticides. This computer method models how a small-molecule ligand fits into a target protein's active site in a key-and-lock fashion. It is based on molecular recognition. Complex intermolecular forces, including as hydrogen bonds, hydrophobic interactions, van der Waals forces, and electrostatic interactions, are what propel this binding. Compounds that generate strongly negative binding scores indicate highly stable, favorable interactions, identifying them as promising candidates for practical pest control [53].

In biopesticide discovery, researchers target essential insect proteins required for survival, metabolism, or development, such as acetylcholinesterase, GABA and voltage-gated sodium channels. High-throughput virtual screening allows libraries containing hundreds of phytochemicals to be evaluated against these targets in a fraction of the time required for traditional laboratory assays. This efficiency significantly reduces research costs and streamlines early-stage screening. Furthermore, analyzing these docking configurations at the atomic level reveals the specific amino acid residues driving the interaction. This structural insight allows researchers to optimize compound design, enhance target selectivity, and minimize potential toxicity toward non-target organisms, providing a strong scientific foundation for developing safer, more effective crop protection tools [54].

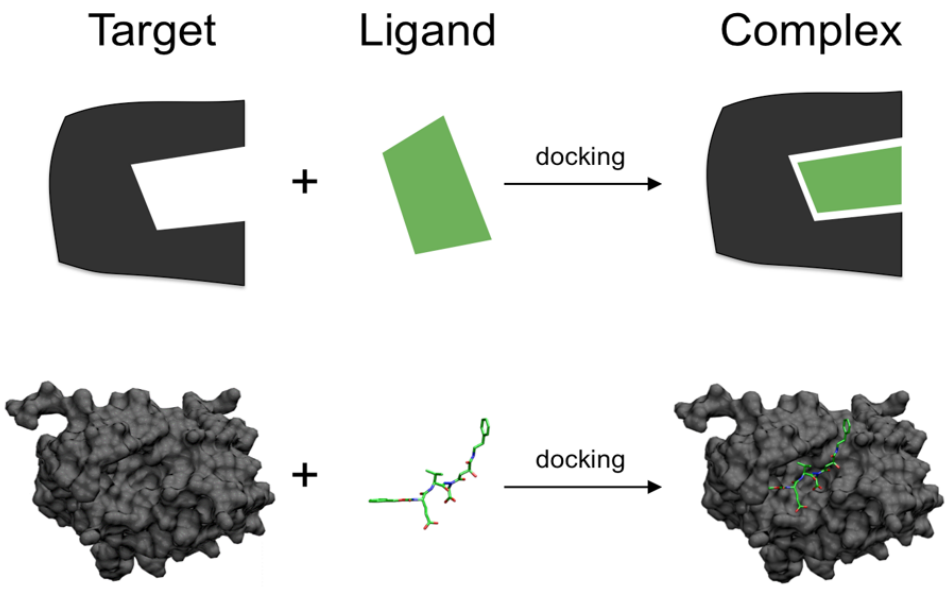


FIGURE 2.12: Molecular docking. In this process ligands attach with target protein, a complex is formed and docking score is calculated and interactions are analyzed [53].

Furthermore, the use of molecular docking in botanical studies helps close the gap between contemporary structural biology and traditional ethnobotanical knowledge. Instead of relying on trial-and-error laboratory screenings, which are time-consuming and resource-intensive, computational frameworks allow for targeted, hypothesis-driven exploration. Researchers can rank candidate molecules based on their estimated binding energies prior to acquiring physical plant extracts. This targeted approach focuses physical validation efforts exclusively on the most

promising compounds, optimizing research workflows and maximizing resource utilization [55].

Conducting an integrated ADMET analysis is essential for evaluating plant-derived compounds, as effective target binding alone does not ensure their agricultural utility. Key parameters evaluate a compound's ability to penetrate biological barriers and its degradation pace, which must balance rapid breakdown with environmental stability. Toxicity profiling ensures safety for humans, livestock, and ecosystems. By combining molecular docking with ADMET screening, researchers can eliminate toxic or unstable molecules early, enhancing the selection of safe and effective *Rhazya stricta* compounds for further validation. High-throughput *in silico* tools also predict potential off-target toxicities, safeguarding future biopesticides for users and the environment [56].

A review of the literature indicates that while *Rhazya stricta* is well-studied for its medicinal benefits, its use in agricultural pest management is underexplored. Research has primarily validated its ethnomedical uses, highlighting its antimicrobial, antioxidant, anti-inflammatory, and anticancer properties. There is a lack of modern computational methods to assess its insecticidal potential. Additionally, existing studies often generalize botanical biopesticides without providing detailed interactions at the molecular level with insect targets like acetylcholinesterase and GABA receptors. This study aims to bridge these gaps by screening bioactive compounds from *Rhazya stricta* through advanced molecular docking and ADMET analysis. By identifying specific phytochemicals that combine strong target binding affinities with favorable safety and environmental profiles, this research establishes a definitive scientific foundation to support the development of sustainable, ecofriendly biopesticides.

Chapter 3

Research Methodology

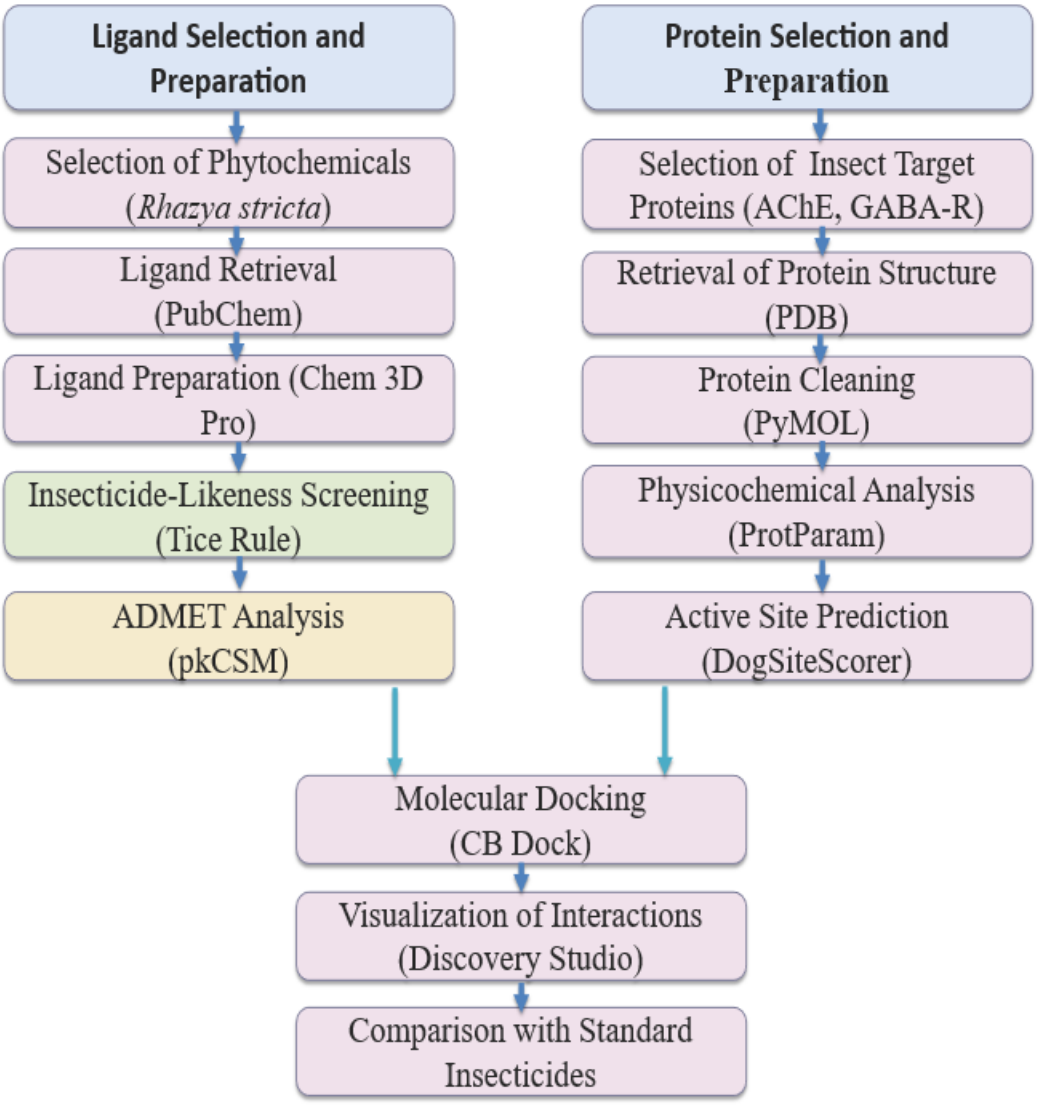


FIGURE 3.1: Flowchart for insecticidal analysis

3.1 Choosing the Proteins to Target

The particular proteins used in adult stage *Anopheles gambiae* and *Apis mellifera*, research are chosen based on their crucial functions in either neurotransmission, metabolism, or regulating growth.

Widely used targets including acetylcholinesterase (AChE) and GABA receptor (GABA-R) are considered, as they are crucial to the functioning of the insect nervous system and detoxification.

AChE regulates nerve impulse transmission by hydrolyzing acetylcholine, while GABA-R controls neuronal inhibition through chloride ion channels. Disruption of either target can impair neural signaling, leading to paralysis and death in insects [57].

3.2 3D Structure Prediction and Cleaning of Proteins

The PDB https://www.rcsb.org/ was used to estimate the target proteins' three-dimensional structures. The same database was also used to obtain the FASTA sequences [58].

Globally, proteins, small molecules, nucleic acid, electron density, diverse surfaces, and trajectories are visualized through PyMOL https://pymol.org/, a free molecular graphics program. The program is also suitable for creating animation and movies, modifying molecules, and ray tracing.

Plugin solutions that enhance the capabilities of this Python programming language are numerous to create and target medication design using the PyMol software. Additional elements related to the protein need to be stripped off after the protein structure has been downloaded; this process was carried out using the free PyMol software [59].

3.3 Evaluation of Physical and Chemical Parameters

These physicochemical properties greatly affect the function of the protein. The software ProtParam expasy was utilized for the estimation of these physicochemical properties of the target proteins http://www.ch.embnet.org/ProtParam. This software is used for calculating number of negative charges (Asp+Glu) and positive charges (Arg +Lys), theoretical isoelectric point, molecular weight, aliphatic index, GRAVY value, instability index, Extinction coefficient (Cysteine included) and Extinction coefficient (without cysteine) [60].

3.4 Determination of Active Sites

The region that the active site of the target protein belongs to is the place where the maximum interaction between the ligand and the protein occurs. Amino acids play an important role in the formation of the complex between the ligand and the protein. Dogsitescorer software was used for the recognition of protein binding pockets. [61].

3.5 Phytochemicals Selection

The phytocompounds selected in this study from Rhazya stricta were chosen based on their reported diverse biological activities and structural diversity as indole alkaloids and related secondary metabolites. These natural compounds have previously shown potential pharmacological properties, including neuroactive and bioactive effects, which make them suitable candidates for interaction with insect neurological targets. These include rhazinilam, aspidospermidine, serpentine, dihydrocorynantheol, tabersonine, vincadifformine, tetrahydroalstonine, vallesiachotamine, eburnamine, luteolin, eburnamonine and quebrachamine [62].

3.6 Obtaining Ligand Chemical Structure and Optimizing Energy

It is important to note that the world's biggest resource of chemical information databases is PubChem https://pubchem.ncbi.nlm.nih.gov/. Consequently, the chemical compound that was selected as the ligand was obtained in SDF format from the PubChem database.

In case the structure of the selected ligand cannot be retrieved, then we will download the canonical smiles from PubChem and use it in the ChemDraw program [63]. Chem3D Ultra was used to decrease the energy of ligands. Before docking, the ligands must be refined; otherwise, the docking scores will be untrustworthy [64].

3.7 Virtual Scanning and ADMET Ligand Assessment

The tice rule is a crucial factor in assessing whether ligands are likely to be insecticides. If a chemical complies with the Lipinski Rule of Five, it may be used as an active pharmaceutical in humans. An online tool called pkCSM https://omictools.com/pkcsm-tool may be used to determine whether or not ligands follow the tice rule. Criterion range of the tice rule includes molecular weight in range of 150-500, log P range is 0-5, hydrogen bond donors confined to three, hydrogen bond acceptors eight and rotatable bonds confined to twelve [65].

The prediction of pharmacokinetic and toxicological parameters came next in the study after the ligands were filtered using the tice rule. During ADMET analysis, the drug's poor candidates were excluded [66]. Potential insecticides can be chosen from the remaining possibilities.

3.8 Docking Molecules and Examining Docked Complexes

The cavity-based blind docking technique, namely CB-dock, was used to perform the docking between the protein and the ligand. The docking site is automatically determined using CB-dock http://clab.labshare.cn/cbdock/blinddock. A protein and ligand docking technique called CB-Dock calculates the center, size, and bonding sites [67].

Docking is carried out after the box size is modified based on the ligand. AutoDock Vina is used for the docking process. Due to the docking process's increased emphasis on cavity binding, its accuracy ratio is higher. For docking, the protein and ligand structures were uploaded and the ligand structure was uploaded in SDF format while the protein structure was in PDB format. Five different docking stances will be the result of the docking process. The lowest docking score will be considered to select the best one. Dock will generate the interactive 3D presentation of the findings in five different postures. In selecting the optimum position of the interaction, the lowest vina score was selected [68].

The interaction of the ligand and the active sites of the protein was calculated in order to interpret the docking results. A number of interactions, including the hydrophobic and hydrogen binding, were studied. Protein-Ligand Interaction was studied by using Discovery Studio 2025 Client software. It will automatically create the diagrammatic representation of the protein-ligand interaction of the desired ligand in the PDB file [69].

3.9 Determination of Lead Compound

Following an analysis of docking score, pharmacokinetics tests, toxicity issues, and the interaction between proteins and ligands, the most active molecule was identified. All of these criteria were satisfied by our lead chemical.

3.10 Choice of Standard Drug

The purpose of this step is to identify the commercial drugs that are already in use as insecticide. Drug Bank https://go.drugbank.com/ database was used for this purpose because it provides details about drugs and their pathways [70].

3.11 Correlation Between Potential Compound and Commercial Drug

Pharmacodynamic factors like docking values, interactions, and pharmacokinetics were studied between the standard drug and the proposed drug candidate.

Chapter 4

Results

4.1 Proteins Structure and Clearance

Structure of the target proteins viz., acetylcholine esterase and GABA receptor was downloaded from the PDB database using PDB IDs 6ARY and 9SHO, respectively. In order to refine the protein structure, ligands and water molecules in the structure were deleted using the PyMol program. For achieving stability in structure, hydrogen atoms were added, and other atoms were eliminated to avoid overlapping. The final refined structure is shown in figure 4.1

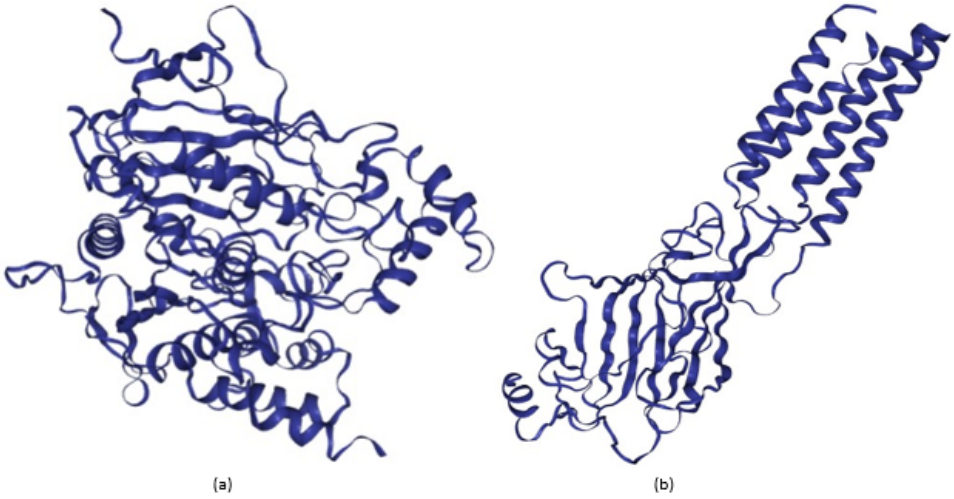


FIGURE 4.1: Selected proteins PDB structures (a) AChE (b) GABA-R

The FASTA sequences have been obtained using PDB with the same IDs mentioned above and depicted in figure 4.2.

>6ARY_1|Chains A|Acetylcholinesterase|Anopheles gambiae
(7165)NDPLVVNTDKGRIRGITVDAPSGKKVDVWLGIPYAQPPVGPLRFRHPRPAEKWTGVLNTTTPPNSCVQIVDTVFGD
FPGATMWNPNTPLSEDCLYINVVAPRPRPKNAAVMLWIFGGSFYSGTATLDVYDHRALASEENVIVVSLQYRVASLGFLFL
GTPEAPGNAGLFDQNLALRWVRDNIHRFGGDPSRVTLFGESAGAVSVSLHLLSALSRDLFQRAILQSGSPTAPWALVSREE
ATLRALRLAEAVGCPHEPSKLSDAVECLRGKDPHVLVNNEWGTLGICEFPFVPVVDGAFLDETPQRSLASGRFKKTEILTG
SNTEEGYYFIIYYLTELLRKEEGVTVTREEFLQAVRELNPYVNGAARQAIVFEYTDWTEPDNPNSNRDALDKMVGDYHFT
CNVNEFAQRYAEEGNNVYMYLYTHRSKGNPWPRWTGVMHGDEINYVFGEPLNPTLGYTEDEKDFSRKIMRYWSNFAKT
GNPNPNTASSEFPEWPKHTAHGRHYLELGLNTSFVGRGPRLRQCAFWKKYLPQLVAATSN
>9SHO_1|Chains A|Gamma-aminobutyric acid receptor subunit beta|Apis mellifera
(7460)DVNISAILDSFSVSYDKRVRPNYGGPPVEVGVTMYVLSISSLSEVKMDFTLDFYFRQFWTDPRLAFKKRTGVETLSV
GSEFIKNIWVPDTFFVNEKQSYFHIATTSNEFIRIHHSGSITRSIRLTITASCPMNLQYFPMDRQLCHIEIESFGYTMRDIRYK
WNEGPNSVGVSNEVSLPQFKVLGHRQRAMEISLTTGNYSRLACEIQFVRSMGYYLIQIYIPSGLIVIISWVSFWLATPARVA
LGVTTVLTMTTLMSSTNAALPKISYVKSIDVYLGTCFVMVFASLLEYATVGYMSDIDKYSSRIVFPVCFVCFNLMYWIIYLH
ISDVVADDLVLL

Figure 4.2: Selected proteins sequences

4.2 Proteins Physical and Chemical Identification

An online tool known as ProtParam was used to predict various parameters, such as molecular and structural features of particular proteins. Physicochemical features of target proteins have been presented in Table 4.1.

Table 4.1: The Proteins Physical and Chemical Identification

Selected Proteins	**AChE**	**GABA-R**
Weight	60113.8	38384.55
Polarity	6.01	7.14
Neg Residues	60	28
Pos Residues	53	28
Extinc Co 1	103165	61685
Extinc Co 2	102790	61310
The Index of Instability	36.97	39.5
The Aliphatic Counts	77.34	98.81
Hydrophobicity	−0.361	−0.268

Proteins possess molecular weights between approximately 38kDa to 60kDa, with isoelectric points ranging from 6.01 to 7.14, thus implying that the proteins could

be solvable in physiological conditions. The instability index values for these proteins are within the normal range, thus implying that they could be stable in physiological conditions. In addition, the volume of aliphatic side chains is measured through the aliphatic index, which is relatively high for all proteins, thus implying that there is a potential for thermal stability. GRAVY score of the proteins is negative, which implies that these proteins are hydrophilic in nature, which is useful in aqueous biological environment.

4.3 Determination of Active Site

The program used to estimate the number of pockets that can be ligated along with other information such as surface area and volume of these pockets is known as dogsitescorer. The colored sections in the following figure 4.3 represent the active sites of the protein.

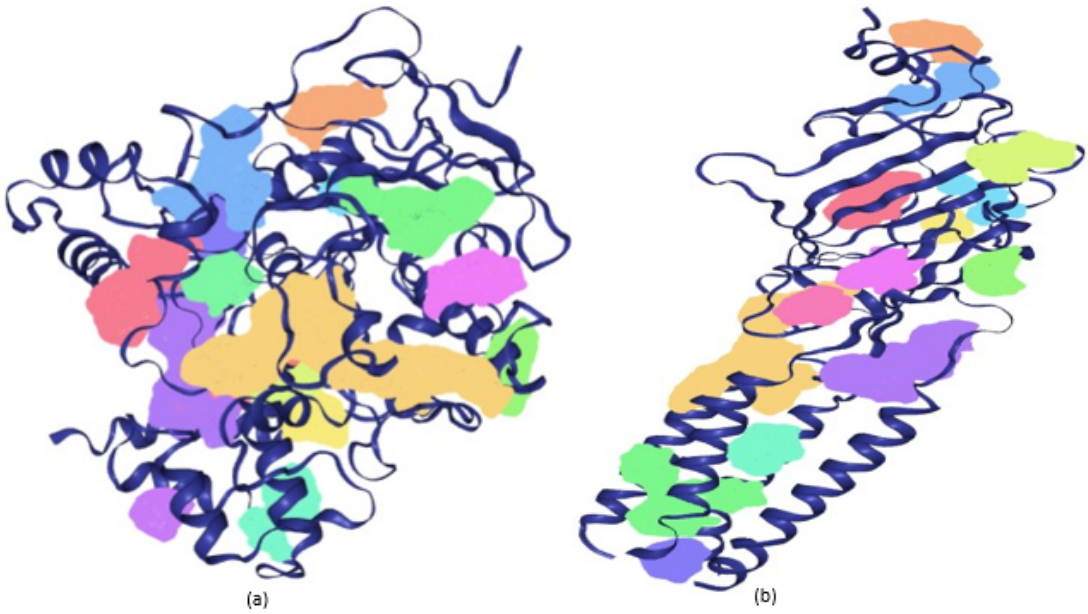


FIGURE 4.3: Selected proteins along with active sites (a) AChE (b) GABA-R

The data from dogsitescorer gives varied number of pockets per protein. The AChE protein has 17 pockets and GABA-R has 14 pockets respectively.

4.4 Ligands Structure Extraction

The choice of ligand depends upon the high-resolution structure considering the crystal-chemical class and the binding affinity. In this, what is important is the conformer selection of the ligand. Ligand has preference towards one of the conformers in the selection process and increases its stability with respect to other conformers and also the protein. The PubChem chemical database, which is the biggest chemical database in the world, was searched for the ligands. The 3D structures of the ligands in SDF format were obtained from the PubChem database. After downloading the structure, the next thing that was done was the energy minimization of these ligands. This is a very important step because the downloaded structure cannot be used because the ligands are very unstable and it can affect the docking vina scores.

TABLE 4.2: Compounds's structure

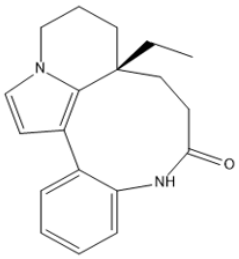
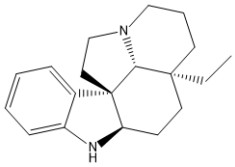
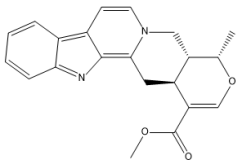
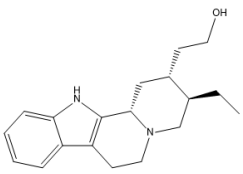
S.No	Name	Formula	PubChem
1	Rhazinilam	$C_{19}H_{22}N_2O$	
2	Aspidospermidine	$C_{19}H_{26}N_2$	
3	Serpentine	$C_{21}H_{20}N_2O_3$	
4	Dihydrocorynantheol	$C_{19}H_{26}N_2O$	

Table 4.2 continued from previous page

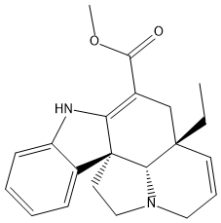
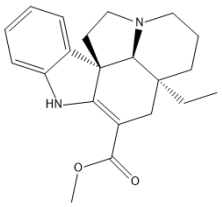
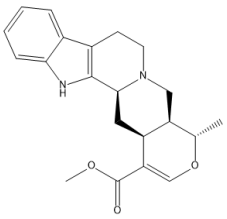
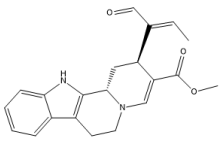
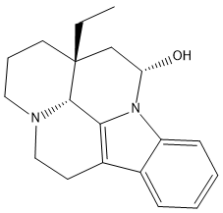
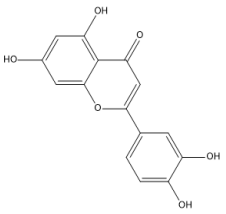
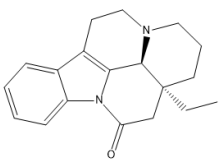
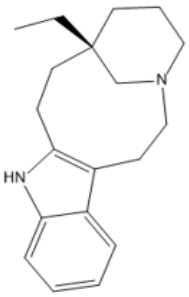
S.No	Name	Formula	PubChem
5	Tabersonine	$C_{21}H_{24}N_2O_2$	
6	Vincadifformine	$C_{21}H_{26}N_2O_2$	
7	Tetrahydroalstonine	$C_{21}H_{24}N_2O_3$	
8	Vallesiachotamine	$C_{21}H_{22}N_2O_3$	
9	Eburnamine	$C_{19}H_{24}N_2O$	
10	Luteolin	$C_{15}H_{10}O_6$	
11	Eburnamonine	$C_{19}H_{22}N_2O$	

Table 4.2 continued from previous page

S.No	Name	Formula	PubChem
12	Quebrachamine	$C_{19}H_{26}N_2$	

4.5 Application of Tice Rule

Before any compound can be regarded as having insecticidal action, virtual screening is done. Drugs that are orally active have to meet these criteria. How a compound is administered determines how drug-like it is. If a compound meets at least three of the criteria, it is a drug, while more than two disqualifying criteria mean poor absorption [71]. Ligand virtual screening is shown in table 4.3.

TABLE 4.3: Ligands of tice rule parameters

S.No	Name	weight	Value of Logarithm P	Bonds which are rotated	Acceptors for H bond	Donors for H bond
1	Rhazinilam	294	4.329	1	2	1
2	Aspidospermidine	282	3.77	1	2	1
3	Serpentine	348	3.4	1	5	0
4	Dihydrocorynantheol	298	3.49	3	2	2
5	Tabersonine	336	3.22	2	4	1
6	Vincadifformine	338	3.44	2	4	1
7	Tetrahydroalstonine	352	3.17	1	4	1
8	Vallesiachotamine	350	3.28	3	4	1
9	Eburnamine	296	3.62	1	3	1
10	Luteolin	286	2.28	1	6	4
11	Eburnamonine	294	3.77	1	3	0
12	Quebrachamine	282	4.14	1	1	1

All ligands adhere to the tice rule. LogP values of all the ligands are below 5. Likewise, molecular weights of all the ligands are also below 500. Hydrogen bond

donor, acceptor, and rotatable bonds of all the ligands fall within the required limits.

4.6 The ADME Evaluation and Toxicity Prediction

In addition, another study was done with the help of an online tool known as pkCSM for the determination of the ADMET properties of ligands as a pharmacokinetic parameter based on the tice rule. The two broad terminologies of pharmacology are pharmacodynamics and pharmacokinetics. Pharmacodynamics is a branch of pharmacology that studies how drugs impact the body. Pharmacokinetics is the study of drug absorption, distribution, metabolization, and excretion [72].

4.6.1 Parameters of Absorption

Important standards for assessing a compound's bioavailability are revealed by the absorption metrics. Better water solubility is indicated by readings larger than -2, which may improve absorption. A good Caco2 permeability value is larger than 0, while values greater than 1 indicate outstanding absorption potential and high permeability. Good absorption is indicated by results above 50%, which are considered beneficial for intestinal absorption. Better skin permeability is indicated by values larger than -2, which is beneficial for topical treatments [73]. Table 4.4 lists the absorption characteristics of ligands.

Table 4.4: Parameters of Absorption

Properties	**Water solubility**	**Caco2 Permeability**	**Intestinal absorption**	**Skin Permeability**
Rhazinilam	−4.395	1.681	93.435	−2.465
Aspidospermidine	−4.056	1.505	92.974	−2.702
Serpentine	−4.538	1.19	97.616	−3.198
Dihydrocorynantheol	−3.483	1.48	93.637	−2.875

Table 4.4 continued from previous page

Properties	**Water solubility**	**Caco2 Permeability**	**Intestinal absorption**	**Skin Permeability**
Tabersonine	−3.516	1.119	93.221	−3.144
Vincadifformine	−4.352	1.099	94.171	−3.142
Tetrahydroalstonine	−3.824	1.126	94.29	−3.303
Vallesiachotamine	−4.103	1.109	94.614	−2.985
Eburnamine	−4.099	1.535	94.219	−2.826
Luteolin	−3.094	0.096	81.13	−2.735
Eburnamonine	−3.896	1.574	95.075	−2.644
Quebrachamine	−4.231	1.333	92.23	−2.621

For insecticides, very high solubility is not always required. Many commercial insecticides have moderate water solubility and good lipophilicity, so values around -3 to -5 are usually acceptable. Almost all ligands have moderate water solubility. For a Caco2 permeability, a value above 0.9 indicates high permeability; except luteolin, all ligands have high permeability. Intestinal absorption is more than 80% for all ligands, which is good. Skin penetration is also moderate for all insecticides.

4.6.2 Parameters of Distribution

The full dosage of the drug that needs to be distributed uniformly to create a concentration comparable to blood plasma is indicated by the theoretical volume. VDss ¿ 0.5 L/kg is a good value and it means that there is an appropriate distribution of the drug in the body tissues. Ideally, fraction unbound (Fu) should be ¿ 0.1 because it means that there is enough of the drug available for its pharmacological action. The blood-brain barrier is protecting the brain from external agents but at the same time limiting their direct passage into it. Log BB values higher than 0 mean that the substance has some capability to penetrate through the blood-brain barrier. Ligand distribution characteristics are presented in Table 4.5 and we can see that there is a safety window for all ligands, which is given below. For CNS permeability, lower values are ideal and they should be ideally higher than -2 [74].

TABLE 4.5: Parameters of Distribution

	ADMET Properties	**VDss**	**Fraction unbound (Fu)**	**BBB permeability log BB**	**CNS permeability log PS**
Distribution	Rhazinilam	0.807	0.135	0.442	−1.887
	Aspidospermidine	1.478	0.237	0.535	−1.776
	Serpentine	0.427	0.09	0.257	−1.883
	Dihydrocorynantheol	1.385	0.223	0.109	−1.628
	Tabersonine	1.314	0.387	0.299	−2.448
	Vincadifformine	1.409	0.281	0.253	−2.26
	Tetrahydroalstonine	1.261	0.228	0.51	−1.989
	Vallesiachotamine	0.645	0.162	−0.056	−2.128
	Eburnamine	1.557	0.265	0.41	−2.318
	Luteolin	1.153	0.168	−0.907	−2.251
	Eburnamonine	1.466	−0.225	0.163	−2.145
	Quebrachamine	1.612	0.227	0.324	−1.665

A value in range of -0.15 to 0.45 shows moderate VDss while ¿ 0.45 shows high distribution. All ligands showed moderate to high VDss value. Fu value ¿0.2 means good availability, all compounds except serpentine values are in range. Values ¿ 0.3 show high BBB penetration, all compounds except vallesiachotamine and luteolin have good value. A value ¿ -2 shows good CNS permeability value, all ligands showed good CNS permeability value.

4.6.3 Parameters of Metabolism

The detoxification process in the liver is controlled by the enzyme cytochrome P450. This enzyme deactivates a lot of medications, but it can also activate others. Inhibitors of this enzyme should not be utilized as they may directly impact the drug's metabolism. Similarly, the metabolism of the medicines is carried out by CYP2D6 and CYP3A4. Pharmacokinetics of the drug used is affected by the inhibition of these [75]. The prediction of ligand metabolism is shown below. Table 4.6 gives the metabolic characteristics of ligands.

TABLE 4.6: Parameters of Metabolism

	Name	CYP2D6 substrate	CYP3A4 substrate	CYP2D6 inhibitor	CYP3A4 inhibitor
Metabolism	Rhazinilam	No	Yes	No	No
	Aspidospermidine	Yes	Yes	Yes	No
	Serpentine	No	Yes	No	No
	Dihydrocorynantheol	Yes	Yes	Yes	No
	Tabersonine	No	Yes	Yes	No
	Vincadifformine	No	Yes	Yes	No
	Tetrahydroalstonine	No	Yes	Yes	No
	Vallesiachotamine	No	Yes	No	No
	Eburnamine	Yes	Yes	Yes	No
	Luteolin	No	No	No	No
	Eburnamonine	Yes	Yes	Yes	No
	Quebrachamine	Yes	Yes	Yes	No

In the case of CYP2D6, “no” is preferred because it represents more metabolic stability. Ligands showed a mixed response. CYP3A4 is the major metabolic enzyme. If ”yes, ” the compound can be metabolized through normal pathways. Except luteolin, all ligands are CYP3A4 enzymes. If a compound inhibits CYP2D6, it may slow metabolism and may increase persistence so “no” is preferred. Ligands showed a mixed response for CYP2D6 enzyme. CYP3A4 is one of the most important metabolic toxicity parameters. If a compound inhibits CYP3A4, it can cause metabolic interactions which may raise safety concerns therefore, “no” is preferred. All compounds showed “no” for CYP3A4 inhibitor.

4.6.4 Parameters of Excretion

Drugs are eliminated by two organs: the kidneys, which are associated with renal elimination, and the liver, which is associated with biliary elimination. Other organs, such as the lungs for volatile or gaseous compounds, might also be part of the process of elimination. Drugs can also be eliminated by sweat, saliva, and tears. The excretion values for the ligands are presented in Table 4.7.

TABLE 4.7: Parameters of Excretion

	ADMET Properties	**Total Clearance**	**Renal OCT2 substrate**
Excretion	Rhazinilam	0.787	Yes
	Aspidospermidine	1.031	Yes
	Serpentine	0.931	No
	Dihydrocorynantheol	0.945	Yes
	Tabersonine	0.902	Yes
	Vincadifformine	0.971	Yes
	Tetrahydroalstonine	0.962	Yes
	Vallesiachotamine	0.815	No
	Eburnamine	1.034	Yes
	Luteolin	0.495	No
	Eburnamonine	1.082	Yes
	Quebrachamine	1.135	Yes

Total clearance tells how fast a compound is removed from the system. Values between 0.5-0.9 show moderate clearance mechanisms. All ligands except luteolin showed best clearance values. If a compound is a substrate of OCT2, it is actively transported into kidney cells and eliminate faster via urine. Ligands showed mixed response to renal OCT2 substrate.

4.6.5 Parameters of Toxicity

We ascertained the ligands' toxicity using pkCSM. The AMES toxicity test uses microorganisms to assess a compound's potential for mutagenesis. The ligand is mutagenic, which can also cause cancer, if it exhibits a positive reaction. The concentration at which the substance may kill 50% of the minnows is represented by the values anticipated in the minnow toxicity test.

A hepatotoxicity test determines whether or not a substance may impair liver function [76]. Table 4.8 lists all of the ligands' toxicity levels.

TABLE 4.8: Parameters of Toxicity

	Name	AMES toxicity	hERG inhibitor	Oral rat acute toxicity (LD50)	Hepato toxicity	Skin sensitization	Minnow toxicity
Toxicity	Rhazinilam	Yes	No	2.336	No	No	0.488
	Aspidospermidine	No	No	2.872	No	No	0.533
	Serpentine	No	No	3.675	No	No	0.332
	Dihydrocorynantheol	No	No	3.092	No	No	1.966
	Tabersonine	No	No	2.933	No	No	0.792
	Vincadifformine	No	No	2.722	No	No	0.566
	Tetrahydroalstonine	No	No	2.974	No	No	−0.641
	Vallesiachotamine	Yes	No	2.271	Yes	No	−1.73
	Eburnamine	No	No	2.905	No	No	0.799
	Luteolin	Yes	No	2.455	Yes	No	3.169
	Eburnamonine	No	No	2.828	No	No	0.545
	Quebrachamine	Yes	No	2.83	No	No	0.734

AMES toxicity should be "no", otherwise it will be mutagenic. Rhazinilam, vallesiachotamine and luteolin showed AMES toxicity. hERG inhibition is related to cardiotoxicity so "no" is safe. All ligands showed "no" for hERG inhibition. LD50 values > 3.0 are considered less toxic. Rhazinilam, vallesiachotamine and luteolin are highly toxic, while others are moderate to less toxic. Vallesiachotamine and luteolin were hepatotoxic, while none of the ligands were skin-sensitizing. Lower minnow toxicity values are preferred because they are environmentally safer. All ligands except luteolin showed low minnow toxicity values.

Along with ADMET properties, ecological concerns of ligands can be checked using several bioinformatics tools including ADMETlab and EcoSAR. These tools tell about safety aspect of potential compound against environment. luteolin has low Caco2 permeability and serpentine has a low fraction unbound value. Vallesiachotamine and luteolin have low BBB permeability. Luteolin also showed no response for the CYP3A4 enzyme. The luteolin total clearance value is also low. Rhazinilam, vallesiachotamine, and luteolin are highly toxic, as their LD50 values are outside the range and they also showed AMES toxicity. Vallesiachotamine and luteolin are also hepatotoxic. The minnow toxicity value of luteolin is also high. Based on ADMET profile of ligands, rhazinilam, vallesiachotamine, and luteolin were not considered further for docking due to safety concerns.

4.7 Docking of Proteins and Ligands

The protein and ligand structures are employed for carrying out docking. In this respect, a web-based auto docking program called CB dock was applied. CB Dock predicts the binding site of proteins and determines the size of the cavity. Once docking was done, CB Dock will give us the top five possess and receptor models. The best one was chosen out of these five considering the cavity size and the vina score [77]. Target proteins and ligands are used for the purpose of molecular docking. Proteins are in the form of PDB while ligands are in the form of SDF. After the validation of input file, CB dock uses Open Babel and MGL tools to convert the files into pdbqt files. The diameter and center of the top five cavities and the cavities of the receptor were calculated through CB dock. The best one among the five best conformations is based on the high affinity score of the protein-ligand interaction [78]. Table 4.9 illustrates the scores achieved after the docking of proteins and ligands.

Table 4.9: Vina scores

Ligands	**Proteins**	
	AChE	**GABA-R**
Aspidospermidine	−8.6	−5.4
Serpentine	−8.7	−6.7
Dihydrocorynantheol	−8.6	−6.4
Tabersonine	−8.7	−6.4
Vincadifformine	−8.5	−5.9
Tetrahydroalstonine	−9.4	−6.3
Eburnamine	−8.6	−6.6
Eburnamonine	−8.9	−6.6
Quebrachamine	−7.0	−5.4

The docking result of receptors with ligands is displayed in Table 4.9. It demonstrates that eburnamonine has a binding score of -8.9 with AChE, whereas tetrahydroalstonine has the greatest binding score of -9.4 with AChE.

4.8 Use of Discovery Studio Software to Check Interactions

Ligand-protein interaction was calculated in order to comprehend docking data. Three major kinds of contact forces were studied and they included hydrogen bonding, alkyl contact force and van der Waals interaction forces. 2025 Discovery Studio Protein-Ligand Interactions were analyzed using a client. Each molecule's saved conformational structure of the ligand and receptor complex was carefully analyzed. This application automatically represents protein-ligand interactions between specified ligands within the PDB database schematically [79]. A large number of interactions have been discovered in the docked data for the nine ligands and two target proteins in PDB form. The docked complexes and ligand-receptor interactions are displayed in the accompanying diagrams. Docking complex is depicted in the (a) section of the figure, and details on the protein-ligand interaction are shown in the (b) section. Alkyl bonds are represented by the color purple, covalent bonds by the color blue, conventional hydrogen bonds by the color dark green, carbon hydrogen bonds by the color light green, and van der Waals interactions by the color green.

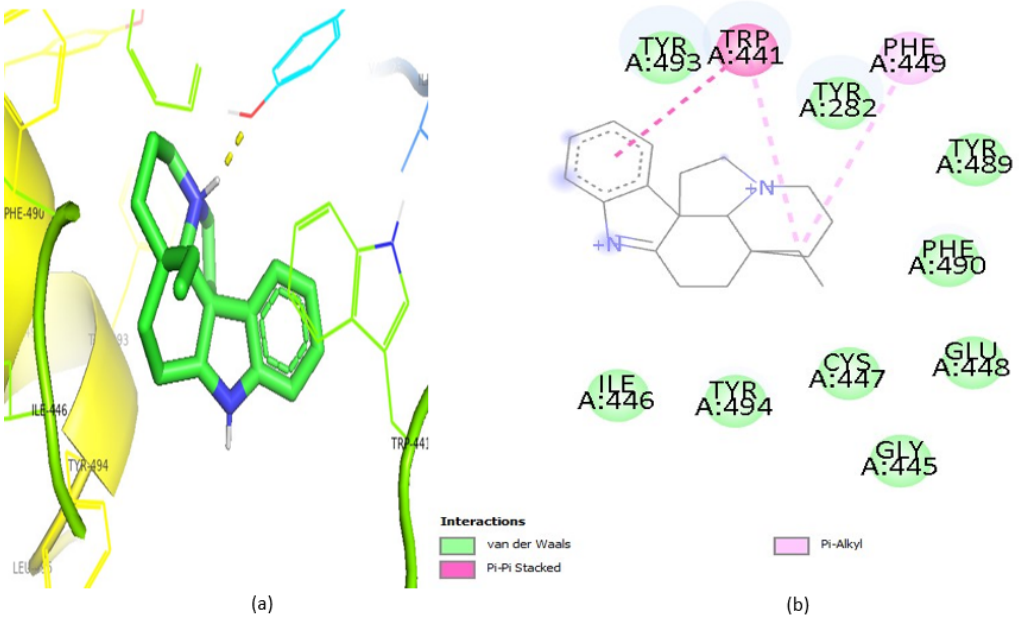


FIGURE 4.4: Aspidospermidine and AChE

Aspidospermidine's interaction with AChE is seen in Figure 4.4. It shows nine types of van der Waals contacts and an alkyl contact.

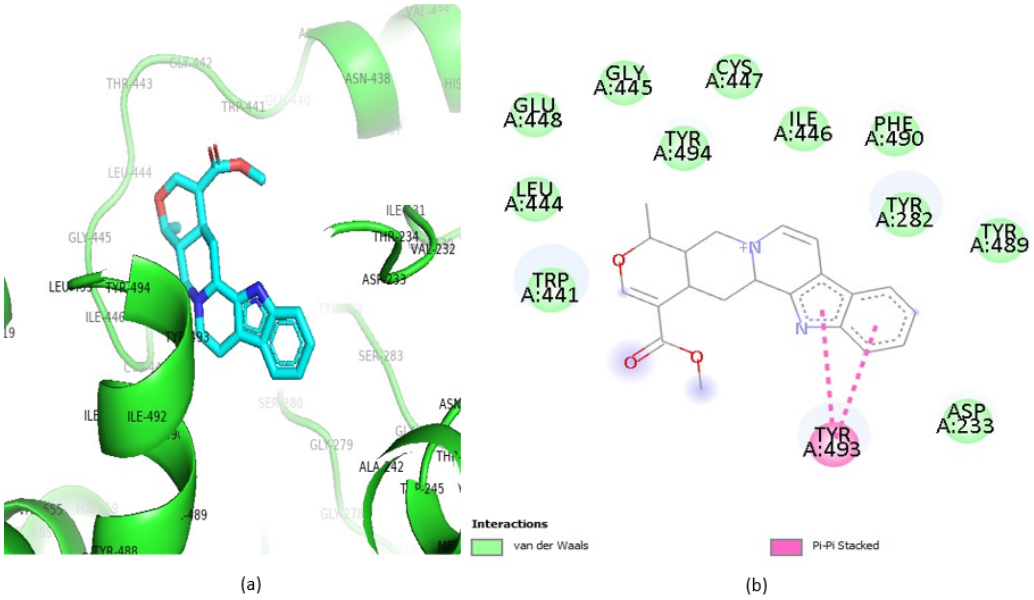


FIGURE 4.5: Serpentine and AChE

The interaction between serpentine and AChE is seen in Figure 4.5. It reveals eleven van der Waals forces and two pi-pi stacking bonds.

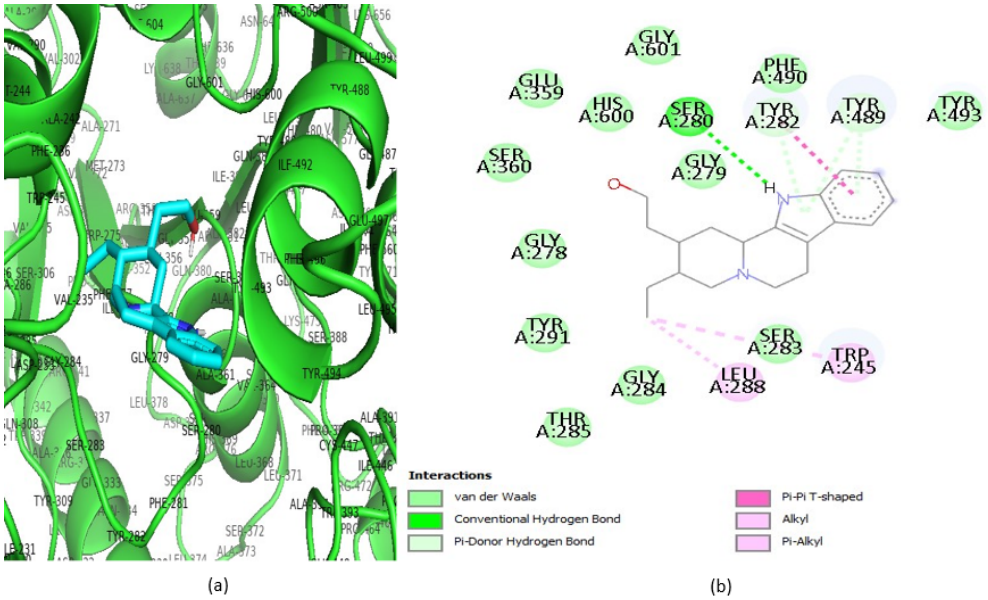


FIGURE 4.6: Dihydrocorynantheol and AChE

Dihydrocorynantheol's interaction with AChE is depicted in Figure 4.6. It displays twelve van der Waals type contacts, one conventional hydrogen bond, two alkyl links, and two pi-donor hydrogen bonds.

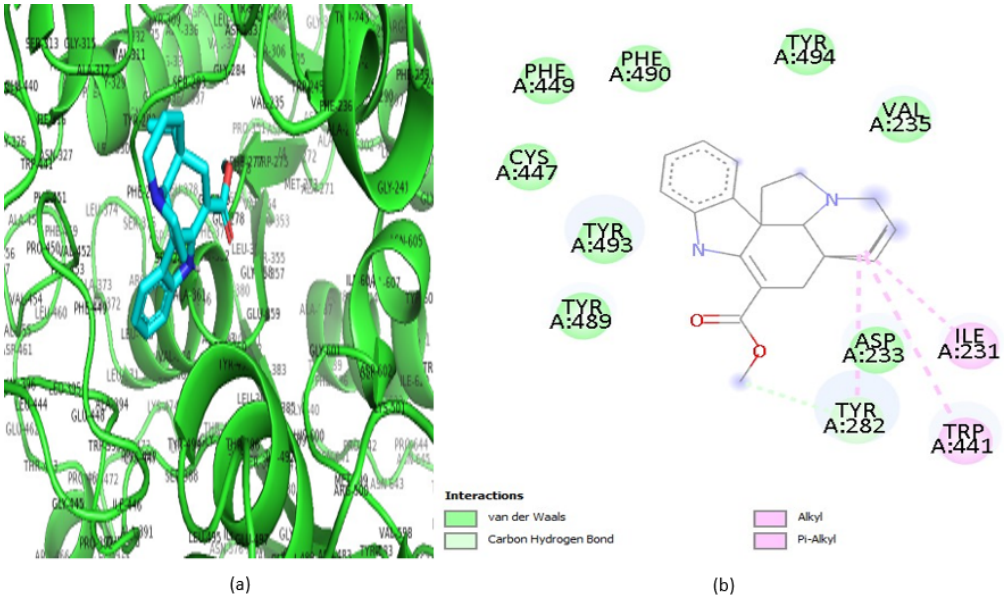


FIGURE 4.7: Tabersonine and AChE

The structure of tabersonine interacting with AChE enzyme is shown in Figure 4.7. It shows eight van der Waals interactions, one carbon-hydrogen bond, and two alkyl interactions.

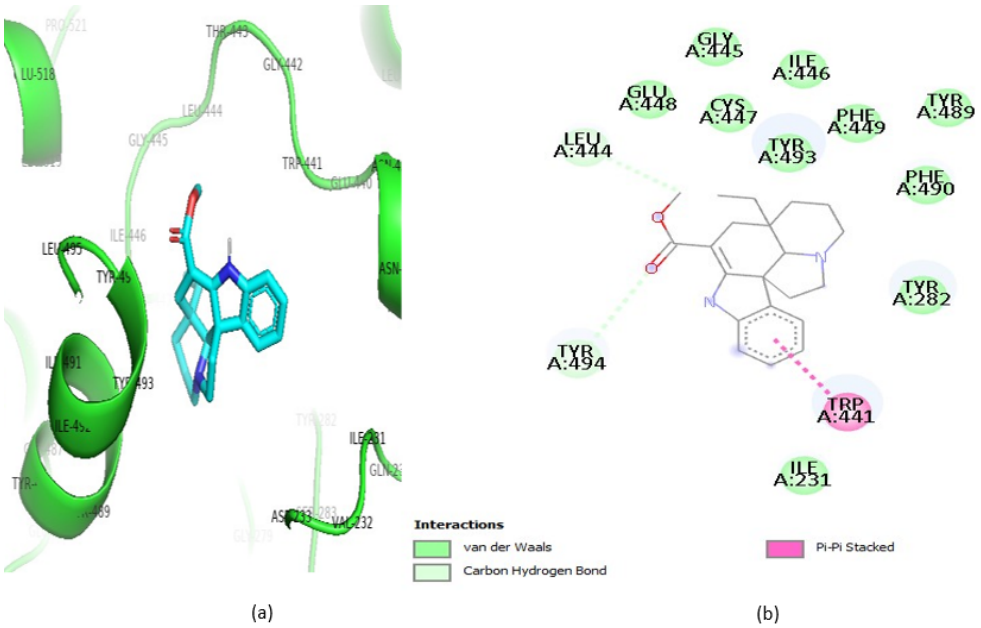


FIGURE 4.8: Vincadifformine and AChE

Vincadifformine's interaction with AChE is depicted in Figure 4.8. The structure shows 10 contacts of van der Waals type and 2 carbon-hydrogen bonds.

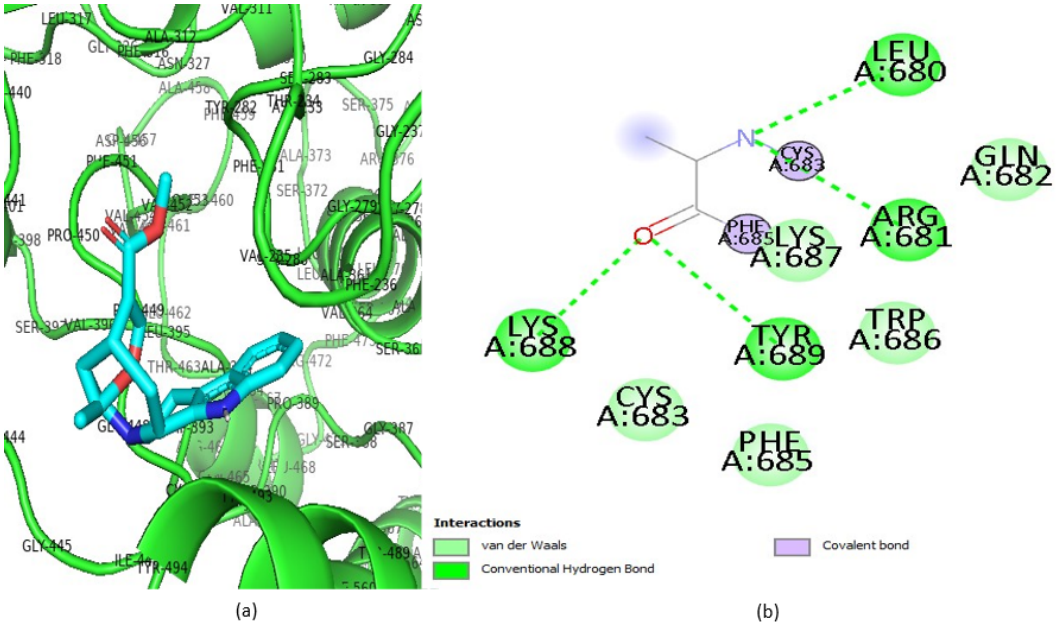


FIGURE 4.9: Tetrahydroalstonine and AChE

Tetrahydroalstonine's interaction with AChE is depicted in Figure 4.9. Five van der Waal forces, four normal hydrogen bonds, and two covalent bonds are shown.

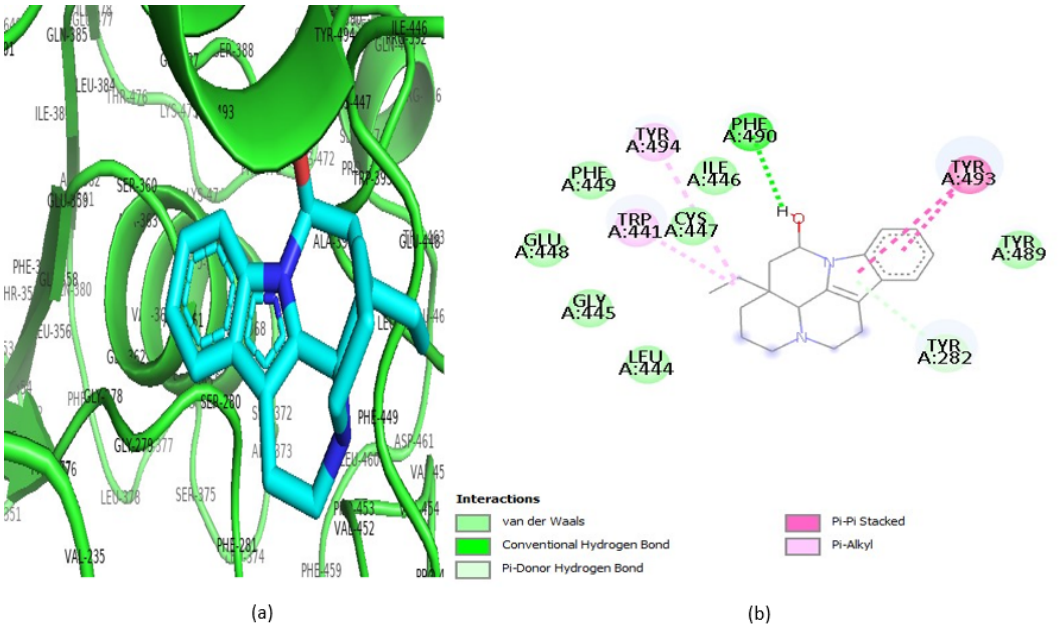


FIGURE 4.10: Eburnamine and AChE

Eburnamine's interaction with AChE is seen in Figure 4.10. It reveals seven van der Waals contacts, one pi-donor hydrogen bond, one conventional hydrogen bond, and two alkyl bonds.

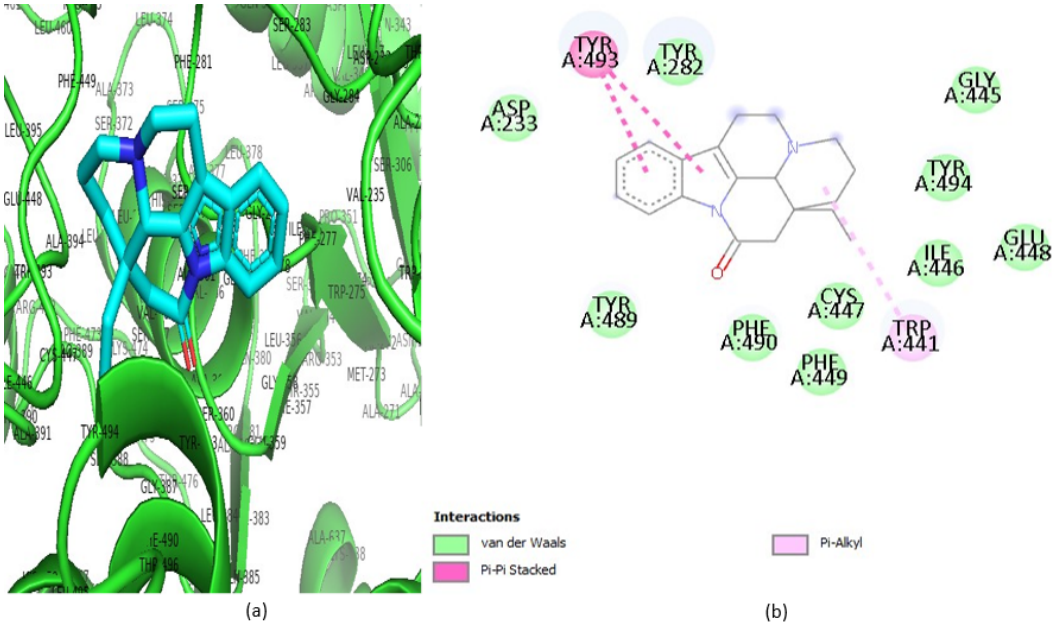


FIGURE 4.11: Eburnamonine and AChE

Figure 4.11 shows the interaction of eburnamonine with AChE. The interactions include ten van der Waals contact points and an alkyl bond.

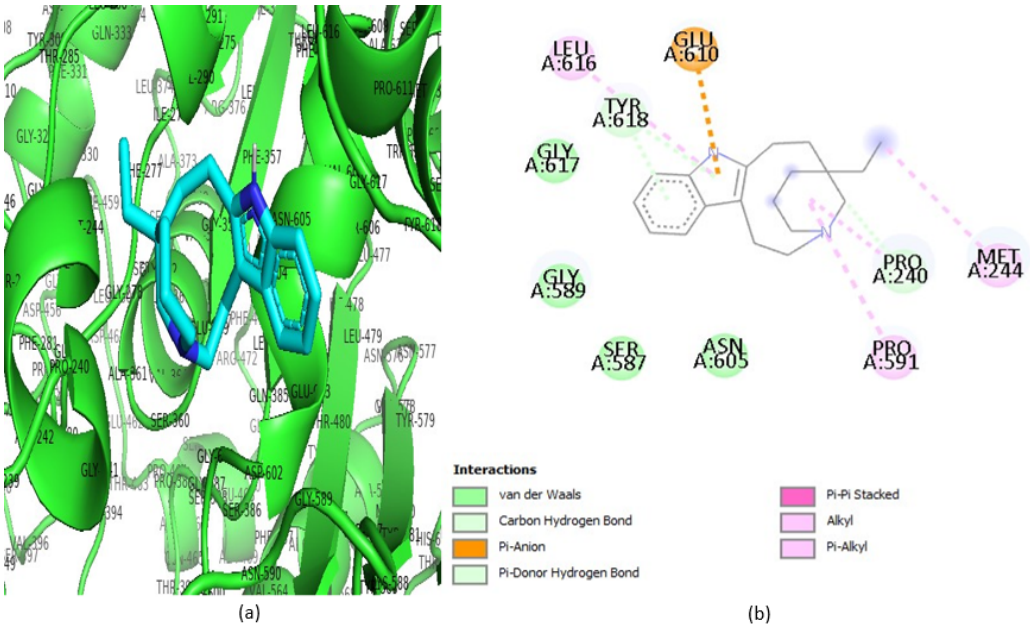


FIGURE 4.12: Quebrachamine and AChE

The binding of quebrachamine to AChE is shown in Fig. 4.12. It displays four van der Waals contacts, one carbon hydrogen bond, one pi-donor hydrogen link, and three alkyl bonds.

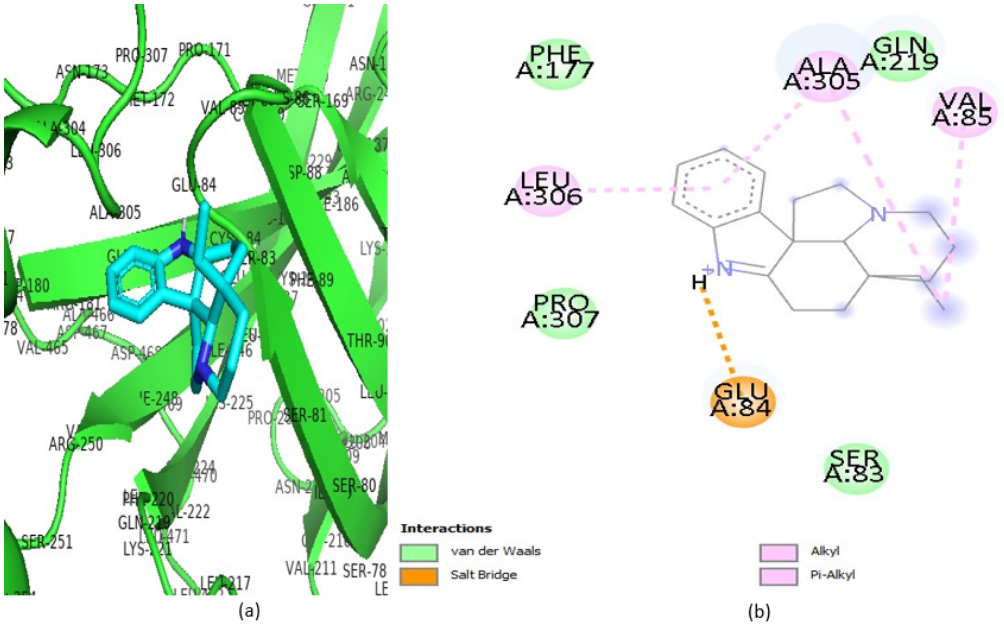


FIGURE 4.13: Aspidospermidine and GABA-R

Aspidospermidine's interaction with GABA-R is seen in Figure 4.13. There are four van der Waals interactions and three alkyl bonds observed in this structure

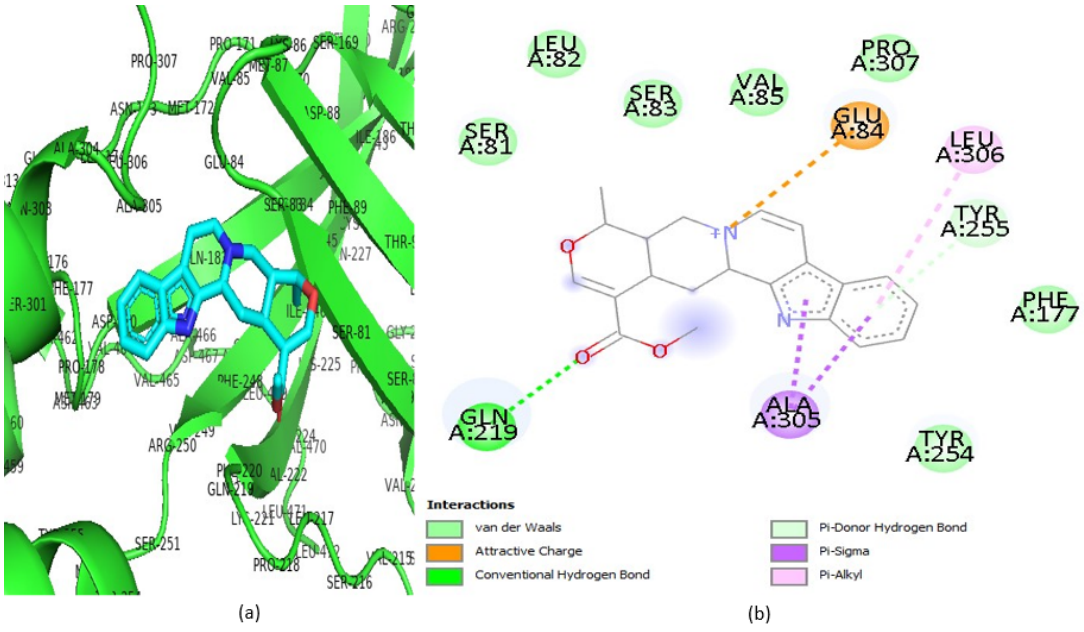


FIGURE 4.14: Serpentine and GABA-R

The interaction between serpentine and GABA-R is shown in Figure 4.14. It displays seven van der Waals type contacts, an alkyl bond, a conventional hydrogen bond, and a pi-donor hydrogen.

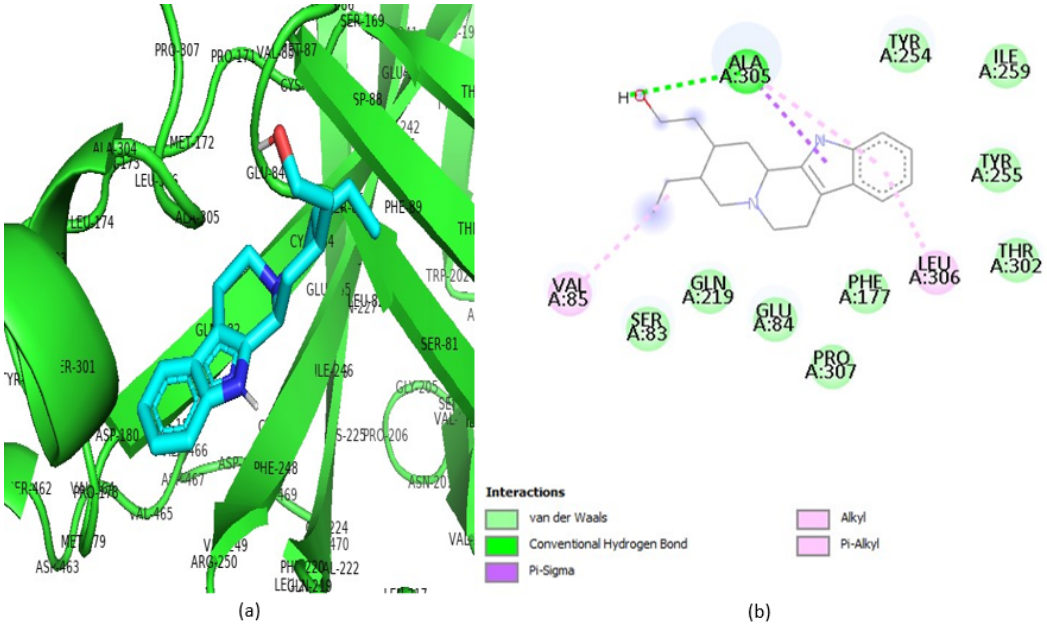


FIGURE 4.15: Dihydrocorynantheol and GABA-R

Dihydrocorynantheol's interaction with GABA-R is depicted in Figure 4.15. This protein has nine Van der Waals contacts, one hydrogen bond, and two alkyl bonds

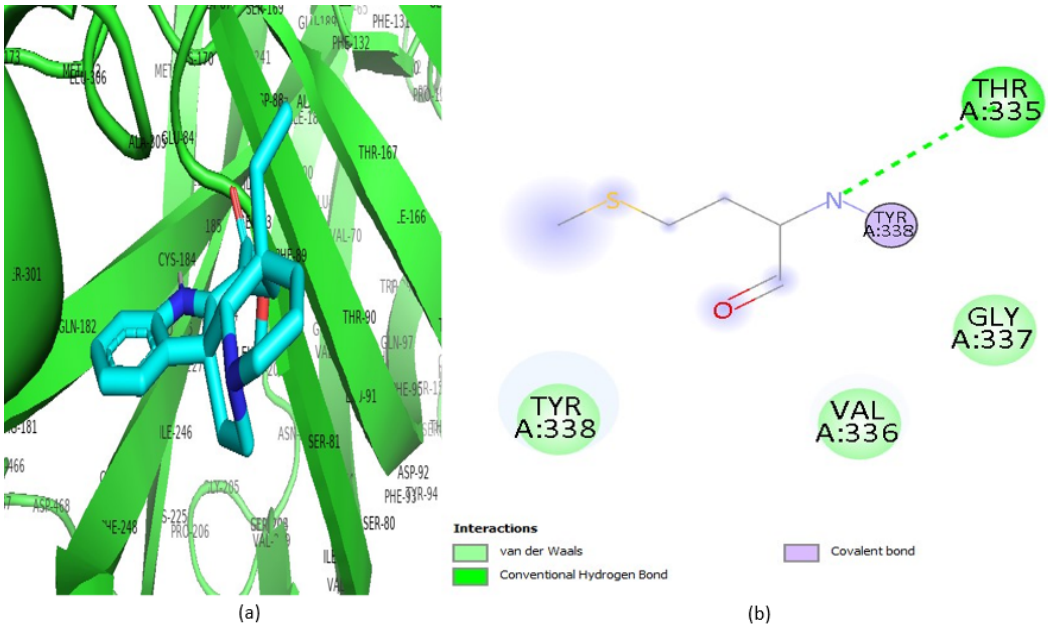


FIGURE 4.16: Tabersonine and GABA-R

Tabersonine's interaction with GABA-R is depicted in Figure 4.16. It demonstrates the presence of three Van der Waals forces, one alkyl link, and one hydrogen bond

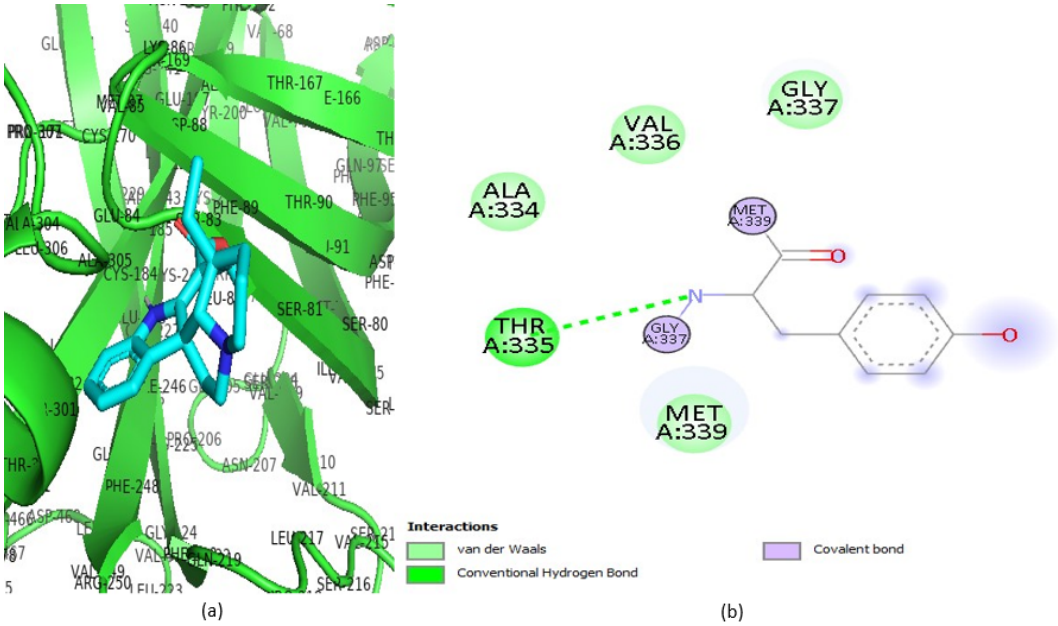


FIGURE 4.17: Vincadifformine and GABA-R

The interaction between vincadifformine and GABA-R is seen in Figure 4.17. It displays four van der Waals interactions that are traditional, one typical hydrogen bond, and two covalent bonds.

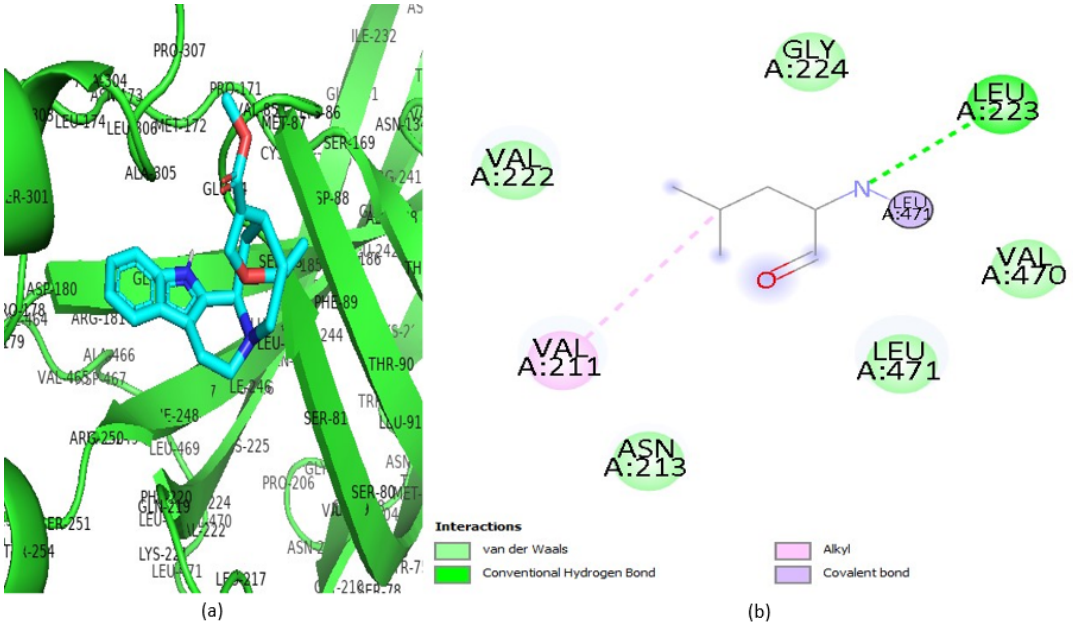


FIGURE 4.18: Tetrahydroalstonine and GABA-R

GABA-R and Tetrahydroalstonine interaction is illustrated in Figure 4.18. It illustrates five van der waals force interactions, one alkyl bond, one covalent link,

and one traditional hydrogen bond.

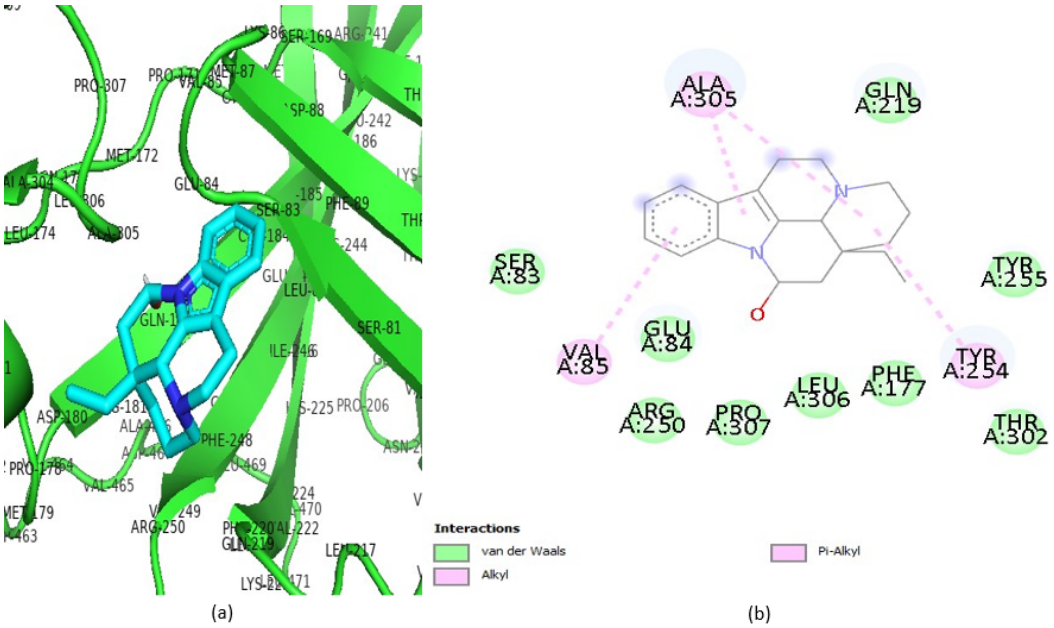


FIGURE 4.19: Eburnamine with GABA-R

The interaction between eburnamine and GABA-R is seen in Figure 4.19. This includes nine common van der Waals interactions and three alkyl bonds.

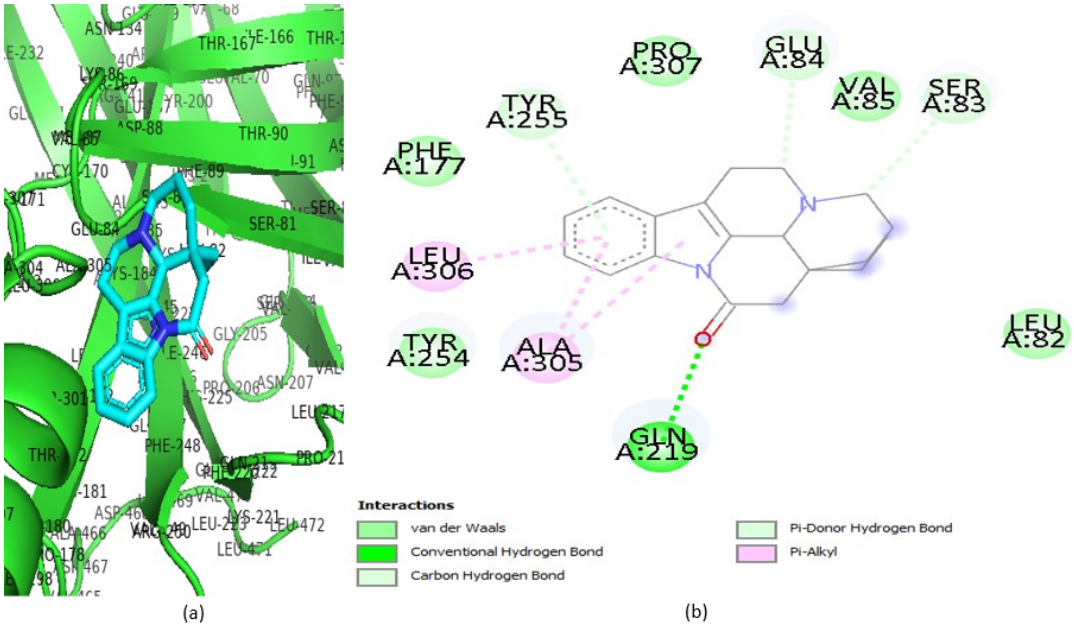


FIGURE 4.20: Eburnamonine and GABA-R

The interaction between eburnamonine and GABA-R is shown in Figure 4.20. It displays five van der Waals type contacts, one conventional hydrogen bond, one carbon hydrogen bond, and two alkyl bonds.

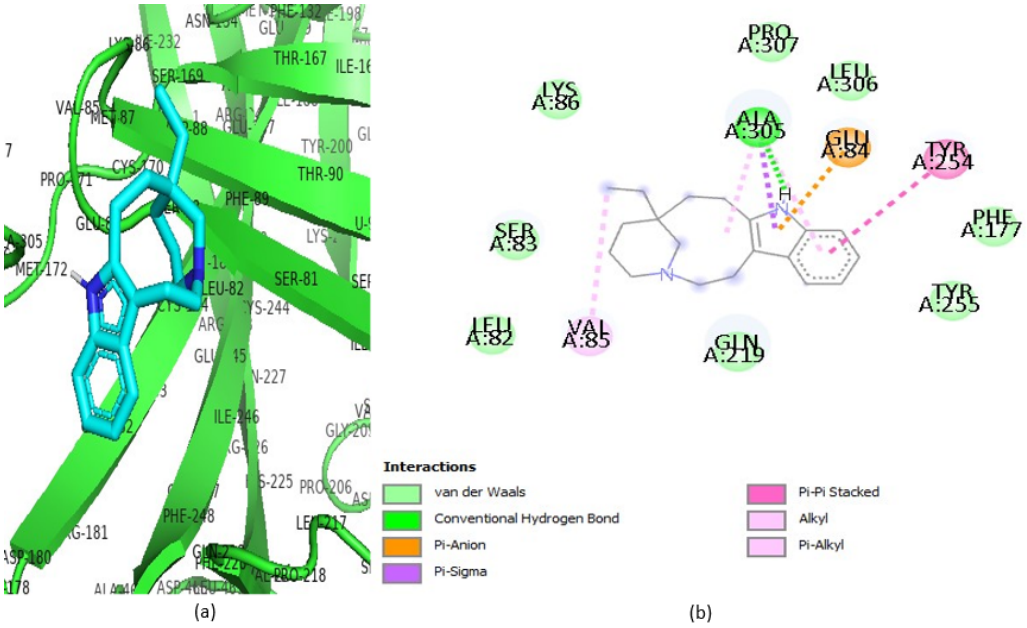


FIGURE 4.21: Quebrachamine and GABA-R

The interaction between quebrachamine and GABA-R is seen in Figure 4.21. There are eight Van der Waal types of contacts, one alkyl linkage, and one regular hydrogen bond.

4.9 Lead Compound Identification

The ligands' fate as insecticidal substances is determined by their physicochemical and pharmacokinetic characteristics. For this identification, the first filter is the Tice rule, and the second is pharmacokinetics. Since every ligand was shown to follow the tice rule, they were all chosen for docking. Pharmacokinetic screening is the next knockout stage, during which three ligands were removed due to safety concerns.

After that, docking was done, and the outcomes were examined. Tetrahydroalstonine was determined to be the lead chemical following a thorough examination

of the docking score and docking complex interactions. It displayed two covalent connections, four hydrogen bonds and five Van der Waals forces and the best vina score -9.4 with the AChE receptor. Tetrahydroalstonine was chosen as the lead molecule because it had the greatest ADMET values in terms of high water solubility, excellent intestine absorption, and minimum toxicity.

4.10 Reference Insecticidal Drug Identification

Malathion was selected as the reference insecticidal compound in the present study due to its well-established efficacy as a broad-spectrum neurotoxic insecticide. Malathion exerts its insecticidal activity primarily through inhibition of acetylcholinesterase, resulting in the accumulation of acetylcholine at synaptic junctions and subsequent disruption of normal nerve transmission in insects [80].

Owing to its extensive use in agricultural pest management and its recognized mode of action on the insect nervous system, malathion serves as an appropriate positive control for comparing the binding affinities and interaction patterns of the selected Rhazya stricta phytochemicals against insecticidal target proteins.

The docking performance of the lead compound was therefore evaluated relative to malathion to assess their potential as alternative insecticidal agents.

4.11 Malathion and Lead Compound Comparison

To identify the most effective insecticide and the best bioactive metabolite, tetrahydroalstonine, and commercial medicine, malathion, were compared. Parameters used in the comparison included ADMET properties, the TICE rule, and docking complex analysis

4.12 Malathion Structure Prediction

In the first place, the malathion structure was downloaded in SDF formate from PubChem. The energy of the malathion structure was then minimized using Chem3D Pro to acquire the refined structure of malathion. The chemical formula of malathion is C10H19O6PS2, and its refined structure can be viewed from figure 4.22

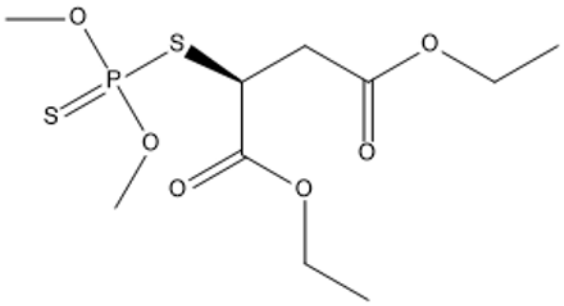


FIGURE 4.22: Malathion Structure from PubChem

4.13 The Comparative Analysis of Tice Rule

The pharmacokinetic characteristics, bioavailability, safety, effectiveness, and drug-likeness of malathion and tetrahydroalstonine were studied in order to see how they responded to the tice rule. Table 4.10 presents the comparison.

TABLE 4.10: The Comparative Analysis of Tice Rule

Name	**Malathion**	**Tetrahydroalstonine**
Value of logarithm P	2.12	3.17
Weight	330	352
Donors for the hydrogen bond	0	1
Acceptors for the hydrogen bond	8	4
Bonds which are rotated	9	1

Both Tetrahydroalstonine and malathion follow the tice rule, as shown in Table 4.10. However, malathion does not have a hydrogen bond donor.

4.14 Comparative Evaluation of ADMET Characteristics

The ADMET properties were used in finding out the medication's and the lead compound's all drug related parameters.

4.14.1 Parameters of Absorption

Comparison between Tetrahydroalstonine and Malathion to Verify Absorbance Models is shown in Table 4.11.

TABLE 4.11: Comparison of Absorption Parameters

Absorption parameters	**Tetrahydroalstonine**	**Malathion**
Water solubility	−3.824	−2.058
Caco2 Permeability	1.126	1.33
Intestinal absorption	94.29	96.63
Skin permeability	−3.303	−1.42

As indicated by Table 4.11, tetrahydroalstonine and malathion exhibit similar absorption characteristics, apart from their skin permeability, which is too low for malathion.

4.14.2 Parameters of Distribution

Tetrahydroalstonine and malathion distribution parameters are given in table 4.12.

TABLE 4.12: Comparison of distribution parameters

	ADMET Properties	**Tetrahydroalstonine**	**Malathion**
Distribution	VDss	1.261	−0.053
	Fraction unbound (Fu)	0.228	0.569
	BBB permeability log BB	0.51	−1.321

Table 4.12 continued from previous page

ADMET Properties	Tetrahydroalstonine	Malathion
CNS permeability log PS	−1.989	−3.089

The distribution features of Malathion and Tetrahydroalstonine have been shown in table 4.12 above. With the exception of VDss, which is too low for malathion, both have similar distribution characteristics.

4.14.3 Parameters of Metabolism

Tetrahydroalstonine and malathion distribution parameters are given in table 4.13.

TABLE 4.13: Comparison of metabolic parameters

Metabolism	Tetrahydroalstonine	Malathion
CYP2D6 substrate	No	No
CYP3A4 substrate	Yes	No
CYP2D6 inhibitor	No	No
CYP3A4 inhibitor	No	No

Tetrahydroalstonine and malathion have similar metabolic patterns, as shown in Table 4.13, with the exception that malathion is not a substrate of the CYP3A4 enzyme.

4.14.4 Parameters of Excretion

Tetrahydroalstonine and malathion excretion parameters are given in table 4.14.

TABLE 4.14: Comparison of excretion parameters

Excretion	ADMET Properties	Tetrahydroalstonine	Malathion
Excretion	Total Clearance	0.962	0.632
	Renal OCT2 substrate	Yes	No

Tetrahydroalstonine is more effectively removed from the body than malathion, as seen by Table 4.14's greater overall clearance rate. Malathion does not appear to depend on the renal OCT2 transporter for renal excretion because it is not a substrate for this route.

4.14.5 Parameters of Toxicity

Tetrahydroalstonine and malathion toxicity parameters are given in table 4.15.

TABLE 4.15: Comparison of toxicity parameters

	ADMET Properties	**Tetrahydroalstonine**	**Malathion**
Toxicity	AMES toxicity	No	No
	hERG inhibitor	No	Yes
	Oral rat acute toxicity (LD50)	2.974	2.378
	Hepatotoxicity	No	No
	Skin sensitization	No	No
	Minnow toxicity	−0.641	1.054

Tetrahydroalstonine and malathion have similar toxicity profiles, according to Table 4.16, as neither medication exhibits AMES toxicity or Herg inhibition, while malathion did exhibit skin sensitization. Additionally, the standard drug's minimum toxicity value is higher than anticipated.

4.15 The Differences in Docking Values

Binding energy was calculated through docking of lead and standard chemical malathion to the target proteins AChE and GABA-R.

The docking energy scores for both the conventional medicine malathion and the lead chemical tetrahydroalstonine are provided in Table 4.16.

TABLE 4.16: Tetrahydroalstonine and malathion docking values

Names of Ligands	**Selected Proteins**	
	AChE	**GABA-R**
Tetrahydroalstonine	−9.4	−6.3
Malathion	−6.1	−4.4

Scores of vina for the major chemical tetrahydroalstonine are much higher than the generic malathion, as table 4.17 illustrates. Tetrahydroalstonine's docking scores against the target proteins AChE and GABA-R are -9.4 and -6.3, respectively, whereas malathion's scores are -6.1 and -4.4. These findings demonstrate that tetrahydroalstonine, a lead chemical, may attach to target proteins more effectively than malathion, a common medication.

4.16 Evaluation of Docking Interactions

The docking data were assessed by Discovery Studio based on the quantity of ligand-protein interactions. The docking analysis of the common medication malathion is displayed in the following graphics.

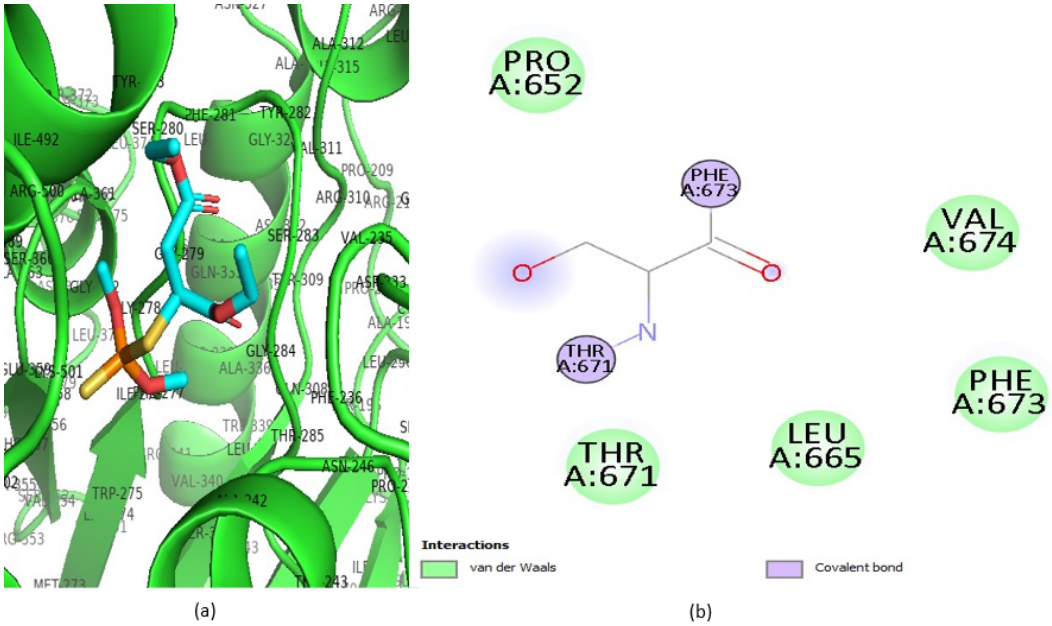


FIGURE 4.23: Malathion and AChE

Interaction of malathion with AChE is shown in Figure 4.23. This figure shows that there are five van der Waals interactions and two covalent bonds in the interaction of malathion with AChE. Interaction of tetrahydroalstonine with AChE occurs through two covalent bonds, four hydrogen bonds, and five van der Waals interactions.

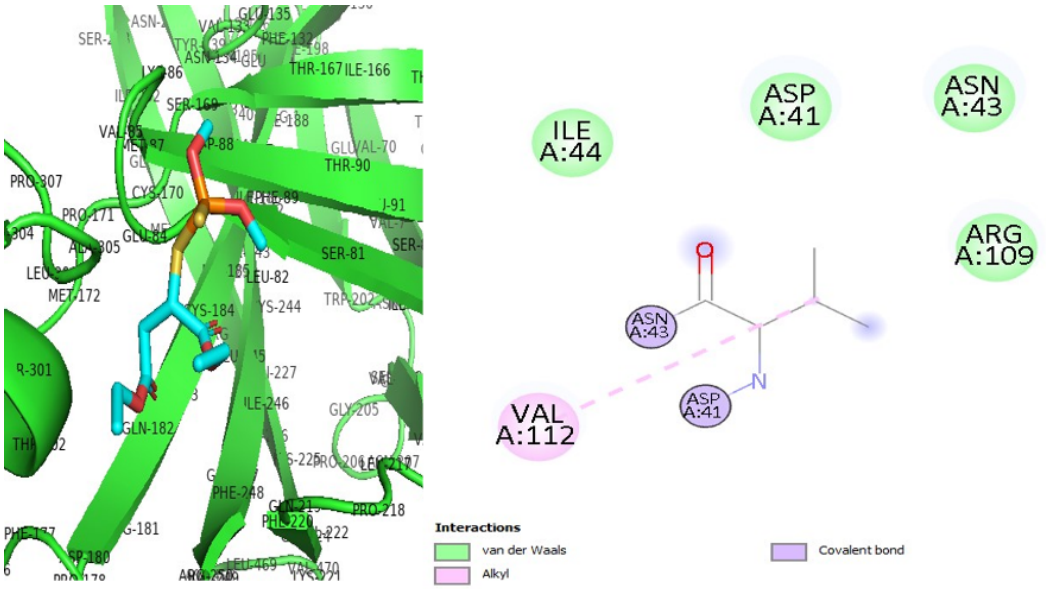


FIGURE 4.24: Malathion and GABA-R

The interaction of malathion with GABA-R is depicted in Figure 4.24. The figure depicts that there are two covalent bonds, one alkyl bond, and four van der waals interactions. On the other hand, the interaction of tetrahydroalstonine with GABA-R includes one alkyl bond, one covalent bond, one conventional hydrogen bond, and five van der waals interactions. The information indicates that in comparison to tetrahydroalstonine and malathion, tetrahydroalstonine exhibits a strong profile of interaction with AChE in terms of docking score and hydrogen bonds. In general, the comparison of tetrahydroalstonine and malathion shows that both of them exhibit similar TICE rules and ADMET analysis. However, the toxicity profile, docking scores, and receptor-ligand interaction profile of tetrahydroalstonine, a lead compound, is better than that of the standard drug, malathion.

Chapter 5

Discussion

This study focuses on the insecticidal potential of compounds found in *Rhazya stricta*, using docking simulations to check how well these chemicals bind against important insect nerve targets like acetylcholinesterase and GABA receptors. With insects becoming resistant to regular insecticides and those synthetics harming the environment, there's a big need for safer options. Because of this, natural plant compounds are getting a lot of interest since they can work in various ways and are super diverse in structure. Research shows that natural compounds like alkaloids, flavonoids, and terpenoids can really knock out insects because of their powerful insecticidal and neurotoxic effects [81].

These compounds often do this by messing with key insect systems. Many studies focus on how these compounds block the enzyme AChE, leading to constant nerve stimulation, paralysis, and ultimately death in insects. Also, blocking GABA receptors causes hyperexcitation and convulsions, which are super harmful for insects. This study shows that phytochemicals from *Rhazya stricta* have different binding affinities and interaction profiles with target proteins. Some compounds, including tetrahydroalstonine and eburnamonine, strongly dock with the active sites of AChE and GABA-R, hinting at their ability to interfere with insect neural signaling pathways. This multi-target activity could be really useful for insecticide design because it might help stave off resistance in insect populations [82].

Along with the already known ways of pest control, the idea of using insecticides that target multiple areas in insects has become really important in recent pest management studies. These compounds can hit more than one neurological spot, which makes it less likely for pests to develop resistance, especially useful in farms where insects get constant exposure to single-target insecticides. In this study, most of the chosen phytochemicals are indole alkaloids. They're valued for their complex structures and flexibility in various biological activities. These chemicals typically include aromatic rings, heteroatoms, and hydrophobic parts, letting them strongly bind to spots on enzymes. Because of this diversity, they could potentially interact with different insect neural receptors, making them great prospects for developing new insecticides [83].

The molecular docking results showed that certain compounds can form stable bindings with AChE and GABA-R. Mainly, they stick around due to hydrogen bonds, hydrophobic contacts, and π–π stacking interactions with important amino acids. This means the compounds could disrupt insect neurotransmission pathways. Additionally, when compared to standard insecticides, these compounds showed competitive abilities. They seem like they could be used as natural substitutes for synthetic insecticides. This is pretty important since compounds from plants tend to break down faster and are safer for non-target organisms than typical insecticides [84].

ADMET analysis backs up the pharmacokinetic suitability of most chosen compounds. They generally show moderate intestinal absorption, acceptable distribution, and manageable metabolism. The toxicity testing showed that several compounds aren't AMES toxic and don't strongly inhibit hERG channels, suggesting they're safer. Yet, some still showed hepatotoxicity or high predicted toxicity, stressing the need for careful picking and tweaking of leads. Additionally, looking at how these compounds affect aquatic life during testing gives us more clues about their eco-safety [85]. Given that insecticides get used a lot in farming, we must consider their impact on water creatures and soil when deciding which to prioritize. Despite these promising computational findings, we need to acknowledge the limitations of *in silico* work. While docking studies predict binding affinities

and interaction patterns, they fall short in replicating biological complexity, things like enzyme kinetics, metabolic breakdown, and organism-level reactions are missing. So, to prove these compounds work against insects, laboratory work is also needed.

Chapter 6

Conclusion and Future Prospects

This study shows that *Rhazya stricta*'s phytochemicals could be really useful for making new insecticides. Through computational screening, it is found that tetrahydroalstonine interact strongly with AChE receptors. This means it can likely interfere with key neurological processes in insects. Also, the ADMET profiling results were pretty good, showing acceptable pharmacokinetics and toxicity levels. This adds more weight to its potential as an effective insecticidal agent. This compound with stable bindings and good ADMET traits might make great leads for future research. Also, being from plants and targeting multiple sites, it could offer a safer, ecofriendly alternative to artificial insecticides. Future research needs to focus on in vitro and in vivo tests to verify its effectiveness. Also, doing structure-activity relationship studies and chemical optimization could limit the potency and selectivity. Environmental safety checks and resistance studies are crucial for real-world use. So, this study gives a solid computational start for creating new plant-based insecticides.

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