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Antioxydant activity and insecticidal effect of *Ziziphus lotus* hydro-ethanolic extract against mosquito species

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بسم الله الرحمن الرحيم

Acknowledgments

At the end of our work, we would like to express our most sincere thanks to God for the grace of health, patience and perseverance that He granted us to complete this dissertation.

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Our warmest thanks go first and foremost to our supervisor, Dr. Seghier Hanane, for her concern, patience, sound advice and cooperation throughout this period.

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Finally, on a more personal note, we would like to express our gratitude to our parents, who have always believed in us and supported us in this dissertation project and throughout our many years of study.

Dedication

First of all, I thank God for everything that I have achieved in my life, including the completion of this memorandum.

I dedicate this modest work to my parents:

My dear father who supported me from a young age, and the best mother for always supporting and stand with me, and all my family members, my brother Mouhamed Islem and Ismail to whom I dedicate my success, which they had a part in.

I thank my friends who are like brothers to me and I wish them the same success

Rania

ملخص

لطالما لعبت النباتات دورًا محوريًا في المجالين الطبي والبيئي، بفضل قدرتها على إنتاج مركبات عضوية فعالة بيولوجيًا .هدفت هذه الدراسة إلى تقييم الخصائص المضادة للأكسدة، والمضادة لمرض السكري، والمبيدة للحشرات في المستخلص المائي-الإيثانولي لأوراق نبات *السدرة*

بلغ مردود المستخلص المائي-الإيثانولي للجزء الهوائي من*نبات السدرة* نسبة.21.17%

تُظهر نتائج النشاط المضاد للأكسدة فعالية ملحوظة للمستخلص، وإن كانت أقل من فيتامين C(حمض الاسكوربيك)
حيث بلغت قيمة EC50 لنبات السدرة حوالي 56.17 **ملغ/** مل مقابل 12.57 **ملغ/** مل لفيتامينC وذلك لتثبيط الجذور الحرة باستخدام اختبار DPPH.

أما في ما يخص النشاط المضاد لمرض السكري، فقد أظهر المستخلص فعالية تفوقت على الدواء المرجعي الميتفورمين
حيث بلغت IC50 للمستخلص7.70 **ملغ/** مل مقارنة بـ 83.46 **ملغ/** مل للميتفورمين، ما يشير إلى إمكانية مساهمته في تنظيم مستويات السكر في الدم.

وفي ما يتعلق بالنشاط المبيد للحشرات، أظهر المستخلص فعالية كمبيد يركي طبيعي ضد *Culiseta longiareolata*، وهي بعوضة منتشرة في الجزائر، خصوصًا في منطقة تبسة، حيث زادت نسبة الوفيات اليرقية بزيادة التركيز وفترة .التعرض

تُبرز هذه النتائج الإمكانات البيولوجية المتنوعة لمستخلص*السدرة*، مما يؤهله ليكون مصدرًا طبيعيًا واعدًا في الاستخدامات الطبية والبيئية.

الكلمات المفتاحية:

Ziziphus lotus، المستخلص المائي الإيثانولي المائي، ، النشاط المضاد لمرض السكري ، النشاط المضاد للأكسدة, النشاط المبيد للحشرات.

Abstract

Plants have always played a vital role in both medical and environmental fields due to their ability to produce biologically active organic compounds. This study aimed to evaluate the antioxidant, antidiabetic, and larvicidal properties of the hydro-ethanolic extract of *Ziziphus lotus* leaves.

The extraction yield of the aerial part of *Ziziphus lotus* was 21.17%.

The antioxidant results showed significant activity, although lower than that of Vitamin C (ascorbic acid). The EC50 value for *Ziziphus lotus* was 56.17 µg/mL, compared to 12.57 µg/mL for Vitamin C, using the DPPH assay to measure free radical scavenging capacity.

For the antidiabetic activity, the extract demonstrated greater effectiveness than the reference drug metformin, with an IC50 of 7.70 mg/mL, compared to 83.46 mg/mL for metformin, suggesting potential for blood sugar regulation.

Regarding larvicidal activity, the extract showed promising natural insecticidal effects against *Culiseta longiareolata*, a mosquito species commonly found in Algeria, especially in the Tebessa region. Larval mortality increased with higher extract concentrations and longer exposure times.

These results highlight the diverse biological potential of *Ziziphus lotus* extract, making it a promising natural source for medical and environmental applications.

Key words: *Ziziphus lotus*, Extrait hydro-ethanolique, Larvicidal activity, Antidiabetic activity, Antioxidante Activity.

Résumé

Les plantes ont toujours joué un rôle central dans les domaines médical et environnemental, grâce à leur capacité à produire des composés organiques biologiquement actifs. Cette étude visait à évaluer les propriétés antioxydantes, antidiabétiques et insecticides de l'extrait hydro-éthanolique des feuilles de *Ziziphus lotus*. Le rendement de l'extrait hydro-éthanolique de la partie aérienne de *Ziziphus lotus* était de 21,17%.

Les résultats de l'activité antioxydante ont montré une efficacité notable de l'extrait, bien qu'inférieure à celle de la vitamine C (acide ascorbique), avec une valeur de EC50 de 56,17 µg/mL contre 12,57 µg/mL pour la vitamine C, en utilisant le test DPPH pour l'inhibition des radicaux libres.

Concernant l'activité antidiabétique, l'extrait a démontré une efficacité supérieure à celle du médicament de référence, la metformine, avec une IC50 de 7,70 mg/mL contre 83,46 mg/mL pour la metformine, suggérant un potentiel dans la régulation de la glycémie.

Pour ce qui est de l'activité insecticide, l'extrait a montré une efficacité en tant qu'insecticide naturel contre Culiseta longiareolata, un moustique largement répandu en Algérie, notamment dans la région de Tébessa. Le taux de mortalité larvaire augmentait avec la concentration et la durée d'exposition.

Ces résultats mettent en évidence le potentiel biologique diversifié de l'extrait de *Ziziphus lotus*, en faisant une source naturelle prometteuse pour des applications médicales et environnementales.

Mots clés: *Ziziphus lotus* Extrait hydro-ethanolique, Activité insecticide, Activité antidiabétique, Activité antioxydante

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List of abbreviations

Abbreviation	**Meaning**
DPPH	2,2-diphenyl-1-picrylhydrazyl
IC50	Inhibitory Concentration at 50%
Z. lotus	*Ziziphus lotus*
AA	Antioxidant Activity
LA	Larvicidal Activity
C. longiareolata	*Culiseta longiareolata*
UV-Vis	Ultraviolet–Visibl Spectrophotometry
Ppm	Parts Per Million
LC50	Lethal Concentration for 50% of larvae

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Introduction

1. Introduction

Since the dawn of human civilization, plants and plant-based products have been widely used as medicine. Today, approximately 75% to 80% of the global population in developing countries relies on herbal remedies for primary healthcare, largely because of their cultural acceptance and minimal side effects (Khanal *et al*., 2020). Scientific research on medicinal plants has significantly contributed to the development of new therapeutic approaches focused on bioactive compounds (Bezerra *et al*., 2022). These compounds include alkaloids, which exhibit antitumor and antiviral activities; flavonoids, known for their anti-inflammatory, antioxidant, antiallergic, and anticancer properties; tannins, used in treating high blood pressure as well as fungal and bacterial infections and burns; saponins, which have antiviral effects and interact with cell membranes; and steroids/triterpenes, natural agents with anti-inflammatory effects (Amanda *et al*., 2020).

These bioactive compounds have increasingly attracted research attention due to their potential in combating various diseases such as cancer, atherosclerosis,cerebrovascular disorders, diabetes, hypertension, and Alzheimer's disease. Many physiological and biochemical processes generate oxygen-centered free radicals and other reactive oxygen species. Antioxidants play a crucial role in neutralizing these free radicals, which can damage important biological macromolecules like DNA, proteins, and lipids within cells and tissues (Kougnimon *et al*., 2018).

Ziziphus lotus, commonly known as jujube or "sedra," is a medicinal plant widely used in traditional medicine across North Africa and the Mediterranean region. Several studies have reported its rich phytochemical composition, including flavonoids, saponins, alkaloids, and phenolic compounds, which contribute to its various biological activities (Boulila *et al*., 2015; Djeridane *et al*., 2006).

These findings highlight the potential of *Ziziphus lotus* as a source of natural antioxidant, antidiabetic, and insecticidal agents with applications in both pharmaceutical and environmental health fields.

Mosquitoes are among the most dangerous insects to human health, as they are responsible for transmitting life-threatening diseases and causing millions of deaths each year (Ghosh *et al*., 2011). The most common mosquito genera serve as vectors for a wide range of illnesses, such as dengue fever, chikungunya, malaria, yellow fever, filariasis, Japanese

encephalitis, Lyme disease, and epidemic polyarthritis (Kalita *et al*., 2013).

Interestingly, only female mosquitoes bite humans, as they require blood for egg development, while males feed on nectar and do not transmit diseases (Dahchar *et al*., 2016).

To control mosquito populations, synthetic insecticides are commonly used, these chemicals, often adapted from agricultural use, belong to several major classes, including organophosphates, pyrethroids, organochlorines, carbamates, and cyclodienes (McAllister and Adams, 2010).

However, these chemicals have caused environmental contamination and side effects to non-target organisms. Moreover, mosquito control techniques pose serious threats due to the emergence of resistance to widely used synthetic insecticides (Ouma and Russell, 2013; Chirchir *et al*., 2013).

Therefore, there is an urgent need to develop new insecticides that are biodegradable and environmentally safe (Pavela *et al*., 2014).

A recent and alternative approach to mosquito control is the use of natural products of plant origin, known as botanical derivatives.

Many of these botanical products are effective, environmentally friendly, easily biodegradable, and inexpensive. They have no negative effects on non-target organisms and possess varied and novel modes of action (George *et al*., 2014).

In this study, we explored the potential of a hydro-ethanolic extract from *Ziziphus lotus* by evaluating three key biological activities: antioxidant, antidiabetic, and larvicidal. The antioxidant analysis aimed to assess the plant's ability to combat oxidative stress by neutralizing free radicals—harmful molecules linked to cell damage and various chronic diseases. We also examined its antidiabetic effect, focusing on how the extract might help regulate blood sugar levels and support metabolic balance. Finally, we tested its larvicidal activity against *Culiseta longiareolata*, a mosquito species of particular concern in Algeria.

Materiel and Methods

2. Materiel and Methods

2.1. Geographical distrubtion of *Ziziphus lotus*

Ziziphus lotus is a drought-resistant shrub native to North Africa, where it is widely distributed across Morocco, Algeria, Tunisia, and Libya (Le Floc'h *et al*., 2010). According to Ghedira (2013), *Ziziphus lotus* (L.) Desf. is a Mediterranean species with limited penetration into the northern Sahara: Morocco, Algeria, Tunisia, and Libya. It then reappears in Yemen, the island of Socotra, the Middle East: Palestine, Syria, Turkey, and Cyprus. It is also found in Greece, Sicily, and southern Spain. In Algeria, *Ziziphus lotus* L. is very widespread in the arid regions of southern Algeria, including Ain Ouessara and Maessad (Djelfa province) with an arid climate, and Taghit (Bechar province) with a Saharan climate (Mounni, 2008 cited by Benammar, 2011).

2.2. Classification

The taxonomic classification of *Ziziphus lotus* reflects its placement within the broader context of flowering plants. It is a member of the family Rhamnaceae, which includes numerous species adapted to dry environments. As part of the order Rosales, *Ziziphus lotus* shares morphological and genetic traits with other dicotyledonous plants. Its classification is based on morphological characteristics such as leaf arrangement, flower structure, and fruit type, as well as molecular data from recent phylogenetic studies. The full taxonomic hierarchy of *Ziziphus lotus* is summarized in the table below (Mabberley, 2017).

According to Ghedira, 2013, *Ziziphus lotus* are classified in the following taxon:

Table 01: Classification of *Ziziphus lotus* (Ghedira,2013)

Kingdom	Plantae (Plant Kingdom)
Phylum	Magnoliophyta (Angiosperms)
Subphylum	Magnoliophytina (Angiosperms)
Class	Magnoliopsida (Dicotyledons)
Subclass	Rosidae
Order	Rhamnales
Family	Rhamnaceae
Genus	*Zizyphus*
Species	*Zizyphus lotus* L

2.3. Botanical description of *Ziziphus lotus*

Ziziphus lotus is a very thorny, slow-growing shrub that can reach a height of 10 meters and a trunk diameter of 50 to 60 cm. It has a rounded shape, twisted branches, green thorny branches, and cracked bark. The branches grow in a zigzag pattern with slender, flexible, branched, tapered, and thorny stems. There are two types of thorns associated in pairs, some being 2.5 cm long, and others being short and easily curving (Mahajan, 2009).

The leaves are small (about 8 x 15 millimeters), short, and oval-oblong, more or less elliptical, measuring 1 to 2 cm in length and 7 mm in width (Bayer and Butter, 2000). They are smooth and shiny on both sides and have three prominent longitudinal veins starting from the petiole (Baba Aissa, 1999). The thorns are stipular and dissimilar; one is large, more or less straight, and directed upwards, while the other is smaller and curved like a hook downwards.

The flowers of *Z. lotus* are very noticeable, yellow in color, with star-shaped sepals, small petals, and a superior bisexual ovary, blooming in June (Baba Aissa, 1999; Nour and Dilmi, 2017). The fruit is oval-elongated, resembling the size and shape of a nice olive, initially green, then yellow, and turns dark red (or orange-brown) when ripe, in October (Bayer and Butter, 2000). The fruit of the jujube is slightly sweet. The seeds, which are more often detached from the pulp, can be up to 1.05 cm long (El Aloui *et al*., 2010).

Z. lotus has a more sparse root system compared to the apple or peach tree (Mengjun, 2006). Thus, this shrub grows very slowly and begins to bear fruit around the age of 4. It can continue to produce fruit for about 20 to 25 years (Maraghni *et al*., 2011). (Figure 1)



Figure 1: *Ziziphus lotus* (1) – Leaves; Flowers (2), Fruits (3).

2.4. Chemical Composition of *Ziziphus lotus*

2.4.1. Terpenes

These are hydrocarbons formed by the assembly of two or more isoprene units (Belhachat,

2019). They constitute a vast group of distinct natural metabolites, many of which have been discovered in plants. The terpenes discovered and isolated from plants have been widely used in food, cosmetics, pharmaceuticals, and various biotechnological applications (Jiang *et al*., 2016).

2.4.2. Alkaloids

Alkaloids are basic compounds containing nitrogen of natural origin, where the nitrogen atom is part of a complex chemical ring. They have a notable pharmacological activity. Additionally, alkaloids have the particularity of reacting with heavy metal salts, thus facilitating their identification using reagents such as Mayer and Dragendorf (Krief, 2003).

2.4.3. Flavonoids

Are natural polyphenolic compounds. They are considered to be almost universal plant pigments. pigments in plants. Structurally, they are generally in free form or in the form of aglycoside. They are found in various plant organs such as roots, stems, wood, leaves, flowers and fruit (Marfak. 2003). (Figure 2)

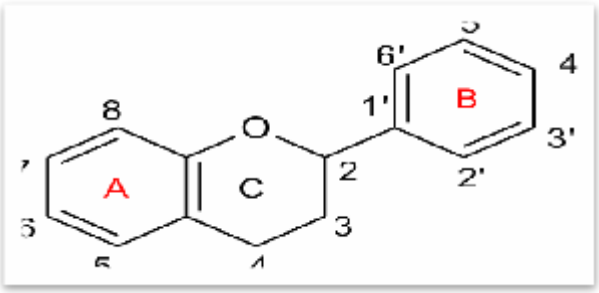


Figure 2: Basic structure of flavonoids **(Harborne, 2000)**

2.4.4. Tannins

Tannins are highly hydroxylated compounds that encompass a wide diversity of chemical structures. These phenolic compounds from higher plants are secondary metabolites with molecular weights up to 20,000 Da, and they share the ability to form complexes with carbohydrates and proteins. They are found either dissolved in the vacuolar sap of certain parenchymal cells or in cells such as idioblasts. They are widely distributed in plant-based foods, particularly in legumes, cereals, fruits, nuts, and vegetables, as well as in various beverages such as wine, tea, cocoa, nuts, and cider (Montes-Ávila *et al*., 2018; Hassanpour *et al*., 2011). (Figure 3)

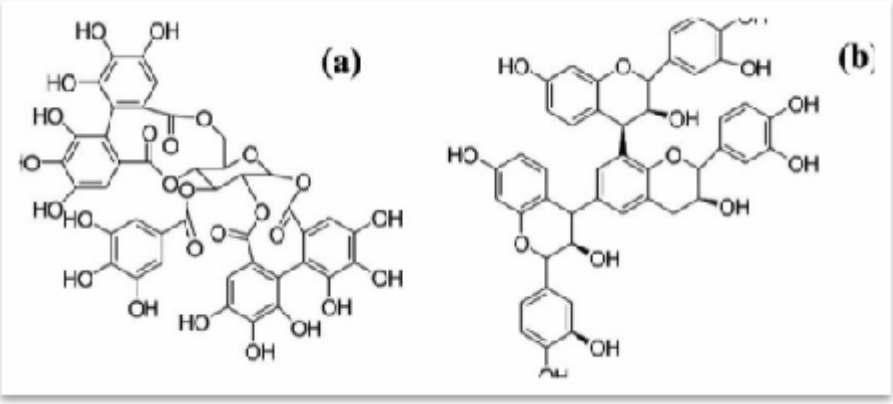


Figure 3: Structure of (a) hydrolysable (b) condensed tannins**.** (Naczk, 2006)

2.5. Uses of the plant

The *Ziziphus lotus* plant has been widely utilized in both traditional practices and medicinal applications.

2.5.1. Traditionally

Its fruits are consumed in various forms: fresh, dried, in preserves, candied, as jam, in liqueur, or as paste. Dried jujubes are especially valued for their long shelf life and are commonly used in confectionery, as well as in pharmacy (Catoire *et al*., 1999). In folk remedies, a mixture of its wood ashes and vinegar was once applied to treat viper bites, while the bark, shoots, and leaves were known for their astringent properties. The powder made from dried leaves and fruits is used in the treatment of boils (Borgi *et al*., 2007), and its fruits are considered soothing, especially for throat and bronchopulmonary issues. Additionally, its seeds are crushed to extract a high-quality oil, and the honey produced from its flowers is highly esteemed for its quality

2.5.2. Medically

Ziziphus lotus is recognized for its abundance of vitamins (A, C, and E) and polyphenols, making it medically useful for potential use in modern phytomedicine. While the therapeutic uses of *Z. lotus* have been documented. More, recent studies establish *Z. lotus* helpful in respiratory health, especially as part of the formulation of cough syrups and throat irritations due to the anti-inflammatory and antimicrobial properties (Benammar *et al*., 2020). Apart from respiratory health, *Z. lotus* also has a wide range of pharmacological activity, with antidiabetic, hepatoprotective, and antimicrobial effects proven by in vitro and in vivo studies (Boukeloua *et al*., 2021). Traditional applications of *Z. lotus* for

diabetes treatments have been clinically supported; root bark extract of *Z. lotus* had appreciable hypoglycemic activity (Ouelbani *et al*., 2016).

2.6. Phytochemical analysis

To identify the different groups of chemical compounds, present in a plant, chemical screening was carried out. Colorimetry and gravimetry were the two main methods used to identify these groups of substances in solution. The Alkaloids, Flavonoids, Saponins, Anthocyanins, Leucoanthocyanins, Tannins, Terpenes and Sterols contained in the plant were identified using the methods described by Harborne (1998).

2.6.1. Tannins

Non-hydrolysable catechic tannins were identified using Stiasny's reagent. 5 ml of the infusion was evaporated to dryness. After adding 15 ml of Stiasny's reagent to the residue, the mixture was kept in a water bath at 80°C for 30 min. The observation of an orange precipitate characterises the catechic tannins.

Gallic tannins, which are hydrolysable, were identified by adding FeCl3. We filtered the previous solution and saturated it with sodium acetate. The addition of 3 drops of 2% FeCl3 produced an intense blue-black hue, indicating the presence of gallic tannins.

2.6.2. Saponins

A 2% decoctate is prepared with 2 g of powder in 100 ml of boiling water and boiled for 30 minutes. the final volume being made up to 10 ml with distilled water. Each tube was shaken for 15 seconds and then left to stand for 15 minutes in an upright position. A persistent foam height superior to 1 cm indicates the presence of saponins.

2.6.3. Flavonoids

10 g of the powder was macerated in 150 ml of 1% HCl for 24 h. NaOH was added to 10 ml of the filtrate to make the solution basic. The appearance of a light yellow colour in the upper part of the tube after 3 hours indicates the presence of flavonoids.

2.6.4. Alkaloids

The presence of alkaloids is established by salt precipitation and revelation using Mayer's reagent. To 5 g of powder add 50 ml of 1% HCl. After macerating for 3 hours at room

temperature, the macerate was filtered. Take 1 ml of filtrate and add 5 drops of reagent. The presence of alkaloids is shown by the appearance of a white precipitate.

2.6.5. Terpenes and steroids

To identify steroids and polyterpenes, we used the Liebermann reagent. 5g of leaf powder was macerated in 20ml of petroleum ether, filtered and then evaporated to dryness in a sand bath at 90°C. The residue was hot triturated in 1 ml acetic anhydride. We added 0.5 ml of concentrated sulphuric acid to the triturate. The appearance of a purple and violet ring at interphase, turning blue and then green, indicates a positive reaction.

2.6.6. Quinones

Moisten 5 g of crushed plant material with a few drops of HCl. Macerate the plant material for one hour or 24 hours in a closed Erlen Meyer containing 10 ml petroleum ether. After filtration, 2 ml of filtrate is stirred with 2 ml of 10% NaOH. A red to violet coloration appeared in the presence of quinones.

2.7. Preparation of the extract

2.7.1. Preparation of hydro-ethanolic extract from Sidr (*Ziziphus lotus*) leaves:

To prepare the extract, we started by mixing ethanol with distilled water in a 1000 mL beaker, stirring well to ensure even blending. We then added dried Sidr (*Ziziphus Lotus*) leaf powder and used a mechanical agitator to stir the mixture, helping to extract the plant's bioactive compounds.

After stirring, we filtered the mixtures using filter paper to remove solid residues, then transferred the filtered extracts to a rotary evaporator set at 55°C to remove the solvents and concentrate the extracts.

Finally, the refined extracts were placed in a drying oven ensuring complete drying and preservation before further testing and analysis. (Figure 4)

The yield of plant extract is the ratio between the weight of the extract and the weight of the dry matter of the plant, evaluated from 4 extractions, it is expressed as a percentage and calculated by the following formula: $Y = (We/Wp) \times 100$

Y = yield of the extract in%

We = Weight of the extract in g

Wp = Weight of the dry matter of the plant in g



Figure 4: Steps of extraction

2.8. Activity antioxydante

The most frequently mentioned characteristic of polyphenols, and of plant phenolic compounds in general, is undoubtedly their recognised ability to trap reactive oxygen species (ROS). This so-called antioxidant capacity is frequently cited as being the key property underlying the prevention and/or reduction of chronic diseases linked to oxidative stress and age-related disorders such as cardiovascular disease (e.g. atherosclerosis), carcinogenesis, neurodegeneration (e.g. Alzheimer's disease), as well as skin deterioration, by dietary plant polyphenols and others (Quideau, 2011).

2.8.1. Free Radicals

A free radical can be defined as any molecule or molecular fragment capable of independent existence that has an unpaired electron in an atomic orbital. The presence of an unpaired electron in an atomic orbital gives rise to a number of common traits shared by most radicals. Many radicals are unstable and highly reactive and can either donate limiting electrons from other molecules, and thus act as oxidants or accept limiting electrons (and react with the electron in the radical) from the oxygen atom that provides the initial oxygen bond. The most important free radicals containing oxygen in many disease states include hydroxyl radical, superoxide anion radical, hydrogen peroxide, oxygen singlet, hypochlorite, nitric oxide radical and peroxynitrite radical. These radicals are highly reactive and can damage biologically important molecules, such as DNA, proteins, carbohydrates, and lipids in the nuclei, and membranes of cells. Free radicals target important macromolecules, leading to damage to these cells, and disruption of homeostasis. Free radicals can attack any cells in the body, and groups of important macromolecules including lipids, nucleic acids, and proteins are the major targets of oxidative processes (Lobo, 2010).

2.8.2. Antioxidant activity by measuring anti-free radical activity on DPPH

To assess the antioxidant activity, we used a method based on the reduction of DPPH (1,1-diphenyl-2-picrylhydrazyl), a stable free radical. When DPPH is combined with a substance capable of donating a hydrogen atom, it gets reduced, leading to a visible color change from deep violet to yellow (Molyneux, 2004). (Figure 5)

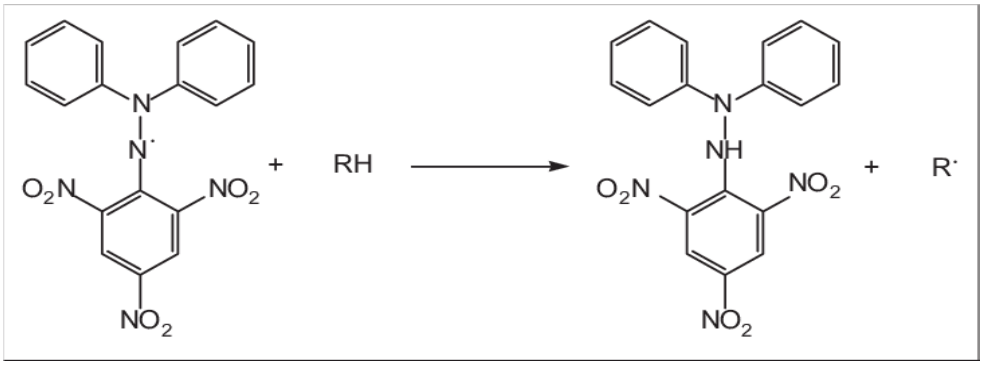


Figure 5: Structure of DPPH and its reduction by an antioxidant (Kunal, 2012)

2.8.3. Dosage method

In our study, this test was evaluated according to the protocol applied by Kuramasamy *et al* (2007). Briefly, 1 ml of a methanolic solution of DPPH (0.2 mM) was mixed with 1ml of different dilutions of plant extracts (0-1 mg/ml). The mixture obtained was then kept protected from light at room temperature for 30 minutes. The absorbance was then measured at 517 nm against a control consisting of 1ml of the DPPH solution and 1ml of methanol. The samples, ascorbic acid and the control were prepared under the same operating conditions. The decrease in absorbance is measured with a spectrophotometer and the % PI (percentage inhibition) is calculated according to the formula below:

$$IP\% = \frac{absorbance\,of\,control - absorbance\,of\,sample}{absorbance\,of\,control} \times 100$$

2.9. Test of the effect of *Ziziphus lotus* extracts on glucose absorption by yeast

Yeast cells were prepared according to the method of Sindhu *et al*. (2013) (modified) with commercial baker's yeast washed by centrifugation (4200 rpm, 5 min) in distilled water (5 ml) until the centrifugation supernatant was clear, made up to 10% (v/v) suspension in distilled water.

Different concentrations of plant extracts (10 to 100 mg/ml) were added to 1 ml glucose solutions (10, 25, and 50 mg/ml), allowed to react for 15 min at 37˚C, before inverting the 100 µl yeast suspension. This was mixed by vortexing and incubated at 37˚C for 60

minutes.

After 1 hour the tubes were centrifuged (2500 rpm, 5 min) and the glucose estimated from supernatants using the iodine reagent (Sinha *et al*, 2013) with metformin as the standard anti-diabetic drug. (Figure 6)

Absorbance was read at 540 nm and the experiments were performed in triplicate.

The percentage increase in glucose uptake by yeast cells was calculated using the formula from Ukwubile and Odugu (2018):

$$\text{Glucose absorption inhibition (\%)} = \frac{(Abs\ extract - Abs\ control)}{Abs\ extract} \times 100$$

- Abs control: absorbance of the control reaction (containing all reagents except the test sample).
- Abs extract: absorbance of the test sample.



Figure 6: Antidiabetic activity dosage

2.10. *Culiseta longiareolata*

2.10.1. Presentation of *Culiseta longiareolata.*

Culiseta longiareolata is a species of mosquito belonging to the Culicidae family and the Culicinae subfamily (Kumar *et al*., 2021). This insect, measuring between 3 and 5 mm in size, thrives in warmer climates and is resistant to complete metamorphosis. It has a small body, long, slender legs, and long, thin, membranous wings (Johnson *et al*., 2020). Identifying this species is relatively straight forward.

This mosquito is considered a vector for avian plasmodia. The larvae are distinguished by a short, conical siphon with irregularly arranged siphuncle teeth. In adult mosquitoes, the presence of at least one dark scale patch on the wing, three longitudinal white stripes on the thorax, and the absence of long, strong bristles on the basal lobe of the gonocoxite are characteristic features. Female mosquitoes of this species may enter diapause during the winter in colder regions, either as adults (imagos) or larvae (in temperate zones). Adults are found year-round, with peak densities occurring in spring and again in autumn

(Bruhnes *et al*., 1999).

2.10.2. Taxonomic position

Table 2: The systematic position of *Cs longiareolata* (Dris, 2019).

Kingdom	Animalia
Sub-kingdom	Metazoa
Phylum	Arthropoda
Sub-phylum	Hexapoda
Super-class	Protostomia
Class	Insecta
Sub-class	Ptrerygota
Infra-class	Neoptera
Super-order	Endopterygota
Order	Diptera
sub-order	Nematocera
Infra-family	Culicomorpha
Family	Culicidae
Genus	*Culiseta*
Species	*Culiseta longiareolata*

2.10.3. Life cycle

Mosquitoes are holometabolous insects whose life cycle includes aquatic and aerial stages (Figure 7) (Dris, 2019).

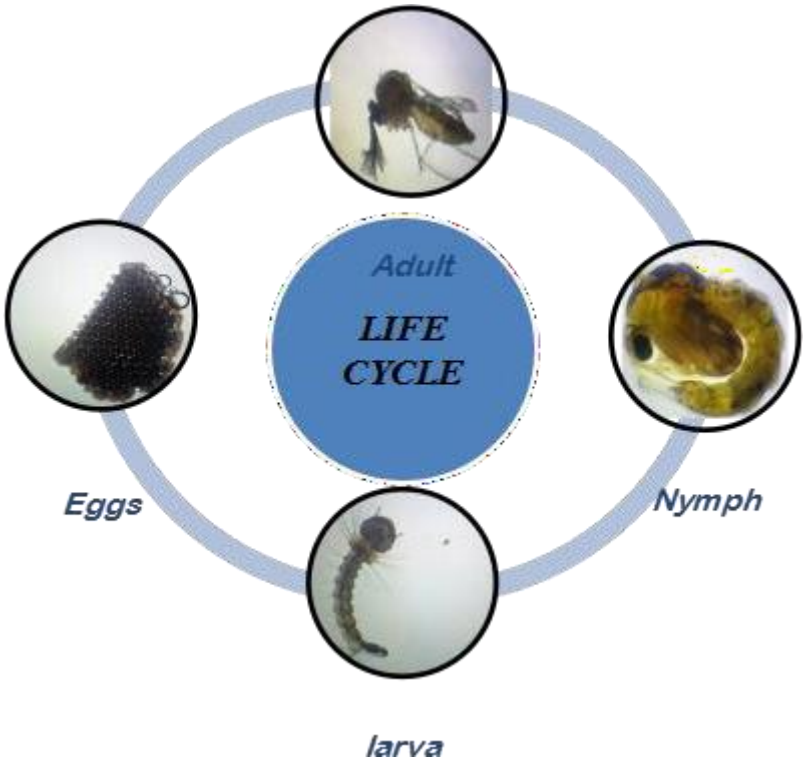


Figure 7: Life cycle of *Culisita langiareolata.*

A. Eggs

Females will deposit their eggs on the surface of multiple aquatic breeding sites (e.g. aquariums, abandoned wells, rock holes, sea, ponds, canals, cisterns, rainwater etc.) where the water is always stagnant and considerably rich in organic matter. The sites can be permanent or temporary, shaded or exposed, filled with fresh or brackish water, clean or polluted (Paul, 2009). The eggs are spindle-shaped and measure from 0.5 to 1 mm in size. The eggs are initially whitish upon oviposition but rapidly darken to black due to oxidation of phenolic compounds in the chorion, a process catalyzed by polyphenol oxidases (PPOs) (KonDo *et al*., 2018; Li *et al*., 2021). (Figure 8).

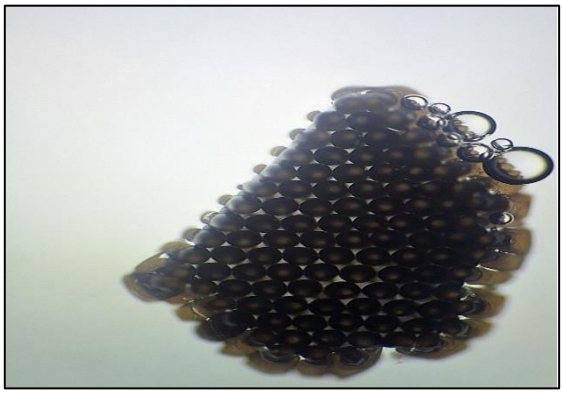


Figure 8: Mosquito eggs

B. Larvae

Mosquito larvae are found in almost all stagnant water bodies. Their habitats are diverse, ranging from surface water to underground shelters, and can exist in both permanent and temporary water sources. These habitats may have natural or artificial characteristics and contain either oligotrophic water (nutrient-poor) or eutrophic water (nutrient-rich). Mosquitoes utilize a wide range of breeding sites, provided they hold water for at least five days, as required by many species (Laëtitia, 2017).

At this stage, larval development is entirely aquatic. The larvae exhibit characteristic wriggling movements and undergo four developmental stages, with sizes ranging from 2 mm to 12 mm (Boulkenafet, 2006). They typically live for about 10 days. The speed of their development influenced by the nutrient content of the water in their breeding site (Peterson, 1980) (Figure 9).

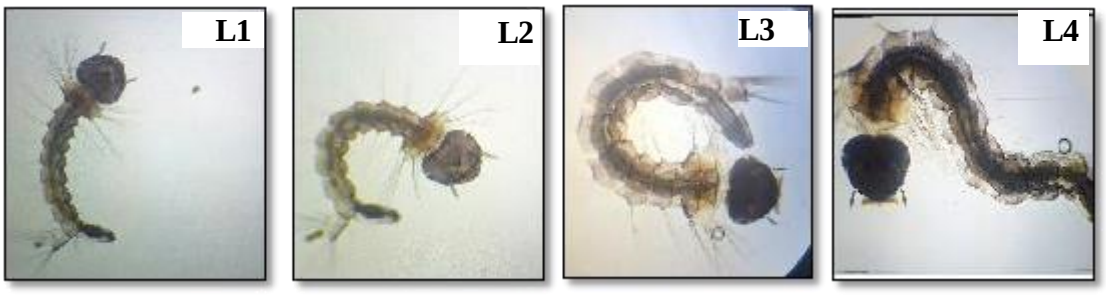


Figure 9: Larval stages of *Culiseta longiareolata*

C. Nymph

After the four larval stages, the pupal or nymphal stage follows, during which metamorphosis allows the insect to transition from the aquatic larval state to the aerial adult state.

Indeed, the larval organs undergo histolysis to form the adult body. The head and thorax of the pupa are fused into a globular cephalothorax, topped with two respiratory trumpets. The segmented abdomen has, at its posterior end, two swimming paddles located on either side of the eighth segment. The pupa is extremely sensitive and dives into the water at the slightest perceived movement (Cachereul, 1997).

This stage is short, lasting no more than four days. The pupa has a comma-shaped form. It does not feed; instead, it relies on the reserves stored during the larval stage (EL Agbani, 1995) (figure 10).

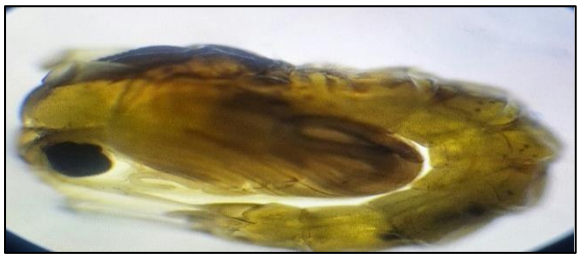


Figure 10: Nymph *culiseta langiarelota*

D. Adult

The adult mosquito, or imago, has an elongated body measuring between 5 and 20 millimeters in length (Rodhain & Perez, 1985). It is generally light brown, with pale anterior stripes on the abdominal tergites. The thorax features three longitudinal white stripes (Bouabida, 2014), and at least one patch of dark scales is present on the wings.

The exoskeleton consists of rigid plates, known as sclerites, which are connected by thin chitinous membranes. Each body segment, or metamer, is structured as a ring composed of three main elements: the tergite (dorsal), the sternite (ventral), and the pleurites (lateral). The mosquito's body is divided into three distinct parts: the head, thorax, and abdomen (Brahmi & Snoussi, 2021)

2.10.4. Collecting and breeding

The mosquitoes larvea are collected from different locations in Tebessa city (1er November, Hammamet). Where we find a dirty water (aquarium, dirty pool). (Figure 12)

The larvea are raised in plastic glasses containing 150 ml of dechlorinated water and feed them with a mixture of 75% biscuits and 25% baking powder. The water is renewed every two days (Dris, 2019). The diet plays a major role in fertility because proteins enable the fimale to lay more eggs than females feed on sugar alone (Wigglesworth, 1972). (Figure 13)



Figure 11: The collect locations

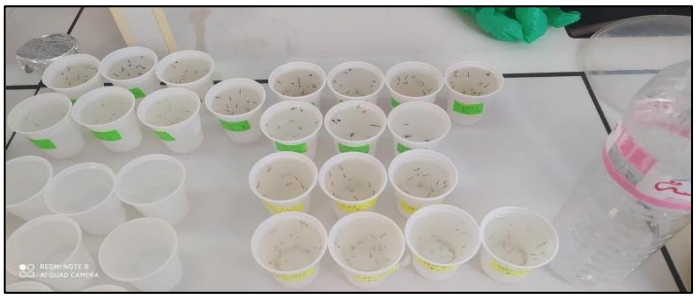


Figure 12: Collection of mosquitoes in the laboratory

2.10.5. Toxicity bioassays

Larval bioassays were carried out to assess the insecticidal potential of hydro-ethanolic extracts from the leaves and fruits of *Ziziphus lotus*. Fourth-instar larvae of *Culiseta longiareolata* were exposed to a series of extract volumes (0.4, 0.6, 1.2, 1.6, 2, and 2.8 mg/ml) for a 24-hour period. Each concentration was tested in triplicate, using 15 larvae per replicate. A negative control group was maintained in distilled water alone, while a positive control group received 1 ml of ethanol diluted in 150 ml of distilled water. Larval mortality was monitored and recorded at 24, 48, and 72 hours after treatment. The mortality data were then analyzed to determine the sub-lethal and lethal concentrations (LC50 and LC90), along with their corresponding 95% confidence intervals. (Figure 14)

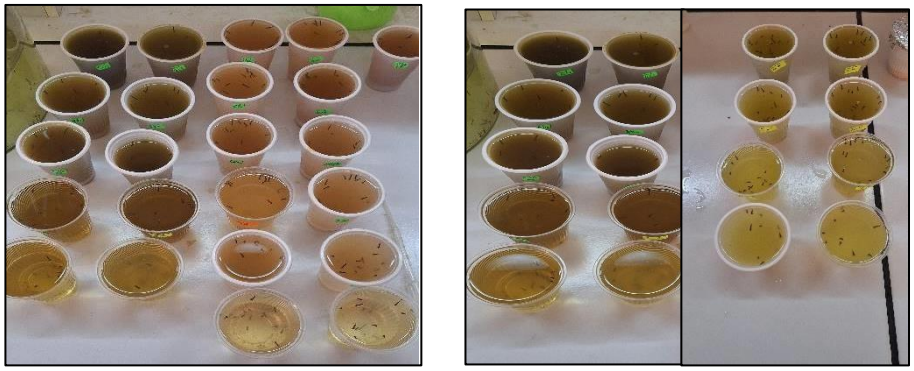


Figure 13: Concentrations applied to mosquitoes

2.11. Statistical Analysis

The number of individuals used in each experimental group is indicated alongside the corresponding results. Data are expressed as mean values ± standard deviation (SD). To assess differences between groups, a one-way analysis of variance (ANOVA) was performed, followed by Tukey's post hoc test for multiple comparisons. Statistical significance was considered at the 5% level ($p < 0.05$). All analyses were carried out using GraphPad Prism version 8.0 for Windows **(**GraphPad Software Inc., www.graphpad.com).

Results and discussion

3. Results and discussion

3.1. Results

3.1.1. Yield of *Ziziphus lotus* hydro-ethanolic extract

The extraction of the leaves of the plant was prepared following the protocol described by Merghem *et al* (1995). The yield of the leaves of *Ziziphus lotus* hydro-ethanolic extract is 21.17%

3.1.2. Phytochimical scrinning of the aerial part of *Ziziphus lotus*

The results of the phytochemical analysis are presented in Table 3. The '+' sign indicates the presence of the group of chemical compounds and the '−' sign a negative reaction. The results indicate that the leaves extract of *Ziziphus lotus* contains flavonoids, alkaloids, steroids, tannins, and saponins, while flavonoids, terpenoids, and quinones were not detected

Table 3: The results of the phytochemical scrinning of the leaves extract of *Ziziphus lotus*

Secondary metabolites	Results (+/-)	Observation
Flavonoids	+	
Quinones	-	

Terpenes **Steroids**	- +	
Alkaloids	+	
Gallic tannins	+	
Catechic tannins	+	
Saponins	+	

3.1.3. Antioxydant activity

Following the method described by Kuramasamy (2007), we determined the EC50 values for both ascorbic acid and *Ziziphus lotus* extracts. The graph illustrates how these substances interact with DPPH, which serves as a free radical in this experiment. Based on our calculations, the EC50 values are presented below:

Table 4: The EC50 values of ascorbic acid and *Ziziphus lotus.*

Antioxydant	**EC50**
Ascorbic acid	**12,57 µg/mL**
Z. lotus	**56.17 µg/mL**

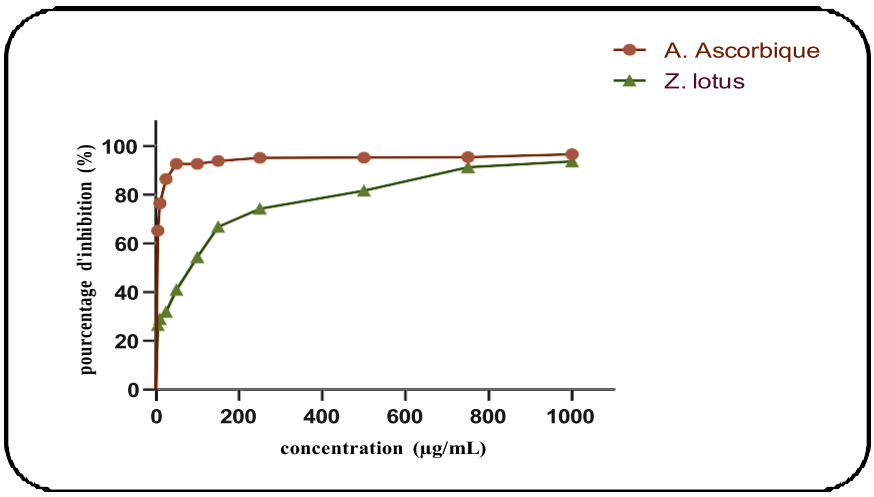


Figure 14: Percentage of DPPH radical efficacy as a function of *Ziziphus lotus* extract concentation.

3.1.4. Antidiabetic Activity of *Ziziphus lotus*

This experiment was conducted to evaluate the inhibitory effect on glucose absorption by yeast cells suspended in glucose solutions of varying concentrations (10-100 mg/ml), in the presence of aqueous and hydro-alcoholic extracts of *Ziziphus lotus* leaves, as well as metformin, tested at different concentrations (10- 100 mg/ml).

Table 05: The IC50 of metformin and *ziziphus lotus*

Antidiabetic	**IC50**
Metformine	**83.46 mg/mL**
Z. lotus	**7.70 mg/Ml**

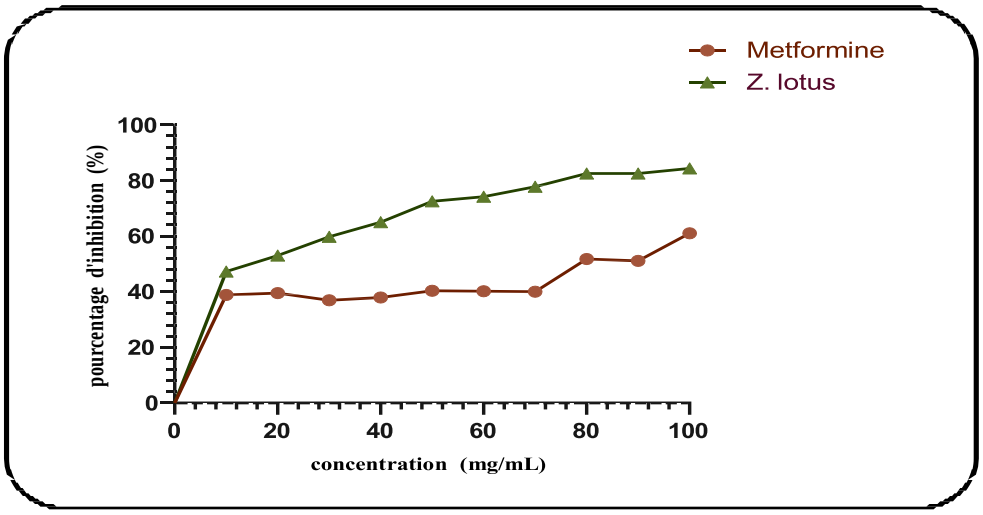


Figure 15: interaction of metformin and *Zizphus lotus* extract with glucose solution

3.1.5. Larvicidal efficacy of *Ziziphus lotus* hydro-ethanolic extract against *Cs. longiareolata*

Toxicological tests were used to determine the efficacy of *Ziziphus lotus* hydro-ethanolic extract on the basis of the mortality recorded in treated individuals after different periods of 24, 48 and 72 hours.

Ziziphus lotus hydro-ethanolic extract was tested on *Cs. longiareolata* larvae 4 at different doses(0.4, 0.6, 1.2, 1.6, 2 and 2.8 mg/ml). The natural mortality recorded in the positive and negative control series was zero. The treatment revealed a larvicidal effect with a dose-response relationship.

Analysis of variance using a classification criterion revealed very highly significant differences ($p < 0.001$) (Table 6). The dose-response curve expressing the percentage of mortality corrected as a function of the logarithm of the dose applied (Figure. 12) was used to determine the values of the various sublethal and lethal concentrations, together with them confidence intervals (Table 07).

Table 6. Efficacy of *Z. lotus* hydro-ethanolic extract applied on fourth instar larvae of *Cs. longiareolata*: corrected mortality (m ± SD, n = 4 replicates each containing 15 larvae)

Time (hours)	0,4mg/ml	0,6mg/ml	1,2mg/ml	1,6mg/ml	2mg/ml	2,8mg/ml	P
24	4.44 ± 3.85	11.11 ± 3.85	28.88 ± 3.85	53.33 ± 6.66	77.77 ± 10.18	95.55 ± 3.85	< 0.0001
48	4.44 ± 3.85	11.11 ± 3.85	28.88 ± 3.85	55.55 ± 3.85	82.22 ± 3.85	100.00 ± 0.00	< 0.0001
72	6.66 ± 0.00	13.33 ± 0.00	40.00 ± 17.64	57.78 ± 3.85	84.44 ± 3.85	100.00 ± 0.00	< 0.0001

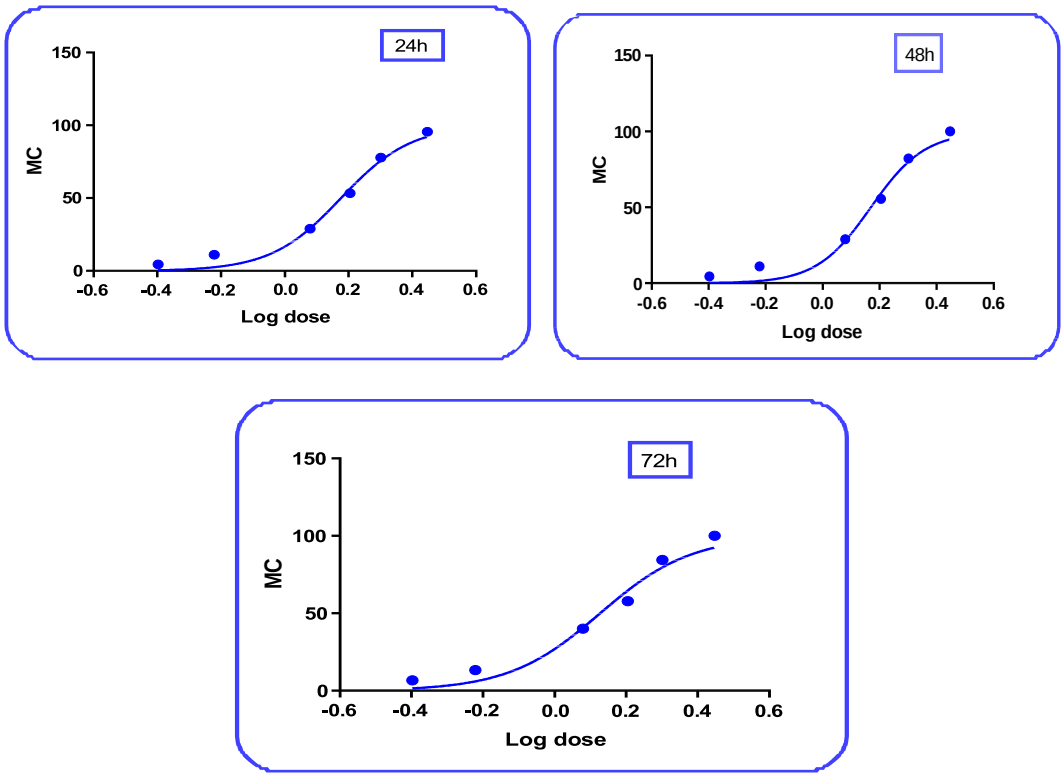


Figure 16: Efficacy of *Z. lotus* hydro-ethanolic extract (mg/mL) applied on fourth instar larvae of *Cs. longiareolata*: Dose-response curve expressing the percentage of mortality corrected according to the logarithm of doses.

Table 7: Sublethal and lethal concentrations (mg/mL, FL) of *Z. lotus* hydro-ethanolic extract against fourth instar larvae *Cs. Longiareolata*.

Time (hours)	*Cs. Longiareolata*			
	LC25, mg/mL (95 % FL)	LC50, mg/mL (95 % FL)	LC90, mg/mL (95 % FL)	r^2
24	1.13 0.88 to 1.32	1.49 1.32 to 1.65	2.61 2.12 to 3.58	0.98
48	1.15 0.88 to 1.36	1.47 1.28 to 1.63	2.38 1.95 to 3.30	0.98
72	0.96 0.65 to 1.25	1.33 1.08 to 1.56	2.55 1.90 to 3.97	0.97

3.2. Discussion

3.2.1. Extract yield

The hydroethanolic extraction of *Ziziphus lotus* leaves resulted in a yield of 21.17%. This shows a good extraction efficiency and aligns well with previous research on this plant and related species. For example, Benammar *et al*. (2020) reported yields 18.5%.

3.2.2. Phytochimical scrinning

According to the results obtained in our study, *Ziziphus lotus* plant is rich in many secondary Metabolites (flavonoids, alkaloids, steroids, tannins, and saponins), and has no or few secondary Metabolites like (terpenoids, and quinones).

- **Flavonoids Test:**

No yellow color was observed, indicating the absence or very low presence of flavonoids. This result confirm that of Chaouche *et al*. (2023), who reported the presence of flavonoids in Algerian *Ziziphus lotus* leaves.

- **Alkaloids Test:**

The formation of a white precipitate confirmed the presence of alkaloids. This agrees with the findings of Mekkiou *et al*. (2022), who also reported alkaloids in *Ziziphus lotus* leaves. Alkaloids are known for their antidiabetic and calming effects (Benammar *et al*., 2020).

- **Tannins Test:**

The solution turned dark green, indicating the presence of catechic and gallic tannins. This matches the results of Belhadj *et al*. (2021), who identified tannins as major components in *Ziziphus lotus*. Tannins are known for their antioxidant and wound-healing properties (Makhloufi, 2013).

- **Saponins Test:**

A persistent foam taller than 1 cm confirmed the presence of saponins. These findings are in line with Boudrahem *et al*. (2019), who highlighted the anti-inflammatory effects of saponins.

- **Steroids Test:**

The purple color that appeared confirmed the presence of steroids. These results are supported by Benammar *et al*. (2020), who noted the antimicrobial activity of steroids (Saad, 2017).

- **Terpenoids and Quinones Test:**

No color change was observed, indicating a negative result for terpenoids and quinones. This contrasts with Belhadj *et al*. (2021), who reported the presence of terpenoids in *Ziziphus lotus*.

3.2.3. Antioxydant activity of *Ziziphus lotus* hydro-ethanolic extract

The results of the antioxidant activity test of *Ziziphus lotus* extract showed that the percentage of free radical inhibition increased proportionally with the concentration of both the plant extract and ascorbic acid.

The EC50 value represents the concentration of an antioxidant required to reduce the initial concentration of free radicals by 50%. A lower EC50 value indicates a more potent antioxidant, as a smaller amount is needed to produce the desired effect. This value is widely used to compare the antioxidant strength of different compounds in experimental studies (Rita, 2019)

The EC50 value of ascorbic acid was 12,57 µg/mL and the EC 50 value of *Ziziphus lotus* extract was 56.17 µg/mL, which proves the effectiveness of *Z.lotus* extract against oxidative stress. The more effective an antioxidant compound is, the lower its EC50 value is. Antioxidant compounds work by donating an electron to free radicals and neutralizing them, preventing oxidative stress, and have high reactivity with free radicals like DPPH, so they can achieve the desired effect at a lower concentration (Molyneux. 2004).

Our Ec50 value at both ascorbic acid and *Ziziphus lotus* extract showed comparable antioxidant activity to Benammar *et al*. (2020) where their Ec50 was estimated (60.1 µg/mL).

Based on our findings and current research, *Ziziphus lotus* exhibits significant antioxidant activity, primarily attributed to its high content of phenolic compounds. These phytochemicals effectively scavenge DPPH radicals. The observed EC50 of 56.17 µg/mL strongly suggests that polyphenolic constituents are principally responsible for this antioxidant action, as they contain multiple hydroxyl groups capable of neutralizing free radicals (Benammar *et al*., 2020). This aligns with the established correlation between phenolic content and radical scavenging activity in medicinal plants (Li *et al*., 2020). Notably, *Z. lotus* leaves have demonstrated superior antioxidant capacity compared to other traditional species, with identified compounds like quercetin-3-O-rutinoside showing

particular efficacy in stabilizing reactive oxygen species (Chaouche *et al*., 2023)."

3.2.4. Antidiabetic activity of *Ziziphus lotus* hydro-ethanolic extract

In our study, the leaf extract of *Ziziphus lotus* demonstrated remarkable antidiabetic activity, with an IC50 value of 7.70 mg/mL for inhibiting α-glucosidase and α-amylase enzymes. This potency is clearly superior to that of the reference drug metformin, which showed an IC50 of 83.46 mg/mL under the same experimental conditions.

Our findings are in line with those of Marmouzi *et al*. (2019), who reported that *Z. lotus* leaf extract exhibited an IC50 of 8.66 ± 0.62 mg/mL against α-glucosidase and 20.40 ± 1.30 mg/mL against α-amylase, highlighting the strong enzyme-inhibitory potential of this plant and supporting its role in slowing carbohydrate digestion and glucose absorption.

Similarly, Benariba *et al*. (2013) found that the methanolic extract of *Z. lotus* leaves had an IC50 of 8.27 mg/mL against α-glucosidase—very close to the value obtained in our study—further supporting the consistency of its biological efficacy across different extraction conditions.

On the other hand, studies such as Bouzenna *et al*. (2017) reported only moderate antidiabetic activity for other types of *Z. lotus* extracts. These differences could be attributed to various factors such as the plant part used (leaves, fruits, roots), the type of solvent employed in the extraction process, or even the environmental and geographical conditions of the sampled plants.

Taken together, these comparative results strengthen the credibility of *Ziziphus lotus* as a promising natural source for the development of complementary or alternative therapies in diabetes management.

3.2.5. Toxicity test

Toxicological tests were made to test larvae sensitivity to the use of insecticides in pest control campaigns (OMS, 1963). The use of chemical insecticides resulted in an ecotoxicological mess together with a spectacular increase in the number of resistant species. The use of natural products continues to be the most beneficial method for the health of the conspecifics and their environment (Benayad, 2008).

The investigation of plant-insect interactions offers the opportunity to develop plant extracts as useful components of pest control. Biopesticides may have several targets: they can target specific sites on the insect nervous system that lead to paralysis and subsequent death, alter the conductance of multiple ion channels (calcium, potassium, sodium), disturb

the organization of lipid bilayers, and act at the presynaptic terminal (Windley *et al*., 2012). The multiple targets of biopesticides are instrumental in hindering the development of resistance in insects. . .

Toxicity, as displayed in what mortality is observed after applying it, is ultimately dose-dependent.

In our study, the hydro-ethanolic extract of *Ziziphus lotus* leaves showed strong toxic effects against mosquito larvae, with LC50 values between 1.28 and 1.65 mg/mL after 48 to 72 hours. This means the extract works well at relatively low concentrations. These results are quite similar to what Bendif and colleagues (2017) found when testing a methanolic leaf extract on *Culex pipiens* larvae, which also showed good effectiveness. On the other hand, Djenane *et al*. (2016) reported a slightly higher LC50 value of 3.5 mg/mL for *Aedes aegypti* larvae with an ethanolic extract, which might be due to differences in mosquito species or the extraction method used.

Benelli and co-authors (2018) explained that plant extracts often target several parts of the larvae's nervous system and ion channels, which could explain why *Ziziphus lotus* extracts are so effective compared to some traditional insecticides that only focus on one target. Supporting this idea, El Bouzidi *et al*. (2020) showed that *Ziziphus lotus* extracts can inhibit larvae's detoxifying enzymes, making the extract even more toxic to the mosquitoes.

Differences in LC50 values across studies are expected because factors like the solvent used for extraction, which part of the plant is tested, how much active compound is present, and the mosquito species can all influence results. For example, Abid *et al*. (2019) found that hydro-ethanolic extracts tend to be more effective than water or other solvents, showing that how we prepare these extracts really matters.

All in all, these studies back up the idea that *Ziziphus lotus* is a promising natural option for creating environmentally friendly and effective mosquito control agents, offering an interesting alternative to chemical insecticides.

3.2.6. Factors Affecting Plant Extraction Yield and Variability of Secondary Metabolites

* **Solvent Type:** The choice of solvent is crucial as different solvents have varied in polarities, which affect the solubility of plant compounds. Polar solvents (like water and ethanol) are more effective for extracting polar compounds, while non-polar solvents (like hexane) are better for non-polar compounds (Stalikas, 2007).

* **Extraction Time:** The duration of the extraction process can affect the yield. Longer extraction times can lead to higher yields, but excessively long times may result in the degradation of sensitive compounds (Proestos & Komaitis. 2008).

* **Temperature Higher:** temperatures generally increase the solubility of compounds and the rate of extraction. However, high temperatures can also degrade thermo-labile compounds (Wang & Weller. 2006).

* **Particle Size:** Smaller particle sizes increase the surface area for solvent interaction, thus improving the extraction efficiency. However, too fine a powder can lead to difficulties in filtration and solvent recovery (Azmir. 2013).

* **Solvent-to-Solid Ratio:** The ratio of solvent to plant material affects the extraction yield . A higher solvent-to-solid ratio typically increases the yield up to a certain point, after which no significant increase is observed**.** (Liu ***et al***., 2004).

* **Agitation or stirring** can enhance the contact between the solvent and the plant material, thus improving the extraction yield**.** (Dai & Mumper. 2010).

***Genetic Factors:** Species and Genotype: Different plant species and even different genotypes within a species can produce distinct secondary metabolites. The genetic makeup controls the enzymatic pathways that synthesize these compounds.

* **Gene Expression:** Variations in the expression of genes involved in metabolic pathways can lead to differences in metabolite profiles. Regulatory genes play a crucial role in controlling these pathways. (Mithöfer & Boland. 2012)

* **Environmental Factors:** Climate and Weather: Temperature, light, humidity, and rainfall can significantly influence the synthesis of secondary metabolites. For instance, high light intensity can enhance the production of flavonoids, which act as UV protectants.

* **Soil Composition:** The availability of nutrients and minerals in the soil can affect the biosynthesis of secondary metabolites. For example, nitrogen availability can impact alkaloid production. Water Availability: Water stress can lead to the accumulation of certain secondary

metabolites like tannins and phenolics, which help plants cope with drought conditions. (Hartmann. 2007).

* **Biotic Interactions:** Herbivory and Pathogens: Plants often produce secondary metabolites as a defense mechanism against herbivores and pathogens. For example, The production of phytoalexins increases in response to pathogen attack. Symbiotic Relationships: Interactions with symbiotic organisms such as mycorrhizal fungi or nitrogen-fixing bacteria can influence the production of secondary metabolites. (Treutter, 2010).

* **Developmental Stage:** The production of secondary metabolites can vary depending on the plant's developmental stage. Seedlings, mature plants, and senescing plants may all exhibit different metabolite profiles. Tissue Specificity: Different parts of the plant (roots, leaves, flowers, seeds) can produce different secondary metabolites, reflecting their specific roles and requirements (Bryant. 1983)

* **Ecological and Evolutionary Factors:** Over evolutionary time, plants adapt to their environments, leading to the development of unique secondary metabolites that provide competitive advantages. Geographic Variation: Plants from different geographical regions often have distinct secondary metabolite profiles due to varying environmental pressures and evolutionary histories**.** (Wink. 2003)

* **Human Influence:** Farming practices, including the use of fertilizers, pesticides, and crop management techniques, can influence the secondary metabolite composition of plants.
Selective Breeding and Genetic Modification: Humans have selectively bred plants for specific traits, including the production of certain secondary metabolites. Genetic engineering can also introduce or enhance pathways for desirable metabolites. (Neilson. 2013).

Conclusion

4. Conclusion

In conclusion, this study revealed significant scientific findings that highlight the therapeutic and environmental potential of the hydro-ethanolic extract of *Ziziphus lotus* leaves. The results demonstrated three remarkable biological properties that position this plant as a promising natural resource.

Firstly, the extract showed noteworthy antioxidant activity (EC50 = 56.17 µg/mL), indicating its ability to neutralize free radicals and prevent oxidative stress-related diseases, although its effect was lower than that of Vitamin C (EC50 = 12.75 µg/mL).

Secondly, the extract exhibited exceptional antidiabetic activity, significantly outperforming the reference drug metformin (IC50 = 7.70 mg/mL vs. 83.46 mg/mL). This opens the door to the development of herbal alternatives or complementary treatments for diabetes, particularly given the plant's lower risk of side effects.

Thirdly, the extract demonstrated strong larvicidal efficacy against *Culiseta longiareolata*, with a mortality rate reaching 95.55% at a concentration of 2.8 mg/mL within just 24 hours. This suggests its potential as a natural larvicide in integrated vector control programs, especially in areas facing resistance to conventional chemical insecticides.

Taken together, these findings underline the broad potential of *Ziziphus lotus* as a natural source for the development of safe, eco-friendly alternatives to synthetic drugs and pesticides. The study paves the way for further scientific exploration in the fields of medicinal plants and vector-borne disease control.

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