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MASTER'S THESIS

For the Completion of the Master's Degree in Biological Sciences

Specialty: Biochemistry

Thesis Title:

GC-MS Characterization of *Melia azedarach* Extracts and In Silico Prediction of Insecticidal Protein Targets

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Abstract

Melia azedarach, commonly referred to as the Chinaberry tree and locally known in Algeria as Zanzalakht, is recognized for its rich composition of bioactive compounds. Although this plant has attracted considerable attention for its pesticidal and therapeutic properties, the molecular mechanisms underlying its effects remain insufficiently understood. This study aims to address that knowledge gap by employing a structure-based in silico approach.

The primary goals of this research are twofold: first, to chemically profile the methanolic fruit extract of *Melia azedarach* through gas chromatography–mass spectrometry (GC-MS); and second, to investigate the interaction potential of its identified constituents with key insect molecular targets—namely Odorant Binding Proteins (OBPs) and Juvenile Hormone Esterase (JHE). These proteins play essential roles in insect sensory processing and endocrine regulation, making them strategic targets for novel bioinsecticide development.

To achieve this, fruits of *M. azedarach* were extracted with methanol, fractionated, and analyzed using GC-MS to determine their phytochemical composition. The resulting compounds were subsequently subjected to molecular docking studies to evaluate their binding affinity with OBP and JHE proteins. Among the detected constituents, compounds such as palmitic acid, phytol, β-sitosterol, and linoleic acid demonstrated significant binding interactions, suggesting their capacity to interfere with physiological pathways crucial to insect survival and development.

This integrative study highlights the potential of *Melia azedarach* as a source of eco-friendly insecticidal agents. By combining chemical analysis with computational modeling, it lays the groundwork for the rational design of plant-derived pesticides that are both effective and environmentally sustainable.

Keywords: *Melia azedarach*, insecticidal activity, GC-MS, bioactive compounds, molecular docking.

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Résumé

Melia azedarach, communément appelé "Chinaberry tree" et localement connu en Algérie sous le nom de Zanzalakht, est reconnu pour sa richesse en composés bioactifs. Bien que cette plante ait suscité un intérêt considérable en raison de ses propriétés pesticides et thérapeutiques, les mécanismes moléculaires sous-jacents à ses effets demeurent encore insuffisamment élucidés. Cette étude vise à combler cette lacune en adoptant une approche in silico basée sur la structure.

Les objectifs principaux de ce travail de recherche sont doubles : d'une part, établir le profil chimique de l'extrait méthanolique des fruits de *Melia azedarach* à l'aide de la chromatographie en phase gazeuse couplée à la spectrométrie de masse (GC-MS), et d'autre part, évaluer le potentiel d'interaction des composés identifiés avec des cibles moléculaires clés chez les insectes — en l'occurrence, les protéines de liaison aux odorants (OBPs) et l'estérase de l'hormone juvénile (JHE). Ces protéines jouent un rôle fondamental dans le traitement sensoriel et la régulation endocrine chez les insectes, ce qui en fait des cibles stratégiques pour le développement de bioinsecticides innovants.

Pour cela, les fruits de *M. azedarach* ont été extraits avec du méthanol, fractionnés, puis analysés par GC-MS afin de déterminer leur composition phytochimique. Les composés identifiés ont ensuite fait l'objet d'études de docking moléculaire afin d'évaluer leur affinité de liaison avec les protéines OBP et JHE. Parmi les constituants détectés, des molécules telles que l'acide palmitique, le phytol, le β-sitostérol et l'acide linoléique ont montré des interactions de liaison significatives, suggérant leur capacité à perturber des voies physiologiques cruciales à la survie et au développement des insectes.

Cette étude intégrative met en évidence le potentiel de *Melia azedarach* comme source d'agents insecticides respectueux de l'environnement. En combinant l'analyse chimique et la modélisation computationnelle, elle jette les bases de la conception rationnelle de pesticides d'origine végétale, efficaces et durables du point de vue écologique.

Mots-clés : *Melia azedarach*, activité insecticide, GC-MS, composés bioactifs, docking moléculaire.

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ملخص

نبات *Melia azedarach*، المعروف عادة باسم "شجرة الخرز الصيني"، والمسمّى محليًا في الجزائر بـ "الزّانزَلَخت"، يُعرف بتركيبته الغنية بالمركبات النشطة بيولوجيًا. وعلى الرغم من أن هذا النبات قد حظي باهتمام كبير بسبب خصائصه المبيدة للحشرات والعلاجية، فإن الآليات الجزيئية الكامنة وراء تأثيراته لا تزال غير مفهومة بشكل كافٍ. تهدف هذه الدراسة إلى سدّ هذه الفجوة المعرفية من خلال اعتماد مقاربة حاسوبية تعتمد على البنية الجزيئية (*structure-based in silico approach*).

تتمثل الأهداف الأساسية لهذا البحث في شقّين :أولًا، التحليل الكيميائي لمستخلص ثمار *Melia azedarach* باستخدام تقنية التحليل بالكروماتوغرافيا الغازية – مطياف الكتلة (GC-MS). وثانيًا، دراسة قدرة المركبات المُكتشفة على التفاعل مع أهداف جزيئية رئيسية لدى الحشرات، وتحديدًا بروتينات ربط الروائح (OBPs) وإنزيم إستراز الهرمون اليافع (JHE). تلعب هذه البروتينات أدوارًا أساسية في معالجة الإشارات الحسية وتنظيم الغدد الصماء لدى الحشرات، مما يجعلها أهدافًا استراتيجية لتطوير مبيدات حيوية جديدة.

لتحقيق ذلك، تم استخلاص ثمار *Melia azedarach* باستخدام الميثانول، ثم تم تجزئتها وتحليلها بتقنية GC-MS لتحديد مكوّناتها الكيميائية النباتية. بعد ذلك، خضعت المركبات الناتجة لدراسات الالتحام الجزيئي (*molecular docking*) لتقييم قوة ارتباطها ببروتينات OBP و JHE. ومن بين المركبات التي تم تحديدها، أظهرت كل من حمض البالمتيك، والفيتول، والبيتا-سيتوستيرول، وحمض اللينوليك تفاعلات ارتباط قوية، ما يشير إلى قدرتها على التأثير في المسارات الفسيولوجية الحيوية لبقاء وتطور الحشرات.

تُسلّط هذه الدراسة التكامليّة الضوء على الإمكانات الواعدة لنبات *Melia azedarach* كمصدر لعوامل طبيعية صديقة للبيئة لمكافحة الحشرات. ومن خلال الجمع بين التحليل الكيميائي والنمذجة الحاسوبية، تمهّد هذه الدراسة الطريق لتصميم مبيدات نباتية فعّالة ومستدامة بيئيًا.

الكلمات المفتاحية : *Melia azedarach* ، النشاط المبيد للحشرات، GC-MS، المركبات النشطة بيولوجيًا، الالتحام الجزيئي.

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Dedication

To my beloved **mother**,
whose endless love, sacrifices, and prayers have been my strength through every step. Your gentle presence and unwavering belief in me are the foundation of all that I have accomplished.

To my dear **father**,
your wisdom, resilience, and silent support have guided me in ways words cannot express. Thank you for teaching me the value of perseverance and dignity.

To my precious **sister Selsabile** and my cherished **brother Abdel Azize**,
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To all my **friends and colleagues**,
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with deep gratitude for your guidance, patience, and encouragement. Your insight and dedication have been essential in shaping the direction and depth of this research. Thank you for helping me bring this work to life.

This work is dedicated to all of you with love, appreciation, and the utmost respect.

List of Abbreviations

Abbreviation	Definition
°C	Degrees Celsius
%	Percent
GC-MS	Gas Chromatography–Mass Spectrometry
OBP	Odorant Binding Protein
JHE	Juvenile Hormone Esterase
RT	Retention Time
m/z	Mass-to-Charge Ratio
3D	Three-Dimensional
TMS	Trimethylsilyl
LC_{50}	Lethal Concentration for 50% of a test population
SMILES	Simplified Molecular Input Line Entry System
NIST	National Institute of Standards and Technology (Database)
PDB	Protein Data Bank
CID	Compound Identifier (PubChem)
SDS	Safety Data Sheet
QSAR	Quantitative Structure–Activity Relationship
PTTH	Prothoracicotropic Hormone
UV	Ultraviolet

Glossary

Bioinformatics: An interdisciplinary field that uses computational tools to analyze and interpret biological data.

GC-MS (Gas Chromatography–Mass Spectrometry): An analytical method used to identify substances within a test sample by separating and detecting chemical compounds.

In Silico: Experiments or simulations performed on computer or via computer simulation.

Insecticidal Activity: The ability of a substance to kill or repel insects.

Molecular Docking: A method that predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex.

OBP (Odorant Binding Protein): A protein involved in the detection of chemical signals (odors) in insects.

JHE (Juvenile Hormone Esterase): An enzyme that regulates the juvenile hormone levels in insects, affecting their development.

Phytochemicals: Bioactive chemical compounds found in plants, which can have health-promoting properties.

TMS Derivatives: Trimethylsilyl derivatives formed during silylation to enhance the volatility of compounds for GC-MS analysis.

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Introduction

Introduction

Melia azedarach is a fast-growing tree from the Meliaceae family widely recognized for its medicinal and pesticidal properties. Traditionally used in various ethnomedicinal systems to treat conditions ranging from skin disorders to digestive issues, *M. azedarach* has recently attracted scientific attention for its ability to act as a source of eco-friendly bioinsecticides (Sharma et al., 2013; Rani et al., 1999).

The increasing environmental and health concerns associated with synthetic pesticides—such as resistance development, non-target toxicity, and ecological imbalance—have emphasized the urgent need for sustainable alternatives. *M. azedarach* offers promising prospects due to its rich phytochemical profile, which includes limonoids, flavonoids, triterpenoids, alkaloids, and phenolic acids (Nahak & Sahu, 2010; Chiffelle et al., 2009). Several studies have demonstrated its broad-spectrum bioactivity, including antifeedant, larvicidal, repellent, and growth-inhibitory effects against major agricultural pests such as *Spodoptera littoralis*, *Sitophilus oryzae*, and *Aedes aegypti* (Carpinella et al., 2003; Senthilnathan et al., 2006; Farag et al., 2011).

Despite interest in *M. azedarach*, little is known about how its compounds work at the molecular level. This gap, especially around validated protein targets, limits optimization and regulatory approval. This study helps bridge that gap using an in-silico target-based strategy.

The main objective of this research is twofold: (1) to chemically characterize the methanolic extract of *Melia azedarach* fruits using gas chromatography–mass spectrometry (GC-MS), and (2) to perform in silico molecular docking to predict interactions between the identified bioactive compounds and insect protein targets such as Odorant Binding Proteins (OBPs) and Juvenile Hormone Esterase (JHE). These targets are crucial in insect development and behavior, and disrupting their function could lead to effective pest control with minimal environmental impact (Spinelli et al., 2012; Laughlin et al., 2008).

By combining chemical analysis with computational prediction, this study contributes to a better understanding of the molecular basis of plant-derived insecticides. It supports the growing scientific effort to replace hazardous synthetic chemicals with plant-based alternatives that are safer for both human health and the environment (Benllia et al., 2020; Mossa et al., 2014).

Chapter I: Literature Review

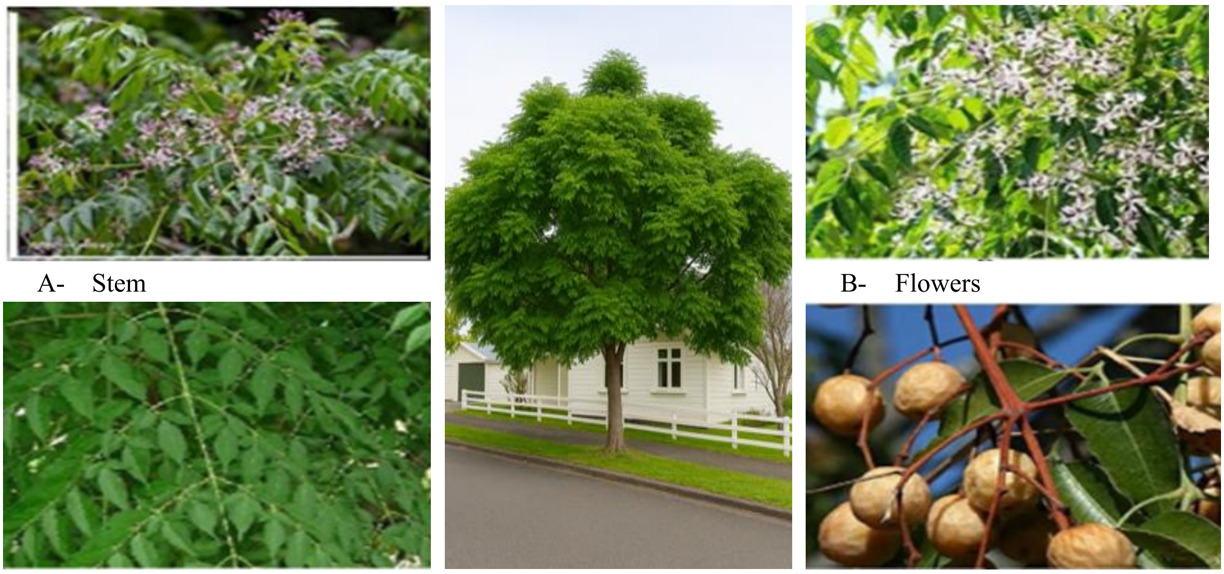
1 *Melia azedarach*

1.1 General Characteristics

Melia azedarach Linnaeus (comonly known as China berry, Pride of China, or Pride of India and locally known in Algeria as Zanzalakhtis**)** a tree in the Meliaceae family with an economic importance. It is native to China and is distributed in mixed forests, fields, and roadside areas between 18 and 40°N, accounting for around one-third of the country's land area (Sivaraj et al., 2018).

This species is 6 to 12 m tall; some rainforest varieties can reach 30 to 45 meters (Oelrichs et al., 1985; Hare et al., 1997). The bark is reddish-brown and cracked on mature trees. Inflorescences in axillary panicles are loose and 10-20 cm long, with purple, fragrant, star shaped flowers 1-2 cm in diameter, with five narrow petals. Mature fruits are yellow and spherical (Figure 1) (Rubae, 2009).

Melia azedarach is a fast-growing species with good timber characteristics for multiple-use, such as construction and furniture, agriculture implements, boats, cars, and musical instrument production (Chen et al., 2014**).** The species' roots, bark, flowers, and fruits have considerable therapeutic value (Angamuthu et al., 2019; Wang et al., 2019). Furthermore, its fruit and leaf extracts can control several agricultural pests and are often utilized as source materials for biological insecticides. (Khoshraftar et al., 2020). The species is a great urban greening tree that is smoke and dust resistant and can absorb a wide range of poisonous and damaging gasses. (Zhang et al., 2023).



A- Stem

B- Flowers

C- Leaves

E- whole plant

D- Fruit

Figure 1: Different parts of *Melia azedarach* (Disha & Karthikeyan, 2024).

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1.2 Taxonomy and Geographical Distribution

Members of the genus *Melia Linnaeus* (Meliaceae: Melioideae: Melieae) are deciduous trees or shrubs that exist in tropical and subtropical areas of the eastern hemisphere, including south of the Yellow River in China (flowering time April to May for *M. azedarach*). The genus Melia has been proposed to comprise either one species (*M. azedarach Linnaeus*) or three species (*M. azedarach*, *M. toosendan* Sieb. et Zucc., and *M. dubia* Cav.) (Sivaraj et al., 2018 ; Liao et al.,2016). Thereby, great controversy continues regarding the classification of this group.

Melia species, particularly *M. azedarach*, are popular in China due to their quick growth and wood's resistance to insects, diseases, and corrosion (Chen et al., 1997). Melia species are medicinal plants used to cure ailments such as diarrhea, diabetes, rheumatic disorders, and hypertension. (Nethmini et al., 2020). Taxonomy of *Melia azedarach* is shown in Table 1.

Melia azedarach, commonly known as chinaberry, is native to South and Southeast Asia and northern Australia. This species lives in Asia's tropics. It is widely available in Pakistan, India, Indonesia, Southeast Asia, and Australia. Naturalization has occurred in the Philippines, the United States, Brazil, Argentina, and other African and Arab states**.** (Sultana et al., 2011; Rubae, 2009).

In Algeria, it was introduced as an ornamental species in the early 1800s and has since become naturalized. Notably, near the town of Skikda, *M. azedarach* has been observed along roadsides consistently from 2014 to 2019 (Sakhraoui, 2019). Additionally, studies have reported the presence of *M. azedarach* in various regions of Algeria, including Blida and Relizane. These observations suggest that *M. azedarach* is distributed across multiple areas in northern Algeria, particularly along roadsides and in urban settings (Sakhraoui, 2019).

Table 1: Taxonomical classification of *Melia azedarach*(Rishi et al., 2003; Azam et al., 2013).

Kingdom	**Plantae**
Subdivision	Angiospermae
Class	Dicotyledonae
Subclass	Polygonae
Order	Geraniales
Family	Meliaceae
Genus	Azedarach
Species	Melia

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1.3 Growing Conditions

Melia azedarach thrives in warm climates and performs well in dry conditions and poor soils, making it a popular avenue tree in arid regions. It propagates through autumn sowing and is suitable for USDA Hardiness Zones 8–12 (Geoff et al., 2005).

In Algeria, *Melia azedarach* is well-adapted to USDA Hardiness Zones 9a to 11a, which include the northern coastal cities such as Algiers and Oran, the Tell Atlas region, and areas near the northern edge of the Sahara like Biskra. These zones offer warm temperatures and relatively dry conditions that suit the species' growth requirements (USDA, 2023). Although it may survive in milder parts of Zone 8a with protection from frost, the species is poorly suited to the cold, high-altitude inland plateaus and the extreme heat and aridity of Zone 12 in the central Sahara (Le Houérou, 1996). These harsher environments typically exceed *M. azedarach*'s tolerance for low moisture and high salinity.

The species prefers deep, well-drained, dry loamy or sandy soils, and it tolerates alkaline conditions and drought. It requires a sunny location but does not tolerate coastal areas or exposure to strong winds. Generally resistant to pests and diseases, its only notable drawback is its structural fragility (Burnie et al., 2013).

1.4 Traditional and Medicinal Uses of *Melia azedarach*

1.4.1 General Use and Gum Applications

Different parts of *Melia azedarach* are widely used for traditional and medicinal purposes. The gum exuded from the trunk is traditionally believed to assist in spleen enlargement, while wood extracts are employed to relieve asthma symptoms (Rani et al., 1999). Ethnomedicinal uses of different parts of M. Azedarach is shown in Table 2.

1.4.2 Bark and Leaf-Based Treatments

A decoction prepared from the bark is used to alleviate symptoms of paroxysmal fever, including thirst, nausea, vomiting, lethargy, appetite loss, and various skin conditions (Dhiman, 2003). Leaf-based applications are also prominent in traditional medicine. For example, a leaf poultice is applied to treat scalp eruptions and tension-related headaches. Leaf decoctions are known for their astringent and stomachic effects and are used in the treatment of hysteria, leprosy, and scrofula (Sharma et al., 2001).

1.4.3 Juices, Flowers, and Fruits

Leaf juice serves multiple purposes, acting as an anthelmintic, diuretic, emmenagogue, expectorant, and vermifuge. The flowers of *M. azedarach* have a variety of properties, including astringent, anodyne, refrigerant, diuretic, emmenagogue, resolvent, and deobstruent effects. The fruits are used to address indigestion, colic, and intestinal catarrh and are also valued for their anti-inflammatory, diuretic, emollient, and purgative properties (Rani et al., 1999; Dhiman, 2003).

1.4.4 Seeds, Oil, and Root-Based Therapies

The seeds are traditionally used to treat typhoid fever, helminthiasis, pelvic discomfort, scrofula, and rheumatism. They are also considered anti-helminthic, expectorant, and aphrodisiac. Seed oil is commonly applied to treat various skin diseases. The roots of the plant are used for their expectorant, febrifuge, astringent, anodyne, emmenagogue, and constipative effects. Root preparations are believed to be beneficial in treating leuoderma, sciatica, lumbago, piles, cough, asthma, ulcers, wounds, diabetes, intermittent fever, and postpartum uterine discomfort (Sharma et al., 2001).

Table 2: Ethnomedicinal uses of different parts of M.azedarach (Sharma et al., 20013).

Plant (Part)	**Ethnic use**
Bark	Anti-diarrhoeal, diuretic, nausea, vomiting, Loss of appetite and pains.
Stem	Treatment of asthama
Root	Antiseptic, cough depressant, reduces fever.
Leaves	Rich in nutritional values, treatment of malaria, diarrhoea, skin diseases and blood purifier.
Fruit	Used as purgative and sweetening agent.
Flowers	In the treatment of inflammation of skin
Seeds	Aphrodisiac in the treatment of typhoid, Helminthiasis.
Whole Plant	Hair growth treatment, and scalp eruption

1.5 Chemical Composition of *Melia azedarach* Extracts

1.5.1 Flavonoid Compounds

Flavonoids are a widespread group of polyphenols found in most plants and are responsible for pigmentation, especially floral colors. They are water-soluble and play various biological roles (Ribérau-Gayon, 1968). Methanolic extracts of *Melia azedarach* leaves are notably rich in phenols and flavonoids but comparatively low in antioxidant activity (Nahak and Sahu, 2010). The plant's depressive effects have been linked to the chemical constituents in its seeds, which include lignans, flavonoids, and limonoids (Meftah et al., 2011).

1.5.2 Alkaloid Cpounds

Preliminary phytochemical analyses of *Melia azedarach* extracts indicate the presence of alkaloids, along with lipids, phenolic compounds, proteins, amino acids, tannins, flavonoids, and carbohydrates (Chiffelle et al., 2009). The Meliaceae family, to which this species belongs, is renowned for its phytosanitary and therapeutic potential due to the pharmacological activities of these alkaloid-rich extracts (Ghnaya et al., 2013).

1.5.3 Triterpenoid Compounds

The seeds of *Melia azedarach* contain meliacarpins, a class of triterpenoids similar but not identical to azadirachtin. These compounds exhibit strong insect growth-regulating effects, functioning by repelling insects and interfering with their developmental cycles rather than acting as immediate toxins. Additionally, they inhibit the release of the prothoracicotropic hormone (PTTH) from neurosecretory cells (Isman, 2006).

1.5.4 Limonoid Compounds

Plants from the Meliaceae family, including *Melia azedarach*, are recognized for their ability to biosynthesize limonoids and triterpenoids. These compounds are associated with significant structural and biological effects (Akihisa et al., 2013). Limonoids, the largest group of terpenes in this family, are valued for their insecticidal properties and have also been used in traditional medicine across various countries (Nakatani, 1994; Bitencourt, 2014).

1.5.5 Other Metabolites

Melia azedarach seeds contain azadirachtin, a compound widely used as a botanical pesticide, especially for controlling aphids and whiteflies (Estephan and Hayar, 2003). In addition, recent phytochemical studies of the fruit have identified a variety of fatty acids, including para-coumaric acid, vanillic acid, chlorogenic acid, syringic acid, caffeic acid, ferulic acid, rutin, catechin, kaempferol, and various saturated and unsaturated fatty acids such as caproic, palmitic, stearic, oleic, linoleic, and linolenic acids (Bitencourt et al., 2014). Other classes of metabolites found throughout the plant include steroids, proteins, saponins, phenols, alkaloids, phytosterols, and volatile compounds (Chiffelle et al., 2009).

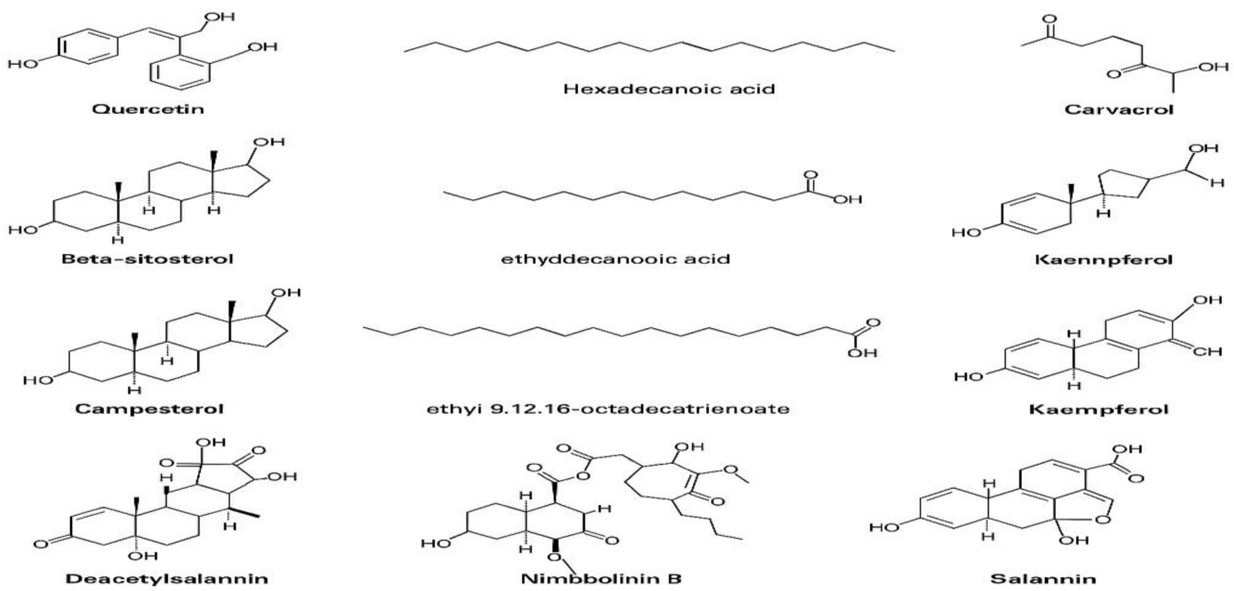

Figure 2: Bioactive Compounds Identified from *Melia azedarach* Extracts via GC-MS Analysis (Naveed et al., 2024).

1.6 Biological Activity of *Melia azedarach* Extracts

1.6.1 Antiviral Activity

Alche et al. (2001) demonstrated that a purified extract of *Melia azedarach* leaves, identified as "Meliacarpin", significantly inhibited the replication of both herpes simplex virus and vesicular stomatitis virus.

1.6.2 Antimalarial Activity

Chaturvedi et al. (2006) investigated the antimalarial potential of methanolic extracts from *M. azedarach* fruit, bark, and leaves in mice infected with *Plasmodium berghei*. The results showed a significant reduction in parasitaemia, particularly in fruit and bark extracts, although their efficacy was lower than that of chloroquine.

1.6.3 Antinephrolithic Activity

Christina et al. (2006) evaluated the aqueous extract of *M. azedarach* for its role in managing ethylene glycol-induced nephrolithiasis in rats. The treatment significantly reduced urinary oxalate, calcium, and phosphate levels, and improved serum and urinary creatinine parameters.

1.6.4 Anthelmintic Activity

Szewezuk et al. (2003) found that ethanol extracts of *M. azedarach* exhibited significant anthelmintic effects on *Taenia solium* and *Pheretima posthuma*. Notably, the extract was more effective against tapeworms than piperazine phosphate.

1.6.5 Antiulcer Activity

Using the Gipsing-restraint stress ulcer model in rats, Moursi et al. (1984) showed that the lipid phytosterol fraction of *M. azedarach* significantly reduced free and total hydrochloric acid and overall gastric acidity, confirming its antiulcer activity.

1.6.6 Antioxidant Activity

Munir et al. (2012) studied the antioxidant properties of *M. azedarach* extracts and found notable levels of total phenolic content (TPC: 74.43–112.10 mg GAE/g DW) and total flavonoid content (TFC: 13.32–28.11 mg CE/g DW). The stem bark extract displayed particularly high antioxidant activity.

1.6.7 Antipyretic Activity

Sultana et al. (2013) demonstrated that hydro-methanolic leaf extracts of *M. azedarach* had significant antipyretic effects in yeast-induced pyrexia models in rats. A 500 mg/kg dose was notably more effective than 250 mg/kg and showed results comparable to paracetamol.

1.6.8 Wound Healing Activity

Vidya et al. (2012) found that topical application of *M. azedarach* leaf extract significantly improved wound healing in alloxan-induced diabetic rats. The healing effect was attributed to the extract's antibacterial properties, using povidone-iodine as a reference treatment.

1.6.9 Antifeedant Activity

El-Lakwah et al. (1995) reported that powdered fruits and petroleum ether/acetone extracts of *M. azedarach* effectively reduced *Sitophilus oryzae* reproduction and adult repulsion. Mortality increased over time during exposure.

1.7 Insecticidal Activity of Plant Extracts

Extracts from *Melia azedarach* have shown insecticidal efficacy comparable to commercial pesticides. Aqueous extracts from leaves and fruits negatively affect insect development, growth, and reproduction. They cause moulting disorders, reduce fecundity, and alter behavior (Senthilnathan et al., 2006; Carpinella et al., 2003; Aoudia et al., 2013). Additional studies confirmed broad insecticidal effects against multiple pest species

(Alessandro and Stella, 1993; Nardo et al., 1997; Valladares et al., 1997; Banchio et al., 2003). The efficacy of extracts derived from the fruits and leaves of *Melia azedarach* L. versus various insects has been well documented in the literature (Carpinella et al., 2002, 2003; Banchio et al., 2003; Valladares et al., 2003). These extracts are particularly known for their antifeedant properties against numerous insect species (Juan et al., 2000; Banchio et al., 2003; Carpinella et al., 2003; Nathan, 2006). Although *M. azedarach* fruits are commonly believed to be toxic,

Toxicity assays conducted on mammals have shown no adverse effects when the extract was administered orally to rats (Carpinella et al., 1999).

The tree *Melia azedarach* has demonstrated significant insecticidal activity against various pest insects. Its extracts, particularly those derived from fruits and leaves, contain a variety of bioactive compounds such as limonoids**,** flavonoids, and fatty acid esters, which act either through ingestion or contact. Several studies have confirmed that the oil from ripe fruits exhibits acute toxicity against the larvae of *Spodoptera littoralis* (Noctuidae), a major crop pest, causing mortality rates of up to 100% at high concentrations, along with larval growth inhibition and strong feeding deterrence (Farag et al., 2011). Additionally, *M. azedarach* has proven effective against other pests such as *Xanthogaleruca luteola* and *Agrotis ipsilon*, through antifeedant mechanisms or by disrupting the insect digestive system Carpinella et al., 2003). These insecticidal properties make *Melia azedarach* a promising ecological alternative to synthetic pesticides, while presenting low toxicity to mammals (Carpinella et al., 1999).

1.8 Toxicology

The toxicity of *Melia azedarach* fruits is primarily linked to tetranortriterpenes, with levels varying depending on environmental conditions and tree maturity (Tam Garland and Barr, 1998). Ingestion of 5–6 fruits by young dogs or 6–8 fruits by children can be fatal (Hare, 1997). Chinese medical literature reports toxicity in humans after consuming 6–9 fruits, 30–40 seeds, or 400 mg of bark (Stuart, 2013). Despite their toxicity, these compounds contribute to plant defense, offering protection against over 400 insect species, including those resistant to chemical pesticides.

2 In Silico Identification of Insecticidal Targets of *Melia azedarach* Compounds

2.1 Exploring In Silico Approaches

Computational modeling has increasingly become central to biological and chemical research. Known as *in silico* methods, these simulations allow scientists to explore how molecules behave, how drugs interact with proteins, and how bioactive compounds from plants like *Melia azedarach* might target specific biological systems. This digital methodology is often more cost-effective than traditional laboratory experiments and can accelerate early-stage discovery (Naik et al., 2009).

2.2 The Role of Bioinformatics in Peptide-Based Research

Bioinformatics offers a suite of tools capable of managing and analyzing large-scale biological datasets, enabling researchers to investigate protein structures, gene functions, and peptide interactions (Mount, 2004). These computational techniques are instrumental in predicting how specific molecules may function as therapeutic agents or insecticides. By using sequence alignment algorithms and structural databases, it is possible to pinpoint biologically active regions within peptides that contribute to their functionality (Lesk, 2019).

2.3 Understanding QSAR in Peptide Screening

Quantitative Structure–Activity Relationship (QSAR) models help scientists connect the chemical structure of a molecule to its biological function. For natural extracts and peptides, QSAR assists in predicting which compounds may be most effective, based solely on computational descriptors and models (Naik et al., 2009). This avoids the need to test every candidate in a lab and is ideal for screening plant-derived insecticidal agents.

2.4 Homology Modeling: 3D Structures Without Crystals

When no crystal structure of a protein or peptide exists, homology modeling provides a valuable alternative. This computational method uses known structures of similar amino acid sequences to construct plausible 3D models of the target protein (Marti-Renom et al., 2000). Platforms such as SWISS-MODEL and AlphaFold are commonly used for automated and accurate structure prediction (Waterhouse et al., 2018; Jumper et al., 2021). The reliability of these predicted models is typically assessed using validation tools such as Ramachandran plots, which evaluate the stereochemical quality of backbone dihedral angles and help confirm proper peptide folding (Laskowski et al., 1993).

2.5 Virtual Screening and Docking

In silico screening plays a key role in drug discovery by identifying compounds with a high likelihood of interacting with biological targets (Lionta et al., 2014). Molecular docking tools simulate how small molecules bind within the active sites of proteins, offering insight into potential binding affinities and conformations (Meng et al., 2011). By evaluating docking scores and binding poses, researchers can prioritize the most promising candidates for experimental validation and further development (Ferreira et al., 2015).

2.6 Chemistry of *Melia azedarach*: Extraction and Profiling

To identify its active compounds, *Melia azedarach* is often extracted with methanol, a solvent known to isolate polar phytochemicals. These extracts typically include limonoids,

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fatty acids, and sterols. The resulting samples are then analyzed using gas chromatography-mass spectrometry (GC-MS). GC-MS identifies individual compounds such as methyl palmitate, β-sitosterol, stigmasterol, phytol, and palmitic acid many of which show biological activity (Ghosh & Chatterjee, 2012).

2.7 Protein Targets

Insecticidal compounds often target critical proteins such as acetylcholinesterase (AChE), which is responsible for the breakdown of the neurotransmitter acetylcholine, and GABA receptors, which modulate nerve signal transmission in the insect nervous system (Casida, 2009). Other compounds interfere with hormonal pathways, particularly those involving juvenile hormones, or block voltage-gated sodium and calcium channels, disrupting neural excitability (Sparks & Nauen, 2015). Disruption of these essential targets can lead to paralysis, disrupted development, and eventually death in insect pests.

2.8 Combining Nature with Simulation

With databases such as PubChem, UniProt, and the Protein Data Bank (PDB) offering extensive structural and chemical data, researchers can employ molecular docking software to simulate how compounds from *Melia azedarach* interact with insect proteins (Kim et al., 2021; Burley et al., 2021; The UniProt Consortium, 2023). Tools such as AutoDock Vina and SwissDock facilitate the prediction of binding affinities and interaction profiles, enabling a structure-based approach to rational pesticide design (Trott & Olson, 2010; Grosdidier et al., 2011).

Chapter II:

MATERIALS AND METHODS

Chapter II: MATERIALS AND METHODS

1 Objectives

This study aimed to evaluate the insecticidal activity of Melia azedarach through a combined chemical and biological approach. The main objectives were to obtain the methanolic extract of the plant, identify key bioactive compounds using GC-MS, and perform molecular docking analyses of the selected compounds against relevant insect protein targets.

2 Materials

The materials required for this research included both natural and synthetic components. The fruits of *Melia azedarach*—mature specimens collected from the Faculty of Natural and Life Sciences at Saad Dahlab University of Blida in February 2025 served as the primary biological substrate. High-grade distilled water was also utilized for washing the plant material and for preparing aqueous suspensions, thereby maintaining experimental sterility and enhancing the reliability of downstream processes. Used for washing and preparation of aqueous suspensions. Non-biological materials are listed in Table 10,11 and 12 Appendice 1.

3 Methods

3.1 Extraction and Fractionation Protocol for Melia azedarach Fruits

3.1.1 Plant Authentication and Collection

Authentication was conducted before harvesting over 3 kg of *Melia azedarach* fruits at the Faculty of Natural and Life Sciences, Saad Dahlab University, Blida in February 2025. Fruits were shade-dried and ground for extraction.

3.1.2 Cleaning of Plant Material

Fruits were rinsed thoroughly with distilled water to remove impurities and avoid contamination.

3.1.3 Drying Process

Fruits were shade-dried at 25–30 °C for 7–10 days or oven-dried at 40 °C. Monitoring was crucial to prevent degradation of sensitive bioactive compounds.

3.1.4 Grinding and Sieving

Dried fruits were ground and sieved through 40 mesh. This increased surface area and extraction efficiency.

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3.1.5 Storage

Powdered material was stored in amber vials at ambient temperature, protected from light and moisture.

3.2 Methanolic Extraction

3.2.1 Sample Preparation

Fifty g of powdered fruit was extracted in a 1 L flask with 500 mL methanol (99.8%) using a 1:10 (w/v) ratio. Methanol is a high-polarity solvent efficient in extracting phenolics, flavonoids, and alkaloids. Due to its toxicity, PPE and proper ventilation were required.

3.3 Soxhlet Extraction

Soxhlet extraction proceeded for 6–8 hours until siphoning solvent became colorless. The methanolic extract was then concentrated.

Figure 3: Soxhlet extraction setup using methanol as solvent.

3.4 Concentration

Methanol was evaporated under reduced pressure at 70 °C to yield a viscous extract.

3.5 Extraction Yield

The extraction yield of the methanolic crude extract was calculated using the following formula:

Extraction Yield (%) = (Mass of dried methanolic extract (g) / Mass of plant material used (g)) × 100

4 Fractionation of the Crude Methanolic Extract

4.1 Aqueous Suspension

The crude extract was dissolved in 75 mL distilled water and stirred until homogeneous.

A B

Figure 4: Steps for the concentration and recovery of the methanolic crude extract of Melia azedarach

A: Concentration of the extract using a rotary evaporator; **B:** Collection of the concentrated crude extract in a Petri dish.

4.2 Sequential Liquid-Liquid Partitioning

Step 1: Hexane Extraction

Performed 3x with 75 mL each to collect non-polar compounds. Combined hexane fractions were concentrated.

A B C

Figure 5: Liquid-liquid partitioning of the methanolic crude extract of Melia azedarach using hexane.

A: Transfer of the diluted crude extract into a flask for liquid–liquid extraction; **B:** Extraction phase in a separatory funnel with the addition of hexane; **C:** Hexane fraction recovered after phase separation.

Step 2: Ethyl Acetate Extraction

Performed 3x with 75 mL each to collect semi-polar compounds. Fractions were concentrated and stored.

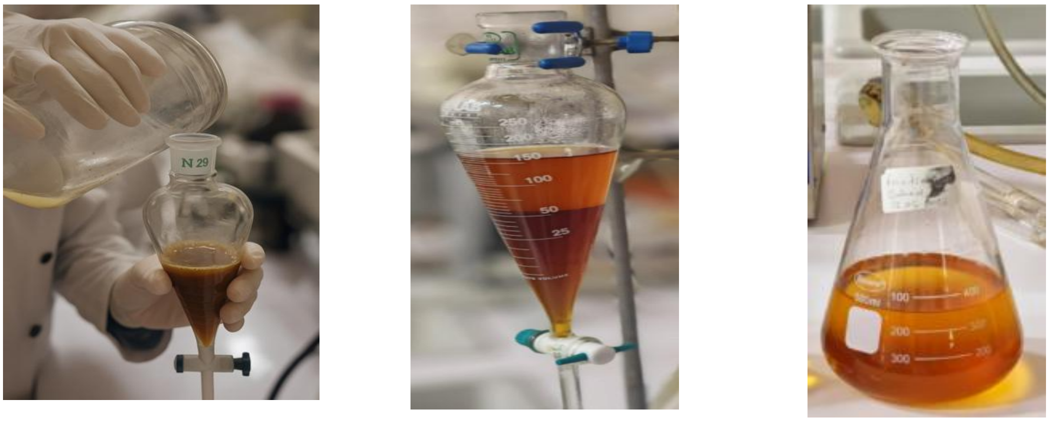
A B C

Figure 6 : Liquid-liquid partitioning of the methanolic crude extract of Melia azedarach using ethyl acetate.

A: Transfer of the diluted crude extract into a separatory funnel; **B:** Phase separation after the addition of ethyl acetate; **C:** Ethyl acetate fraction collected after decantation.

Step 3: n-Butanol Extraction

Performed 3x with 75 mL each to isolate polar compounds. Fractions were concentrated and stored.



A B C

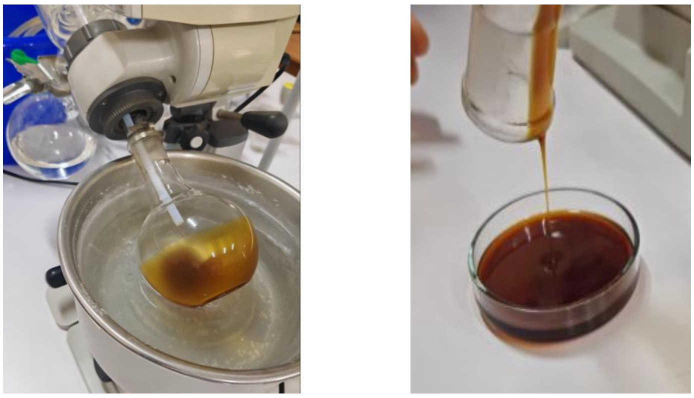
Figure 7: Liquid-liquid partitioning of the methanolic crude extract of Melia azedarach using ethyl acetate.

A: Transfer of the diluted crude extract into a separatory funnel; **B:** Phase separation after the addition of *n*-butanol; **C:** *n*-Butanol fraction collected after decantation.

Fractions were stored at -20°C until GC-MS analysis.

4.3 Final Concentration

Each fraction was evaporated under reduced pressure.



A B

Figure 8: Final concentration of solvent fractions

A: Evaporation of the hexane fraction using a rotary evaporator; **B:** Recovery of the dried concentrated extracts in labeled containers for storage

4.4 Storage

Dried extracts were sealed in amber glass vials and stored at −20°C, away from light, air, and humidity.

5 Gas chromatography–mass spectrometry

5.1 Sample Preparation and Silylation

A total of 200 µL of BSTFA (N,O-bis(trimethylsilyl)trifluoroacetamide) was mixed with 200 µL of the methanolic extract and 50 µL of pyridine in a glass vial with a septum. The mixture was vortexed thoroughly for homogenization and incubated in a water bath at 80 °C for 60 minutes to ensure complete silylation of phenolic and thermolabile constituents.

5.2 GC-MS Conditions

GC-MS analysis was carried out using an Agilent 6890 system equipped with an HP-5MS column (30 m × 0.25 mm × 0.25 µm, 5% phenyl / 95% methylsiloxane). The injector and detector temperatures were maintained at 250 °C. Helium (99.999%) was used as the carrier gas at a flow rate of 1.0 mL/min. The injection volume was 1 µL.

5.3 Oven Temperature Program

The oven was programmed as follows:

- Initial temperature of 60 °C, held for 1 minute
- Ramp of 5 °C/min up to 260 °C
- Further increase of 10 °C/min to 280 °C, followed by a final hold of 1 minute

5.4 Compound Identification

Metabolites were identified based on mass spectral data obtained from GC-MS, retention indices (Kovats index), comparison with published literature, and spectral library matches from NIST and Wiley databases. All analyses were conducted in duplicate to verify accuracy and reproducibility.

6 Molecular Docking

6.1 Ligand and Protein Preparation

Ligands were initially drawn and optimized in Avogadro, then saved in .pdb format. Each ligand file was opened in UCSF Chimera, where hydrogens were added using *Tools → Structure Editing → AddH*, and charges were assigned via the Gasteiger method (*Add Charge* function). Proteins in .pdb format were obtained from the RCSB Protein Data Bank. Water

Molecules and co-crystallized ligands were removed manually or via command-line input. Polar hydrogens were added, and charges were assigned using the AMBER ff14SB force field. Both ligands and proteins were saved as prepared .pdb files for docking.

6.2 Target Protein Selection

To evaluate the potential insecticidal interaction of fatty acid-based compounds from *Melia azedarach*, two functionally important insect protein targets were selected for in silico docking studies. These targets are involved in key physiological processes such as olfaction and hormone regulation, making them suitable for assessing behavioral and developmental disruptions in insect species:

- **Odorant Binding Protein (OBP)**: This protein is crucial for odor perception and host-seeking behavior. It was selected for *Musca domestica* and *Phlebotomus papatasi*, both of which rely on olfactory signals for survival and reproduction. Due to the absence of a resolved OBP structure for these species, the OBP of *Aedes aegypti* (PDB ID: 5V13) was used as a homologous model, previously validated in *Anopheles gambiae* (Spinelli et al., 2012).

- **Juvenile Hormone Esterase (JHE)**: This enzyme regulates juvenile hormone levels, thereby controlling development and molting in insects. It was selected for *Anopheles gambiae*, with the crystal structure available under PDB ID: 3N7H. This protein is a well-established molecular target for juvenile hormone analogs and inhibitors (Laughlin et al., 2008).

These proteins were chosen based on functional relevance, cross-species structural availability, and suitability for ligand docking using AutoDock Vina.

The selected insect species—*Musca domestica*, *Phlebotomus papatasi*, and *Anopheles gambiae*—are recognized vectors of medically important diseases in Algeria. *M. domestica* serves as a mechanical vector for enteric pathogens such as *Salmonella* and *Escherichia coli*, contributing to diarrheal disease transmission. *P. papatasi* transmits *Leishmania major*, the causative agent of zoonotic cutaneous leishmaniasis, which remains endemic in various Algerian regions. *A. gambiae* is a primary malaria vector (Capinera, 2008); although Algeria was declared malaria-free in 2019, the species persists in the south, posing a reintroduction risk. The use of bioinsecticidal agents targeting key physiological proteins in these species is therefore crucial to vector control and public health strategies.

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Table 3: Insect Proteins Used In Molecular Docking.

Protein Name	Function	Species	PDB ID	Purpose in Study
Odorant Binding Protein (OBP)	Detects odor molecules; essential for insect behavior (e.g., mating, feeding)	*Aedes aegypti*	5V13	Model for OBPs of *Musca domestica* and *Phlebotomus papatasi* (olfactory disruption)
Juvenile Hormone Esterase (JHE)	Regulates juvenile hormone; controls insect development and reproduction	*Anopheles gambiae*	3N7H	Target for disrupting hormone metabolism and insect growth

6.3 Binding Site Coordinates and Docking Grid Definition

To define the binding site, the ligand's geometric center was determined using the Chimera command:

```
measure center sel
```

This provided the X, Y, Z coordinates used to center the docking grid. A cubic grid box of 20 × 20 × 20 Å was used around the centroid to accommodate ligand flexibility during docking. These coordinates were entered in the AutoDock Vina dialog within Chimera (Figure 9).

6.4 Binding Site Definition and Grid Box Calculation

For each protein–ligand complex, the binding site was defined based on the spatial position of the ligand within the three-dimensional structure. The combined protein–ligand PDB files were loaded into UCSF Chimera, and the ligand residue was identified using the residues command or by inspecting HETATM entries in the PDB file. The ligand was selected using its residue name (e.g., select :LIG), and the centroid of the ligand was calculated using the Chimera command:

```
measure center sel
```

This command returns the Cartesian coordinates (X, Y, Z) of the geometric center of the selected ligand atoms, which was used to define the center of the docking box. The result was obtained from the Reply Log after executing the command.

A cubic docking box of 20 Å × 20 Å × 20 Å was applied around the centroid to allow adequate spatial flexibility for ligand translation, rotation, and conformational sampling. This box size was selected to ensure complete coverage of the ligand binding pocket while preserving computational efficiency. All binding site coordinates and grid dimensions were recorded and subsequently used in AutoDock Vina configuration files for docking simulations.

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6.1 Docking Procedure

Docking was conducted using AutoDock Vina integrated into Chimera (*Tools* → *Surface/Binding Analysis* → *AutoDock Vina*). The prepared protein and ligand were loaded, the grid box was set as described, and the exhaustiveness parameter was set to 8. The number of modes was kept at default (9). Docked output files were automatically loaded into Chimera for visualization.

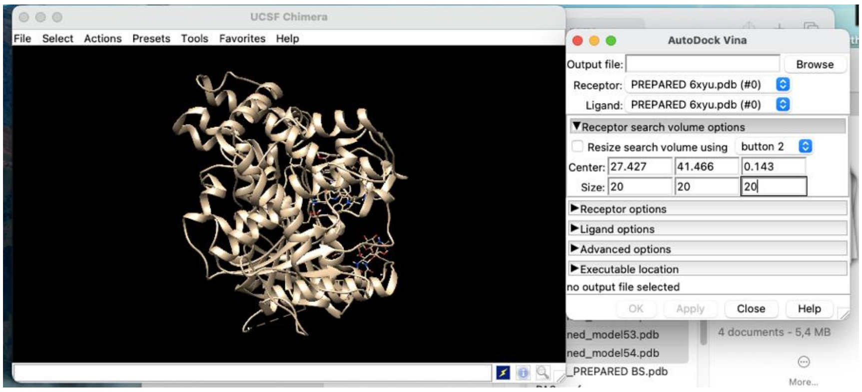

Figure 9: Docking setup using UCSF Chimera integrated with AutoDock Vina.

The protein–ligand complex is visualized in ribbon representation, with the ligand positioned in the active site. The AutoDock Vina dialog shows the selected receptor and ligand files (PREPARED 6xyu.pdb), and the search grid is centered at coordinates X = 27.427, Y = 41.466, Z = 0.143 with box dimensions set to 20 × 20 × 20 Å, defining the docking search space used for simulation.

6.2 Post-Docking Analysis

The post-docking analyses were performed in Chimera. The binding energy (kcal/mol) was extracted from the Reply Log after docking and the torsion count (number of rotatable bonds) was noted from the. pdbqt output or Vina log remarks.

Chapter III: Results and Discussion

Chapter III: Results and Discussion

1 Yield of Methanolic Extract of *Melia azedarach*

The yield obtained from the methanolic extraction of *Melia azedarach* fruits was **45.3%**, which is considered relatively high for plant-based bioactive extractions. This high yield suggests a significant presence of polar and semi-polar secondary metabolites such as phenolics, flavonoids, and limonoids, known for their biological activities including insecticidal, antimicrobial, and antioxidant effects.

Previous studies have also reported efficient extraction of bioactive compounds from *Melia azedarach* using methanol as a solvent (Nasri et al., 2016; Bachir et al., 2017), supporting the effectiveness of this method.

2 GC-MS Results of Methanolic Extract

Preliminary GC-MS analysis of the methanolic extract of *Melia azedarach* revealed the presence of several bioactive compounds with potential insecticidal properties. Three chromatograms (CGMS1, CGMS2, and CGMS3) were interpreted based on their retention times (RTs), mass fragment ions (m/z), and comparison with literature data and known spectral patterns. Silylation was used to enhance compound volatility and thermal stability, which enabled detection of polar compounds such as fatty acids, alcohols, phenolics, and limonoids.

Key fragment ions such as m/z 286, 253, 218, 204, 147, 116 in CGMS1 and CGMS2 are consistent with TMS-derivatized fatty acids and alcohols like palmitic acid, phytol, and linoleic acid. CGMS3, which spanned a broader RT range (7.6–25.7 min), showed ions m/z 144, 174, 203, 214, indicative of flavonoids, limonoids, and other oxidized terpenoids. These interpretations are summarized in Table 4, which outlines the retention time ranges, dominant ions, likely compound classes, and corresponding biological activities.

Table 4: Identification of GC-MS main peaks after Silylation.

RT Range (min)	Dominant m/z	Silylated Compounds	Functional Group	Bioactivity
17.4–27.7	286, 253, 218, 204, 147	Palmitic acid-TMS, Phytol-TMS	–COOH, –OH	Insecticidal, repellent
7.6–15.6	204, 147, 116	Methyl linoleate-TMS, phenol-TMS	–OH, –COOH	Larvicidal, antioxidant
15–25	203, 174, 144	Limonoids, flavonoid-TMS	Multiple (–OH, –COOH)	Antifeedant, growth inhibitor

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Discussion

The GC-MS profiles confirm the presence of various bioactive compounds in *Melia azedarach* methanolic extract that are widely recognized for their insecticidal and repellent activities. For instance, palmitic acid, methyl linoleate, and linoleic acid have been reported as effective larvicides and insect growth regulators (Pavela et al., 2016; Al-Rajhy et al., 2003). The detection of phytol and caffeic acid further supports the insecticidal potential due to their antioxidant, repellent, and antimicrobial actions (Mossa et al., 2014; Jeyasankar et al., 2012).

A detailed list of the detected compounds, including their PubChem CIDs, retention time ranges, biological relevance, and docking suitability, is presented in Table 5. These compounds form a strong chemical basis for downstream molecular docking studies and bioassay validation against target insect species.

Table 5: Bioactive Compounds Identified from GC-MS (Parent Forms)

#	Compound Name	PubChem CID	Detected via RT / m/z	Biological Activity
1	Palmitic acid	985	RT ~20–25 min / m/z 286 (TMS)	Insecticidal, ovicidal, repellent
2	Phytol	5280435	RT ~23–26 min / m/z 296 (TMS)	Larvicidal, antimicrobial, repellent
3	Linoleic acid	5280450	RT ~20–24 min / m/z 294 (TMS)	Repellent, larvicidal, cytotoxic
4	Caffeic acid	689043	RT ~12–15 min / m/z 342 (diTMS)	Antioxidant, larvicidal, anti-feedant
5	Methyl palmitate	8182	RT ~19–22 min / m/z 270–286 (TMS)	Larvicidal, toxic to adults
6	β-Sitosterol	222284	RT ~27+ min / m/z ~414 (TMS)	Ovicidal, growth inhibitor
7	Methyl linoleate	5284421	RT ~17–21 min / m/z ~296	Larvicidal, mosquito control agent
8	Campesterol	173183	RT ~26–28 min / m/z ~400+ (TMS)	Antifeedant, hormonal mimic
9	Stigmasterol	5280794	RT ~28 min / m/z ~412+ (TMS)	Antifeedant, growth regulator

3 GC-MS Results of Methanolic Extract

GC-MS analysis of the *Melia azedarach* methanolic extract revealed several bioactive compounds. Three chromatograms (CGMS1–CGMS3) exhibited characteristic retention times and mass fragment patterns consistent with silylated fatty acids, alcohols, phenolics, flavonoids, and limonoids (see Table 6).

Notable fragment ions—m/z 286, 253, 204, 147, 116—observed in CGMS1 and CGMS2 correspond to TMS-derivatized palmitic acid, phytol, and linoleic acid. CGMS3 showed ions m/z 144, 174, 203, 214, suggesting the presence of silylated flavonoids or limonoids derivatives (Table 6).

Table 6: Identification of GC MS Peaks after Silylation.

RT Range (min)	Dominant m/z	Likely Silylated Compounds	Functional Group	Bioactivity
17.4–27.7	286, 253, 218, 204, 147	Palmitic acid-TMS, Phytol-TMS	–COOH, –OH	Insecticidal, repellent
7.6–15.6	204, 147, 116	Methyl linoleate-TMS, phenol-TMS	–OH, –COOH	Larvicidal, antioxidant
15–25	203, 174, 144	Limonoids, flavonoid-TMS	–OH, –COOH	Antifeedant, growth inhibitor

Identified compounds are well-supported in insecticidal research:

- **Palmitic acid**, shown to interact with mosquito sterol carrier protein-2 (SCP-2), has been implicated in disrupting lipid transport and causing larval mortality (Radek et al., 2010; Mosquitocidal study).
- **Phytol**, a diterpene alcohol, has proven larvicidal and repellent effects in *Aedes aegypti* larvae (Renjana & Thoppil, 2013; phytocompounds review).
- **Linoleic acid** has been reported to exhibit potent larvicidal activity, with LC_{50} values around 18 ppm (Rahuman et al., 2008)
- **Caffeic acid**, confirmed to impair larval growth and antioxidant status in *Spodoptera litura*, supports the insect-growth inhibitor profile (Punia et al., 2023).

4 Bioinformatic Analysis

To assess the insecticidal potential and molecular properties of selected phytochemicals, a comprehensive bioinformatic analysis was conducted, beginning with the physicochemical characterization of each ligand. Table 7 summarizes key parameters.

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Table 7: Ligand Physicochemical Properties and Reported Bioactivities

Ligand	Molecular Weight	LogP	Solubility	Bioactivity	Reference
Palmitic acid	256.42 g/mol	7.17	Practically insoluble in water (0.04 mg/L at 25 °C); soluble in ethanol, acetone, benzene	Moderate insecticidal activity (non-specific)	Abdul Rahuman et al. (2008)
Phytol	296.5 g/mol	8.2	Practically insoluble in water; soluble in ethanol	Larvicidal effect on stored-product pests	Benllia et al. (2020)
Linoleic acid	280.4 g/mol	7.05	Insoluble in water (1.59 mg/L at 25 °C); freely soluble in organic solvents	Larvicidal activity against *Spodoptera littoralis* (LC_{50} = 4.78–9.11 g/100 mL)	Yousef, El-Lakwah, & El Sayed (2013)
Caffeic acid	180.16 g/mol	1.2	Sparingly soluble in cold water, freely soluble in hot water and alcohol	Activity against *Spodoptera litura* (LC_{50} = 3125 ppm)	Punia et al. (2023)
Methyl palmitate	270.5 g/mol	7.9	Insoluble in water; highly soluble in ethanol, acetone, ether	Acaricidal activity against *Tetranychus cinnabarinus* (LC_{50} = 1.7 mg/mL)	Wang et al. (2009)
β-Sitosterol	414.7 g/mol	9.3	Very low water solubility (~10 mg/mL)	Larvicidal potential against *Aedes aegypti*, *Culex quinquefasciatus*	Abdul Rahuman et al. (2008)
Methyl linoleate	294.5 g/mol	6.9	Insoluble in water (~0.02 mg/L at 25 °C); soluble in organic solvents	Not available	—
Campesterol	400.7 g/mol	8.8	Insoluble in water (≈0.00015 mg/L at 25 °C); soluble in organic solvents	Not available	—
Stigmasterol	412.7 g/mol	8.6	Very soluble in benzene, ethyl ether, and ethanol	Not available	—

5 Docking Grid Analysis and Ligand-Receptor Interaction Zones

The ligand docking coordinates revealed distinct clustering patterns based on molecular properties. Table 8 summarizes the grid center values (X, Y, Z) for each ligand-protein complex.

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Table 8: Docking Grid Centers for Ligands Docked with 5V13

File	Ligand	Protein Target	Center_X	Center_Y	Center_Z
combined_model19.pdb	Palmitic acid	5V13	171.986	-3.608	257.3
combined_model20.pdb	Phytol	5V13	163.659	-2.661	240.74
combined_model21.pdb	Linoleic acid	5V13	169.918	-3.604	254.549
combined_model22.pdb	Caffeic acid	5V13	203.621	-4.273	305.132
combined_model23.pdb	Methyl palmitate	5V13	169.22	-3.553	253.193
combined_model24.pdb	β-Sitosterol	5V13	147.516	-3.093	221.376
combined_model25.pdb	Methyl linoleate	5V13	167.19	-3.505	250.569
combined_model26.pdb	Campesterol	5V13	149.626	-3.13	224.529
combined_model27.pdb	Stigmasterol	5V13	148.96	-3.123	223.452
combined_model37.pdb	Palmitic acid	5V13	11.532	9.371	10.565
combined_model38.pdb	Phytol	5V13	12.079	9.547	8.377
combined_model39.pdb	Linoleic acid	5V13	11.251	9.263	10.475
combined_model40.pdb	Caffeic acid	5V13	12.92	10.713	12.074
combined_model41.pdb	Methyl palmitate	5V13	11.368	9.249	10.429
combined_model42.pdb	β-Sitosterol	5V13	9.824	8.307	9.338
combined_model43.pdb	Methyl linoleate	5V13	11.073	9.179	10.374
combined_model44.pdb	Campesterol	5V13	9.957	8.408	9.462
combined_model45.pdb	Stigmasterol	5V13	9.948	8.37	9.407

6 Molecular Docking Results

Molecular docking results are depicted in Table 9. Binding affinity analysis further revealed key differences in molecular interactions among the tested ligands. The most potent binders were stigmasterol (-13.9 kcal/mol), β-sitosterol (-12.62 kcal/mol), and campesterol (-11.5 kcal/mol), indicating strong interaction energy with the protein target (5V13). These sterols share a planar sterol backbone conducive to hydrophobic binding and likely interact within deep lipidic pockets.

Moderate binders included linoleic acid (-7.8 kcal/mol) and caffeic acid (-7.5 kcal/mol), with caffeic acid's relatively low number of torsions (n=5) suggesting a favorable entropic contribution. Methyl linoleate, methyl palmitate, and phytol also showed mid-range binding energies (-7.2 to -6.3 kcal/mol), reflecting balanced hydrophilic–lipophilic interaction profiles.

Notably, palmitic acid consistently showed the weakest binding energy (-4.3 kcal/mol in both poses), despite a flexible alkyl chain. This suggests limited electrostatic and hydrogen bonding opportunities with the receptor site.

Interestingly, methyl linoleate yielded a slightly positive docking score (0.1 kcal/mol) in one pose, possibly due to an unfavorable conformation or steric clashes—highlighting the need for experimental confirmation.

These findings align with earlier studies indicating phytosterols and unsaturated fatty acid esters as promising bioinsecticidal agents (Benllia et al., 2020; Abdul Rahuman et al., 2008).

Table 9: Binding energies and torsional flexibility of ligands docked with 5V13 and 3N7H protein targets.

Ligand	Protein Target	Binding Energy (kcal/mol)	Number of Torsions
Palmitic acid	5V13	-4.3	15
Phytol	5V13	-6.2	14
Linoleic acid	5V13	-6.5	15
Caffeic acid	5V13	-7	5
Methyl palmitate	5V13	-5	15
β-Sitosterol	5V13	-12.62	7
Methyl linoleate	5V13	0.1	15
Campesterol	5V13	-11.5	6
Stigmasterol	5V13	-13.9	6
Palmitic acid	3N7H	-4.3	15
Phytol	3N7H	-6.3	14
Linoleic acid	3N7H	-7.8	15
Caffeic acid	3N7H	-7.5	5
Methyl palmitate	3N7H	-7.2	15
β-Sitosterol	3N7H	-9.2	7
Methyl linoleate	3N7H	-7.3	15
Campesterol	3N7H	-7.6	6
Stigmasterol	3N7H	-9.5	6

Conclusion

Conclusion and perspective

This research aimed to evaluate the insecticidal potential of *Melia azedarach* through an integrated chemical and computational approach. The main objectives were to extract and identify bioactive compounds from the plant using GC-MS and to predict their interaction with insect-specific protein targets via in silico molecular docking.

To achieve this, methanolic extracts of *Melia azedarach* fruits were obtained and subjected to gas chromatography–mass spectrometry, which revealed a diverse range of bioactive compounds, including fatty acids, flavonoids, and phytosterols. These compounds were then analyzed using molecular docking techniques to assess their potential binding affinities with key insect proteins such as Odorant Binding Proteins (OBP) and Juvenile Hormone Esterase (JHE), which are essential for insect development and behavior.

The study stands out for its originality in combining phytochemical profiling with predictive bioinformatics to identify natural compounds with insecticidal potential. This multidisciplinary methodology contributes to the broader effort of developing environmentally sustainable alternatives to chemical pesticides.

In conclusion, this work lays a solid foundation for further experimental validation and formulation studies. Future research could focus on in vivo bioassays, toxicity assessments, and the development of bioinsecticide formulations derived from *Melia azedarach*, contributing to effective and eco-friendly pest management strategies.

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Appendices

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Appendices

1.Solvents and Chemicals

Table 10: Laboratory glassware and equipment

Equipment	Use
Round-bottom flask (1 L)	For Soxhlet extraction
Complete Soxhlet apparatus	Continuous extraction via reflux
Beakers (500 mL)	For aqueous suspensions and intermediate handling
Separatory funnel	Liquid-liquid partitioning
Rotary evaporator	Concentration under reduced pressure
Drying trays	Shade-drying of fruits
Amber glass bottles with Teflon caps	Storage of solvent fractions and extracts
Mortar and pestle / Mechanical grinder	Grinding of dried fruits
40 mesh sieves	Granule size homogenization
Magnetic stirrer or glass rod	Formation of homogeneous suspension

Table 11: Solvents and Chemicals Employed

Solvent / Product	**Specific Use**
Methanol	Extraction of polar and semi-polar metabolites
Hexane	Extraction of non-polar lipophilic compounds (terpenoids, sterols)
Ethyl acetate	Extraction of semi-polar compounds (flavonoids, esters)
n-Butanol	Extraction of polar compounds (glycosides, alkaloids, saponins)

Table 12: Tools for Molecular Docking

Tools	**Specific Use**
Avogadro	draw, optimize, and save molecules before docking.
chimera	Add charges and hydrogens, prepare ligands and proteins.
AutoDock Vina	Perform docking simulations to predict binding affinity

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For the Completion of the Master's Degree in Biological Sciences

Specialty: Biochemistry

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Presented by: Sidiachour Thara

Jury Members:

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Academic Year 2025 / 2026