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Extraction and evaluation of some pharmacological and biological activities of medicinal plants "*Ruta montana* and *Ruta chalapensis*"

Realized by:

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Thesis defended on: 18/06/2025

In front of the jury composed of:

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Dedication

To our dear parents; grand fathers and grand mothers

Words cannot express the depth of my gratitude for your unwavering support, prayers, love, and guidance throughout our journey. thank you for your encouragement and for everything you have done for us. May they find in this work the fruit of their endless sacrifices and the proof of our eternal gratitude

To our sisters and brothers

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Résumé

La *Ruta montana* est une plante aromatique et médicinale traditionnellement utilisée pour ses propriétés anti-inflammatoires, antioxydantes et antispasmodiques. Cependant, peu d'études ont exploré sa composition chimique et son innocuité.

L'objectif de cette recherche est de valoriser cette plante à travers une étude phytochimique approfondie et une évaluation de sa toxicité et de son activité antioxydante. Après extraction de l'huile essentielle par hydrodistillation, une analyse par chromatographie en phase gazeuse couplée à la spectrométrie de masse (GC-MS) a permis d'identifier ses constituants principaux : 2-undécanone (32,739 %), 1H-phosphole, 2,5-dihydro-1-méthyle (17,113 %) et 2-nonène-4-one (10,976 %). Les tests de toxicité aiguë réalisés chez la souris ont révélé des effets secondaires modérés (sédation, paralysie des pattes, baisse de l'activité locomotrice) sans cas de mortalité, indiquant une toxicité relativement faible. Par ailleurs, l'activité antioxydante évaluée par le test DPPH a montré un effet modéré avec une valeur IC_{50} de 108,3 mg/ml.

En conclusion, bien que le rendement en huile essentielle soit faible (0,26 ml/100g), la *Ruta montana* présente une composition chimique riche et des propriétés biologiques intéressantes, justifiant son potentiel pour des applications pharmacologiques, sous réserve d'un usage maîtrisé.

Mots-clés :

Ruta montana, huile essentielle, GC-MS, toxicité aiguë, activité antioxydante, 2-undécanone, phytothérapie

Abstract

Ruta montana is an aromatic and medicinal plant traditionally used for its anti-inflammatory, antioxidant, and antispasmodic properties. However, few studies have investigated its chemical composition and safety.

The objective of this research is to enhance the value of this plant through an in-depth phytochemical study and an evaluation of its toxicity and antioxidant activity. After extracting the essential oil by hydrodistillation, an analysis using gas chromatography coupled with mass spectrometry (GC-MS) identified its main constituents: 2-undecanone (32.739%), 1H-phosphole, 2,5-dihydro-1-methyl (17.113%), and 2-nonen-4-one (10.976%). Acute toxicity tests performed on mice revealed moderate side effects (sedation, limb paralysis, decreased locomotor activity) without any mortality, indicating relatively low toxicity. In addition, antioxidant activity evaluated by the DPPH assay showed moderate activity, with an IC_{50} value of 108.3 mg/ml.

In conclusion, although the essential oil yield was low (0.26 ml/100 g), Ruta montana exhibits a rich chemical composition and interesting biological properties, supporting its potential for pharmacological applications, provided it is used with caution.

Keywords:

Ruta montana, essential oil, GC-MS, acute toxicity, antioxidant activity, 2-undecanone, phytotherapy

ملخص

تُعد نبتة Ruta montana من النباتات العطرية و الطبية التي تستخدم تقليديا لخصائصها المضادة للالتهابات و الاكسدة و التشنجات . و مع ذلك لم تتناول الا دراسات قليلة تركيبتها الكيميائية و سلامتها .

يهدف هذا البحث إلى تثمين هذه النبتة من خلال دراسة فيتوكيميائية معمقة وتقييم سميتها ونشاطها المضاد للأكسدة. بعد استخراج الزيت العطري بالتقطير المائي، تم إجراء تحليل باستخدام كروماتوغرافيا الغاز المقترنة بمطيافية الكتلة (GC-MS) مما سمح بتحديد مركباتها الرئيسية:

2-undecanone (32.739%)

1H-phosphole, 2,5-dihydro-1-methyl (17.113%)

2-nonen-4-one (10.976%)

أظهرت اختبارات السمية الحادة التي أجريت على الفئران آثارًا جانبية معتدلة (تهدئة، شلل الأطراف، انخفاض النشاط الحركي) دون تسجيل أي وفيات، مما يشير إلى سُمية منخفضة نسبيًا. كما أظهر تقييم النشاط المضاد للأكسدة باستخدام اختبارDPPH نشاطًا متوسطًا بقيمة IC_{50} بلغت 108.3 ملغ/مل

ختامًا، بالرغم من انخفاض مردود الزيت العطري (0.26 مل/100غ)، تُظهر النبتة تركيبة كيميائية غنية وخصائص بيولوجية واعدة، مما يدعم إمكانيّة استعمالها في التطبيقات الدوائية شرط استخدامها بحذر

الكلمات المفتاحية: روتا مونتانا، زيت عطري، كروماتوغرافيا الغاز-مطياف الكتلة، سمية حادة، نشاط مضاد للأكسدة، 2-أونديكانون، علاج بالنباتات

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List of abbreviation

RM: *Ruta montana*

EO : Essentiel oil

DL50: Lethal dose50

DPPH: 2,2-diphenyl-1-picryl-hydrazyl

IC50: Inhibitory concentration at 50%

I%: Percentage of inhibition

kg: kilogram

MG: milligram

ML: milliliter

H&E: Hematoxylin and Eosin stain

NOAEL: No –observed-adverse-effect level

OCDE: Organization for economic co-operation and development

AFNOUR: French Association for Standardization

GC-MS: Gas chromatography coupled with mass spectrometry

HD: Hydrodistillation

Tween: polioxyethylene (20) sorbitan monooleate

Introduction

Introduction

For thousands of years, man has been able to rely on nature to meet his basic needs, such as food, shelter, clothing, and also for his medical needs. Plants possess extraordinary therapeutic virtues, which are utilized for treating various diseases in living beings, particularly humans. He gradually succeeded in identifying the medicinal and/or toxic properties of plants. Thus, on each continent, different traditions and rituals developed from plants that were passed down and enriched over time (Svoboda and Svoboda, 2000; Merad and Mahiout, 2019).

Medicinal plants are any plant that contains substances in one or more of its organs that can be used for medicinal purposes. They are the foundation and an integral part of modern medicine and pharmacology, both as active ingredients exclusively extracted from plants and as raw materials in the chemical synthesis of drugs, but also as excipients (M. Joël Labé, 2018; Allouni, 2018).

They provide a multitude of active molecules used in various sectors: industry, nutrition, cosmetics, and the medical and pharmaceutical sectors. Among these molecules are coumarin, alkaloids, phenolic acids, tannins, lignans, terpenes, and flavonoids, as well as essential oils: It has anti-inflammatory, anticancer, antioxidant, antifungal, and antimicrobial properties. However, that doesn't mean it can't become harmful or toxic if not used properly.

Algeria has a rich and diverse plant flora; however, these potential plant resources and the valorization of its species have only been partially studied. The genus *Ruta* belongs to the Rutaceae family and is widely distributed, especially in mountainous regions. Many species of this type are used in traditional medicine because they contain several molecules with therapeutic activities; among the most well-known species is *Ruta montana*, commonly called *Fidjel*. This plant is widely used as a medicinal plant for its abortive and anti-fertility properties, antispasmodic, analgesic, anti-rheumatic, emmenagogue, antiparasitic, antifungal, anti-inflammatory, disinfectant, and antipyretic effects (Oliva and al., 2003; Duke and al., 2008; Kambouche and al., 2008; Belkassam and al., 2011).

This plant was chosen for our work due to its abundance in Algeria and also for its medicinal properties recognized since antiquity.

Our work consists of two parts:

The first part presents comprehensive bibliographic research on the origin of the plant and its distribution in Algeria, its description, classification, extraction, therapeutic properties, uses,

and toxicity. The second part presents the experimental section containing the materials and methods used and result and discussion and focuses on:

The extraction of essential oil from *Ruta Montana*.
The identification of the chemical composition of the essential oil by gas chromatography coupled with mass spectrometry (GC-MS).
The evaluation of antioxidant activity (DPPH).
The evaluation of acute toxicity in male Wistar albino mice.
The conduct of a histological study.

A conclusion is provided at the end of this work by highlighting the main results obtained, which could stimulate further research efforts aimed at serving and enhancing the national heritage in the field of medicinal plants.

Chapter I

literature review

1 Definition

Ruta montana and *Ruta chalepensis* belongs to the Rutaceae family, and it is one of the herbs used in alternative medicine. It possesses numerous medicinal properties and benefits, but it is a toxic and dangerous plant if not used with caution (Amabye, 2015). It spreads by seeds that can reach up to 1 meter in length (Allouni, 2018). It is present in several regions of the world, abundantly in the Mediterranean regions (Nahar et al., 2021). One of the most renowned species within this genus is *Ruta Montana* and *Ruta chalepensis* commonly recognized as "*Fijel*" in Algeria (Mohammedi and al., 2020; Benkhaira and al., 2022) .

1.1 Common names

Common names The *Ruta montana* (RM) species comprises different common names. It is known as '*Fijel*' in Arabic and 'Awermi' in Berber. It is called 'Rue des montagnes' in French and 'Mountain Rue' in English. It was concluded that the vernacular name of this plant varies according to each country or region. Indeed, RM can take various vernacular nominations including Fidjel, Fijel, El Fijel, Aourmi or Awermi (Morocco, Tunisia, and Algeria), Sedef (Turkey), Arruda (Portugal), and Ruda de jardín (Spain) (Benkhaira and al., 2022).

1.2 The family

Rutaceae is a large family of trees and shrubs, predominantly tropical and subtropical. It consists of 150–162 genera and 1500–2096 species with three main centers of diversity: Tropical America, southern Africa, and Australia (Groppo and al.,2012). This family has long been economically important for edible fruits (especially citrus, with many varieties of oranges, lemons, tangerines, etc.), aromatic oils (Boronia and Ruta), drugs (e.g., Pilocarpus, source of pilocarpine, used against glaucoma), and bitter beverages used to treat fevers (Angostura, Galipea). Species of Flindersia, Zanthoxylum, Balfourodendron, and Euxylophora are sources of timbers. More recently, the antimicrobial and antifungal properties of rutaceous compounds are being exploited as natural pesticides, herbicides, and antimicrobials, while others are medically useful (Oliva and al., 2000). Ruta is the richest genus of the rutaceae family. It features mainly shrubby plants, native to the Mediterranean region and present in traditional medicine of this region since Antiquity. But now, genus Ruta is cultivated in many parts of the world (Parray and al.,2012). The three most diffused species, *Ruta chalepensis L., Ruta graveolens L., and Ruta montana L*., are morphologically poorly differentiated and were probably interchangeably used during Antiquity (Pollio and al., 2008). Rutaceae are often woody plants possessing secretory cavities of a type not found in any other family, known as schizolysigenous cavities (Ozenda, 2000).

1.3 Botanical description

1.3.1 The aerial part

Stems: Straight, cylindrical, very branched, glabrous and glaucous, 2 to 5 feet tall.

Leaves: Petiolate, alternate, scattered, compound, with a glaucous green color, with oval, obtuse, thick leaflets, slightly toothed on the edges or entire.

Flowers: Yellow, with five concave petals that contain ten stamens much longer than the petals and ending in almost round anthers, peduncled in a terminal corymb.

Fruits: Globular capsules with rounded lobes and a short peduncle (4 mm) that end in 4 or 5 apparent rounded lobes, releasing small dark seeds upon maturity.

Seeds: Reniform, with an embryo enclosed in fleshy albumen.

Odor: foul, and flavor: warm and bitter.

1.3.2 Underground parts

Roots: White, fibrous, and with numerous rootlets.

1.4 Classification

Kingdom: Plantae.

Subkingdom: Tracheobionta.

Super division: Spermatophyta.

Division: Magnoliophyta.

Subdivision: Angiospermae.

Class: Magnoliopsida.

Subclass: Rosidae.

Superorder: Rutanae.

Order: Sapindales.

Family: Rutaceae.

Genus: *Ruta montana L.* (Wiart, 2006; Takhtajan, 2009).

1.5 Geographical distributions in Algeria

Rue grows spontaneously in rocky, arid places, old walls, and dry hills, and it is abundant in limestone terrains in Mediterranean regions. It is present in rocky pastures and lawns of the tell, commonly found in mountainous areas from the interior to the Saharan Atlas (Quezel and al., 1963).

1.6 Main uses of *Ruta montana* in traditional medicine

Rutamontana is an exceptional therapeutic plant, with the most commonly used parts being the leaves and roots. A pinch for a week is the most recommended dosage to avoid potential intoxications (Table 01) (Daoudi and al., 2016). *Rutamontana* is prescribed to treat a variety of conditions: dermal, pulmonary, dental, urogenital, oral-dental, traumatic, gastrointestinal, and neurological (Najem and al., 2018). Their uses were primarily as an abortifacient and emmenagogue and as a hypoglycemic, anti-rheumatic, vermifuge, anti-epileptic, anti-spasmodic, diuretic, and antipyretic (Table 02) (Mohammedi and al., 2020). It has the potential to be used as potassium channel blockers in the treatment of neuromuscular diseases, anxiety, and dysmenorrhea (Nahar and al., 2021). It is also recorded as a remedy against snake venom (Mohammedi and al., 2020).

Table 1:Some traditional uses of ruta montana (Alloun,2013; Lakhdar,2015).

Species	Pays	Used part	Way	Usage	References
Ruta montana	Spain	whole plant	Oral	Fever-Emmenagogue-Abortive-Antispasmodic against intestinal worms	(Alloun, 2013).
	Algeria	Aerial part		Emmenagogue Antispasmodic Rubefacient powder Echarrodic	(Alloun, 2013).

	Moroco	Aerial part	Against stomach aches, respiratory tract conditions, and liver diseases	(Lakhdar, 2015).

Table 2:Types of use of ruta montana (Hammiche and al.,2013)

Internal use	**External use**
As a powerful emmenagogue, for painful periods, difficult childbirths, and, in high doses, as an abortifacient and aphrodisiac.	The decoction in oil, when used for friction, relieves rheumatism and muscle soreness, and when applied to the skin, is reputed to improve vitiligo and psoriasis.
For severe respiratory conditions, gastralgias, intestinal disorders, spasms, gout, epilepsy, nervous disorders, paralysis, and as an anthelmintic.	The infusion as eye drops is used against corneal ulcers, as ear drops for otitis and ear ringing; nasally, the drops treat ozena.
In vaginal injections as an abortifacient, in enemas as an anthelmintic	As well as the fevers and vomiting in infants and young children.

1.7 Chimical compotition

Ruta species are sources of various chemicals such as essential oils (aliphatic ketones), coumarins (rotarin, furanocoumarins: psoralen, pregabalin, antitoxins), furoquinolines and acridone derivatives, flavonoids (rutoside), and tannins (Allouni and al., 2018). According to studies conducted on six alkaloids of *Ruta montana*, two of which were known as 1-methyl-4-methoxy-2-quinolone and evolitrine. The new compounds were 2-(nonan-8-one)-(1H)-4-quinolone , 2-(nonan-8-one)-4-methoxy-quinoline, 2-(nonan-8-one)-N-methyl-4-quinolone (3), and 2-(decan-9-one)-N-methyl-4-quinolone. (Touati and Ulubelen, 2000). The study by

Belkassam and al. (2011) revealed that the main components of essential oils from the aerial parts of *Ruta montana* were 2-undecanone, 2-nonanone, monoethylhexyl phthalate, decanone, 2-acetoxytridecane, and 2-tridecanol.

1.8 Toxicity of the plant

The use of *Ruta montana* as a remedy does not mean that it is always beneficial for human health (Masri and al., 2015). It is a plant to be handled with caution because its essential oil is toxic; it contains alkaloids, flavonoids, vitamin C, and furocoumarins. The leaves are irritating and vesicant, properties due to the essential oils, particularly methylnonylketone, which is a rubefacient (Allouni, 2018). As a general rule, external uses are preferred because the toxicity of the plant is widely recognized. (Pollio and al., 2008).

It should not be consumed by pregnant women, as high doses can lead to abortion (Parray and al., 2012). Taking an infusion (3 to 4 minutes) of *Ruta montana* leaves and stems for a week at high doses (3 infusions of 200 ml per day) caused acute poisoning in a woman in Tunisia. Therefore, random doses can lead to harmful health effects (Masri and al., 2015). Random doses can lead to adverse health effects (Masri and al., 2015).

Skin contact with the plant's leaves causes erythema, sometimes severe bullous dermatitis, and burning sensations, which differ from photoallergic reactions due to the presence of photosensitizing furanocoumarins (Alouni, 2018). Skin contact with the plant's leaves causes erythema, sometimes severe bullous dermatitis, and stimulates burns, which differ from photoallergic reactions due to the presence of photosensitizing furanocoumarins (Alouni, 2018). Skin irritations are caused by the presence of methyl nonyl ketone, one of the properties of the essential oil (Belazizia and Askri, 2020).

2 Essential oils

2.1 Definition

Essential oils (Burt) (etheric or volatile oils) are known as essences, volatile oils, ethers, or aetheroleum. They are found in aromatic plants in the form of a mixture of volatile components. Products such as secondary metabolites (Mossa, 2016) are natural volatile compounds that are largely produced from plant raw materials or plant organs such as flowers, seeds, buds, leaves, fruits, wood, roots, bark, and twigs. They give a distinctive flavor, smell, or fragrance to an aromatic plant. For example, essential oils derived from citrus fruits, which include a large group of fruits from the citrus genus, are the most well-known natural essential oils and

represent the widest range of commercially available natural fragrances and flavors, perfumes, and aromas.

2.2 Characteristics of Essential Oils

According to Echchaoui (2018), the main characteristics are:

Essential oils are composed of aromatic molecules of very low mass.

Liquids at room temperature, highly flammable.

Contain volatile and rarely colored principles.

Density lower than that of water.

2.3 Role of essential oils

Some scientists say that essential oils act as chemical signals between parasites and microbes, and most of them believe it is a plant hormone. Essential oils protect aromatic plants from viruses (Willem, 2009).

2.4 Localization

Essential oils develop within the cytoplasm of certain cells and are separated by synaeresis in the form of small droplets that then converge into more or less diffuse areas, and secretory cells are found in all parts of the plant (vegetative organs or reproductive organs). (Kahoul and Salim, 2017).

2.5 Toxicity of essential oils

Essential oils are not products that can be used without risk. Some essential oils are dangerous when applied to the skin, due to their irritating properties (essential oils rich in thymol or carvacrol), allergenic potential (oils rich in cinnamaldehyde), or photo-toxicity (citrus oils containing furanocoumarins). Other oils have a neurotoxic effect (ketones like α-thujone are toxic to nerve tissues). The toxicity of essential oils is quite poorly understood. Most of the time, under the term toxicity, accumulated experimental data are described in order to assess the risk associated with their use. (El-Haciet and al.,2014).

2.6 Extraction methods

Several essential oil extraction techniques are currently being implemented; they are guided by the plant material, the considerable sensitivity of certain plants, and the use of essential oils (Bozin and al., 2006) Table3.

Table 3: Extraction Methods of EO

Méthode	Principe	Aplication
Hydrodistilation	This process corresponds to heterogeneous distillation and involves immersing the plant raw material in a water bath. (El-Haci., 2014).	Extracting essential oils.
Steam distilation	The principle of this technique lies in the fact that the plant material to be distilled, supported by a grid or a perforated plate, is not in contact with the water but is traversed by steam (Jang and al.,2010).	Extracting essential oils

2.7 Analysis method

Gas chromatography coupled with mass spectrometry (GC-MS) The significant development of mass spectrometry in the identification of complex constituents has been made possible by the coupling of gas chromatography directly with mass spectrometry (GC-MS). This coupling allows for the acquisition of an interpretable mass spectrum for substance quantities ranging from micrograms to milligrams (Richard and al.,1992).

The electron impact ionization (EI) mode allows the bombardment of substances with a beam of electrons with an energy on the order of 70 eV, causing their ionization and fragmentation. The positive ionic fragments then form the characteristic mass spectrum of the compound. The mass spectra thus obtained are compared with those of reference products obtained from commercial computerized libraries such as the NIST Mass Spectral Library (National Institute of Standards and Technology 1999) and the Wiley Registry of Mass Spectral Data (Mc Lafferty and al.,1994).

2.8 Property of EO of *ruta montana* ,chimical compunds and biological activities

Essential oils (EOs) extracted from RM are characterized by a strong nauseous odor and predominated by 2-ketones, such as 2-undecanone and 2-decanone. (zeraiband and al., 2021),Moreover, many in vitro and in vivo works have shown that RM, especially its EOs, exhibit various biological activities such as antibacterial ,antifungal ,antioxidant, antidiabetic, anticancer, anti-fertility, antihypertensive, insecticidal and larvicidal properties (Mouhamadi hichem,2019; Bengalit and al.,2020; Hammami, I, and al.,2015; Gibka, J and al.,2009; Merghem, M and al.,2020; khadhri, A, and al.,2017; Farid, O and al.,2017; Ali, wk and al.,2016; Meghrem and al., 2017; El ouady, F and al.,2021; Boutoumi, H and al., 2009; Bouzeraa, H and al., 2019).

3 Methods for determining acute, subacute, and chronic toxicity

Toxicity of substances can be tested through three kinds of studies: acute, subacute, and chronic. Acute toxicity is determined following a single or short administration and is used to estimate the DL_{50} (lethal dose for 50% of the animals), according to the guidelines OCDE 423 or 425. Subacute toxicity, over 28 days (OCDE 407), is a repeated daily administration to observe cumulative effects and to determine the NOAEL (no observable adverse effect level). Then, chronic toxicity is examined over an extended period (90 days to 24 months) according to guidelines OCDE 452 or 453 to unveil long-term effects like organ damage or a carcinogenic potential. These normalised techniques are widely used in the evaluation of the safety of natural or artificial substances (OCDE, 2001; Casarett and Doull, 2013).

Target organs of *ruta montana* EO

Table 4:Target organs of ruta montana EO (Harchouche I,Khabaza N;2023).

Target organs	Effect
The liver	Mass reduction
The heart	Vascular rupture
The uterus	Hemorrhage
The stomach	Swelling

The brain	Vasodilatation

4 Biological activity

Table 5:Biological activity

Antibacterial activity	The researchers results indicate that the antibacterial activity is related to the chemical composition of the essential oil (Nahar and al., 2021). Phenols, terpenes, or terpenoids are the most important molecules characterized by their antibacterial activity and their broad spectrum (Lakhdar, 2015).
Antifungal activity	According to Allouni (2018), the results of the antifungal study of various extracts of *Ruta montana* indicate that the sensitivity of Aspergillus niger and Fusarium oxysporum increases with the concentration of the crude RM extract. All extracts of *Ruta montana* exhibited moderate antifungal activity against the tested fungi.
Antioxydant activity	Accumulation of the free radicals in body organs or tissues can cause oxidative damage to biomolecules and membranes of cells, eventually leading to many chronic diseases, such as inflammatory, cancer, diabetes, aging, cardiac dysfunction, and other degenerative (Wang, E.A.Konorev, andal,2004) . Antioxidants are capable of stabilizing or deactivating free radicals before they attack cells (A. Sisein,2014). Reactive Oxygen species can be eliminated by a number of enzymatic and non-enzymatic antioxidant mechanisms, among these antioxidants, we mention ascorbic acid (vitamin C) and superoxide dismutase (Wang, E.A.Konorev, andal,2004) .

Chapter II

Materials and Methods

5 Objectives of the work

The plant we tested is called *Ruta montana* L. and *Ruta chalepensis*, commonly known as "mountain rue" or "*Fidjel*" and belongs to the Rutaceae family. The Algerian Mediterranean plants of our endemic flora possess medicinal and biological properties due to their rich secondary metabolism. Due to the widespread use of *Fidjel* in traditional medicine, it can have undesirable, even toxic, effects if taken in high doses. Our study therefore focused on the effects of the lethal dose of *Ruta montana* and on the in vitro examination of the antioxidant activity of this oil. In this context, the acute toxicity of the essential oil from the aerial parts of *Ruta montana* was studied *in vivo* in Wistar albinos mice.

6 Vegetal Material

The collection of the plant *Ruta montana* and *Ruta chalepensis* (mountain rue) takes place between December and March 2024-2025: a time when the plant only has leaves (young stage) in the region of Miliana, wilaya of Aïn Defla—North Central Algeria, specifically at Djebel Zaccar in a semi-arid Mediterranean climate. It was identified based on morphological characteristics (Quazel and Santa, 1963). The aerial part of the *Rutamontana and Ruta chalepensis* plants was dehydrated at room temperature without light or humidity for a period of 15 days Figure1.

Following the drying process, we transported the plant to the analysis laboratory of the Faculty of Natural and Life Sciences at Saad Dahleb Blida 1 University. The extraction of the essential oil began over a period of three months (from December to mid-March) during the 2024-2025 academic year.

Figure 1: Drying of Ruta montana and Ruta chalepensis (personal photo)

6.1 Extraction of essential oil from *Rutamontana*

6.1.1 Experimental extraction protocol

First, the apparatus was rinsed with distilled water before starting the extraction process to avoid any contamination of the essential oil. The extraction of the essential oil was carried out using a Clevenger-type hydrodistillator. We performed 3 separate extractions:

We weighed 400g, 350g, and 140g of the dried plant material and then placed them in 1 and 2-liter boiling flask, adding 1450ml, 1200ml, and 600ml of distilled water; then the whole setup was placed on a heating mantle for boiling point for 2 to 3 hours. The water charged with essential oil vapor, passing through the condenser, condenses and falls into the oil-water separating funnel, separating according to density. Finally, the essential oil was collected with a syringe, then measured and stored in sterile glass bottles covered with aluminum foil in a refrigerator at 4°C Figure2.

Figure 2: Extraction of EO by Clevenger (personal photo)

6.1.2 Extraction by infusion of *Ruta montana*

We weighed 10 g of *Ruta montana* plant material and we placed a beaker containing 100 ml of water on a magnetic stirrer heating until boiling for 25 minutes. After boiling, we added 10 g of plant material and let it cool with a gentle manual stirring and we covered the contents with aluminum foil for 24 hours.

6.1.3 Yield of essential oil

Oil Yield calculation: We refer to yield as the ratio between the weight of the extracted essential oil and the weight of the plant to be processed. The percentage yield (R) is calculated using the following formula (Attou, 2011):

ROu = (M/Bm) × 100

M: the mass of the extracted oil (g)

Bm: is the initial plant biomass (g).

The yield "EO/plant raw material" can vary greatly depending on the plants. In general, essential oils have a low yield (Nowicki, 2019).

6.1.4 The Organoleptic Characteristics

The organoleptic characteristics of *Rutamontana* essential oil consist of verifying (appearance, color, odor, and flavor). The organoleptic characteristics were studied and compared to the AFNOR standards, 2006, and other researchers' studies.

7 Gas chromatography coupled with mass spectrometry

The GC/MS analysis was performed using a Hewlett Packard Agilent 6890 gas chromatograph coupled with a Hewlett Packard Agilent 5973 mass spectrometer. The system is equipped with an electron impact ionizer with a filament intensity of 70 eV. The source temperature is 230 °C while the interface temperature is 270 °C. The injection was performed in split mode (1:50), with a volume of 0.2 µL injected and an injector temperature of 250 °C.

The capillary column is of the HP-5MS type (30 m length, 0.25 mm internal diameter, 0.25 µm film thickness) with a stationary phase of 5% phenyl and 95% dimethylpolysiloxane. The oven temperature program starts at 45 °C for 8 minutes, reaching 250 °C at a rate of 2 °C/min and remaining at that temperature for 10 minutes. Very high purity helium (6.0) is the carrier gas used at a constant flow rate of 0.5 mL/min. The total analysis time is 120 minutes. The spectra are acquired in scan mode after a solvent delay of 3 minutes Figure 3.

Figure 3: Gas chromatography coupled with mass spectrometry (personal photo)

8 Antioxidant activity

We can use various tests to measure the antioxidant activity of substances of natural origin. The majority of these analyses rely on the coloration/decoloration of a reactive agent in the reaction environment. In this research work, we used one test, namely DPPH˙.

8.1 DPPH˙ (2,2-diphenyl-1-picrylhydrazyl) anti-radical act

The DPPH˙ test is a stable violet radical in solution, with a maximum absorbance at 517 nm (Brand-Williams and al.,1995). The routinely applied protocol is based on the disappearance of this maximum due to the reduction of DPPH˙ by an antioxidant compound (Figure5), causing a yellowish discoloration. The concentration of an antioxidant required to eliminate 50% of the initial concentration of DPPH˙ (IC_{50}) is widely used as a measure of antioxidant activity (Atoui and al.,2005) Figure 4.

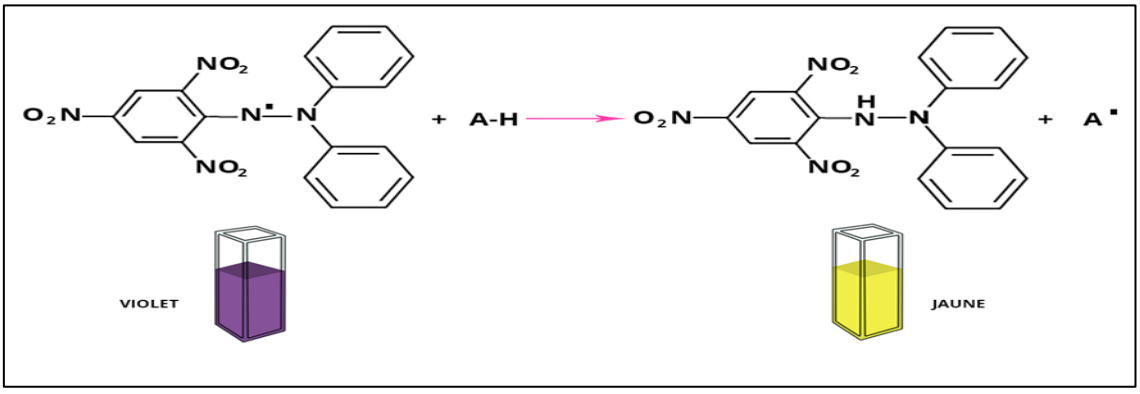


Figure 4 : Antioxydant activity (Chimactiv – AgroParisTech, s.d.)

8.1.1 Preparation of the alcoholic DPPH solution

We took 10.14 µmol of DPPH, which corresponds approximately to 4 mg of product, and dissolved it in 100 ml of methanol. The solution must be prepared in advance because solubilization is difficult, and it does not last more than 24 hours in the dark.

8.1.2 Preparation of a stock solution for sampling

We dissolved 200 mg of *Ruta montana* essential oil from the Ain Deffla region in 0.3 ml of methanol to achieve a concentration of 333 mg/ml. From this stock solution, we prepare diluted solutions of lower concentrations that will be used to provide concentrations lower than 333 mg/ml (166,6 mg/ml; 66,66 mg/ml; 13,33 mg/ml; 2,67 mg/ml; 0,53 mg/ml; 0,11 mg/ml).

8.1.3 Preparation of positive witness

The results are always compared to one or more positive controls. These controls are often synthetic antioxidants that are currently used in the agri-food industry. The antioxidant we used as a positive control is vitamin C. Regardless of the positive control used, its preparation and experimentation are identical to those of other samples.

We dissolved 50mg of ascorbic acid (vitamin C) in 10 ml of methanol. From this stock solution, we prepare diluted solutions of lower concentrations (1 mg/ml; 0,2 mg/ml; 0,04 mg/ml; 0,008 mg/ml; 0,0016 mg/ml).

8.1.4 Experimental protocol

for a given sample and a given concentration, we proceed as follows: we take 3 ml of the DPPH solution and measure its absorbance (A0) at 517 nm using a UV-visible spectrophotometer, then 200 µL of the sample at different concentrations are added to 2800 µL of a methanolic $DPPH^{\cdot}$ solution. After incubation (30 min) in the dark at room temperature, the absorbance (A1) was measured using the same spectrophotometer. Ethanol is used as a negative control.

The percentage of inhibition was calculated according to the following equation:

DPPH scavenging activity (%) = (A 0 - A 1) / A 0 × 100

A0: the absorbance of the control

A1: the absorbance of the sample.

The linear regression analysis of the dose-response curve, which represents the percentage of inhibition as a function of concentrations, allowed us to determine the effective concentration required to eliminate 50% of the $DPPH^{\cdot}$ radical (IC_{50} value).

9 Pharmacological study

The pharmacological research process begins with the examination of toxicity, particularly acute toxicity. In this research work, we sought to evaluate the acute toxicity of the volatile essential oil of *R. Montana* via oral administration, as this is gener ally the route used under normal conditions for a human.

9.1 Toxicological study

The in vivo experiment was carried out on male Swiss albino mice weighing 30 ±2 g. The animals were bred from the PASTEUR INSTITUTE KOUBA on 24 February 2025.The animals are housed in plastic enclosures in an animal house at the experimental station of the University of Blida 1, where they benefit from appropriate rearing conditions with a temperature of 25°C, constant lighting for 12 hours a day, and a humidity level of 55%. With a balanced diet of pellets and drinking water is provided as part of the diet. Our study focused on the toxic effects and histopathological study of liver and brain in male mice after administration of our *Ruta montana* essential oil-based emulsion for 21 days. 48 healthy mice were divided into 8 groups, each containing 6mice (n=6), on the basis of their homogenous mean body weight.

9.1.1 Adaptation period

Prior to experimentation, the mice underwent a 15-day adaptation period to stabilize in a new environment and to adapt to the handlers, in order to avoid them being factors that could modulate our results Figure 5.

Figure 5 : Adaptation period (personal photo)

9.1.2 Experimental design

- the maximum dose volume cannot be exceeded. The maximum volume of the liquid that can be administered at one time depends on the size of the test animal.

- the volume must not exceed 0.5ml of body weight, we will calculate the average body weight.
- The animals must be fasting before the administration of the substance, for the mice we withhold food, but not water, for 3 to 4 hours.
- The test substance is administered in a single dose using a gastric tube.
- The doses must be prepared just before administration.
- After the administration of the substance, the animals can be deprived of food again, for 1 to 2 hours for the mice.
- Animals must be observed individually at least once during the first 30 minutes and regularly during the first 24 hours after treatment. Particular attention is required during the first 4 hours and daily for 14 days after the administration of the substance (OECD, 2001) Figure 6.

Figure 6: Preparation of doses and gavage method (personal photo)

The serie of groups and doses:

- **Group 1:** the control mice receive 0,5ml of physiological water.
- **Group 2:** mice receive0, 5ml of emulsion (water+tween+EO) (500mg/ 1Kg) .
- **Group 3:** mice receive0, 5ml of emulsion (water+tween+EO) (1000mg/1Kg).
- **Group 4:** mice receive0, 5ml of emulsion (water+tween+EO) (1500mg/1kg).
- **Group 5:** mice receive0, 5ml of emulsion (water+tween+EO) (2000mg/1kg).
- **Group 6:** mice receive0, 5ml of emulsion (water+tween+EO) (2500mg/1kg)
- **Group 7:** mice receive0, 5ml of emulsion (water+tween+EO) (3000mg/1Kg).
- **Group 8:** mice receive 0, 5ml of infusion.

9.1.3 Animals sacrifice and organ extraction

At the end of the experimental period, 1 mouse from each group was sacrificed by decapitation. Afterdissection, the livers and the brains were immediately removed, weighed, and quickly fixed for 48 hours in10% buffered formalin solution to preserve the cells and tissue components in a "near-life state." Then, the samples are placed in labeled cassettes for their histological examination Figure 7.

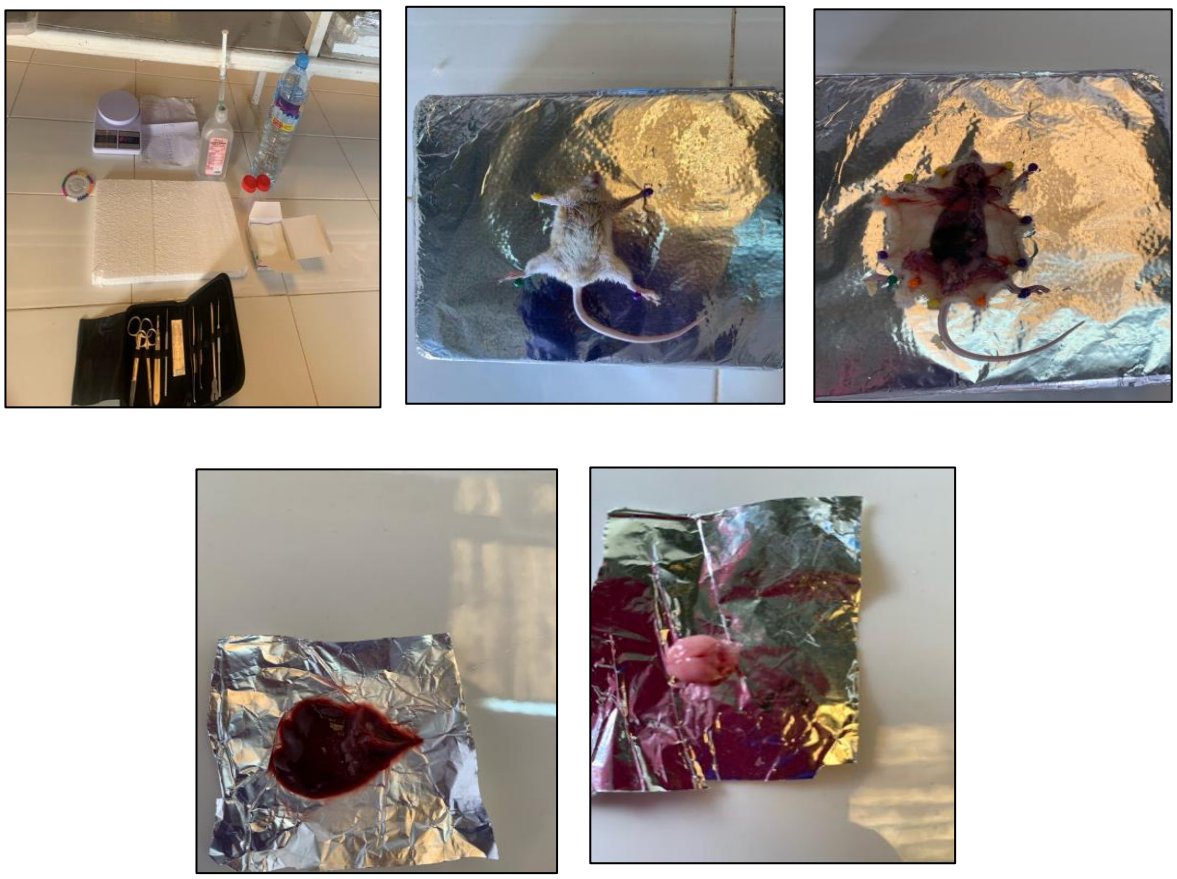


Figure 7: Animals sacrifice and organs extraction (personal photo)

10 Histopathological examination

The organs of the treated mice were removed and examined for macroscopic changes. Similar samples were fixed in a 10% formalin solution, dehydrated in 70-90% alcohol, cleared with xylene, and embedded in paraffin. For the histological examination of tissues, sections 5 to 6 µm thick were prepared using a microtome (Leica, RM 2145). These sections were then deparaffinized in xylene, passed through 70–90% alcohol, and stained with hematoxylin and eosin (H&E) and trichrome. The slides prepared by this process were observed under an optical microscope Figure 8.

Figure 8: Histology of organs (personal photo)

Chapter III

Results and Discussion

11 Yield of essential oil from Ruta montana

The hydrodistillation process of the dried aerial part of the Ruta montana plant, harvested in Djebel Zaccar (Ain Defla), carried out with a Clevenger apparatus, produced an average essential oil (EO) yield of 0.26% and zero yield for *Ruta chalepensis*. The table 6 summarizes the yield in essential oils (EO) as well as the yields reported in the literature for the same species.

Table 6:Yield in EO of ruta montana

Provenance	**Used part**	**Extraction method**	**Yield%**	**Reference**
Algeria (Ain Defla)	A.P	HD	0.26	*
(Bouira)	A.P	HD	0.62	(Mohammedi, H.2019)
(Mila)	A.P	HD	4.5	(Zellagui, A and al,2012)
(Tizi ouzou)	A.P	HD	0.38	(Mohammedi and al, 2020)
(Oum El Bouaghi)	A.P	HD	1.5	(Kambouche, N andal,2008)
(Oran)	Leaf	HD	1.63	(Khadhri, A and al,2014)
(Tipaza)	Leaf + Stem	S.D	0.6-0.97	(Hammami, I and al,2015)
Tunisia	Stem	HD	0.66	(Tanker, N and al,1980)
	Leaf	HD	0.66	(Tanker, N and al,1980)
	Leaf	HD	0.26	(Mohammedi, H and al,2019)
Turkey	A.P	S.D	2.76	(Rajabi, Z and al,2014)

* : Sample studied ; HD : Hydrodistillation ; S.D : Steam distillation ; A.P : Aerial part.

The extraction yield (EY) of the dried aerial part of *Rutamontana* that we discovered is 0.26%. This value is comparable to those mentioned in several studies (Tanker, N and al,1980; Hammami, I and al,2015; Mohammedi, H.2019; Mohammedi, H and al,2019; Mohammedi and al, 2020) but remains lower than those recorded in other areas of the country (Kambouche, N and al,2008; Zellagui, A and al,2012; Khadhri, A and al,2014). Furthermore, the steam distillation extraction of *Ruta montana* from Turkey yielded an essential oil with a high yield (2.76%) (Rajabi, Z and al,2014).

Several factors can influence this variation in (EO) yield, including: weather, geographical and seasonal conditions, the timing of the harvest, the part of the plant used, the state of plant utilization, and the extraction method applied. This provides an explanation for the observed diversity in these results (Allouni, 2018; Yosra and al., 2019; Bourahla and al., 2020).

12 The kinetics of the oil

Table 7:Kinetics of EO

T/min	10	20	30	40	50	60	70	80	90	100	110	120	140	150
V/ml	0,04	0,06	0,08	0,12	0,14	0,16	0,18	0,20	0,22	0,24	0,24	0,26	0,26	0,26

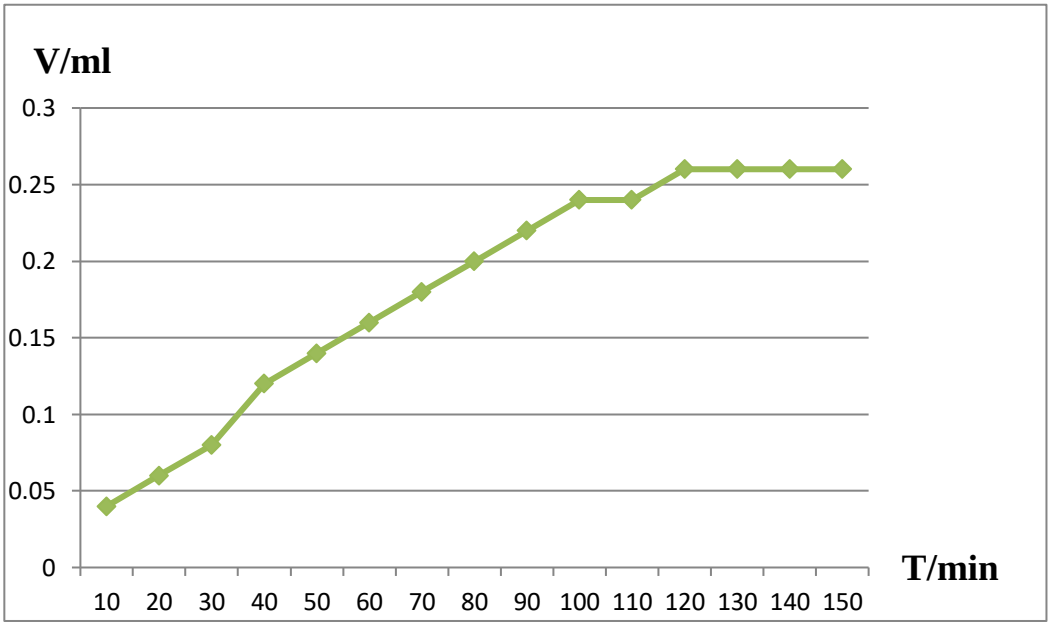


Figure 9 : Graph of the kinetics

Our graph showing the volume of oil as a function of time per minute. Typically, the curve shows 3 phases:

- The initial phase: extraction begins slowly, as the accessible compounds are released (10 to 30 minutes).
- The Acceleration Phase: the extraction rate increases by 0.02 ml every 10 minutes (30 to 120 minutes).

- The Stabilization Phase: the extraction slows down and reaches a plateau, indicating that the majority of the oil has been extracted (120;150).

13 The organoleptic characteristics of essential oil

The essential oil extracted by hydrodistillation from the plant material of *Rutamontana* is a pale yellow liquid that emits a very pronounced and distinctive scent. The table 8 summarizes the results.

Figure 10: EO of Ruta montana (personal photo)

Table 8:Organoleptic characteristics of EO of Ruta montana

Characteristics organoleptic	**Aspect**	**Color**	**Odor**
Alloun, 2013	Liquid Mobile	pale yellow	strong pronounced and characteristic
AFNOR, 2006	Liquid Mobile	pale yellow	strong pronounced and characteristic
Essential oil studied	Liquid Mobile	pale yellow	strong pronounced and characteristic

The organoleptic parameters of the (EO) are in accordance with those listed in the standards (AFNOR, 2006) and similar to that found by (Alloun, 2013).

14 Gas chromatography coupled with mass spectrometry (GC/MS) of essential oils

The GC-MS analysis of *Ruta montana* essential oil revealed the presence of fifty-four chemical compounds; among these constituents, three are major compounds.

The figure11 below presents the results of the GC-MS analysis of Ruta montana essential oil.

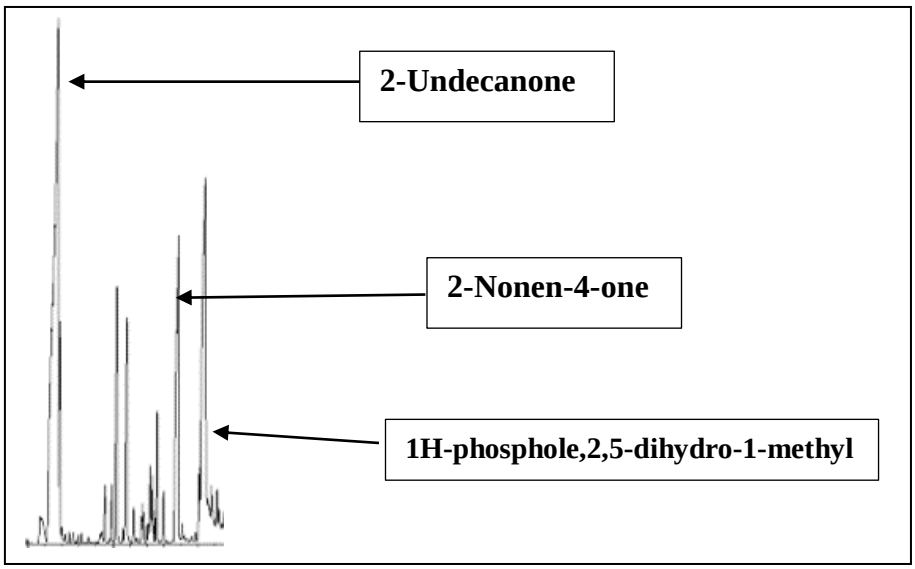


Figure 11: Result of GC-MS of EO of Ruta montana

According to the results presented in the table, the major compounds of *Ruta montana* essential oil are: 2-Undecanone (32.739%), 1H-phosphole,2,5-dihydro-1-methyl (17.113%), and 2-Nonen-4-one (10.976%). This essential oil is mainly dominated by ketones: 2 – Undecanone. According to these results, among the chemical compounds of this essential oil, the percentage of the major components is high (60.828%). These results are well illustrated in the Diagram below:

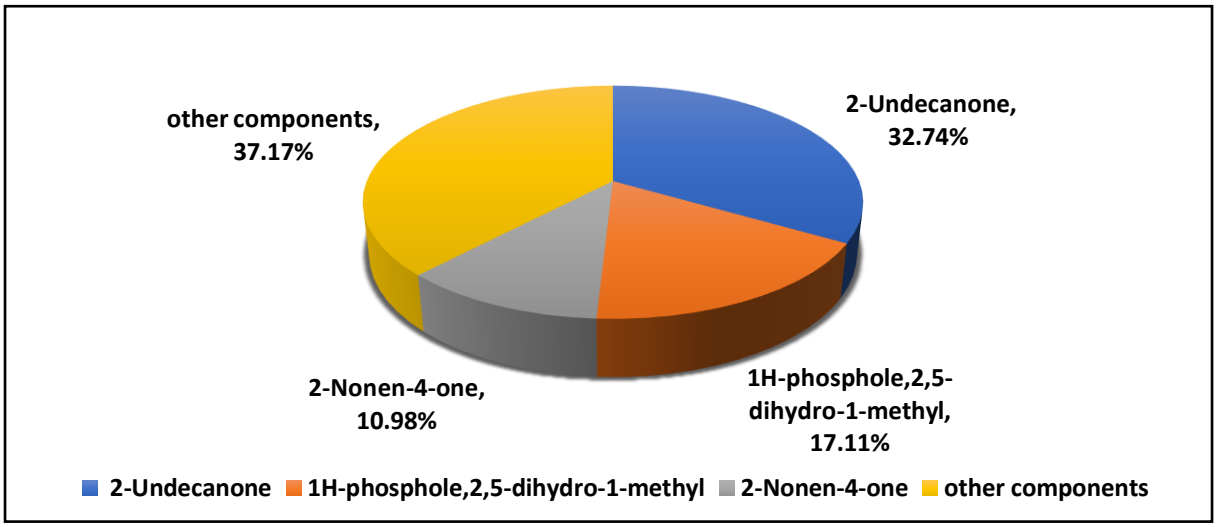


Figure 12: Percentage of the major components of EO of Ruta montana

For example, (Zellagui and al., 2011), who studied *Ruta Montana L.*, found that the percentage of 2-undecanone (60.19%), 2-nonanone (08.63%), monoethylhexyl phthalate (6.46%), decanone (06.26%), 2-acetoxytridecane (3.38%), and 2-tridecanol (3.37%).While (Chaibeddra,2014) discovered that the percentages of 2-undecanone (16.20%), 2-tridecanol (16.07%), tetramethyl succinimide (15.51%), 1-dodecanone, 1-cyclopropyl (15.43%), tridecane-2,4-dione (11.33%), and 2-acetyloxy-tetradecane (10.06%).We observe that the rate of 2-undecanone is higher in the first situation, while it is lower in the second situation compared to the one found in our case (32.74%).

15 Acute toxicity (evaluation of theLD50)

The evaluation of the acute toxicity of *R. montana* essential oil in albino mice showed that oral administration of doses (500, 1000, 1500, 2000, 2500, and 3000 mg/kg) did not cause any mortality during the 14-day observation period but resulted in significant differences in physical signs such as sedation, paralysis of the hind legs, decreased locomotor activity, convulsions, and closed eyes Table 9. These signs appeared over a duration of 6 to 10 days. Therefore, the essential oil was not eliminated from the body within four hours following its administration, as is the case with the vast majority of synthetic drugs. This observation allows us to take the precaution of not administering multiple doses of the essential oil over short periods to avoid any accumulation of compounds that could poison living tissues. These results prove that this oil seems to be low toxic up to the higher dose of 3000mg/kg, thus LD50 3000 mg/kg. And regarding the oral administration of the *R. montana* infusion, the results obtained are similar to the oil.

Table 9:The symptoms of toxicity of EO of Ruta montana

	15 first minute	**4th hour**	**1st day to 3**	**7th day**	**14th day**
Group 1: (500mg/kg)	-Sedation -Closed eyes -Decrease in locomotor activity -Straightening of the hair -No mortality	-Sedation -Closed eyes -Decrease in locomotor activity -Straightening of the hair -No mortality	-Sedation -Closed eyes -Decrease in locomotor activity -Straightening of the hair -No mortality	No signe No mortality	No signe No mortality
Group 2: (1000mg/kg)	-Sedation -Closed eyes -Decrease in locomotor activity -Straightening of the hair -No mortality	-Sedation -Closed eyes -Decrease in locomotor activity -Straightening of the hair -No mortality	-Sedation -Closed eyes -Decrease in locomotor activity -Straightening of the hair -No mortality	No signe No mortality	No signe No mortality
Group 3: (1500 mg/kg)	-Sedation -Closed eyes -Convulsion -Decrease in locomotor activity -Straightening of the hair -No mortality	-Sedation -Closed eyes -Decrease in locomotor activity -Straightening of the hair -No mortality	-Sedation -Closed eyes -Decrease in locomotor activity -Straightening of the hair -No mortality	No signe No mortality	No signe No mortality
Group 4: (2000mg/kg)	-Sedation -Closed eyes -Decrease in locomotor activity -Straightening of the hair	-Sedation -Closed eyes -Decrease in locomotor activity -Straightening of the hair	-Sedation -Closed eyes -Decrease in locomotor activity -Straightening of the hair	No signe No mortality	No signe No mortality

	-No mortality	-No mortality	-No mortality		
Group 5: (2500mg/kg)	-Sedation -Closed eyes -Paralysis of the legs -Decrease in locomotor activity -Convulsion -Straightening of the hair -No mortality	-Sedation -Closed eyes -Paralysis of the legs -Decrease in locomotor activity -Convulsion -Straightening of the hair -No mortality	-Sedation -Closed eyes -Paralysis of the legs -Decrease in locomotor activity -Convulsion -Straightening of the hair -No mortality	No signe No mortality	No signe No mortality
Group 6: (3000mg/kg)	-Sedation -Closed eyes -Paralysis of the legs -Decrease in locomotor activity -Convulsion -Straightening of the hair -No mortality	-Sedation -Closed eyes -Paralysis of the legs -Decrease in locomotor activity -Convulsion -Straightening of the hair -No mortality	-Sedation -Closed eyes -Paralysis of the legs -Decrease in locomotor activity -Convulsion -Straightening of the hair -No mortality	No signe No mortality	No signe No mortality
Group 7: infusion	-Sedation -Closed eyes -Paralysis of the legs -Decrease in locomotor activity -Convulsion -Straightening of the hair -No mortality	-Sedation -Closed eyes -Paralysis of the legs -Decrease in locomotor activity -Convulsion -Straightening of the hair -No mortality	-Sedation -Closed eyes -Paralysis of the legs -Decrease in locomotor activity -Convulsion -Straightening of the hair -No mortality	No signe No mortality	No signe No mortality
Group 8: control	No signe	No signe	No signe	No signe	No signe

All species of the genus Ruta are considered potentially toxic at high doses; this characteristic is mainly due to their chemical composition (De Sa and al. 2000; Gonzalez-Trujano and al. 2006; Hammami and al. 2015). According to Gonzalez-Trujano and al., the ethanolic extract of *Ruta chalepensis* showed no lethal effect in mice at doses up to 5000 mg/kg during the observation period (14 days). However, the oral administration of *Ruta chalepensis* infusion to pregnant mice resulted in significant differences in physical signs such as weight gain in the mice and histological modifications of the fetal placenta.

And according to M. Mohamedi Hichem (2019), his results show that the mortality of mice mainly depends on the administered dose. The oral administration of *Ruta montana* essential oil at doses up to 750 mg/kg (maximum altered doses) did not cause any mortality in the mice; however, a single mortality was observed after 10 days of administration at a dose of 1000 mg/kg. The severity of toxicity increases with the increase in dosage. During the first day of observation, three deaths were observed at the 1500 mg/kg dose, and six deaths were confirmed in the early hours after administering the 2000 mg/kg dose. The DL50 value of *Ruta montana* oil is 1500 mg/kg, which classifies this essential oil as slightly toxic on the Hodge and Sterner scale.

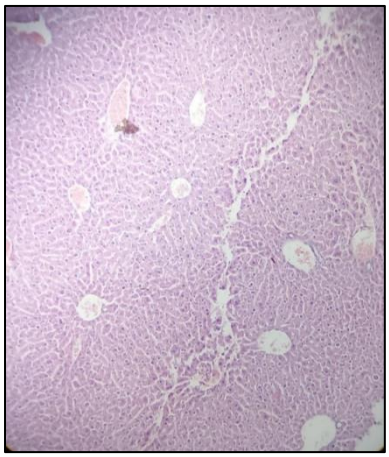
16 Histopathological examination

Microscopic observations in the control and treated groups revealed a normal hepatic and cerebral histomorphology, characterized by hepatocytes separated by sinusoids in the liver and an intact neuronal parenchyma, composed of well-organized glial cells, with evenly distributed cerebral capillaries in the brain.

Histological observations showed slight vasodilation in the groups treated with low doses of *Ruta montana* essential oil (500 and 1000 mg/kg) and no notable modification of the tissue structures. With the increase in doses (1500 mg/kg and 2000 mg/kg), this phenomenon intensifies, and the dilation becomes evident in the case of cerebral vessels and hepatic sinusoids, where the vessels appear engorged with blood. At the highest dose (3000 mg/kg), histopathological examination revealed a more pronounced vasodilation in the cerebral tissue, associated with a slight inflammatory infiltrate, and a heavily dilated sinusoid was observed in the hepatic tissue Figure 13.

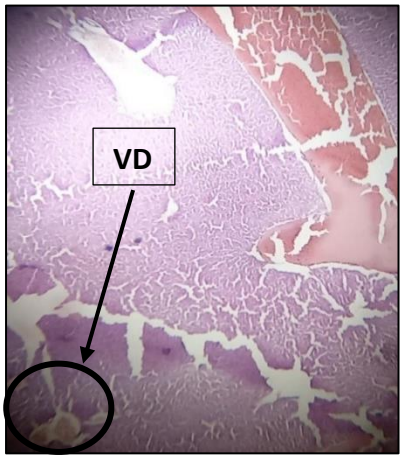
Liver:

Negative witness

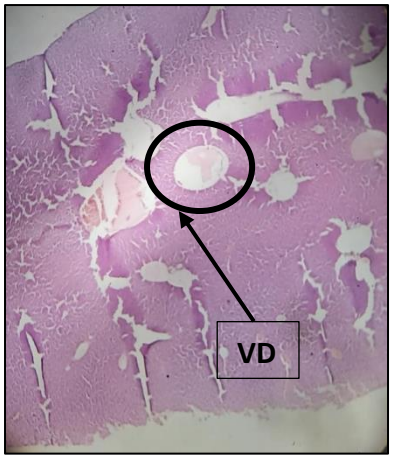


Infusion

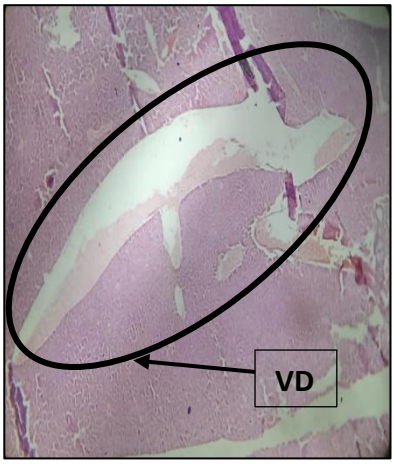
Dose of: 500 mg/ml



Dose of :2000 mg/ml

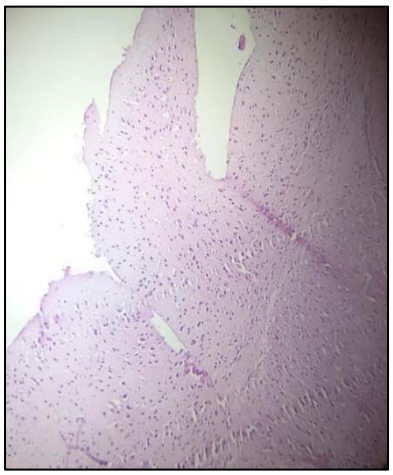


Dose of: 3000 mg/ml



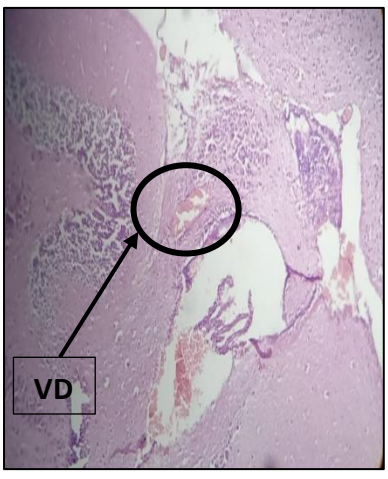
Brain:

Negative witness

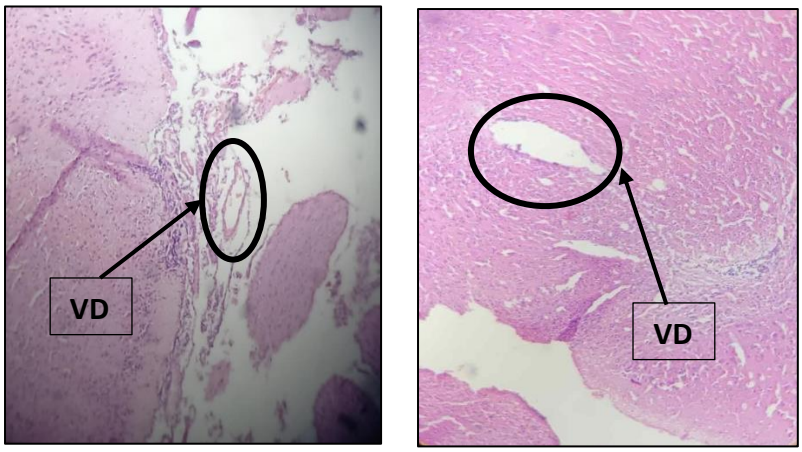


Infusion

Dose of: 500 mg/ml



Dose of :2000 mg/ml **Dose of: 3000 mg/ml**



VD = Vasodilation

Figure 13: Result of histopatological examination of the brain and liver

Histological examination is the gold standard for evaluating treatment-related pathological changes in tissues and organs (Subramanion and al., 2011). In the present study, histopathological evaluation of the acute oral administration of *Ruta montana* L. indicated that the essential oil did not cause toxicity to the organs, as there were no structural damages to the liver and brain organs of the mice.

The liver is the main target organ of toxicity where exposed to the foreign substances being absorbed in intestines and metabolized to other compounds, which may or may not be hepatotoxic to the mice (Rhiouani and al., 2008). In this study, the liver histology revealed normal. Hepatocytes did not show any alteration in the structure in treated animals compared to control. Except vasodilation. Also, there was no necrosis, fibrosis, or local fatty degeneration observed in liver and the arrangement of cell structure was almost similar to the organs of mice in control groups. In contrast, the histological examination study conducted by Merghem, M. (2018) showed a normal liver and kidneys histomorphology of control and treated rats with presence of vascular congestion in treated rats with doses of 100, 300 and 600 mg/kg and with no histological changes observed in the brain and ovary in all treated groups compared with control group.

Conversely, the histological examination by Shama and al. (2014) revealed that after four weeks of treatment with daily oral doses of *Ruta graveolens* seed extracts, lesions were observed in the liver and kidneys of rats administered ethanolic extract at 200 mg/kg/day and aqueous extract at 200 mg/kg/day. Fatty cytoplasmic vacuolation of centrilobular hepatocytes, cellular necrosis, and haemorrhage were observed in the livers of rats administered 200 mg/kg/day of

aqueous extract, alongside glomerular alterations, dilatation, and fatty changes in the kidneys at a dose of 200 mg/kg of ethanolic extract.

17 DPPH free radical scavenging

The results can be expressed as a percentage of radical scavenging activity or a percentage of remaining DPPH or can also be expressed using the IC50 parameter, which is defined as the concentration of the substrate that causes a 50% loss of DPPH activity (Markowicz Bastos., 2007).

Our results from the study of the antioxidant activity of EO of *Ruta montana* compared to the antioxidant standard vitamin C, expressed as a percentage of DPPH* radical inhibition, are represented in the figure 14 and 15.

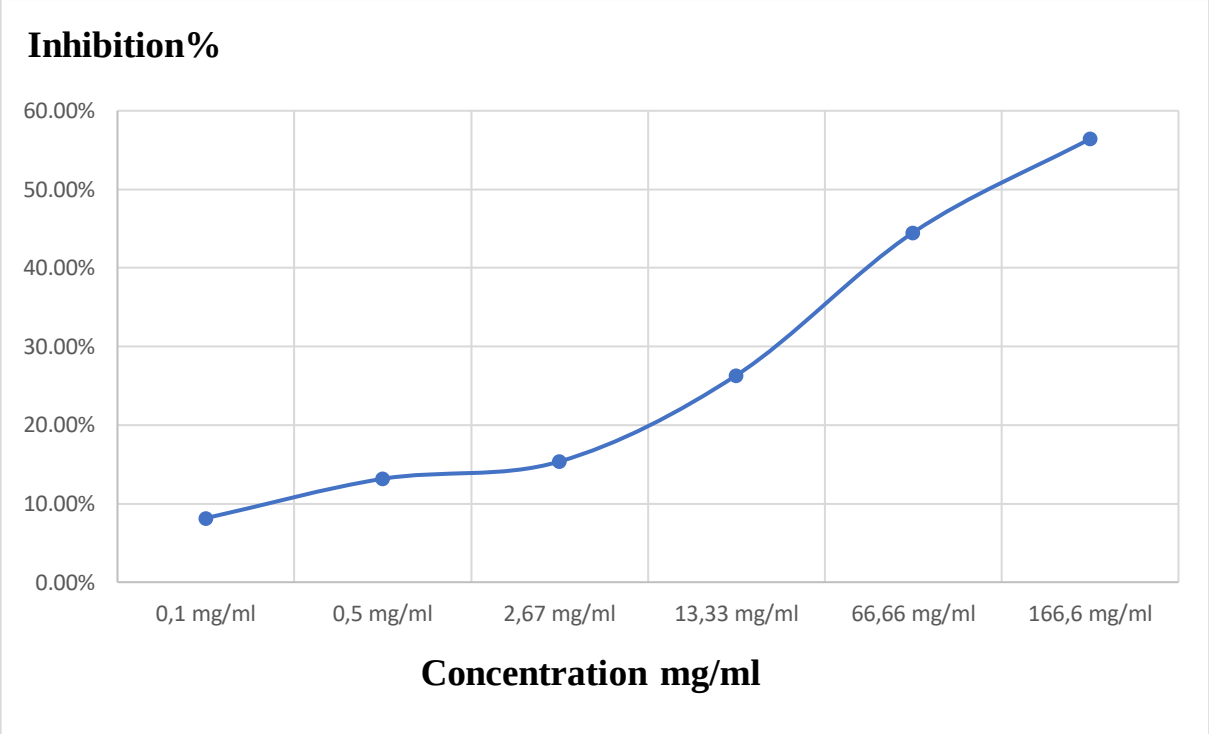


Figure 14: Graph of antioxydant activity of EO of ruta montana

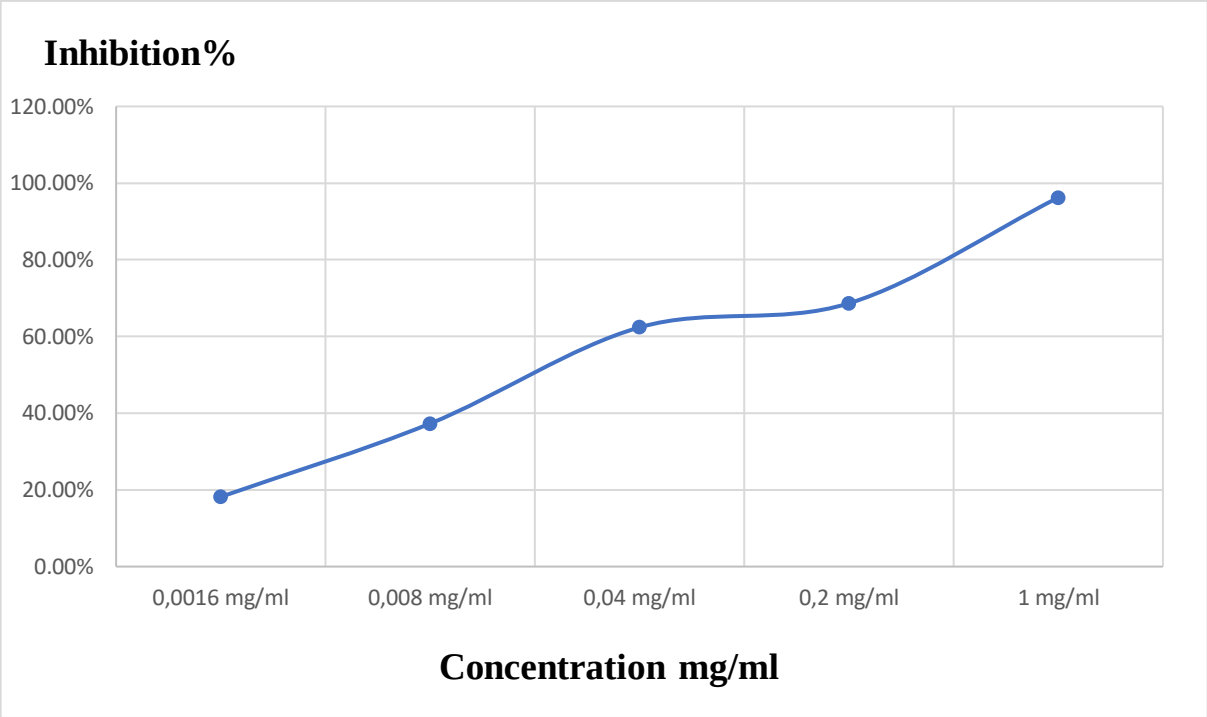


Figure 15: Graphe of antioxydant activity of Vitamin C

Our results indicate that the essential oil of *Ruta montana* has a significant capacity to reduce the DPPH radical, thus a greater inhibition of the DPPH radical. Indeed, at the lowest concentration of 0.1 mg/ml, the inhibition percentage is 8.15%, and at the highest concentration of 166.6 mg/ml, it reaches a value of 56.42%.

Due to the importance of the IC50 in evaluating the antioxidant activity of a substance, the IC50 value of the EO of each sample was determined and presented in the table 9. The results show that our sample exhibits a very interesting antioxidant effect against the DPPH radical with an inhibitory concentration (IC50) of 108,3 mg/ml. This activity is certainly less important than that of the positive control vitamin C (IC50 = 0.025 mg/ml) but has the immense advantage of being natural and would thus reduce the risk of chemical toxicity of these synthetic antioxidants.

This antiradical effect is probably attributed to the presence of monoterpenes, sesquiterpenes, coumarin, aromatic compounds, and organophosphorus compounds in our essential oil, recognised for its ability to trap reactive oxygen species (ROS).

Table 10:The result of IC50 of EO and Vitamin C

Essential oil		Vitamin C	
IC50(mg/ml)	**I%**	**IC50(mg/ml)**	**I%**
108,3mg/ml	50%	0,025mg/ml	50%

The data reported in the literature are not coordinated. Indeed, Kambouche and al. reported significant antioxidant activity of the essential oil of *R. montana* during the DPPH* radical scavenging assay (IC50=16.7 µL/L). On the other hand, Zellagui and al. indicated that the essential oil of this plant was not capable of reducing the DPPH* free radical by 50% (maximum reduction = 11.62%).

Conclusion

Conclusion

At the end of our study conducted on albino mice, evaluating the acute oral toxicity in vivo of the essential oil of *Ruta montana* "*Fidjel*," we can conclude that During the extraction of *Ruta montana*, the yield of extraction by hydrodistillation is low (0.26%).The chemical composition of this oil is dominated by 2-undecanone (32.739%), 1H-phosphole, 2,5-dihydro-1-methyl (17.113%), and 2-nonen-4-one (10.976%).The analysis of the acute oral toxicity of *Ruta montana*, administered in doses ranging from 500 mg/kg to 3000, revealed toxic symptoms manifested by neurological indicators and paralysis. After seven days, the mice exhibited normal behavior comparable to that of the control mice, with no mortality observed. The antioxidant test conducted with DPPH reveals that *Ruta Montana* essential oil exhibits remarkable antioxidant activity (IC50 =108,3mg/ml). The analysis of the biological characteristics of *Ruta Montana* essential oil revealed that it is low toxic, allowing its use in clinical trials or pharmaceutical formulations.

Outlook:

- Perform skin toxicity tests
- Complete biological activity tests
- Develop pharmaceutical formulations
- Explore other pharmacological activities

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People's Democratic Republic of Algeria

Ministry of Higher Education and Scientific Research

University of Blida

Faculty of Natural and Life Sciences

Department of Biology

END-OF-STUDIES THESIS

TOWARDS OBTAINING A MASTER'S DEGREE

Specialty: Phamacotoxicology

Sector: Biological Science

Field: Natural and Life Sciences

Theme

Extraction and evaluation of some pharmacological and biological activities of medicinal plants "*Ruta montana* and *Ruta chalapensis*"

Realized by:

Matari Sara

Khelifa Nadjlaa

Avis favorable

D. RAHIM

Thesis defended on: 18/06/2025

In front of the jury composed of:

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MÉMOIRE DE FIN D'ÉTUDES
EN VUE DE L'OBTENTION D'UN MASTER

Spécialité : Pharmacotoxicologie
Filière : Sciences biologiques
Domaine : Sciences de la nature et de la vie

Thème

Extraction et évaluation de quelques activités pharmacologiques et biologiques des plantes médicinales « *Ruta montana* et *Ruta chalapensis* »

Réalisé par :
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Khelifa Nadjlaa

Avis favorable
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Thèse soutenue le : 18/06/2025

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