People's Democratic Republic of Algeria

Ministry of Higher Education and Scientific Research

Echahid Cheikh Larbi Tebessi University –Tébessa

Faculty of Exact Sciences and Natural and Life Sciences

Department of Living Beings

End of study Dissertation

Presented for obtaining the master's degree

Field: Natural and life sciences

Sector: Biotechnology

Option: Plant biotechnology

Titled:

Antioxydant activity and insecticidal effects of *Citrullus colocynthis* hydro-ehanolic extract against mosquito species

Presented and supported by:

Ms. BOUTARFA Mariem Wissal

Ms. CHIKH Dhouha

Before the jury:

Pr. BOUABIDA Hayette	Pr	Université de Tebessa	President
Dr. SEGHIER Hanane	SLA	Université de Tebessa	Raporter
Dr. DRIS djemaa	SLA	Université de Tebessa	Examiner

Date of defence: 09/06/2025


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.

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

I also thank all the members of the jury: Pr. Bouabida Hayette and Dr Dris Djemaa, for accepting to evaluate this work and for discussing our theses.

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

In the name of Allah, the most gracious the most merciful, all praise to him without him this work would never been done.

With all my love, I dedicate this work to:

My beloved parents whose supported and encouraged me a lot during these years of study. No words can fully thank you, but I know you feel it.

My beloved sisters: Hiba, Imene and, Baya, you are the home that never fades.

To my best friends: Anfel, Chahd, Affaf and Dhouha, your laughter was a lifeline, and your presence meant more than any explanation.

And to everyone who stood with me,

To those whose may never appear in the spotlight, but whose support as in every success...Thank you for being there, exactly when I mattered most.

Marieme Wissal

Dedication

All praise is due to Allah, by whose grace good deeds are completed, and through whose guidance this humble work has been achieved. To Him belongs all thanks, in every time and every state.

To the dearest people in my life : my beloved father and mother,
Thank you for your endless support, your prayers, and your unconditional love. You have been my strength and my light.

To my cherished brothers and sisters :Wissam, Fouad, Zouheir, Anouar, Siham, and Souria, your support has meant the world to me. Thank you for being my constant encouragement and a source of strength throughout this journey.

To my precious friends : Aya, Chourouk, Chiraz,, Manel, Chaima, Shahed, Afaf, Mariam, Rania, Maram, and Hadjar, you have been my companions through both hardship and joy. Thank you for your kindness, love, and unwavering belief in me.

And finally,
A special thank you to myself…
For never giving up, for staying strong despite the challenges, for believing even when it was hard. Thank you for the patience, the perseverance, and the courage to keep going.

Dhouha

ملخص

تهدف هذه الدراسة إلى تقييم الخصائص البيولوجية للمستخلص الكحولي المائي لنبات الحنظل, مع التركيز على نشاطه المضاد للأكسدة, و المضاد لمرض السكري, و تأثيره الحشري ضد نوع من أنواع البعوض.
كان محصول المستخلص المائي-الايثانولي لثمرة الحنظل19,21.
أظهرت اختبارات النشاط المضاد للأكسدة قدرة محدودة على التخلص من الجذور الحرة, و تم تاكيد النشاط المضاد للسكري من خلال تثبيط انزيم الفا-غلوكوزيداز كما اظهر المستخلص فعالية ملحوظة كمبيد حشري طبيعي, مما يشير إلى إمكانية استخدامه في المجالات الصيدلانية و الحشرية.

الكلمات المفتاحية الحنظل, مستخلص كحولي مائي, النشاط المضاد للاكسدة, النشاط المضاد للسكري, التاثير الحشري, البعوض.

Abstract

This study investigates the biological properties of the hydroethanolic extract of *Citrullus colocynthis*, focusing on its antioxidant, antidiabetic, and insecticidal activities against mosquito species.

The yield of the hydro-ethanolic extract of the fruit of *Citrullus colocynthis* was 19.21%. Antioxidant assays showed moderate free radical scavenging ability. The antidiabetic activity was confirmed through inhibition of α-glucosidase enzyme. Additionally, the extract exhibited significant insecticidal potential, highlighting its possible use in pharmaceutical and entomological fields.

Keywords: *Citrullus colocynthis*, hydroethanolic extract, antioxidant activity, antidiabetic activity, insecticidal effect, mosquitoes.

Résumé

Cette étude vise à évaluer les propriétés biologiques de l'extrait hydro-éthanolique de *Citrullus colocynthis,* en mettant l'accent sur son activité antioxydante, antidiabétique et ses effets insecticides contre une espèce de moustiques.

Le rendement de l'extrait hydro-ethanolique de Fuit de *Citrullus colocynthis* est de 19,21%.

Le test antioxydant a révélé une capacité modéré de piégeage des radicaux libres, tandis que l'activité antidiabétique a été confirmée par l'inhibition de l'enzyme α-glucosidase. Par ailleurs, l'extrait a démontré une efficacité notable comme insecticide naturel, suggérant son potentiel pour des applications pharmaceutiques et entomologiques.

Mots-clés : *Citrullus colocynthis,* extrait hydro-éthanolique, activité antioxydante, activité antidiabétique, effet insecticide, moustiques.

Liste of figure

Figure 1: *Citrullus colocynthis* 3
Figure 2: Basic structure of flavonoids 4
Figure 3: Structure of saponines 5
Figure 4: Steps of the extraction 8
Figure 5: Structure of DPPH and its reduction by an antioxydant 9
Figure 6: Breeding technique 13
Figure 7: Toxicity technique 13
Figure 8: Percentage of DPPH radical efficacy as a function of *Citrullus colocynthis* extract concentration 16
Figure 9: Interaction of Metformin and *C.colocynthis* extracts with glucose solution.....17
Figure 10: Efficacy of *C.colocynthis* hydro-ethanolic extract (mg/ml) applied on fourth instar larvae of *Cs. longiareolata* after 48h: Dose response curve expressing the percentage of mortality corrected according to the logarthm of dose 18
Figure 11: Efficacy of *C.colocynthis* hydro-ethanolic extract(mg/ml) applied on fourth instar larvae of *Cs. longiareolata* after 72h: Dose response curve expresing the percentage of mortality corrected according to the logarithm of dose 19

Liste of table

Table 1: Classification of *Citrullus colocynthis* 4
Tableau 2: Systematic position of *Culiseta longiareolata* mosquito species.....11
Table 3: The results of the phytochemicals scrinning of Citrullus colocynthis fruit.....14
Table 4: The EC50 values of Ascorbic acid and *C.colocunthis* 15
Tableau 5: The IC50 values of Metformin and *C.colocynthis* 16
Tableau 6: Efficacy of *C.colocynthis* hydro-ethanolic extract applied on fourth instar larvae of *Cs. longiareolata* corrected mortality(m ± SD, n = 4 replicates each containing 15 larvae).....18
Tableau 7: Sublethal and lethal concentrations(mg/ml,FL) of *C.colocynthis* hydro-ethanolic extract against fourth instar larvae *Cs. longiareolata* 19

Liste of abbreviation

C.colocynthis: *Citrullus colocynthis*

EC 50: Half Maximal Effective Concentration

DPPH: 2,2-Diphenyl-1-Picrylhydrazyl

µg: Microgram.

m ± SD: Mean ± Standard Deviation

Cs : Culiseta

FL: Field Laboratory

°C : Degrees Celsius

Summary

1. Introduction 1

2. Materials and methods 3

2.1. Geographic distribution of *Citrullus colocynthis* (L.) 3

2.2. Botanical description of *Citullus colocynthis* (L.) 3

2.3. Classification of *Citullus colocynthis* (L.) 3

2.4. Chemical composition of *Citullus colocynthis* (L.) 4

2.4.1. Flavonoids 4

2.4.2. Alkaloids 4

2.4.3. Saponines 5

2.5. Conventional uses of *Citullus colocynthis* (L.) 5

2.5.1. Traditional uses of *C. Colocynthis* (L.) 5

2.5.2. Medicinales uses of *C. colocynthis*(L.)Schard 5

2.6. Phytochemical analysis 6

2.6.1. Tannins 6

2.6.2. Saponins 6

2.6.3. Flavonoids 6

2.6.4. Alkaloids 6

2.6.5. Terpenes and steroids 7

2.6.6. Quinons 7

2.7. Preparation of the extract 7

2.8. Antioxydant activity 8

2.8.1. Free radicals 8

2.8.2. Antioxidant activity by measuring anti-free radical activity on DPPH 9

2.8.3. Dosage method 9

2.9. Anti diabetic activity 9

2.10. Description of mosquito species used 10

2.10.1. Systematic position of *Culiseta longiareolata* mosquito species 11

2.10.2. Life cycle of *Culiseta longiareolata* 11

2.10.4. Collecting and breading 12

2.10.5. Toxicity test 13
3. Result 14
3.1. Results 14
3.1.1. Yield of *Citrullus colocynthis* 14
3.1.2. Phytochemical scrinning of the fruit of *Citrullus colocynthis* 14
3.1.3. Antioxydant activity of *Citrullus colocynthis* 15
3.1.4. Antidiabetic Activity *Citrullus colocynthis* 16
3.1.4. Larvicidal efficacy of *Citrullus colocynthis* hydro-ethanolic extract against Cs. longiareolata 17
3.2. Discussion 20
3.2.1. Extract yield 20
3.2.2. Phytochemical scrinning 20
3.2.3. Antioxydante activity of *Citrullus colocynthis* hydro-ethanolic extract 21
3.2.4. Antidiabetic activity of *Citrullus colocynthis* hydro-ethanolic extract 21
3.2.4. Larvicidal efficacy of *Citrullus colocynthis* hydro-ethanolic extract against Cs. longiareolata 22
4. Conclusion 23

Introduction

1. Introduction

Medicinal plants have been a cornerstone of healthcare systems worldwide for thousands of years, offering natural remedies for a wide range of ailments. Today, they continue to play a vital role, not only in traditional medicine but also as a key source for modern drug development. According to the World Health Organization **(WHO, 2023)**, an estimated 80% of people in developing countries rely on plant-based medicines for primary healthcare. Recent research highlights their potential in managing chronic diseases such as diabetes, cardiovascular disorders, and even cancer, thanks to their rich bioactive compounds **(Fabricant & Farnsworth, 2023).**

For instance, many of plants have demonstrated potent anti-inflammatory, antioxidant, and antimicrobial properties **(Ekor, 2022).**

Plants are also renowned as rich sources of natural antioxidants, playing a vital role in neutralizing harmful free radicals and reducing oxidative stress in the body. Many medicinal plants contain bioactive compounds like polyphenols, flavonoids, and vitamin C, which help prevent cellular damage linked to chronic diseases such as cancer and cardiovascular disorders. These natural antioxidants are increasingly preferred over synthetic ones due to their safety and additional health benefits **(Pandey & Rizvi, 2021).**

Diabetes mellitus remains a significant global health challenge, and numerous plants have demonstrated promising antidiabetic effects by improving insulin sensitivity and reducing blood glucose levels. Compounds like alkaloids, terpenoids, and glycosides found in plants have been shown to enhance glucose metabolism and protect pancreatic β-cells. These findings support the use of plant-based therapies as complementary approaches in diabetes management **(Kumar *et al*., 2022).**

With growing concerns over synthetic pesticide resistance and environmental toxicity, plant-derived insecticides offer a sustainable alternative. Many plants produce secondary metabolites which effectively repel or kill insects while being biodegradable and less harmful to non-target organisms **(Isman, 2020).**

Among these plants, bitter apple *Citrullus colocynthis* (L.) Schard has garnered significant attention due to its traditional use in treating various ailments and its rich phytochemical profile. Recent studies have highlighted its potent antioxidant, antidiabetic, and insecticidal properties **(Alagawany *et al*., 2021).**

The antioxidant activity of *C. colocynthis* is primarily attributed to its high content of polyphenols and flavonoids, which are effective in scavenging free radicals and mitigating oxidative stress a key factor in the pathogenesis of chronic diseases such as diabetes

mellitus. In vitro analyses have demonstrated that ethanolic extracts of *C. colocynthis* exhibit significant free radical scavenging activities, suggesting their potential in preventing oxidative damage at the cellular level **(Fetni & Bertella, 2021).** Furthermore, the antioxidant capacity of the plant varies with environmental factors, such as the season of fruit collection, indicating that these factors can influence the concentration of bioactive compounds **(Al-Mahmoud *et al*., 2022).**

In the context of diabetes management, *C. colocynthis* has shown promising antidiabetic effects. Experimental studies involving diabetic Wistar rats have revealed that administration of *C. colocynthis* extracts leads to notable improvements in blood glucose levels, lipid profiles, and renal function markers **(Sansri *et al*., 2022).** These effects are believed to stem from the plant's ability to enhance insulin sensitivity and modulate carbohydrate metabolism, thereby offering a natural alternative for diabetes treatment **(Jat & Jat, 2021).**

Furthermore, with the rise of insecticide resistance among mosquito populations, especially vectors responsible for transmitting diseases such as malaria, dengue, and Zika virus, the search for eco-friendly and effective alternatives has intensified. The insecticidal potential of *Citrullus colocynthis* has been documented in several ethnobotanical surveys and preliminary bioassays, highlighting its efficacy against various mosquito species. Extracts from this plant contain saponins, alkaloids, and essential oils that may interfere with insect nervous systems or reproduction cycles **(Elango *et al*., 2012; Bakkali *et al*., 2008; WHO, 2005).**

In light of these considerations, this study aims to evaluate the antioxidant capacity and insecticidal effectiveness of the hydro-ethanolic extract of *Citrullus colocynthis* against selected mosquito species. Through phytochemical screening and biological assays, we hope to uncover natural agents capable of addressing pressing public health concerns while also offering safer alternatives to synthetic chemicals.

Materials and methods

2. Materials and methods

2.1. Geographic distribution of *Citrullus colocynthis* (L.)

Citrullus colocynthis (L.) is extensively found across North Africa, the Middle East, India, and various other arid regions. In Algeria, it thrives mainly in the Saharan and steppe areas, especially within sandy and semi-arid environments. Here, it plays a significant role in traditional folk medicine and helps maintain ecological balance **(Benmehdi *et al*., 2012).**

2.2. Botanical description of *Citullus colocynthis* (L.)

A perennial herbaceous plant with long creeping stems that spread across the ground, reaching over one meter in length **(Benchalah *et al*., 2000; Chehma, 2006).**

Citullus colocynthis has deeply lobed alternate leaves that appear bright green after rainfall. The entire plant is covered with short, stiff hairs **(Benchalah *et al*., 2000).**

The flowers are monoecious, small, solitary, axillary and greenish- yellow in color. The fruit are ovoid, smooth and green with striped patterns when young, turning yellow at maturity. They contain a fleshy yellowish pulp that is very bitter, with small dark seeds **(Mujahid *et al*., 2021) (Figure 01).**



Figure 1: *Citrullus colocynthis* **(Guittonneau *et al*., 2011)**

2.3. Classification of *Citullus colocynthis* (L.)

Citrullus colocynthis (L.) belongs to the Curcubitaceae family, which includes several economically important edible and medicinal plant soecies. Taxonomically, it is classified as follows **(Benariba *et al*., 2021) (Table 1)**

Table 1: Classification of *Citrullus colocynthis* **(Benariba *et al*., 2021)**

Kingdom	Plantae
Phylum	Magnoliopsida
Order	Curcurbitales
Family	Curcurbitaceae
Genus	*Citrullus*
Species	*colocynthis*

2.4. Chemical composition of *Citullus colocynthis* (L.)

2.4.1. 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) (Figure02).**

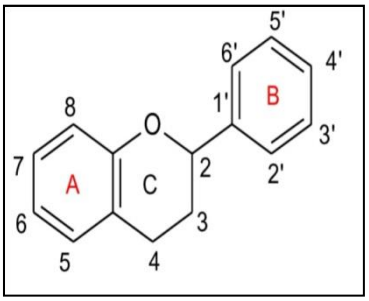


Figure 2: Basic structure of flavonoids **(Harbone, 2000)**

2.4.2. Alkaloids

The word "alkaloids" is nearly synonymous with the word "drug" **(Serge, 2003),** All alkaloids contain nitrogen(N), most often present in a heterocycle, Most of these compounds have an alkaline response; in the natural state, they are usually salted by organic acids (tartrates, malates) or combined with tannins (**Marghem, 2009)**.

2.4.3. Saponines

The term saponine stems from soapwort (Saponaria), which earlier served as a substitute for soap **(Serge, 2003)**. Are heterosides, can be found in the following forms triterpene and steroid **(Robinet, 1951)**. They have surface-active properties **(William, 2003).**

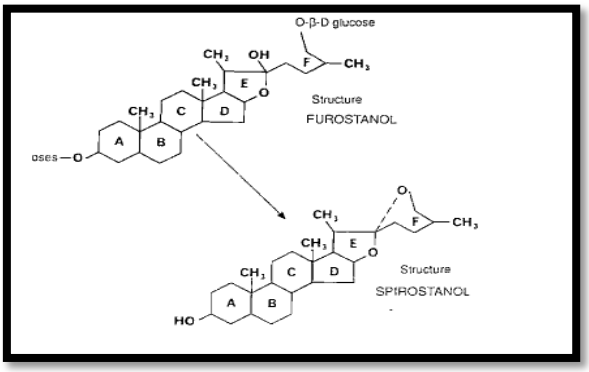


Figure 3: Structure of saponines **(Messian *et al*., 1993)**

2.5. Conventional uses of *Citullus colocynthis* (L.)

2.5.1. Traditional uses of *C. Colocynthis* (L.)

In North African and Arabian traditional medicine, the dried fruit pulp of *Citrullus colocynthis* is widely recognized for its potent laxative and purgative properties. Due to its high toxicity, it is traditionally administered in minimal doses under strict guidance **(Alqahtani *et al*., 2021).**

Decoction or extracts from the leaves and roots are traditionally applied to traet skin infections, ulcers, and also used as an anthelminitic **(Al-sanafi, 2016).**

In veterinary folk practices, the plant is occasionally used to expel intestinal parasites from livestock or as a tropical traetment for wounds (**El Ghouizi *et al*., 2022).**

2.5.2. Medicinales uses of *C. colocynthis* (L.)

The plant is valued for its anti-inflamatory and analgesic properties, especially for relieving joint pain and swelling **(Marwat *et al*.; 2012).**

The seeds are considered antidiabetic and are used in various cultures to help regulate blood sugar levels **(Huseini *et al*., 2009).**

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, 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. Quinons

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

Plant material of *Citrullus colocynthis* (L.) fruit was collected from Negrine, the south of Tébessa city, Algeria during fruit maturation season. Plant identification was conducted at the laboratory of plant biology and physiology at the faculty of exact sciences and natural and life sciences in university of Tébessa.

The fruit of *C.colocynthis* (L.) were dried at room temperature in the shade, then ground using an electric grinder and sieved to obtain a fine powder. The powders were packed in bags and stored until needed.

In a 1000 mL capacity beaker, a mixture comprising ethanol and distilled water (80/20) was prepared, followed by thorough agitation. Subsequently, plant powder (*C.colocynthis*) was introduced into the solution, and the amalgamation was subjected to agitator. Following complete homogenization, the solution was then subjected to filtration using filter paper into another receptacle to isolate the liquid fraction. Post-filtration, the solution was transferred to a rotary evaporator set at 45°C for further processing. The resultant extract was stored in an electric evaporator apparatus to obtain the final extract **(Merghem *et al.*, 1995) (Figure04)**.

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 3 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

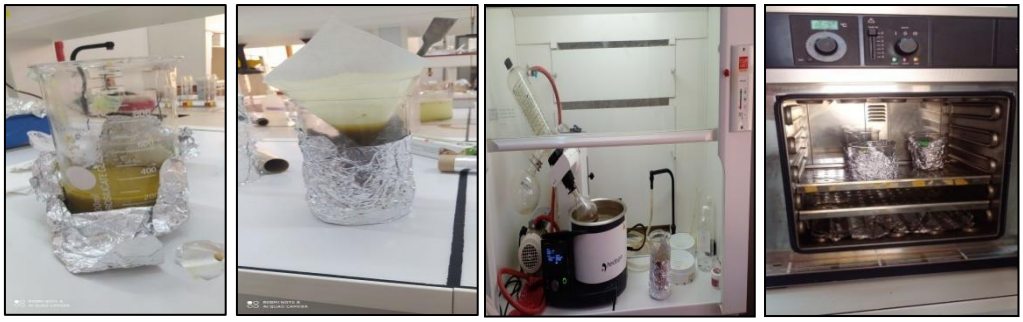


Figure 4: Steps of the extraction

2.8. Antioxydant activity

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 molecular species capable of independent existence that contains an unpaired electron in an atomic orbital. The presence of an unpaired electron results in certain common properties that are shared by most radicals. Many radicals are unstable andhighly reactive. They can either donate an electron to or accept an electron from other molecules, therefore behaving as oxidants or reductants. The most important oxygencontaining free radicals in many disease states are hydroxyl radical, superoxide anion radical, hydrogenperoxide, oxygen singlet, hypochlorite, nitric oxide radical, and peroxynitrite radical. These are highly reactive species, capable in the nucleus, and in the membranes of cells of damaging biologically relevant molecules such as DNA, proteins, carbohydrates, and lipids. Free radicals attack important macromolecules leading to cell damage and homeostatic disruption. Targets of free radicalsinclude all kinds of

molecules in the body. Among them, lipids, nucleic acids, and proteins are the major targets **(Lobo, 2010)**.

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

The use of this radical as a reagent is based on the fact that as soon as the DPPH radical reacts with an anti-radical compound, the latter releases hydrogen after reduction, thus leading to a change in color (from dark purple to light yellow. Anti-radical activity can therefore be visualised very quickly, simply following a change in color of the radical (DPPH) **(Figure 05) (Meriane, 2018).**

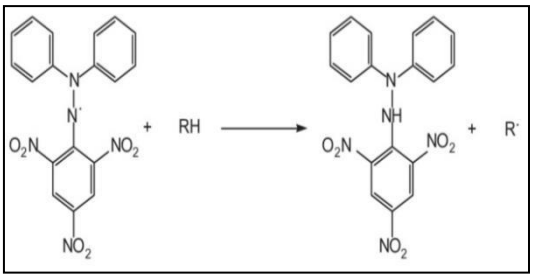


Figure 5: Structure of DPPH and its reduction by an antioxydant **(Kunal, 2012)**

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:

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

2.9. Anti diabetic activity

Yeast cells were prepared according to the method of **Sindhu *et al.*, (2013)**, with modifications. Commercial baker's yeast (1g) was washed by centrifugation (4.200 rpm,

5min) in distilled water (5ml) until the supernatant became clear, and a 10% (v/v) suspension was prepared in distilled water. Various concentrations of plant extracts (10to 100mg/ml) were added to 1 ml of glucose solution (10, 25 and 50 mg/ml), and the mixture was incubated for 15 min at 37°C. The reaction was initiated by adding 100µl of the yeast suspension, followed by vortexing and further incubation at 37°C for 60 min. After 1 hour, the tubes were centrifuged (2.500 rpm, 5 min), and glucose content in the supernatant was estimated using the ioding reagent.

Metformin was used as the standard anti diabetic medicines. Absorbance was measured at 540 nm, and all experiments were performed in triplicate. The percentage increase in glucose uptake by yeast cells was calculated using the following formula **(Ukwubile et odugu, 2018):**

$$\text{Inhibition of glucose absorbance (\%)} = \frac{\text{Abs extract} - \text{Abs control}}{\text{Abs extract}} \times 100$$

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

Extract Abs: the absorbance of the test sample.

2.10. Description of mosquito species used

Culiseta longiareolata is a species belonging to the family Culicidae, within the subfamily Culicinae **(Khaligh et al., 2020).** This insect undergoes complete metamorphosis and thrives in warmer climates. As a member of the order Diptera and the family Culicidae, it measures between 3 and 5 mm in length, with a small body, long slender legs, and narrow, membranous wings **(Ferede et al., 2022).** The identification of this species is relatively easy due to its distinctive features: non-plumose antennae, a short, nearly conical siphon, and an anal saddle with an interrupted segment **(Chorfi & Sedira, 2016).**

2.10.1. Systematic position of *Culiseta longiareolata* mosquito species

Tableau 2: Systematic position of *Culiseta longiareolata* mosquito species **(Aitken, 1954)**

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

2.10.2. Life cycle of *Culiseta longiareolata*

Culiseta mosquitoes complete a total metamorphosis with four stages **(Figure 06)**:

2.10.2.1. Egge stage

Culiseta females place their eggs in rafts on calm or static water bodies. As rough rule of thumb, they tend to hatch in the range of 1-3 days given adequate temperature and moisture **(Becker *et al*., 2010).**

2.10.2.2. Larval stage

Colloquially referred to as "wrigglers," mosquito larvae are primarily aquatic and feed on filamentous algae and microorganisms. This developmental phase typically lasts between 5 to 14 days, depending on environmental conditions **(Harbach, 2023).**

2.10.2.3. Pupal stage

Obligatory non- feeding while mobile, the pupae or "tumblers" have a transitional period of about 1-4 days before changing into an adult mosquito **(Becker *et al*., 2010)**

2.10.2.4. Adult stage

Post the pupal stage, the adult *Culiseta* mosquitoes rest on the water surface. *Culiseta* males sustain themselves on plant juices, whereas females need a blood diet prior to laying subsequent eggs. The adult mosquitoes could last from few days to a couple of weeks **(Darsie & Ward, 2005).**

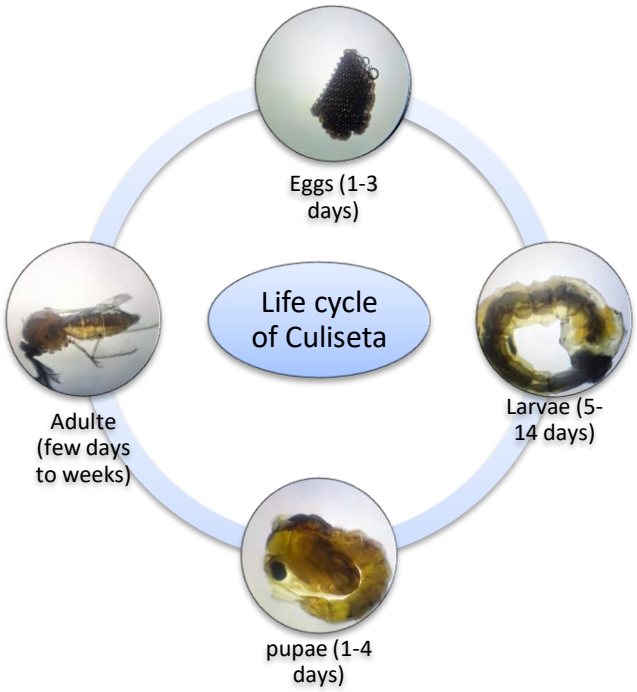


Figure 06: Life cycle of *Culisita langiareolata*.

2.10.4. Collecting and breading

Mosquito species were collected from an agricultural plant nursery located in El Hammamat (Tebessa). Groups of 15 mosquitoes were placed in separate plastic cups, each containing 150 ml of dechlorinated water. A standardized diet consisting of 75% yeast and 25% biscuit was provided to ensure proper developpement **(WHO, 1963)**. The water is renewed every two days **(Dris, 2019).**



Figure 7: Breeding technique

2.10.5. Toxicity test

The hydroethanolic extract dissolved in distilled water were applied to fourth instar larvae of *Culiseta longiariolata*. Following a preliminary screening, the hydroethanolic extract of *C.colocinthys* was tested at different concentration. Three replicates, each consisting of 15 larvae, were conducted for each concentration. The treatment was applied in plastic cups containing 150ml of dechlorinated water and food. Larval mortality was monitored at 24, 48, 72 hours post-treatments. The observed mortality percentage was corrected using Abbott's formula (1925), which eliminate natural mortality and determines the actual toxicity of the biopesticide. The determination of sublthal and lethal concentrations (LC25, LC50, LC90), as well as their 95% confidence intevales (95%Cl), was carried out using Graph Pad Prism 8 software **(Figure 08).**

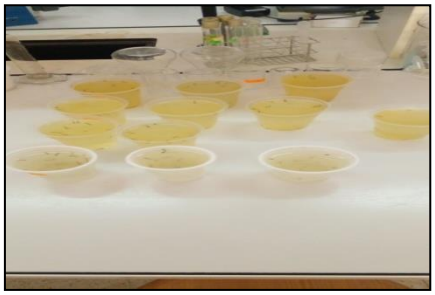


Figure 8: Toxicity technique

2.11. Statistical analysis

The number of individuals tested in each series is given with the results. Data are presented as the mean ± standard deviation (SD). The significance between different series was tested using one-way analysis of variance (ANOVA) at 5% level followed by Tukey's multiple comparaison test. All statistical analyses were performed using Prism 8.0 for Windows (GraphPad Software Inc., www.graphpad with a significant level $p<0.05$).

Results and Discussion

3. Results and Discussion

3.1. Results

3.1.1. Yield of *Citrullus colocynthis*

The extraction of the fruit of the plant was prepared following the protocol described by Merghem et al (1995). The yield of the fruit of *Citrullus colocynthis* hydroethanolic extract is 19.21%

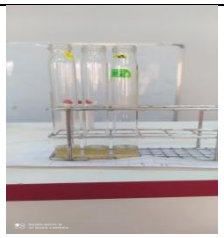
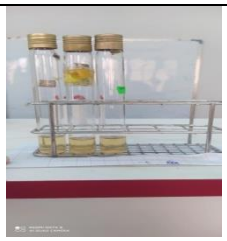
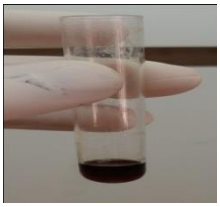
3.1.2. Phytochemical scrinning of the fruit of *Citrullus colocynthis*

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 fruit of *Citrullus colocynthis* contain flavonoids, alkaloids and steroids, and no saponins, catechic and gallic tannins, terpenoids and quinones.

Table 3: The results of the phytochemicals scrinning of Citrullus colocynthis fruit

Secondary metabolites	**Results**	**Observation**
Flavonoids		+
Saponines		-
Alkaloids	 	+

Catechic tannins		-
Gallic tannins		-
Steroids Terpenoids		+ -
Quinones		-

3.1.3. Antioxydant activity of *Citrullus colocynthis*

By following the protocol described by Kuramasamy (2007), we calculated the Ec50 for ascorbic acid and *C. colocynthis* extracts and we obtained these results.

This curve shows the interaction of ascorbic acid and *C.colocynthis* extracts with DPPH, which acts as a free radical in this experiment. After calculation, the EC50 is as below:

Table 4: The EC50 values of Ascorbic acid and *C.colocunthis*

Antioxydant	**EC50**
Ascorbic acid	**12.57 µg/ml**
C. colocynthis	**834.1µg/ml**

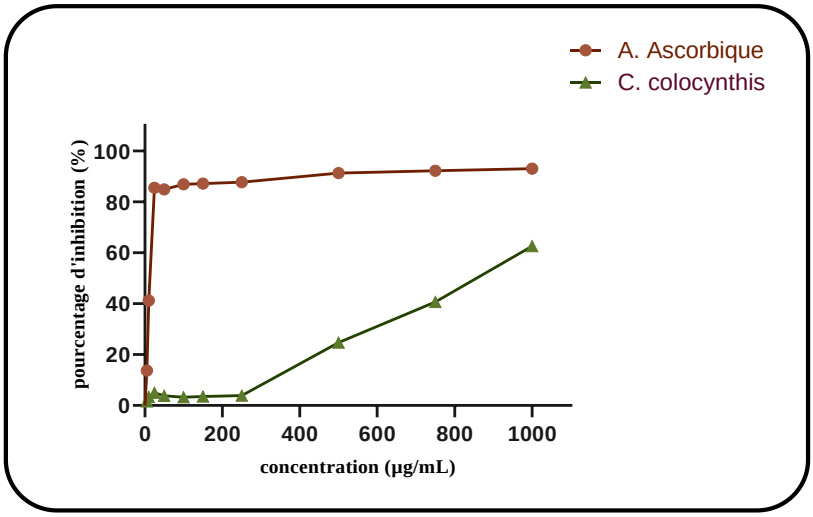


Figure 9: Percentage of DPPH radical efficacy as a function of *Citrullus colocynthis* extract concentration

3.1.4. Antidiabetic Activity *Citrullus colocynthis*

This test is conducted to study the inhibitory effect on glucose uptake by yeast suspended in a glucose solution of varying concentrations, in the presence of hydro-ethanolic extracts of *C.colocynthis* fruit and Metformin at different concentrations (10- 100 mg/ml).

This curve shows the interaction of Metformin and *C.colocynthis* extracts with glucose solution. After calculation, the IC50 is as below:

Table 5: The IC50 values of Metformin and *C.colocynthis*

Antidiabetic	**IC50**
Ascorbic acid	**83.46mg/mL**
C. colocynthis	**7.66 mg/mL**

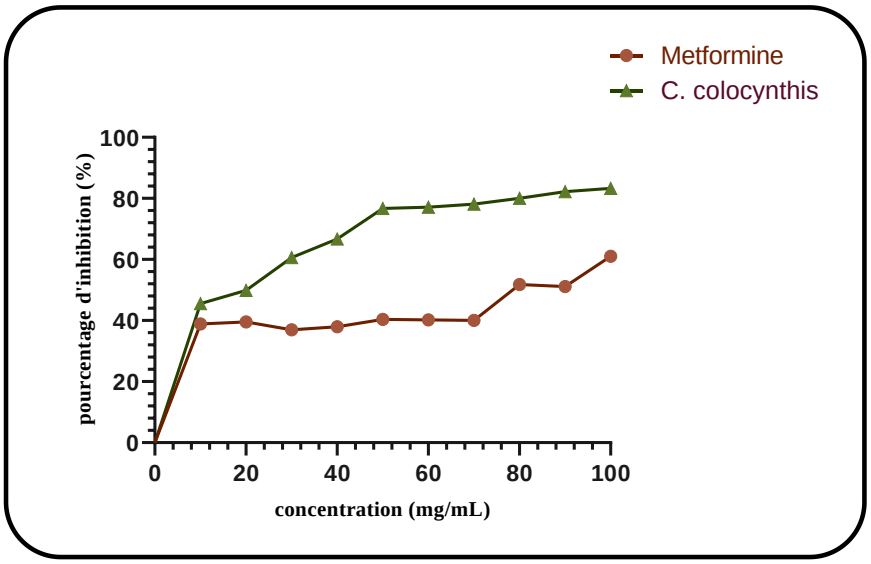


Figure 10: Interaction of Metformin and *C.colocynthis* extracts with glucose solution

3.1.5. Larvicidal efficacy of *Citrullus colocynthis* hydro-ethanolic extract against Cs. longiareolata

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

C.colocynthis hydro-ethanolic extract was tested on *Cs. longiareolata* larvae 4 at different doses (0.2mg/ml, 0.4mg/ml, 1.2mg/ml, 1.6mg/ml, 2mg/ml, and 2.8mg/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 06).** The dose-response curve expressing the percentage of mortality corrected as a function of the logarithm of the dose applied **(Figure 11)** was used to determine the values of the various sublethal and lethal concentrations, together with their confidence intervals and the HillSlope **(Table 07).**

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

Time (hours)	0,2mg/ml	0,4mg/ml	1,2mg/ml	1,6mg/ml	2mg/ml	2,8mg/ml	P
48	8.88 ± 3.85	20.00 ± 0.00	26.66 ± 0.00	44.44 ± 3.85	66.66± 6.66	91.11± 10.18	< 0.0001
72	8.88 ± 3.85	20.00 ± 0.00	31.11 ± 3.85	48.89 ± 7.69	68.88 ± 3.85	100.00 ± 0.00	< 0.0001

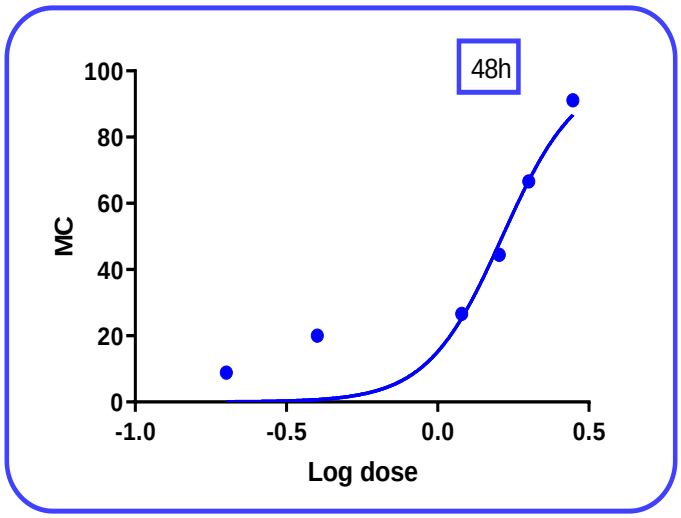


Figure 6: Efficacy of *C.colocynthis* hydro-ethanolic extract (mg/ml) applied on fourth instar larvae of *Cs. longiareolata* after 48h: Dose response curve expressing the percentage of mortality corrected according to the logarthm of dose

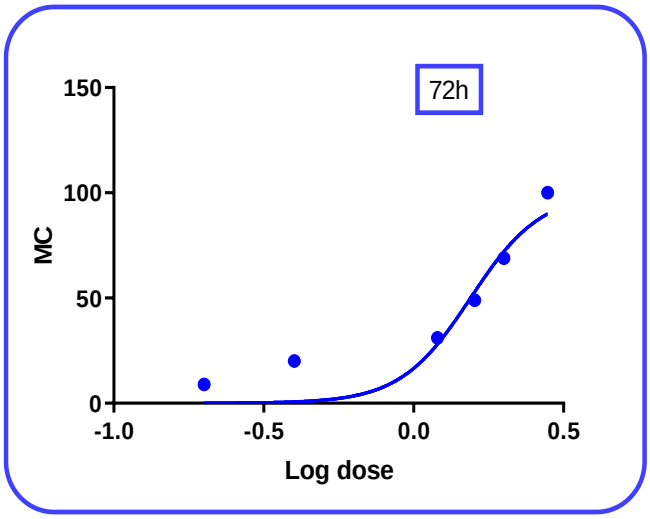


Figure 7: Efficacy of *C.colocynthis* hydro-ethanolic extract(mg/ml) applied on fourth instar larvae of *Cs. longiareolata* after 72h: Dose response curve expresing the percentage of mortality corrected according to the logarithm of dose

Table 7: Sublethal and lethal concentrations (mg/ml,FL) of *C.colocynthis* 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
48	1.19 0.33 to 1.60	1.63 1.00 to 2.06	3.07 2.03 to 22.57	0.90
72	1.15 0.28 to 1.58	1.54 0.84 to 1.95	2.80 1.87 to 18.05	0.90

3.2. Discussion

3.2.1. Extract yield

The hydroethanolic extraction of *Citrullus colocynthis* fruits yielded 19.21%. This percentage reflects satisfactory extraction efficiency and is consistent with previous studies on the species and family. For instance, **Bourhia *et al***. (2020) reported yields ranging from 18% to 21% in the same species *Citrullus colocynthis*, however **Kumar *et al*., (2023)** found the yield of *Curcubita pepo* a plant belongs on the Curcubitaeae family 15% to 20%.

3.2.2. Phytochemical scrinning

According to the results obtained in our study, *Citrullus colocynthis* plant is rich in many secondary Metabolites (flavonoids, Alkaloids, Steroids), and has no or few secondary Metabolites like (quinones, Terpenoids, Saponins, Tannins, quinines).

-Flavonoids test, the color of the solution changed to yellow color, indicating their presence, our results are consistent with the findings by **Almeer *et al* (2020)** in *C.colocynthis.* Flavonoids are well-established antioxidants and are known to reduce oxidative damage by scavenging reactive oxygen species **(Saini & Yadav, 2020)**

-The appearance of a precipitate was confirmed the presence of Alkaloids. These results are in line with those by **Benariba *et al* (2021)** and **Almeer et al (2020)** in *C.colocynthis.* Alkaloids have a possess antimicrobial and anti-inflammatory properties and contribute significantly to the biological activity of the plant (**El-Keblawy *et al*., 2022).**

-The presence of Steroids was confirmed by changing the color of the solution to purple. These results are consistent by the results obtained by **Hussain *et al* (2020)** in *C.colocynthis.* The properties of steroids are antibacterial and cardiotonic **(Saad. 2017; Makhloufi. 2013).**

-The test of Quinones, Terpenoids and tannins, the results were negative, the color of the solution did not changed to red, indicating their absence in *C.colocynthis,* our results are agree with the results of **Benariba *et al* (2021)** and incompatible with those obtained by **Almeer et al., (2020)** and **Hussain *et al*., (2020)** where they found them.

-As for the Saponins test, the persistant moss does not appeared indicating their absence, our results are conflicting with the results obtained by **Rahmani *et al*., (2021).**

3.2.3. Antioxydante activity of *Citrullus colocynthis* hydro-ethanolic extract

The results obtained when testing the antioxidant activity of *C.colocynthis* extract showed that the percentage of free radical inhibition increases with the concentration of the plant extract and the concentration of ascorbic acid.

The EC50 value indicates the concentration of the antioxidant needed to reduce the initial concentration of free radicals by 50%. A lower EC50 value signifies a more potent antioxidant, as a smaller amount is required to achieve the same effect. This value is used to compare the efficacy of different antioxidants in various experiments **(Rita, 2019).**

The EC50 value of ascorbic acid was 12.57µg/ml and the EC50 value of *C.colocynthis* extract was 834.1µg/ml, which indicate that a much larger concentration is needed to reach the same antioxydante effect, which suggest moderate antioxydante activity of the plant. 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 12.57µg/ml and *C.colocynthis* 834.1µg/ml are consistent with those obtained by **Al-Snafi *et al*, 2022,** where their Ec50 was estimated (789.5µg/ml) in the fruit extract.

Based on all our results and research, we can say that *Citrullus colocynthis* has moderate antioxydante activity but is not effective as synthetic antioxydante **(Hussain *et al*., 2021).**

3.2.4. Antidiabetic activity of *Citrullus colocynthis* hydro-ethanolic extract

The antidiabetic activity results revealed that *C.colocynthis* extract exhibits considerable inhibitory potential compared to metformin, with an IC50 value of 7.66 mg/ml versus 83.46 mg/ml for metformin. These findings indicate that the natural extract can inhibit carbohydrate-digesting enzymes (such as α-amylase and α-glycosidase) at significantly lower concentrations than the reference medicine. The lower IC50 value reflects high biological efficacy. Our IC50 value at *C.colocynthis* extract is similar with that obtained by **Benariba *et al*., 2023** were their IC50 was 8.27 mg/ml. These findings support the potential of this plant as a promising natural source for diabetes management or as a complementary agent to conventional therapy and also validate the traditional use of the plant in folk medicine for treating glycemic disorders.

3.2.4. Larvicidal efficacy of *Citrullus colocynthis* hydro-ethanolic extract against *Cs. longiareolata*

Toxicological tests were introduced to test the sensitivity of larvae to insecticides used in control campaigns **(OMS, 1963).** The use of chemical insecticides leads to an ecotoxicological mess, accompanied by a spectacular increase in the number of resistant species. The use of natural products remains the most beneficial method for the health of living beings and their environment **(Benayad, 2008).**

The research into plant-insect interactions has led to the potential use of plant extracts for pest control. Biopesticides have multiple targets: they can affect the insect nervous system, causing paralysis and subsequent death, alter the conductance of several ion channels (calcium, potassium, sodium), disrupt the arrangement of lipid bilayers, and also act at the level of presynaptic terminals **(Windley *et al.*, 2012).** These multiple targets of biopesticides work against the development of resistance in insects...

Our study evaluates the larvicidal effect of *Citrullus colocynthis* hydro-ethanolic extract on fourth instar larvae of *Culiseta longiareolata,* the results of which showed larvicidal activity with a dose-response relationship, reaching 100% at 2.8 mg/mL after 72 hours. The calculated LC_{50} value (1.15 mg/mL at 72 h) confirms the extract's efficacy, with a strong correlation coefficient ($r^2 = 0.90$), indicating a consistent and reliable dose-response relationship.

These results align with previous findings highlighting the insecticidal properties of *C. colocynthis* due to its rich composition in bioactive compounds such as cucurbitacins, flavonoids, and alkaloids, known to disrupt insect physiology and behavior (**Ahmed et al., 2021; Sadeghi-Aliabadi et al., 2020).** In particular, cucurbitacins have been reported to act as feeding deterrents and to impair the digestive and nervous systems of mosquito larvae **(Moustafa *et al.*, 2023).**

Moreover, the mortality observed in the current study is comparable or even superior to that reported in earlier works involving *C. colocynthis* extracts tested on other mosquito species such as *Aedes aegypti* and *Culex quinquefasciatus* **(Al-Harbi *et al.*, 2022)**. For instance, **Yadav *et al.* (2021)** reported an LC_{50} of 2.4 mg/mL against *Culex* species, suggesting that *Cs. longiareolata* may be more susceptible, or that the hydro-ethanolic extraction method used here yields a more potent phytochemical profile.

The hydro-ethanolic extract of *C. colocynthis* shows promise as a natural, eco-friendly larvicide.

Conclusion

4. Conclusion

As part of our investigation into the biological activities of the ethanolic extract of *Citrullus colocynthis*, we assessed its antioxidant, antidiabetic and insecticidal properties, along with analysis of its phytochemical composition. Based on the findings obtained, we were able to draw the following conclusion.

The yield of ethanol extraction was 19,21%.

The results also showed that the plant is rich in many secondary metabolites such as flavonoids, alkaloids and steroids.

Based on the DPPH assay results the extract exhibited a lower antioxidant activity with EC50 value of 834.1 µg/ml in comparaison with the ascorbic acid with EC50 value of 12.57 µg/ml. This suggests that while the plant extract possess compounds with antioxidant potential, their efficiency in scavenging free radicals is considerably lower than that of ascorbic acid.

These result of the antidiabetic activity of *C. Colocynthis* revealed a notable inhibitory effect. The extract exhibited a strong inhibitory activity with an IC50: 7.66 mg/ml, which was significantly lower than that of the standard meformin, which showed an IC50: 83.46 mg/ml.

These findings support the potential of this plant as a promising natural source for diabetes management or as a complementary agent to conventional therapy and also validate the traditional use of the plant in folk medicine for treating glycemic disorders.

The extract showed also a significant insecticidal action against mosquito species.

References

References

- Aitken, T. H. G. (1954). The culicidae of sardinia and corsica (diptera). bull. ent. res., 45 (3): 437-494

- Al-harbi, M. , Khalaf, A. A. , & Al-Mekhlafi, F. A. (2022). Mosquito larvicidal activity of C*itrullus colocynthis* seed extracts against A*edes aegypti* and C*ulex quinquefasciatus*. saudi journal of biological sciences, 29(3), 1856–1862

- Almeer, R. S., Kassab, R. B. , Albasher, G. , Alarifi, S. , Alkahtani, S. , Ali, D. , & Abdel moneim, A. E. (2020). C*itrullus colocynthis* extract protects against lead-induced hepatotoxicity and oxidative stress. Environmental science and pollution research, 27(21), 26041–26049

- Alqahtani, A. S., Hidayathulla, S. , Rehman, M. T. , Elgamal, A.A. , El-Massarani, S. , Razmovski-Naumovski, V. , Alqahtani, M. S. M, El Dib, R. A. , & Alajmi, M. F. (2021). C*itrullus colocynthis* (L.) schrad. : a review on phytochemistry, pharmacology, and toxicology. journal of ethnopharmacology, 265, 113310

- Al-snafi, A. E. (2016). The chemical constituents and pharmacological effects of *citrullus colocynthis* - an overview. International journal of pharmacological screening methods, 6(1), 7–15.

--Ahmed, E. A., Salama, A. K., & El-Sayed, A. (2021). Phytochemical constituents and biological activities of C*itrullus colocynthis*. a review. egyptian journal of basic and applied sciences, 8(1), 30–45.

-Aissani, N , Seghiri, R. , & Mekkiou, R. (2022). Phytochemical profiling and bioactives of curcubita pepo seeds. industrial crops and products, 178,114602

Alagawany, M. , Abd El-Hack, M.E. , Farag, M.R. , Tiwari, R. , Dhama, K. , & Karthik, K. (2021). C*itrullus colocynthis* (l.) schrad (bitter apple fruit): promising traditional uses, pharmacological effects, aspects, and potential applications. frontiers in pharmacology, 12, 791049

Al-mahmoud, M.E . , al-Marzouqi, A.H. , & Al-Mahmoud, M. A. (2022). Phenolic contents and antioxidant activity of *citrullus colocynthis* fruits, growing in the hot arid desert of the uae, influenced by the fruit parts, accessions, and seasons of fruit collection. antioxidants, 11(4), 656

-Al-snafi, A.E., et al. (2022). Anti-inflammatory potential of C*itrullus colocynthis* fruit extracts. saudi journal of biological science, 29(4), 1030-1038

-Altemimi, A. , et al. (2017). Optimization of ultrasound assisted extraction of bioactive compounds from C*itrullus colocynthis* using response surface methodology. molecules, 22(8), 1406

-Al-Yhia, M. , et al. (2022). Comparative metabolomics of C*itrullus colocynthis* organs: unraveling the secrets of its bioactivity. saudi pharmaceutical journal, 30(8), 1123-1135

-Azwanida, N. N. (2015). Areview on the extraction methods and stability bioactive compounds. journal of applied science & process engineering, 2(1), 8-14

- Bakkali, F. , Averbeck, S. , Averbeck, D. , & Idaomar, M. (2008). Biological effects of essential oils–a review. food and chemicaltoxicology, 46(2), 446–475

- Becker, N. , Petric, D. , Zgomba, M. , Boase, C. , Madon, M. ; Dahl, C. , & Kaiser, A. (2010). Mosquitoes and their control. springer science & business media

- Benariba, N. , Djaziri, R. , Bellakhdar, W. , Belkacem, N. , Kadiata, M. , Malaisse, W. J. , & Sener, a. (2021). Phytochemical screening and free radical scavenging activity of *citrullus colocynthis* seeds extracts. asian pacific journal of tropical biomedicine, 11(1), 20–27

- Benariba, N.J , Djaziri, R. , Bellakhdar, W. , Belkacem, n. , Kadiata, m. , Malaisse, W. J. J, & Sener, A. (2021). C*itrullus colocynthis* (L.) schrad. : phytochemical review and medicinal potential. plants, 10 (12), 2657

- Benchelah A. C., Bouziane. H., Maka M. et Ouahés. C., (2000). Fleurs du saharavoyage ethnobotanique avec touaregs du tassilli. ibis press, paris (france), p62

- Bourhia, M., et al. (2020). *Citrullus colocynthis* (l.) schrad: chemical characterization, scavenging and cytotoxic activities. open chemistry, 18(1), 986–994.

-Benariba, N. , et al. (2023). Antidiabetic potential of citrullus colocynthis: in vitro and in silico studies on α-amylase and α-glucosidase inhibition. plants, 12(4), 789

-Benayad, N. (2008). Les huiles essentielles extraites des plantes medicinales marocaines :moyen efficace de lutte contre les ravageurs des denrees alimentaires stockees. 63 p

-Benmehdi, H. , alloui, N., & meskine, M. (2012). Contribution to the ethnobotanical study of some medicinal plants from el oued region (algerian sahara). journal of medicinal plants research, 6(7), 1232–1235

- Chehma A., 2006 - Catalogue des plantes spontan2es du sahara septemtrionel algerien. labo -eco-sys, ouargla, p75.

-Chorfi D, Sedira H, 2016. Effet des huiles essentielles extraites de deux plantes, mentha piperita et ocimum basilicum sur les biomarqueurs biochimiques des larves de cutiseta longiareolata, mémoir, biochimie et biologie moléculaire, faculté des science exactes et science de la nature et de la vie, université de larbi tébessi_ Tébessa. 81 page.

- Darsie, R. F., & Ward, R. A. (2005). Identification and geographical distribution of the mosquitoes of north america, northof mexico. university press of florida.

-Dris, D. (2019). Etude de l'activité larvicide des extraits de trois plantes : *Mentha piperita, lavandula dentata* et *Ocimum basilicum* sur les larves de deux espèces de moustiques *Culex pipiens* (linné) et *Culiseta longiareolata* (aitken)*. thèse de doctorat. biologie animale. université badji mokhtar Annaba. p 181

- Ekor, M. (2022). The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. frontiers in pharmacology, 14, 254

- El Ghouizi, A., Boutahiri, S., & Bourhim, N. (2022). C*itrullus colocynthis* in moroccan traditional medicine: uses, toxicity and pharmacological activities. journal of ethnopharmacology, 285, 114876

- Elango, G., Bagavan, A., Kamaraj, C , Abduzzahir, A., & Abdul Rahuman, A. (2012). Efficacy of medicinal plant extracts against mosquito larvae. asian pacific journal of tropical biomedicine, 2(2), 98–103

- El-keblawy, A., et al. (2022). Phenolic contents and antioxidant activity of citrullus colocynthis fruits from uae deserts. antioxidants, 11(4), 656

- Fabricant, D. S., & Farnsworth, N. R. (2023). The value of plants used in traditional medicine for drug discovery. environmental health perspectives, 119(7), 109–114

- Ferede, G., Tiruneh, M., Abate, E., Kassa, W. J., Wondimeneh, Y., Damtie, D., & Tessema, B. (2022). Morphological characterization and phylogenetic analysis of culicidae species (diptera) in tropical climates. parasites & vectors, 15(1), 45.

-Fetni, S. , & Bertella, N. (2021). Cytotoxic and antioxidant effect of the ethanolic extract of citrullus colocynthis l. plant fruit. genetics & biodiversity journal, 5(2), 93–102.

-Ghasemzadeh, A. , Ghasemzadeh, N. (2023). Environmental factors shaping the phytochemical diversity of medicinal plants : a comprehensive review. frontiers in plant science, 14, 1128956

- Guittonneau G. g., (2011). La flore et la végétation de la tunisie méridionale. société botanique de france, france : 283-359.

-Hussain, A. I. , et al. (2021). Phytochemical profiling and antioxydant activity of *citrullus colocynthis* (L.) schrad. extracts. molecules, 26(9),2567

-Jat, R. k. , & Jat, R. (2021). Evaluation of antidiabetic activity of ethanolic extract of *citrullus colocynthis*. tropical journal of pharmaceutical and life sciences, 9(3), 1–6

- Harbach, R.. (2023). Mosquito larvae (diptera: culicidae): ecology, identification, and control. academic press

- Harborne, J. B. , 1998. Phytochemical methods. london: chapman and hall

- Huseini, H. F. , Darvishzadeh, F. , Heshmat, R. , Jafariazar, Z. , Raza, M. , & Larijani, B. (2009). The clinical investigation of C*itrullus colocynthis* (l.) schrad fruit in treatment of type ii diabetic patients: a randomized, double blind, placebo-controlled clinical trial. phytotherapy research, 23(8), 1186–1189

- Hussain, A. I. , Rathore, H. A. , Sattar, M. Z. , Chatha, S. A. , Ahmad, F. , & Ahmad, A. (2020). Phytochemical and antioxidant profiling of C*itrullus colocynthis* (l.) schrad. journal of herbs, spices & medicinal plants, 26(3), 291–303

- Isman, M. B. (2020). Botanical insecticides in the twenty-first century—fulfilling their promise? annual review of entomology, 65, 233-249

-Khaligh, F. g., Naghian, A. , Soltanbeiglou, s. & Gholizadeh, s. (2020). Autogeny in C*uliseta longiareolata* (culicidae: diptera) mosquitoes in laboratory conditions in iran. bmc research notes. 5(1)

- Kumar, S., Narwal, S., & Prakash, O. (2022). Medicinal plants with antidiabetic properties: a review of mechanisms and clinical evidence. journal of ethnopharmacology, 285, 11479.

-Makhloufi, C. (2013). Evaluation of antioxidant and antibacterial properties of algerian plant extracts. africain journal of traditional, complementary and alternative medicine, 10(4), 100-108

-Messian G. M., Cohat JJ. , Leroux J. P. , Pichon m. et Beyries A., (1993). Les allium alimentaires. edition irna, paris : 228

-Molyneux, P. 2004, The use of the stable free radical diphenylpicrylhdrazyl (DPPH) for estimating antioxidant activity songlanakarin journal of science and technology, 26 (2), 221-2019.

-Moustafa, A. A., El-Awady, N. A., & Abdel-Farid, I. B. (2023). Larvicidal and growth inhibitory effects of cucurbitacin-containing plant extracts against mosquitoes. pesticide biochemistry and physiology, 193, 105360

- Marfak A. 2003. Radiolyse gamma des flavonoïdes. Etude de leur réactivité avec les radicaux issus des alcools : formation de depsides. thèse de doctorat. université de limoges. 199p

- Marwat, S. k. , Khan, m. a. , Khan, m. a. , Ahmad, m. , Zafar, m. , & Sultana, s. (2012). Fruit and seed morphology of *Citrullus colocynthis* from pakistan. middle-east journal of scientific research, 11(3), 356–362

- Mujahid. m. , Singh, p. p. , & Yadav, s. (2021). Phytochemicaland pharmacological review on a tropical medicinal plant: *Memordica charanthia* l. journal of ethnopharmacology, 280, 114471

- Pandey, K. B. , & Rizvi, S. I. (2021). Plant polyphenols as dietary antioxidants in human health and disease. oxidative medicine and cellular longevity, 2021, 1627435.

-Rahimi, Y., et al.(2021). Genetic diversity and metabolic profiling of *citrullus colocynthis* from arid and semi-arid regions. industrial crops and products, 170, 113803

-Robinet, F.G. (1951). Saponosides stéroïdes et triterpéniques de synthèse. ecole polytechnique fédérale, zurich.

- Rahmani, A. H. , Aly, S.M. , Ali, H. , Babiker, A. Y. , & Aldebasi, y. h. (2021). phytochemical, antioxidant, and anticancer potential of *Citrullus colocynthis* (L.) schrad. molecules, 26(4). 10-37

- Rahuman, A. A. , Gopalakrishnan, g. , Venkatesan, P. , & Geetha, k. (2008). Isolation and identification of mosquito larvicidal compound from abutilon indicum (linn.) sweet. parasitol research, 102(5), 981–988

- Sadeghi-aliabadi, H. , Mohammadi, R. , & Gholamrezaei, F. (2020). Cucurbitacins and their derivatives as promising natural insecticides. natural product research, 34(21), 3041–3052.

-Saad, A. (2017). Phytochemical screening and biological activities of medicinal plants in algeria. journal of medicinal plants research, 11(3), 45-52

-Sansri, S , Kalbaza, A.Y. , & Bairi, A. E.M. (2022). Treatment of diabetes with a medicinal plant “citrullus colocynthis” in wistar rats. agricultural science digest, 42(6), 780–784

-Serge , R , (2003). Physiologie végétale. 2ème édition de boeck, bruxelles : 514.

- Saini, S. , & Yadav, J.P. (2020). Antioxidant activity and the estimation of total phenolic

- World Health Organization (who). (2005). Guidelines for laboratory and field testing of mosquito larvicides. geneva: who.

-William G., Hopkins M, 2003. Physiologie végétale. traduction de la 2ème éditionaméricaine par serge rambour, bibliothèque nationale, paris, 268-273

-Windley, M.J., Herzig, V. , Dziemborowicz, S. A. , Hardy, M. C. , King, G. F. & Nicholson,G. M. (2012). Spider-venom peptides as bioinsecticides. toxins 4: 191-227

- Yadav, R. S., Rivastava, R. , & Singh, S.k. (2021). Evaluation of larvicidal potential of indigenous plants against mosquito vectors. environmental science and pollution research, 28(3), 3434–3442