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Faculty of Mathematics and Material Sciences, Department of Chemistry

A memorandum submitted for obtaining academic master's degree branch chemistry.

Option: Applied Chemistry

The title:

Chemical study of a medicinal plant of meliaceae family.

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***The Day: 17* / 06 / 2023.**

In front of the jury composed of:

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Academic year: *2022-2023*.

ABSTRACT

The aim of this work is the chemical study of the fruits of *Azadirachta indica* A. Juss, which is one of the tree species that belongs to the Meliaceae family and is endemic and located in the south of the Algerian desert in the city of Tamanrasset. The *Azadirachta indica* A. Juss tree, called the neem tree, was known by several names as a result of its therapeutic and medicinal role. It was also used in traditional medicine, which made it the focus of attention and research by some Scientists.

The study revolves around the phytochemical study of the fruit extracts of *Azadirachta indica* A. Juss, the use of some chromatographic techniques (TLC and CC) to separate and purify some compounds, the determination of phenols and their antioxidant activity.

Keywords: *Azadirachta indica* A. Juss, Meliaceae, chromatography, antioxidant activity**.**

ملخص

الهدف من هذا العمل هو الدراسة الكيميائية لشجرة *Azadirachta indica* A. Juss والذي يعد من أصناف الشجر الذي ينتمي لعائلة Meliaceae المُستوطِن والمُتواجد في جنوب الصحراء الجزائرية بمدينة تمراست .

عُرِفت شجرة *Azadirachta indica* A. Juss والمسماة بشجرة النيم بِأسماء متعددة نتيجة دورها العِلاجي والطبي ،كم أُستُعمِلت في الطب التقليدي مما جعلها محط اهتمام وبحث بعض العلماء.

تمحور الدراسة حول الدراسة الفيتو كيميائية لمستخلصات فاكهة شجرة *Azadirachta indica* A. Juss، استخدام بعض تقنيات الكروماتوغرافيا للفصل (TLC و CC) كما تم تنقية بعض المركبات و كذلك تقدير الفينولات وفعالية المضادة للأكسدة.

كلمات مفتاحية :*Azadirachta indica* A. Juss، Meliaceae، الكروماتوغرافيا ،فعالية مضاد للأكسدة .

Thanks and gratitude:

Praise and thanks be to ***Allah*** first and foremost, who helped me and inspired me to complete this research and come up with it in such a good way.

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We thank every person who helped us and whose name did not mention

DEDICATION

I dedicate this precious and humble work to those who were the reason for my existence, ***my father*** *and* ***my mother****, may* ***Allah*** *prolong their lives.*

To everyone who taught me a letter in this world and encouraged me to complete my academic career.

To my dear brothers ***Ossama****,* ***Abdel Hamid*** *and* ***Hisham****.*

To my dear sisters ***Hafsa****,* ***Ayaa*** *and* ***Marwa****.*

To the loved ones, family and all the staff and members of Kasdi Merbah University.

Elbarka Ines

إهداء

أُهدي هذا العمل الثمين المُتواضع إلى من كانوا سببا في وجودي والدي و والدتي أطال الله في عمرهما .

إلى كل من علمني حرفا في هذه الدنيا وشجعني على إكمال مسيرتي الدراسية .

إلى إخواني الأعزاء أسامة ،عبد الحميد و هشام .

إلى أخواتي الكريمات حفصة ،آية و مروى .

إلى الأحباب و الأهل وجميع طاقم وأفراد جامعة قاصدي مرباح .

البركة إيناس

Summary

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Introduction general

Introduction general

Plants and herbs have been used since ancient times, in almost all cultures as a source of medicine and for the wide use of herbal remedies and health care products, as plants were the first pharmacy known to man, so he relied on them in various fields throughout the ages as his food and treatment of diseases that he was exposed to And with the passage of time, modern man discovered diseases that were not known and widespread before, so he knew the value of medicinal plants, benefited from them in his various treatments and faced most diseases with them, as he strived to value and transfer those treatments from one generation to another, which is credited with the development of various medical sciences[1].

Most plants, herbs and plant foods contain significant amounts of secondary metabolite products, which are very effective materials that are used in the manufacture of various medicines to produce some chemical compounds, which are the source of chemical manufacturing for some important pharmaceutical materials[2].

After the development of the field of pharmacological and therapeutic techniques and the development of the science of chemistry, the field of cupping became dependent on manufactured drugs only, which made man enter the era of chronic and dangerous diseases, the era of the spread of many diseases, side effects and side complications during treatment with manufactured medicines in addition to causing fetal deformities and congenital deformities. ..etc. This is due to human distancing from natural pharmacy. All these symptoms made people gradually return to medication with medicinal herbs, herbs and plants, which made scientists reconsider the study of most plants and herbs, knowing that they found that one plant contains many effective secondary metabolism products[3] .

In this work we are interrested to study the fruits of *Azadirachta indica* A. Juss.Our study is based on a phytochemical screening study followed by an extraction by solvents of increasing polarities. Then a chromatographic study on fruit extracts has been carried out, an evaluation of the antioxidant activity by DPPH was carried out as well as the content of flavonoids and polyphenols is reached. Some products was isolated by column chromatography.

Our study revolves around the phytochemical study of the fruit of the *Azadirachta indica* A.Juss plant, as well as its antioxidant activity. This research is focused on three chapters that include:

The first part includes two chapters, the first on the bibliographical study and the second is on the secondary metabolites.

The second part contains a single chapter on the experimental study.

The third part contains a single chapter on the results obtained and their discussions.

The Theoretical Part

Bibliographic study

Chapter I

I. 1.The Introduction:

The neem tree was known since ancient times with the scientific name is *Azadirachta indica* A. Juss. It is considered one of the most promising trees in the future, due to the success of its cultivation in dry environments and lands that are poor in its content of nutrients, and for its many benefits. Now, many researches and scientific study have been devotes to it. Due to the many uses of this plant, it is considered the tree of the twenty-first century and a pharmacy of nature and human [4]. Recently, it invaded (global) spread, thus taking the status of global citizenship. It is cultivated today in more than 65 countries, especially in the tropics and subtropics, even in our Arab world for commercial purposes, medical, therapeutic and industrial. In relation to the properties of this medicinal and therapeutic tree, it become a source of Millions types of industries related to the care of human, animal and plant health. It used in cosmetics industries and in the industries based on wood and others, their markets are still in their developing stage [4].

I. 2.The Meliaceae Family:

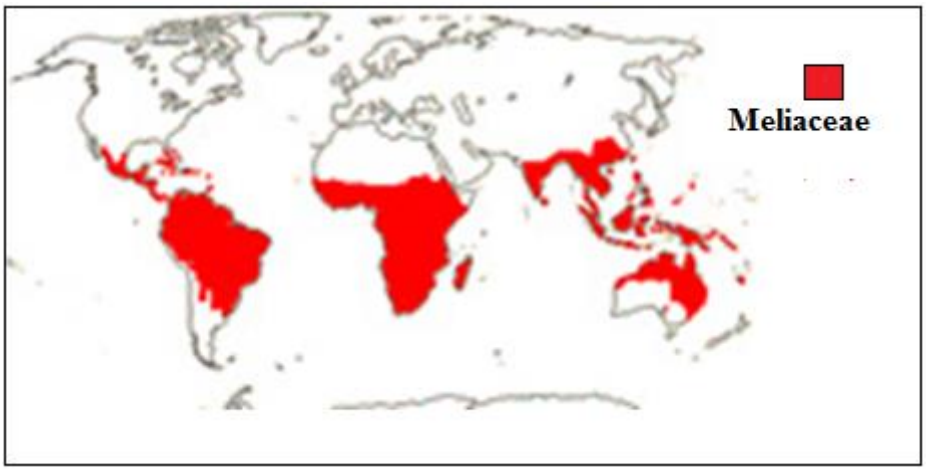
The Meliaceae family represents flowering plants with 53 genera and about 600 listed species, of the order Sapindales. They consist mostly of deciduous trees or shrubs and sometimes herbaceous plants that grow in tropical, subtropical, semi-arid, and sometimes in temperate regions. Meliaceae are characterized by alternate leaves without stipules and bisexual leaves, grouped in groups or in whorls, leaves, obtuse, alternate, rarely opposite, pinnate, rarely simple because they are monocotyledonous or dicotyledonous (milia). The leaf has entire margins and rarely lobed or toothed. The most Meliaceae are evergreen [6,7].

- **the reproduction:**

The flowers are radial and asexual, and more bisexual. There is dioecy, polygamy or currency. They are grouped in whorls, clusters or axillary spikes, rarely in bundles or solitary. Sometimes there is cauliflower or epiphylly (chisochiton) [6].

I. 3.Geographical distribution of the Meliaceae family:

Neem tree was distributed in tropical and subtropical regions worldwide, with a few species found in more temperate regions [9].



Figure(I-1) Geographical distribution of the Meliaceae family around the world.

I. 4.Some plants belonging to the Meliaceae family :

Acalypha indica L.

Dillenia indica L.

Duchesnea indica Focke

Ipomoea indica (Burm.F.) Merr

Quisqualis indica L.

Hyophorbe indica Gaertn

Tamarindus indica L.

Lagerstroemia indica L.

Mangifera indica L.

Saraca indica L.

Acalypha indica L.

Dillenia indica L.

Duchesnea indica Focke

Hyophorbe indica Gaertn

Ipomoea indica(Burm.F.) Merr

Lagerstroemia indica L.

Mangifera indica L.

Figure (I-2) Some of the Meliaceae's families[8,5].

I. 5. Azadirachta indica A. Juss. (The neem)

I. 5.1-Origin and history:

The exact origin of neem is unknown. Some believe that neem originated in the vast region of Assam-Burma. It is distributed naturally in the Indian subcontinent. It has been cultivated for a long time in the Malay and Indonesian peninsulas and in Thailand where it is completely naturalized. In the 19th century it was planted in Africa. It was recently planted in tropical South America, Philippines, northern Australia. It is one of the most planted trees in the tropical zone of the planet [8].

In the traditional culture of India, neem is considered sacred and protective plant. It was an integral part of the Harappa culture, one of the greatest civilizations that developed in the Indus Valley (north-west India) from the sixth millennium BC. J.-C. [5].

Neem is a common ingredient in Ayurvedic medicine. The World Health Organization has recognized it as a system of traditional medicine based on religious belief (Dhanvantari is the Hindu deity associated with Ayurveda). These pearls of ambrosia would have germinated to produce this marvelous tree. In the countryside of India, it is called "the village pharmacist [8].

Figure (I-3): The neem tree[7].

I. 5.2- Systematic classification of Azadirachta indica (The neem)[3,7,8]:

The Scientific name: ***Azadirachta indica* A. Juss.**

The Common name: **The Neem.**

The English name: **The neem tree is called by several foreign names including: Margosa, Neem, Imba, Mindi, Indian Lilac**.

The French name: **Parasio de india, Neem.**

The Arabic name : **This tree is called several Arabic names, including: Shereesh, Meraimarah, and Neem...etc.**

- ***Taxonomic positions of neem :***

Table (I-1): Classification of *Azadirachta indica* (the neem).

Kingdom	**Plantae**	**Royaume**
Under Reign	Trachebionta	Under Reign
Division	Magnoliophyta	Division
Class	Magnoliopsida	Class
Sub- Class	Rosidae	under- Class
Order	Sapindales	Order
Under -Order	Rutineae	under- Order
Family	Meliaceae	Family
Sub -family	Meloideae	under- family
Gender	*Azadirachta*	Gender
Species	*Azadirachta indica* A. Juss	specie

A. Gender *Azadirachta*:

Flowering plants are represented by the Meliaceae family, which includes 53 genera and about 600 different species, of the order Sapindales, among which *Azadirachta indica A. Juss*, consists mostly of trees, shrubs, and sometimes herbaceous plants that grow in tropical, subtropical, arid regions, semi-arid and sometimes temperate parts of the world [7].

B. Species :

Azadirachta indica A. Juss (Neem) is a fast-growing shrub by tree that can reach a height of 15–20 m (49–66 ft) and rarely 35–40 m in height and can reach in shady evergreen surroundings and grow densely (115–131 ft). In a severe drought, most or almost all of its leaves may fall off. The branches are wide and extended. It has a straight leg. Its bark is hard, rough, scaly even in young trees, grayish-brown. The leaves are alternate, consisting of several leaflets with serrated edges. Its flowers are small and white in color, it grows from July to August, and its edible fruits are oval and round with a thin skin, Holds 150 to 250 flowers. The bisexual protodendros flowers and the male flowers are present on the same haploid tree [9].

C. Botanical Nomenclature :

The neem -*Azadirachta indica* A. Juss (Neem) -tree is a tree known since ancient times grows quickly. It is a perennial tree whose age reaches one hundred and eighty years and its life in the forest may extend to two hundred years. It's ability to purify the soil from salts. It is known for its multiple medicinal uses, as the whole parts of the tree are used in veterinary vaccines and in the manufacture of medicines and cosmetics .It is known by its different names in most people:

It is called in the United Arab Emirates and Oman (Al Shereesh), in Iran (Neeb) and (Nib), in Yemen (Meraimarah), in Pakistan (Nimmi), (Nimuri) and (Limbo), in Sri Lanka and

Singapore. It is called (Kohumba), in Kenya (Muarubaini), in Spain (Margosa) and (India de Parasio), in Malaysia (Baby Baypay) and (Dawoon Mambu), in Indonesia (Intaran), (Mempheuh), (Imba), (Mindi) and (Mimbo), and it is called in India by more than twenty names, including: The Neem, The Nind, Bal-Bimb, Olly, Adji and Venu. The neem tree is called in Latin America, Central America, Germany, Madagascar, Australia, Britain and Bangladesh (the neem), with the possibility of another known name, as is the case in Britain, where it is known as neem as it is called (Lilac Indian), The neem tree has been known by many names as a result of its therapeutic and medical role, so it is called (the tree of wonders, the tree of a thousand remedies, the rich gold mine, the cure for disease, the village pharmacy, and the plant of forty remedies) [4,8].

D. **Characteristics of the neem tree :**

Previous studies showed that the neem tree, is characterized by features that distinguish it from other trees, represented in:

Trunk:

Its bark is brown and cracked vertically. It contains 14% tannins, a percentage similar to that of classic tannin-producing trees (such as: Acacia decurrens). Also, the bark yields strong coarse fibers commonly plaited into ropes in Indian villages. Neem's wood is hard, heavy and red-brown in color. Its grain is rough/coarse. Its density is 720 to 930 kg/m^3. It is easy to dry. Ease of working (sanding, polishing, nailing, screwing...) but its polishing is quite delicate because of its coarse grain [8].

Figure (I-4): Trunk 'the wood[2,11]

Foliage:

Its foliage is persistent but can become obsolete in the event of severe drought [8].

Figure (I-5): The Foliage [2]

Sheets :

Its leaves are compound, penninervate and oddpinnate (odd number of leaflets) and somewhat resemble those of the ash (Fraxinus excelsior) and a lot like those of the soap tree (Sapindus mukorossi). The leaves are alternate, clustered near the end of the branches, 20-40 cm long (on average, 20-31 cm). The leaflets are opposite light to dark green, 3-8 centimeters long. Young neem leaves are often red [6,7].

Figure (I-6): The Leaves [2]

Bloom:

Flowering period: Trees may begin flowering and fruiting at 4-5 years of age, but economic quantities of seed are produced only after 10-12 years. Pollination is done by insects, such as bees. In East Africa (with a marked wet and dry season) flowering and fruiting are restricted to distinct periods [6].

Flowers:

The flowers (white and fragrant) are arranged axillary, with normally more or less drooping panicles, up to 25 centimeters in length. The inflorescences, which branch, carry from 150 to 250 flowers. An individual flower is 5-6 millimeters long and 8-11 millimeters wide. The flowers are bisexual and male flowers, protandrous, exist on the same individual. Axillary inflorescence, numerous three-flowered, up to 30 cm long; minute and deciduous bracts; flowers bisexual or male on same tree, actinomorphic, small, pentamerous, white or pale yellow, slightly sweet scented; calyx lobes imbricate, broadly ovate and slender, puberulous inside; petals free, overlapping, spatulate, inside ciliate (with cilioles, i.e. small eyelashes). The flowers are fragrant, abundant and melliferous [6,7].

Figure (I-7): Flowers [5]

Fruit:

The fruit has a smooth (glabrous), olive drupe that varies from an elongated oval to a rounded shape. When ripe, it is 1.4-2.8 centimeters by 1.0-1.5 centimeters. The skin of the fruit (epicarp) is thin and the bittersweet pulp (mesocarp) is yellowish-white, greenish-yellow to yellow and is very fibrous. The exocarp is thin. The mesocarp is pulpy (0.3-0.5 cm thick), and the endocarp cartilaginous. The white, hard inner shell (endocarp) of the fruit encloses one, rarely two or three, elongated seeds (kernels) with a brown integument. Some isolated trees do not bear fruit, suggesting the presence of self-incompatibility. The fruits ripen in about 12 weeks from anthesis (period when the flower is functional) [5]

The fruits are an important food source for some wildlife, especially birds and bats, although they only digest the pulp, not the seed [7].

Figure (I-8): Fruits [4]

Seed:

At maturity, the neem tree can produce up to 50 kg of fruit, which is equivalent to 30 kg of seeds.Egg-shaped or spherical seeds have a pointed top. The seeds (kernels) are elongated with a brown seed coat. Its fruits and seeds are the source of neem oil. No seed pre-treatment is needed, although pulping and cleaning the seeds greatly improves the germination rate .In Senegal, neem is productive around 4 or 5 years. It reaches its full maturity around its 10th year.

Storing seeds:

Seed storage behavior (viability) is probably intermediate. The viability is reduced from 85% to 60% after 1 month, in hermetic storage, in dry air, at temperature ambient and 45% at 6°C.

Seed germination:

Without pre-germination treatment, the germination rate is reached at 75 to 90% after 20 to 30 days (one week minimum). The seeds lose their germinative power fairly quickly, falling from 85% to 45% after a few months [8].

Figure (I-9): The seeds of neem [21]

I. 5.3-Geographical distribution of *Azadirachta indica* (The neem) :

The original home of the neem tree and its discovery Neem trees grew on Earth 475 million years ago in the giant continent of Pangea at the time, which was divided over time into the northern continent and the southern continent, and there were different sayings about the place of origin of the original neem, either in Burma or the Indian subcontinent, or dry forests In South Asia and Southeast Asia, such as: Malaysia, Indonesia, Pakistan, and Sri Lanka [6].

Adapted to poor soils, it tolerates high temperatures and low rainfall of less than 500 mm. It is found in arid and semi-arid areas of tropical Asia, Africa and Australia and semi-humid areas. Today, it is increasingly cultivated in South and Central America (in Mexico), in the West Indies and even in Europe (on the Côte d'Azur), where it is appreciated as an ornamental tree. The species adapts to lean, stony or sandy soils [21].

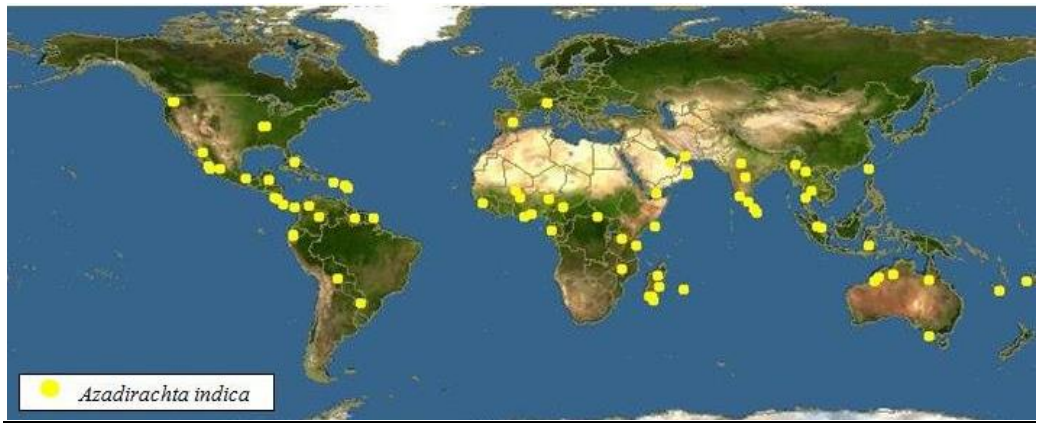


Figure (I-10): Distribution of *Azadirachta indica* in different countries [10].

I. 5.4- Uses of *Azadirachta indica* (The neem):

All parts of the *Azadirachta indica* plant are widely used in the life of mankind.

- ***Therapeutic and pharmaceutical uses :***

a) Antibacterial and antimicrobial agent (leaf), **b)** Antifungal agent, **c)** Antiviral compounds, **d)** An antiplasma **[12].**

- ***Antioxidant and anti-inflammatory :***

a) Anti-inflammatory,

Nimbidin, a component of *Azadirachta indica*, has been shown to be an effective anti-inflammatory and anti-arthritic agent. Nimbidin, a compound isolated from the seed oil of *Azadirachta indica* A. Juss. **[20].**

b) Antioxidants, **c)** Neuroprotection, **d)** Inhibition of nitric oxide production, **e)** Anti-anxiety ,

- ***It used in other activities:***

a) Immune stimulatory, b) Anti- allergic, c)Cures Asthma, d) Antipyretic properties,
e) Anti-diabetics, f) In HIV AIDS, g) Anti-cancer

- ***Animal nutrition and health:***

The leaves and seeds are used as food for animals, but it has been observed that the use of neem in high concentrations affects and poisons some animals, but some countries (such as Kenya) use neem powder manufactured in farms for food security for domestic animals, to control viral and microbial diseases and various types of bacteria that infect poultry And it was found that the aqueous extract containing 10 percent of the fresh leaves has the ability to form reactions against viruses that cause viral livestock diseases such as smallpox [1].

- ***Control of agricultural pests and diseases:***

- **In the field of agricultural pest control :**

The neem oil extracted from seeds can be used as effective pesticide

a) Soil fertilization**,** b) Anthelmintic for small ruminants [1, 9] .

- ***Biofuel production:***

Vegetable oils have become more attractive for the biofuel industry due to their environmental benefits, in addition to being a renewable resource and an inexhaustible resource. The neem tree is considered one of the most important candidate trees for biodiesel extraction, so the standards for fuel produced from neem were considered among the recommended biofuel standards. This fuel can be used in any of the diesel engines without the need for modification, which makes it a good alternative to fossil diesel fuel [1].

I. 5.5 -Previous Studies on *Azadirachta indica* (The neem) :

Neem (*Azadirachta indica*. A.Juss) has a therapeutic effect in preventing and treating diseases. But the exact molecular mechanism in preventing pathogenesis is not fully understood. Azadirachta indica is believed to show a therapeutic role due to the rich source of antioxidants and other valuable active compounds such as azadirachtin, nimbolene, nimbin, nimbidin, nimbidol, salanine and quercetin. The possible mechanism of action of *Azadirachta indica*. A .Juss is presented as follows **[12]:**
Parts of neem plants (*Azadirachta indica*. A.Juss) exhibit an antibacterial role through inhibitory effect on microbial growth/ability to degrade cell wall. *Azadirachtin*, a quaternary compound limonoid found in the seeds, is the main component responsible for both antitrophic and toxic effects in insects. The results indicate that the ethanol extract of neem leaves showed in vitro antibacterial activity against both Staphylococcus aureus and MRSA with the largest areas of inhibition observed at 100% concentration.

(1) Neem plays a role as a free radical scavenger due to its rich source of antioxidants. *Azadirachtin* and *nimbolide* showed concentration- and reductase-dependent antiscavenging activity in the following order:
nimbolide > azadirachtin > ascorbate.

(2) Neem appears to be instrumental in stimulating cancer by regulating cell signaling pathways. Neem modulates the activity of various tumor suppressor genes (eg: p53, pTEN), angiogenesis (VEGF), transcription factors and apoptosis .

(3) Neem also plays a role as an anti-inflammatory by reactivating the activities of pro-inflammatory enzyme including cyclooxygenase (COX) and lipoxygenase (LOX).

I. 5.6 -Chemical scanning of the tree :

Previous chemical study of compounds isolated from neem plant (chemical survey of *Azadirachta indica* plant):
Chemical screening of neem extract led to the detection of a variety of metabolically active compounds that have health effects. Among these main active compounds include **[12,20]:**

b) Limonoids:
Plant compounds responsible for antibacterial, antifungal and antihelminthic activity. These plant compounds include nimbine, azadiractin and myeliazin.

c) Triterpenoids:
Metabolic compounds with anti-inflammatory and anti-tumor activity. This type includes compounds such as: nemoline, nemoctone and nimpidine.

d) Flavonoids:
The neem plant, like other plants, is rich in a variety of flavonoids, so that these compounds are considered antioxidants and anti-inflammatory. We mention: quercetin, kaempferol and nimbin.

e) Azadirachtic acid:

This acid is an important compound found in the neem plant, as it contains antibacterial, anti-inflammatory and anti-tumor properties.

These compounds and others contribute to the multiple medical benefits of the neem plant, as this plant is powerful and useful from a medical point of view, taking into account the correct doses and warnings when used in medical and self-treatment.

Chemical structure of madjor constituents of neem.

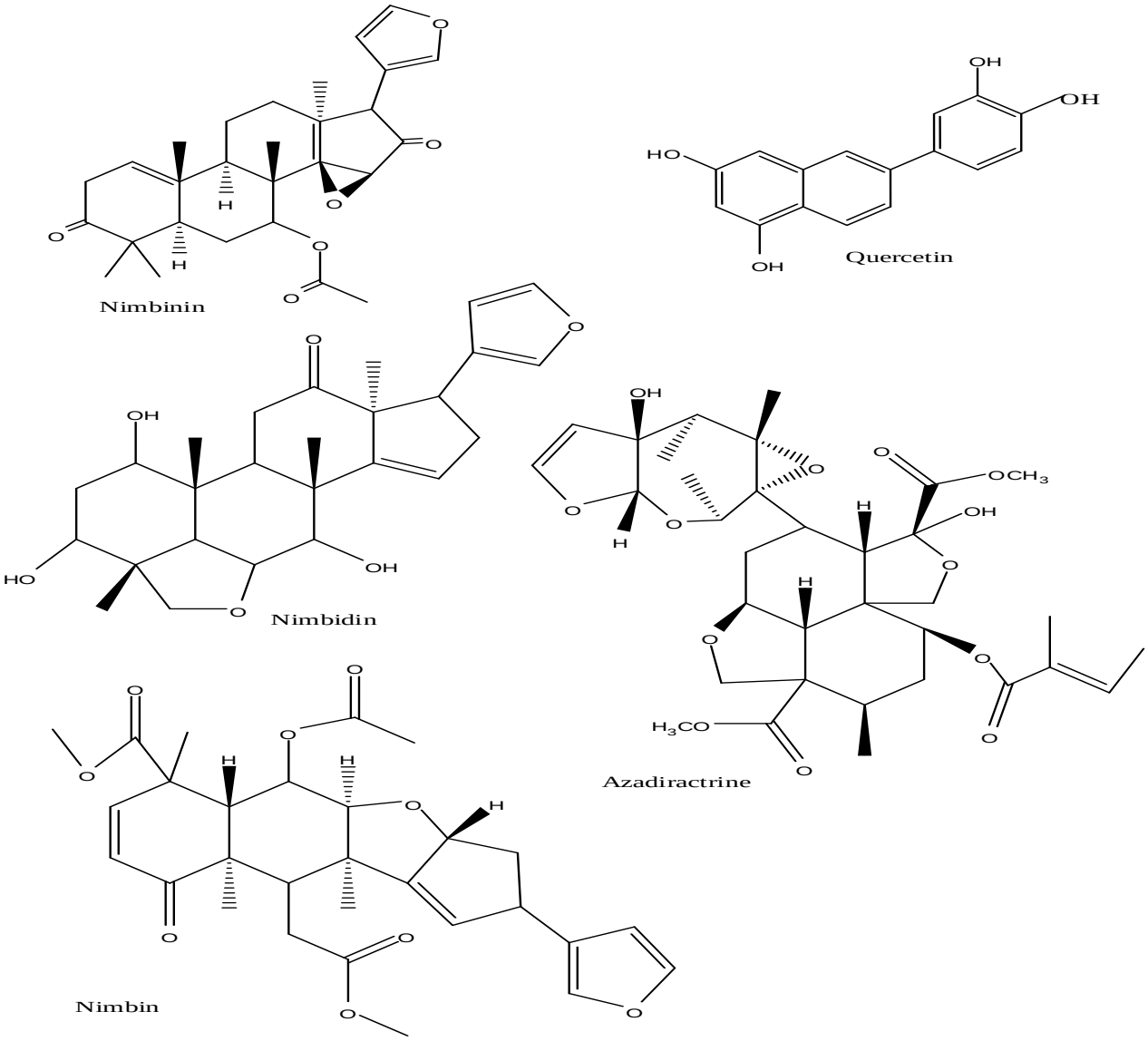


Figure (I-11): some structure isolated from neem.

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Chapter II

Products of secondary metabolism

II. 1 Products of secondary metabolism:

They are products produced through secondary metabolism processes using intermediate compounds, produced during primary metabolism processes (carbohydrates, proteins), and include: polyphenols, terpenes, alkaloids ... etc., they represent a second line of defense for plants, they are considered as a source: plant dyes, vitamins Essential oils and plant hormones are also of great importance to humans, as they are used in many important industries such as: Soap industry, leather dyeing, pharmaceutical industries, essential oils extraction...etc.

II.1.1 Phenolic Compounds:

A. *Definition:*

They are second products of secondary metabolism [01], containing phenolic rings with one or more hydroxyl groups directly attached to the benzene ring [02,03], widespread in the plant kingdom, these compounds include many molecules and represent one of the most important groups in existence In the plant kingdom, this definition is not sufficient to limit most of the phenolic compounds, due to the presence of other compounds that are not phenolic but contain in their chemical composition a hydroxyl group that is directly linked to the benzene ring, such as[03]:

Alkaloids such as: Morphine

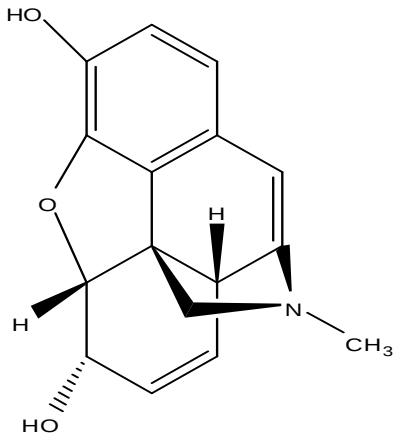


Figure (II-1) The Morphine**.**

Terpenes such as: Thymol

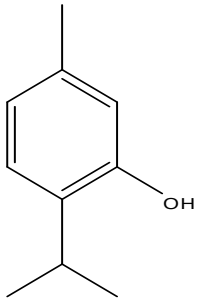


Figure (II-2) Thymol.

"It is a large and heterogeneous group of phytochemicals [01], having one or more benzene rings, bearing a free hydroxyl group in addition to its bonding to another free function, and its aromatic rings formed from either shikimic acid or a polyacetate" [03].

In recent years, phenolic compounds have been considered one of the most important biologically active secondary metabolite compounds against various diseases, including cardiovascular disorders, neurodegenerative disorders and some types of cancer. Anti-inflammatory [02], and their role in removing free radicals and their anti-inflammatory properties, these substances can be used to treat various types of conditions associated with metabolic disorders, many symptoms of the metabolic syndrome, including obesity, dyslipidemia, atherosclerosis, and high blood sugar. blood, accelerate aging, liver toxicity, high blood pressure, as well as cancer, prevent platelets from aggregating to form a clot, maintain the integrity of vascular tissues, are significantly mitigated by dietary phenols [02].

It is also an essential part of various chemical industries for the production of commodity chemicals, food additives, cosmetics, and paints. It has multiple therapeutic effects, potential health benefits in vivo and in vitro, in addition to reported clinical studies [02].

B .Classification of phenolic compounds :

Phenolic compounds comprise a large space in the plant kingdom, about eight thousand polyphenolic compounds have been isolated and identified at the present time, they can be classified by the presence of many phenolic groups, and they are also classified on the basis of the chemical complexity of their phenolic structure to [04]:

Flavonoids: Cyanidin, Flavonons...etc.

Non-Flavonoids compounds: mention: coumarins, tannins, phenolic acid...etc.

It is also classified into several classes according to its carbon structure:

- **Phenolic acids**
- **Flavonoids**
- **Tannins**
- **Stilbenes**
- **Lignes**
- **Saponines**
- **Phytosterols**

- The most important of which are: **Phenolic acids, Flavonoids** and **Tannins**. They are more abundant in addition to dietary phenols.

The main classifications of polyphenols:

Table (II-01) The Major classifications of polyphenols.

The formula	**The class**
C_6	Simple phenols
C_1-C_6	Phenolic acids and their derivative compounds
C_2-C_6	Acetophenone / Phenylacetic acid
C_{15}	Flavonoids
C_{30}	Biflavonyls
C_6-C_1-C_6	Benzophenone / Xanthones / Stilbene
C_6 ,C_{10}, C_{14}	Quinones
C_{18}	Betacyanins
Lignanes / Newlignanes	Dimers / Oligomers
Lignanes	Polymers
Tannins	Accumulated / Decomposed

C. Found in plants:

Polyphenolic compounds constitute an important chemical group in the plant kingdom, and several hundred different polyphenolic compounds are found in plant foods (fruits, vegetables, legumes, grains, plant-derived beverages and chocolate), and approximately 100 mg of polyphenols are identified In a cup of coffee, tea or herbs, and in different types of berries. These compounds play a major role in growth, protection from ultraviolet radiation

and against pathogens, and are responsible for pigmentation and colors (green, yellow, red and orange) in vegetables and fruits [03].

II. 2 Flavonoids:

II.2.1 Definition:

They are multiple phenolic compounds, one of the most important sections of phenolic compounds, with a low molecular weight. Scientific research began on the term flavonoids in 1936 AD [05], so it is a term of Latin origin, derived from the word "FLAVUS" meaning "favus" meaning yellow, the phrase Yellow plant pigments distributed on all parts of the plant are responsible for the colors of flowers, fruits and sometimes leaves [06,03].

II.2.2 The composition of flavonoids :

More than 4000 natural flavonoid compounds have been extracted and isolated, all of which have a unified basic structure consisting of 15 carbon atoms, with the formula: C6-C3-C6, consisting of two aromatic rings C6 (A and B), linked through a heterocyclic pyran ring (C).) that give the basic structure of flavonoids, as in the figure [06] (Fig.II-3):

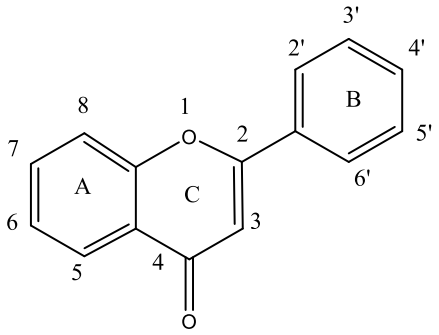


Figure (II-3) The basic structure of flavonoids.

II.2.3 Distribution in plants:

Flavonoids are considered the most abundant in the polyphenolic groups, as they are secondary metabolite products, distributed to all parts of the plant, but are excessively concentrated in the aerial part of the plant, their concentration increases according to exposure to sunlight [06,03], that is, they are found in: leaves, flowers Seeds of all kinds, stems, fruits, vegetables, tea...etc., are present in the vacuoles at the cell level in the form of terozides soluble in water, due to the sugar part attached to them. They are concentrated in the dermis of the leaves, or they may be distributed between the dermis and the middle layer. In the case

of flowers, they are concentrated in cells, present in the leaves in the form of crystals in the cell in dry plants. As for flavonoids (which dissolve in non-polar solvents) such as polymethoxyl flavonoids (they are concentrated in the cytoplasm of the cell) [08], they are also found in Our daily diet, in the peels of citrus fruits.

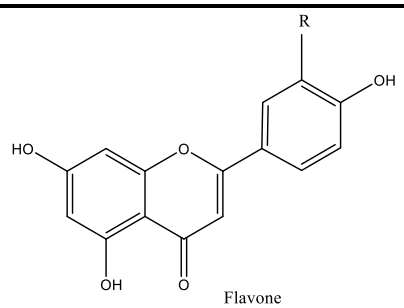
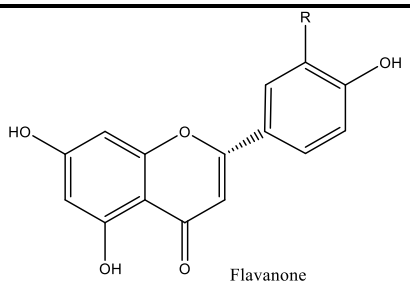
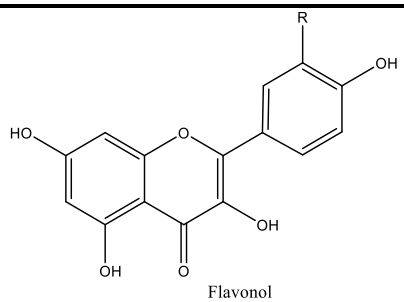
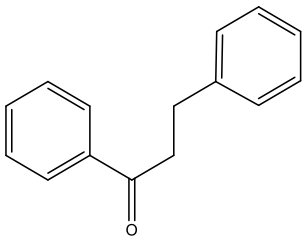
II.2.4 Classification of flavonoids:

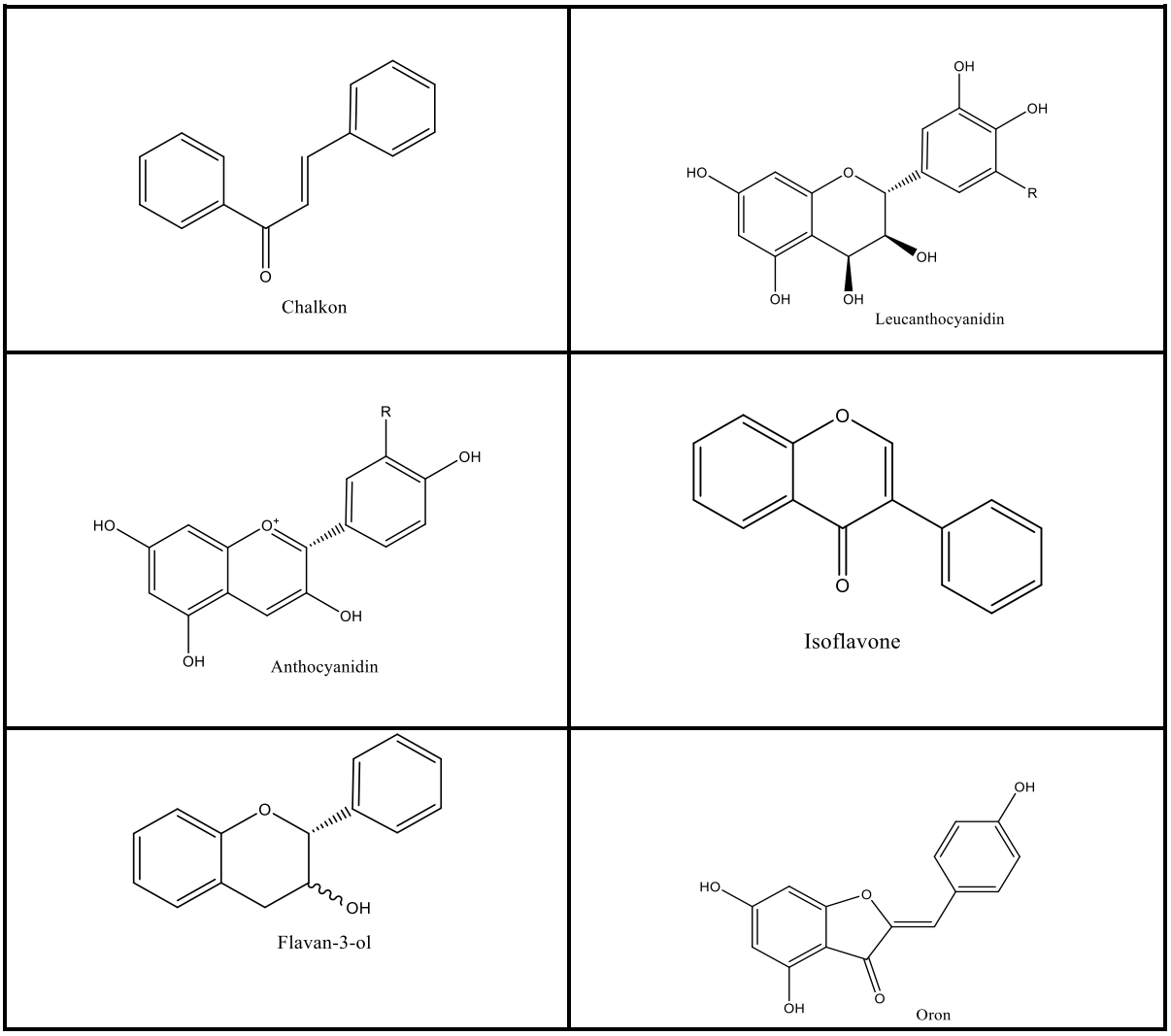
These compounds are divided into different classes according to:

- ✓ Number of substitutes.
- ✓ Position of substituents (functional variation at loci 2, 3 and 4 in the C ring).
- ✓ The nature of the substitutes.
- ✓ According to the oxidation level of the heterocyclic "pyran ring" (C).

However, the classification of flavonoids into 05 main sections according to the degree of oxidation of the pyran ring (ring C), the following table shows the various of these sections, [06,03,07]: (Table No.II-02)

Table (II-2): Different classes of flavonoids.

 Flavone	 Flavanone
 Flavonol	 Dihydrochalcone



II.2.5 Chemical study of flavonoids:

- **Extraction:**

The extraction of flavonoids depends on various methods, devices and specific solvents, knowing that most of the analyzes of flavonoids are located on the aerial part of the plant of all kinds.
Extraction methods can be followed according to the type and properties of compounds and chemical compositions, but the most used method in the extraction of flavonoids is the traditional method: solid-liquid extraction by polarity graded solvents (from less polar to higher polar) [10,06] .

- **Initial detection:**

The most important means of detection on the nature of the flavonoid structure in most of the extracts, which is considered one of the most important means of detection for many

compounds, are: paper chromatography, thin layer and fluorescence color under UV lamp at (365 nm).
Most flavonoids are not visible and do not appear on paper or thin film chromatograms. We resort to some reagents (Table No.03) that give distinctive colors according to the type of flavonoids and related substituents, showing the relationship between the color of the compound and its chemical structure under UV rays [10]:

Table (II-03) Different of flavonoids after showing.

Spot color without reagent	***The detector***	***Spot color after the detector***	***Potential flavonoid compositions***
Purple, black, pale blue, pale green, blue	$AlCl_3$05 %	yellow or orange	All flavonoids
Blue, green, pale blue, yellowish green	H_2SO_4 Center	yellow or orange	All flavonoids
Pale green, green, blue	Vanillin solution-HCl 5%	Red in the case of or after heating	All flavonoids
Green, brown, pale blue, green	Neu	Red or yellow	Flavonols
Violet - black	NH_3	Yellow or greenish yellow Slight or no color change	5-OH Flavones
Blue		Greenish yellow or greenish blue	Flavonols
Bright yellow or pale yellow		Slight or no color change	Flavonols , Flavones

- **Separation and purification:**

The basic technique and the optimal application for the separation and purification of organic compounds is chromatography in its various sections. It has been widely used to separate flavonoid compounds and contribute to determining their structural structure, in addition to various physicochemical analysis methods.

Among the methods used for separating the series chromatography:

- Thin layer chromatography (TLC)
- Paper chromatography CP
- Column chromatography (CC)

As for the physiochemical analysis methods, we mention:

- Uv-Vis spectroscopy
- IR spectroscopy
- SM mass spectrometry
- RMN (^{1}H) or RMN (^{13}C) magnetic resonance spectroscopy
- Correlation homonucleaire and heleronucleaire spectroscopy

All these techniques give sufficient information about the structure and chemical structure of the compound.

II.3 Terpenes:

Terpenes are among the products of secondary metabolism. They are a group of major organic compounds that include 3000 compounds, which are produced by most plants, and secreted by some insects (termites and some types of butterflies). They are unsaturated hydrocarbon compounds, with different structures, which may be open chain or Cycloidal, monocyclic, bicyclic or tricyclic. They are considered main components of essential oils (volatile) in aromatic plants, which are monoterpenes (Monoterpene) and triple (Sesquiterpenes) [10] In addition to this, terpenes constitute an important source of biologically active compounds that have succeeded as effective drugs such as the anti-cancer compound Taxol and the anti-cancer compound Aretmesin for malaria and terpenes are important renewable sources as products of great commercial importance such as solvents (detergents, adhesives, packaging materials, polymers (rubber) and as intermediates in chemical synthesis and others**.**

II.3.1 Composition of terpenes [10,11]:

In the early twentieth century, Ruzicka was able to identify the common structural unit in turbines. It is built from small units called isoprene (Isoprene), i.e. (3-Methyl buta-1,3-diene)

with the overall chemical formula C_5H_8, and the molecular formula of terpenes is a multiple of $(C_5H_8)_n$, referring to the number of isoprene units, the latter can form They are linked with each other "head-tail" to form linear chains or arranged to form rings:

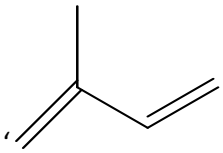


Figure (II-4) Isoprene structural unit.

Isoprene units are mostly linked to each other via a C1 (head) unit in combination with another C4 (tail) isoprene unit.

II.3.2 Types of terpenes:

The resulting terpenes are divided into groups according to the number of isoprene units included in their composition. Table (II-04) summarizes this division:

Table (II-04) Classification of terpenes.

Classification	***n the number of isoprene units***	***Number of carbon atoms***
Hemiterpene	1	5
Monoterpenes	2	10
Sesquiterpenes	3	15
Diterpenes	4	20

And so on. As for multi-terpenes, they consist of a large number of isoprene units, ranging from 500 to 50,000 units, with open chains only. They are also called **High terpenes**, such as:

- **Monoterpenes**

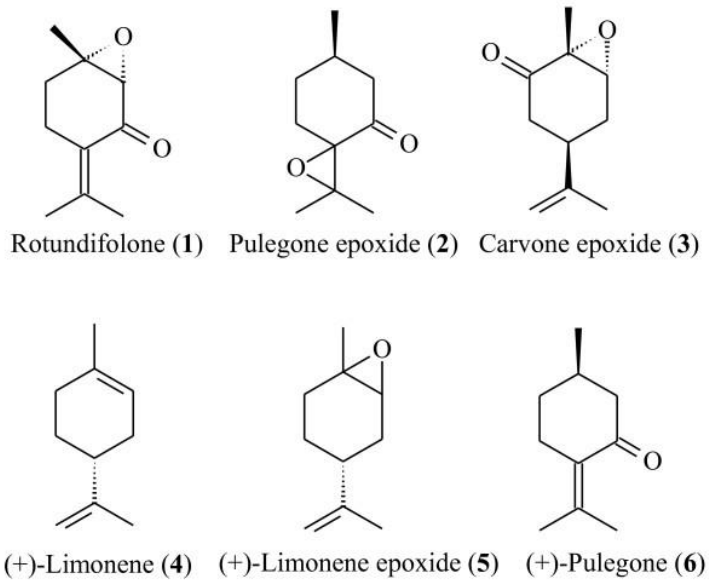
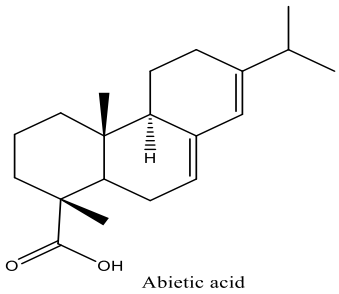


Figure (II-5) monoterpenes structures

- **Sesquiterpenes**



Abietic acid

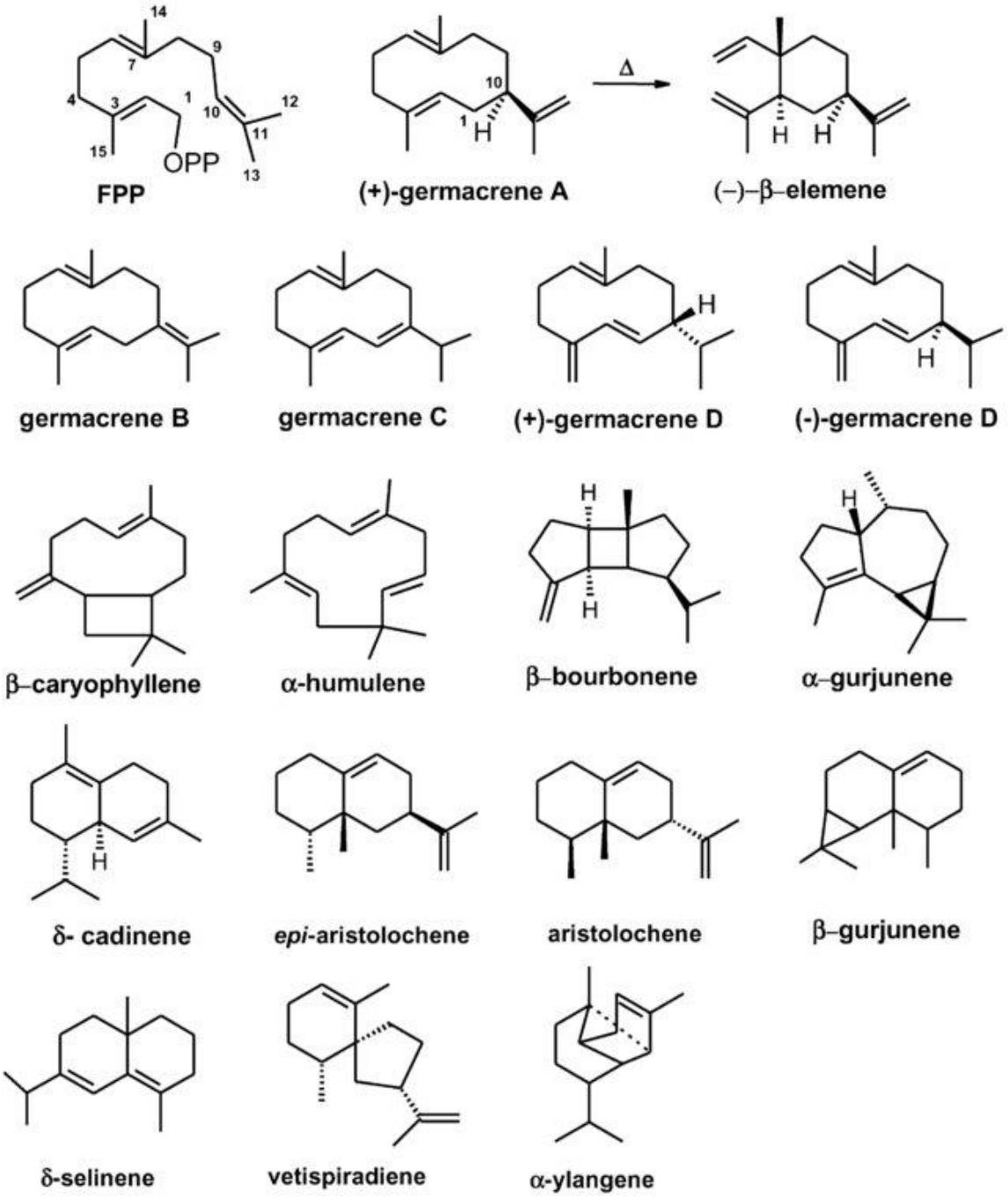


Figure (II-6): sesquiterpenes structures.

- **Diterpenes**

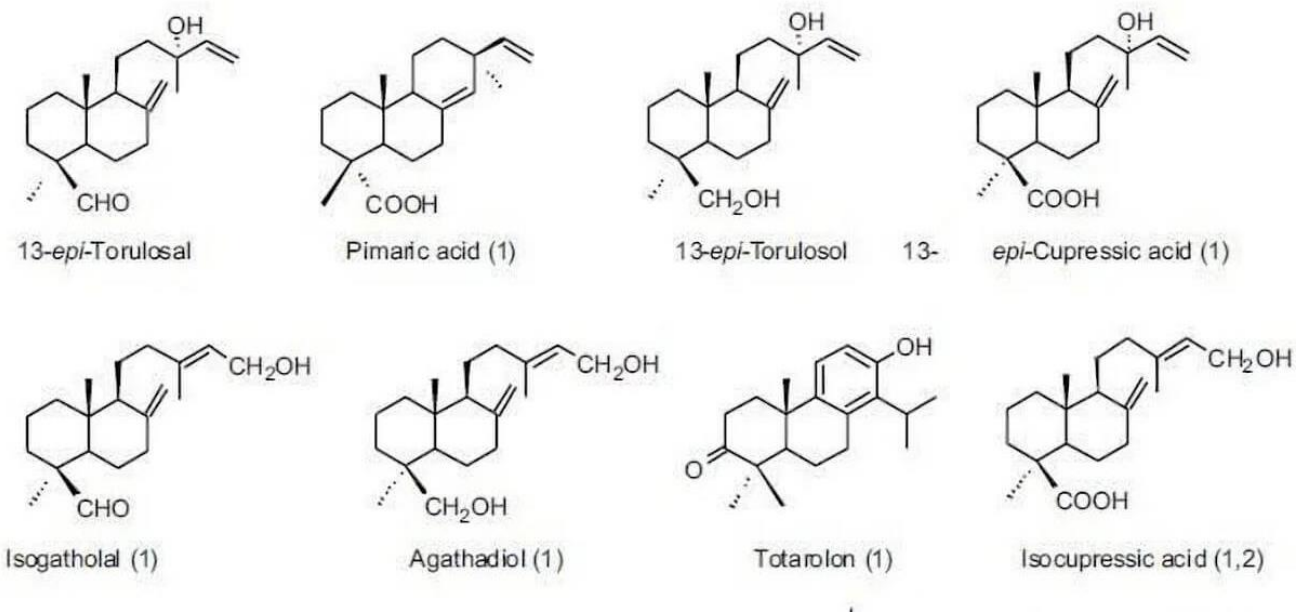


Figure (II-6) Diterpenes structures .

II.3.3Chemical study of Terpenes:

- ***Extraction*:**

The traditional extraction methods for turbines are based on the use of continuous, discontinuous or mixed methods.

- **Discontinuous methods**: include extraction with organic solvents or water. such as cold soaking extraction.

- **Continuous methods**: such as steam distillation and distillation under vacuum.

- **Mixed ways:**
  Extraction-Simultanious distillation
  Soxley extraction

- ***Initial detection*:**

The method mentioned in Harpon-1984 was followed - by dissolving 1 mg of the methanolic extract in a little chloroform and adding to it (Lieberman-Berjad reagent) a drop of anhydride acetic acid and then adding a drop of concentrated sulfuric acid to it. The brown color is evidence that the extract contains terpenes. Detection can also be used by magnetic resonance

spectrometers RMN ^{1}H and RMN ^{13}C, but the real detection is not done except after a series of successive separations followed by a spectroscopic study.

- ***Separation and purification:***

The process of separating turbines is a sensitive process and it is not an easy matter, so that if they are found in a significant quantity, they are in the form of mixtures with significant molecular masses. Therefore, it can be said that obtaining pure compounds is not an easy and quick matter.
This type of compound is separated by gas chromatography to separate the volatile mixture due to the high separation capacity of the columns and the availability of general detection devices.
Most of the terpenes have weak absorbance in Uv-Vis spectroscopy due to the lack of chromophores, and detection by paper chromatography is sometimes valuable to distinguish many terpenes in addition to preparatory chromatography, but the detection of this type of compounds is done by HPLC technology, and the inexpensive TLC technique is widely used In the quantitative and descriptive analysis of various terpenes.

- ***Designation and structural diagnosis*:**

The mating technique (CG - MS) occupies the ideal method for the diagnosis and identification of terpene compounds. It is also possible to use the mating techniques (CG - MS) and RMN ^{13}C. The ionization methods allow obtaining the molecular mass, the nature and methods of association and the number of carbons, in addition to the use of the IR technique that gives different bands for functional groups.

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The Applied part

Chapter III

Methods and Material

III. Methods and Material

III.1 Materials

Glassware:

Pistons of different capacities (5, 10, 40), burette 10 ml, graduated tester 10 ml, graduated tester 50 ml, separating funnels, two separation flasks for the rotary evaporator, Pasteur pipettes, Arlene, conical flasks 250 ml, glass filter funnels of different sizes pipette, flask, test tubes, 2D CP glass cell.

Thin layer chromatographic paper (TLC), mortar mash, spoon, stand, filter paper N° 01 and 02, magnetic rod, medium porosity sieve, pear, metal tongs, distilled water bottle, test tube holder , Wutman paper N°02 .

Appliance:

Rota vaporizer, sensor scale, Uv-Vis spectrophotometer, heating and shaking device.

Chemicals products:

Table (III-01) Chemicals produits.

The scientific name	***Chemical formula***	***Molar mass (g/mol)***	***Density D (g/ml)***	***Purity %***	***the manufacture company***
Ammoniac	NH_3	17.03	0.91	25	Sigma-Aldrich
Sulfric acid	*H_2O_4S*	*98.08*	*1.840*	*95-97*	Sigma-Aldrich
Ninhydrin	*$C_9H_6O_4$*	*178.14*	/	/	BIOCHEM chemopharma
Iron (III) chloride hexahydrate	$FeCl_3,6H_2O$	270.30	/	/	BIOCHEM chemopharma
Formic acid	HCOOH	46.03	1.22	99-100	CHEM-LAB NV
Silica gel 60	O_2Si	60.09	/	/	MERCK
n-Hexane	C_6H_{14}	86.18	/	95	BIOCHEM chemopharma
Vanillin	$C_8H_8O_3$	152.15	/	/	MERCK
Ethyl acetate	$C_4H_8O_2$	88.11	0.902	99.5	Honeywell
Methanol	*CH_3OH*	*32.04*	*0.79*	*99.7*	Honeywell
Butanol	*$C_4H_{10}O$*	*74.12*	*0.8-0.82*	*98.5*	*RECHTAPUR*
Acetone	*C_3H_6O*	*58*	*0.79*	*99.5*	Honeywell
Acetic acid	*$C_2H_4O_2$*	*60.04*	*1.048-1.051*	*99-100*	BIOCHEM chemopharma
Chloroform	*$CHCl_3$*	*119.38*	*1.476-1.483*	*99-99.4*	*Sigma-Aldrich*
***Dichloromethan*e**	CH_2Cl_2	84.93	1.32-1.33	99.9	Honeywell

Acetic Anhydride	$C_4H_6O_3$	102	/	98.0	Fluka Sigma-Aldrich
Petroleum ether	C_6H_{14}	82.2	0.65	90	Honeywell
Ethanol	C_2H_5OH	45.04	0.78	99.8	Honeywell
Toulene	C_7H_8	92.14	0.87	99.8	Sigma-Aldrich
Distilled water	H_2O	18	1	93-49	NASSEH

Reagents of TLC and screening phytochemical :

Table (III-02) Used some reagents**.**

Name of reagent	Uses
Fehling A	detection of carbohydrates
Fehling B	detection of carbohydrates
Dragendorff	detection of alkaloids
iodine beads	Detection of spots in thin layer chromatography after migration
Folin-Ciocalteu	Detection of the phenolic content in the sample extract
Ninhydrin	Detection of peptide bonds, especially the group of amines I and II.
Vanilline sulfric	Detection of spots in thin layer chromatography after migration

III.2 Methods

III.2.1 Sample preparation:

The fruits of the neem tree *Azadirachta indica* (The neem tree) are almost spherical in shape at the beginning, and when ripe they turn yellow and become oval in shape, and the length of the fruit reaches 02 cm. The fleshy shell of the fruit must be removed immediately after harvest, for fear of rotting the fruits, and the fruits are usually planted for multiplication during the first week of harvesting, with age, its ability to germinate weakens, and this tree is planted in many tropical, dry and semi-arid regions.

- **Picking and collecting fruits :**

The process of harvesting the fruits is one of the most important steps. The appropriate season of the year must be chosen to collect the fruits, so that neem trees often bear fruit

once a year and may bear fruit twice in humid areas [03], and these seeds carry effective substances throughout the year. This material changes from season to season.
The seeds were harvested from the outskirts of Tamanrasset, which is about 1,600 km away, in late July 2022.
The harvesting process is carried out with extreme caution, as the fruits that are not well dried rot during storage, and during the rainy season, the fruits must be covered well or moved to a covered place so as not to be exposed to rain. Dried fruits must also be stored in air-permeable containers such as: baskets or burlap bags. Tightly covered nylon bags are not suitable.

- **Drying :**

After the selection process, we start the drying process directly, purifying it from impurities and dust, and placing it in a place that leaves space for ventilation between them. The drying process takes place in the shade, away from sunlight and moisture, turning over from time to time (both the dandruff of the fruit and the pulps are dried separately)

Note: The fruit can be dried until the desired degree of drying, and then the skin is separated from the pulp.

- **Grinding and mashing :**

Before proceeding with the milling process, first:

- Peeling the fruits and separating the outer shell of the fruit from the inner core of the latter.
- Grinding the outer shell of the fruit alone and the inner pulp alone using a mortar masher.
- Sifting the two samples using a medium porosity sieve.

Thus obtaining the following two weights:
Table (III-03) Weight of samples

The sample	Weight in grams
Pulps of the fruit	8.0628 g
Dandruff of the fruit	8.0725 g

- **Soaking :**

The samples are cold soaked in solvents of different polarities (from the least polar to the most polar).

III.2.2 Maceration and Extraction:

The extraction process is considered the first and most important stage to obtain the highest concentration of the active substance from a specific part of the plant to be studied by means of graduated polarity solvents. In the extraction process, we used equal weights for a part of the aerial parts of the neem plant, which are as follows:

Fruit:
Dandruff: 8.0725 g
Pulps: 8.0628 g

One of the separation techniques, which depend on the use of two unmixed phases to transfer the materials to be separated from one phase to another, and it is of two types:

- Solid-liquid extraction
- Liquid-liquid extraction

The extraction process depends on the distribution of the material between the two phases until reaching an equilibrium state**.[06]**

Preparation of the extract (The protocol):

Since the fruits are dry, we went through the following extraction procedure:

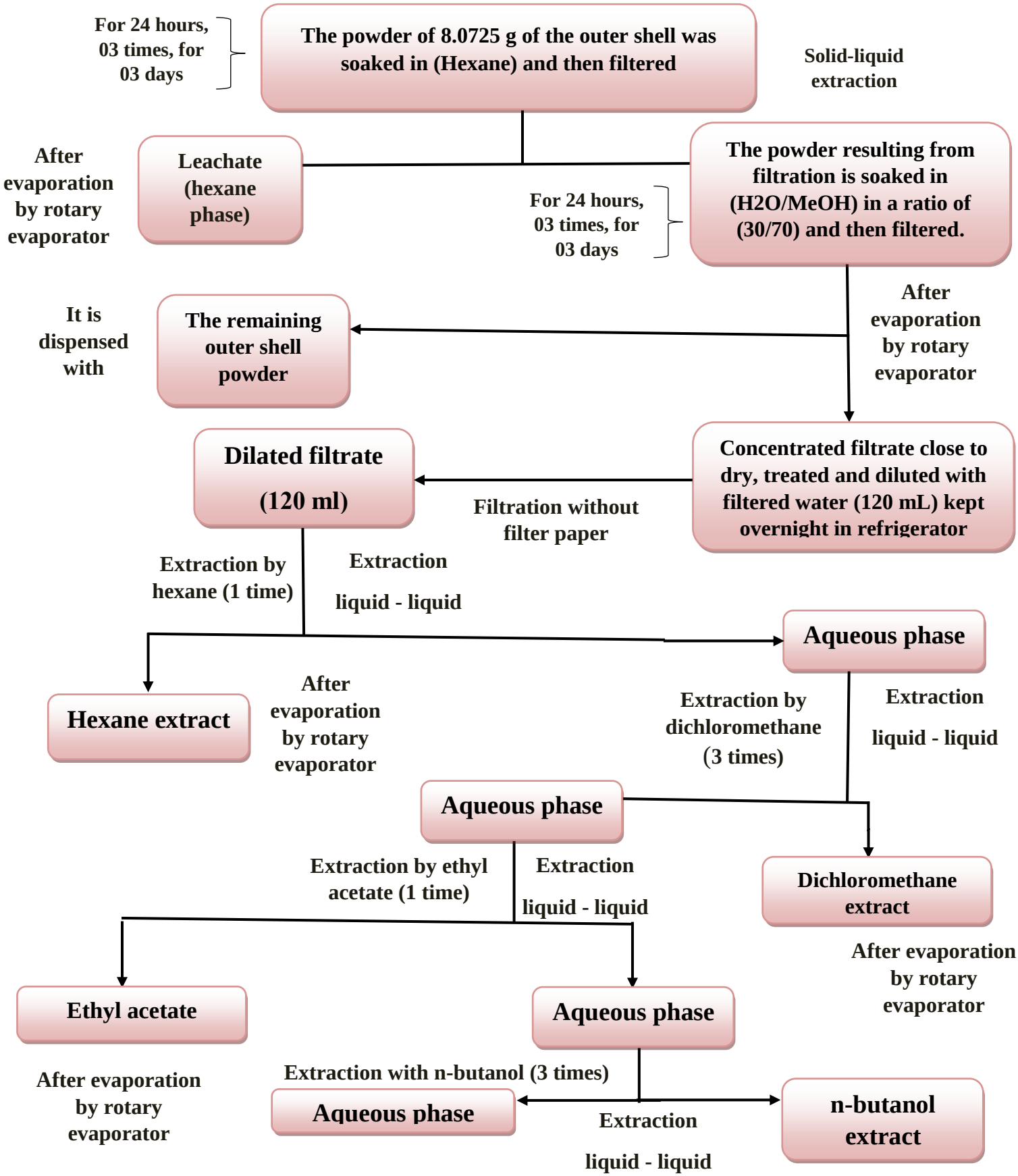
- Where each of the dandruff and the pulps of the fruits were soaked separately in Hexane three times for a period of 24 hours for three days, each time filtered and then the filtrate was collected (the solvent was renewed each time).

- We soak each of the previously filtered dandruff and pulps powders in mixture (eau /MeOH) (30/70 V/V) respectively, the samples being dry three times for a period of 24 hours for three days, each time filtering and then collecting the filtrate with the solvent being renewed each time .

- The previous filtrate is evaporated in the Rota vaporizer, in order to get rid of the (MeOH) solvent and obtain two crude extracts.

- We treat the two crude extracts with distilled water close to boiling (filtered water) so that (every 500 ml of filtered water is in 10 kg of the sample), they are left overnight in the refrigerator, after which they are filtered without the two filter papers, so we get two aqueous phases.

- Extraction operations are carried out on the two aqueous phases using solvents of different polarities, also called liquid-liquid extraction. While water is the other phase [01]. The extraction process begins with hexane, then dichloromethane, then ethyl acetate, and finally n-butanol. In the end, we obtain eight 08 extracts of the outer shell and inner pulp of the neem tree fruit. We summarize the extraction process in the following diagram **(the same protocol of pulps).**



Scheme (III-1) Stages of extracting the dandruff**.**

III.2.3 Phytochemical screening :

The goal:

Determine the presence of various flavonoids in the extracts: methanolic, aqueous and non-methanolic: (aqueous, acetone, hexane and ethyl acetate), and confirm the type of solvent (water, methanol, acetone, hexane or ethyl acetate) suitable for extracting most of these compounds [02] .

- **Preparation of methanolic and non-methanolic extracts:[05,04]**

02g of the dry powder of the outer shell was weighed and placed in five conical flasks with a capacity of 250 ml (the same for the inner core), adding 100 ml of the following solvents (putting each solvent in a flask separately): methanol, methanol/water, hexane, Ethyl acetate, acetone (cold soaking).

Keep the flasks in a dark place for 7 days, stirring every 24 hours. Filtering the extracts using filter papers and drying the extracts at room temperature, then measuring the weight of the extracts after drying them [5].

- **Preparation of the aqueous extract:**

Take 2 g of dry powder for the dandruff and 02 g and place them in two conical flasks with a capacity of 250 ml each, add 100 ml of boiled distilled water to the two flasks and soak for 15 minutes (hot soaking), the two mixtures are filtered by filter papers and rinsed with a little vinegar hot water.

Chemical tests were carried out using the previous extracts to determine whether the components were present or not, so that these tests depend on qualitative analysis, either by forming insoluble complexes (using precipitation reactions) or forming colored complexes (using coloring reactions) [04,05].

Prepare the extracts and then start the detection processes and preliminary phytochemical tests.

- **Test for carbohydrates and glycosides : (Fehling's test)**

Boil 10 ml of the crude extract in a water bath with 10 ml each of Fehling's solutions A and B (a red precipitate indicates the presence of sugar) **[**04].

Fehling A:
Copper sulfate (34.66 g) was dissolved in distilled water and made up to 500 ml using distilled water.

Fehling B:
It is prepared by placing potassium sodium titrate (173 g) and sodium hydroxide (50 g) in water, to a volume of 500 ml.

- **Alkaloids test (Dragendorff test):**

"Tests are performed by precipitation reactions with a Dragendorff reagent."
Enter 10 g of dry vegetable powder into a conical flask, to which add 50 ml of H_2SO_4 acid diluted 1/10 with distilled water. This mixture was stirred and left for 24 hours, then in 1 ml of the filter add 5 drops of Dragendorff's reagent.
(The appearance of prominent orange precipitates in the test indicates that it is positive and the presence of alkaloid compounds) [05].

- **Glycosides cardiques test :**

2ml of chloroform $CHCl_3$ is added to 1 ml of the extract, the appearance of a reddish-brown color after the addition of H_2SO_4 indicates the presence of Glycosides cardiques [05].

- **Tannins test :**

The presence of tannins was shown by adding 10 ml of each extract, 10 ml of water and 10 to 20 drops of a 10% diluted $FeCl_3$ solution. (A dark green or blue-green appearance indicates the presence of tannins) [05] .

The appearance of a dark green color indicates the presence of catechic tannins.
The appearance of a blue-green color indicates the presence of gallic tannins.

- **Quinones libres test:**

01g of dry crushed plant material is placed in a tube with 15-30ml of Ether de petrole, after stirring for 24 hours, the extracts are filtered in a rotary steamer. The presence of free quinones is confirmed by adding a few drops of NaOH (1/10), when the aqueous phase turns yellow, red or purple [04] .

- **Mucilages test:**

Put 10 ml of decocte in a test tube, and then add 50 ml of Absolute alcohol. (A thin sediment after shaking indicates the presence of gum) [04].

- **Test Derive's anthracenics liberes :**

Anthracene derivatives were determined by the following procedure:

Free anthracene is detected by adding 10 ml of chloroform extract $CHCl_3$ and 10 ml of diluted NH_4OH to a test tube. After shaking and stirring, (a slightly red color indicates the presence of free anthracene) [04].

- **Cyanogenic compounds test :**

Three grams of fresh plant material is moistened with a few drops of chloroform $CHCl_3$ in a test tube, after which a strip of filter paper impregnated with sodium bicarbonate is

inserted, and the tube is placed in a water bath at 35 °C for 3 hours (red strip indicates the presence of Composes cyanogens) [04**]**.

- **Saponines test :**

In a series of 10 test tubes numbered from 01 to 10, enter, respectively, 01, 02, 03 to 10 ml of the solution to be analyzed, prepared by decoction in a water-alcohol medium. Adjust the volume of each tube to 10 ml with distilled water. Shake each tube lengthwise for 15 seconds at a rate of one shake per second. Leave the tubes standing in the stand for 15 minutes and measure the height of foam produced in each tube [**0**4**]**.

The foaming index (I) is calculated by the following formula:

$$\mathbf{I} = \frac{\mathbf{1000}}{\mathbf{N}} \dots\dots\dots\dots\dots\dots\dots (\mathbf{I})$$

N: is the tube number where the foam height is 1 cm.

- **Ninhydrin test:**

Two drops of Ninhydrin solution (10 mg of Ninhydrin in 200 ml of acetone) were added to 2 ml of the crude extract.
(The distinctive purple color indicates the presence of amino acids) [05] .

- **Phenolic compounds tests :**

- **Alkaline reagent:**

The aqueous solution of the extracts was treated with 10% ammonium hydroxide solution (yellow fluorescence indicates the presence of flavonoids) [05].

- **Gums and Mucilages test:**

The extract (100 mg) was dissolved in 10 ml of distilled water and to this was added 25 ml of absolute alcohol with continuous stirring. (White or cloudy deposition indicates the presence of gum and mucilage) [05**]**.

- **Terpenoids test (Salkowski test)**:

Five milliliters of the extract was mixed with 20 milliliters of chloroform $CHCl_3$ and concentrated sulfuric acid H_2SO_4 to form a layer. (The appearance of a reddish-brown color indicates the presence of a terpenoid) [05].

- **Phytosterols test (Libermann-Burchards test):**

The extract was dissolved 100 mg in 20 ml of acetic anhydride. For this a drop or two of concentrated sulfuric acid was slowly added along the sides of the test tube (a band of changing colors shows the presence of phytosterols)[05**]** .

- **Phlobatannins test:**

The sample was boiled with 01% aqueous hydrochloric acid to produce (a red precipitate indicating the presence of Phlobatannins) [05**]**.

- **Fixed oils and fats test :**

Spot test:

We squeeze a small amount of the extract between two filter papers. (The oil stain on the paper indicates the presence of fixed oils.)[05] .

- **Proteins and Amino Acids Test:**

We dissolved the 100 mg extract in 10 ml of distilled water and filtered it through Whatman N°1 filter paper. The filtrate was subjected to protein and amino acid tests [05].

III.2.4 Separation and purification:

III.2.4.1 TLC thin layer chromatography Screening:

After extraction and before proceeding with the separation operations, we conducted a TLC test with several systems on the extracts of the dandruff and the pulps of the fruits:

The TLC System tested:

Hexane and dichloromethane extracts for dandruff and pulps

- Hexane/ $CHCl_3$(2/1),(4/1),(6/1),(8/1).
- Hexane/ $CHCl_3$ /Acetic acid (8/1/1), (7/1/0.5).
- Ethyl acetate / Petroleum ether (1/1),(2/1),(1/6), (1/4), (1/2).
- $CHCl_3$/Acetone (1/1), (1/2), (1/4), (1/6), (2/1)
- $CHCl_3$/Acetone / Acetic acid (3/1/0.1) , (10/1/0.1), (30/1/0.1).
- Hexane/ CH_2Cl_2/ Ethyl acetate (2/2/1),(15/5/1),(1/1/2),(4/4/1) .
- CH_2Cl_2 + Two drops of methanol.
- $CHCl_3$ / MeOH (40/1),(20/1),(10/1),(1/1).
- Hexane /CH_2Cl_2 /Ethyl acetate (4/2/1),(2/2/1) .

Ethyl acetate and n-butanol extracts for dandruff and pulps

- Hexane/ $CHCl_3$ (2/1), (4/1), (6/1), (8/1).
- Hexane/ $CHCl_3$ /Acetic acid (8/1/1), (7/1/0.5).

- Ethyl acetate / Petroleum ether (1/1),(2/1),(1/6),(1/4),(1/2).
- $CHCl_3$/Acetone (1/1),(1/2),(1/4),(1/6),(2/1).
- $CHCl_3$/Acetone /Acetic acid (3/1/0.1),(10/1/0.1),(30/1/0.1).
- Hexane/ CH_2Cl_2/ Ethyl acetate (2/2/1),(15/5/1),(1/1/2),(4/4/1).
- CH_2Cl_2+Two drops of methanol .
- $CHCl_3$ / MeOH (40/1),(20/1),(10/1),(1/1).
- Hexane /CH_2Cl_2 / Ethyl acetate /Acetic acid (4/2/1/0,5),(2/2/1/0,5),(6/1/1/0,5).
- n-butanol / Acetic acid /water (4/1/1), (2 /$\frac{1}{2}$/ $\frac{1}{2}$), (4 /$\frac{1}{2}$/ $\frac{1}{2}$).
- Ethyl acetate /Formic acid / Acetic acid/water (10/1.1/1.1/2.6),(7.25/0.75/0.75/1.25),(10/1.1/0.5/1),(10/1/1/2.6),(10/1,1/1,1/2).

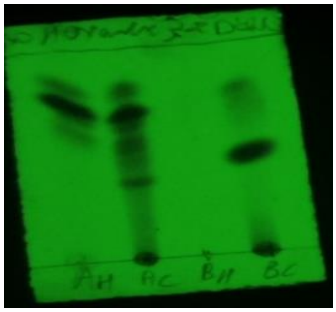
Note:

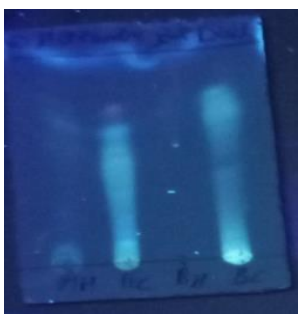
Some systems contain 4 solvents which are used as a moving phase, the fourth solvent represents acetic acid, it is a polar solvent used to separate and show well the separated spots in the sample from each other in thin layer chromatography (TLC).
By achieving the principle of "**the likeness dissolves the like**", non-polar systems for non-polar extracts (Hexane and DCM) and polar systems for polar extracts (Ethyl acetate and N-butanol) were used.

Photographs were taken under ultraviolet (UV) light of some experimental systems at the following wavelengths:

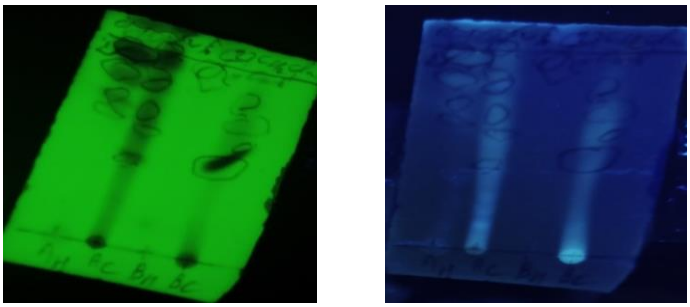
- λ_1 = 245 nm.
- λ_2 = 365 nm .

by mode:
(A: dandruff of the fruit) and (B: pulps of the fruit).
A_E: Ethyl acetate extract "dandruff"
A_B: butanolic extract "dandruff"
A_H: hexane "dandruff" extract
A_{Cl}: dichloromethane extract "dandruff"
B_E: ethyl acetate extract "pulps"
B_B: butanolic "pulps" extract
B_H: "pulps" hexane extract
B_{Cl} : dichloromethane "pulps" extract



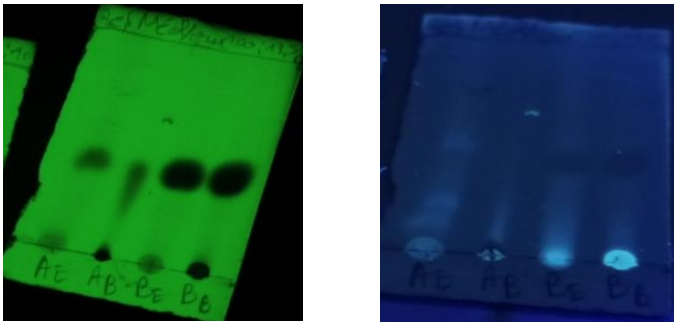


The hexane system and then dichloromethane.

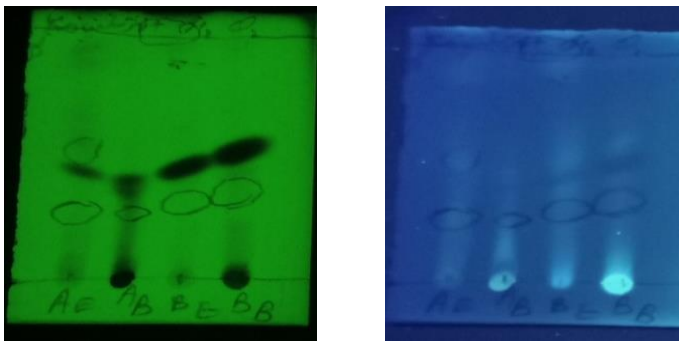


Hexane system, then dichloromethane system + two drops of methanol.

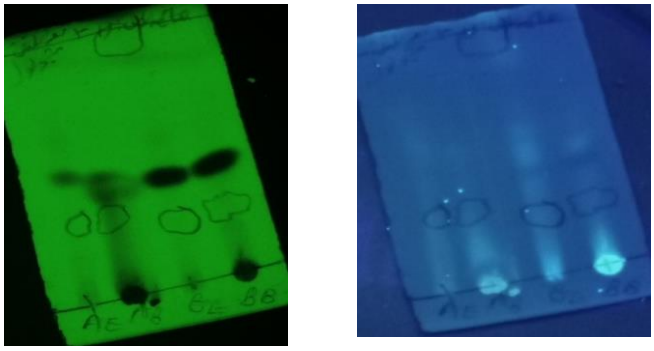
Figure (III-4) Some systems -1-



Ethyl acetate / MeOH / H_2O system(10/ 13,5 / 100)



Dichloromethane system + 2 drops methanol



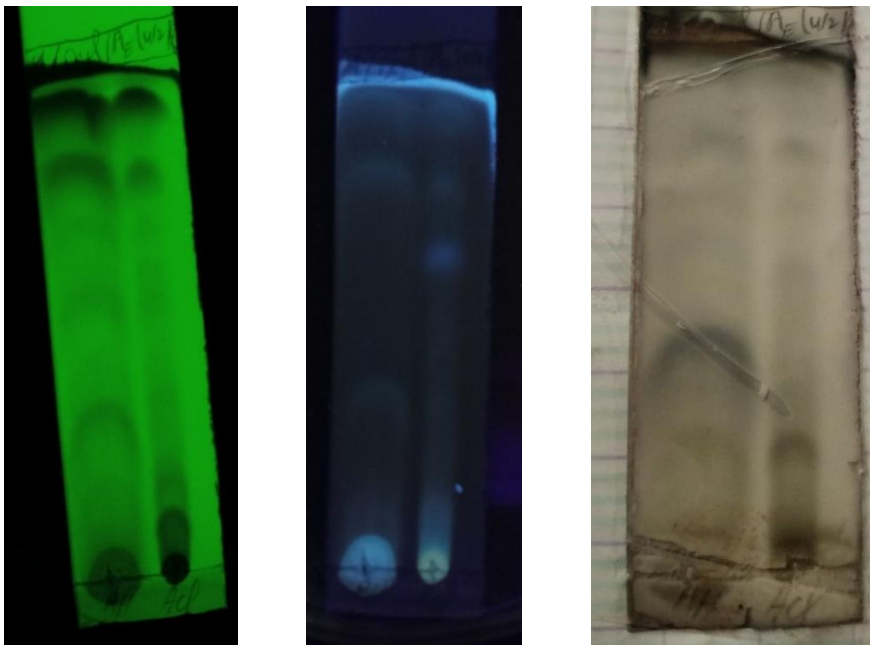
Dichloromethane system + 2 drops methanol

Figure (III-4) Some systems.-2-

The process of separation of the hexane extract A_H and the dichloromethane extract A_{Cl} of the dandruff of neem fruit was carried out using thin layer chromatography (TLC), and the appropriate system was found as a mobile phase, which is:

- Hexane/CH_2Cl_2/Ethyl acetate (4/2/1).
- Hexane/CH_2Cl_2/Ethyl acetate (2/2/1).
- Hexane / CH_2Cl_2 / Ethyl acetate / Acetic acid (6/1/1/0.5).

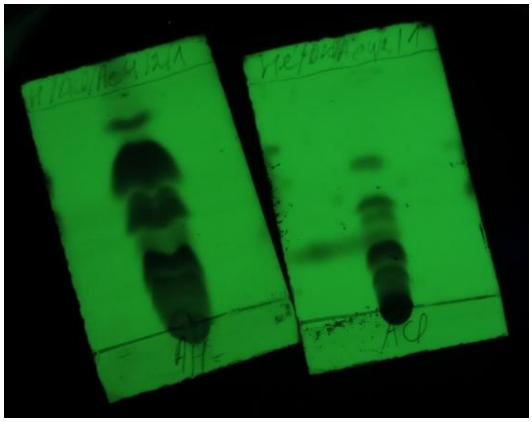
<u>-System 01-</u> The system of Hexane/ CH_2Cl_2/ Ethyl acetate (4/2/1)



λ = 245 nm λ = 365 nm After showing (vanillin sulfuric)

Figure (III-5) The TLC of the hexane and dichloromethane extracts of the dandruff of the fruit system 01.

Separating each extract separately, we get:

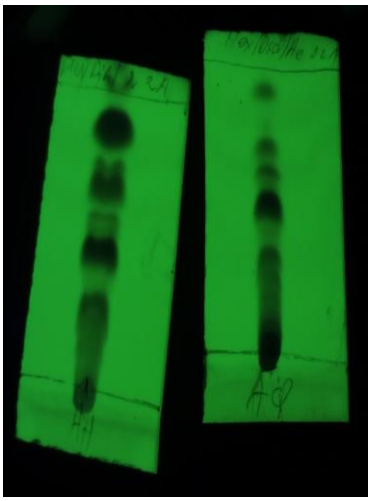


Before showing (λ = 245 nm)

After showing

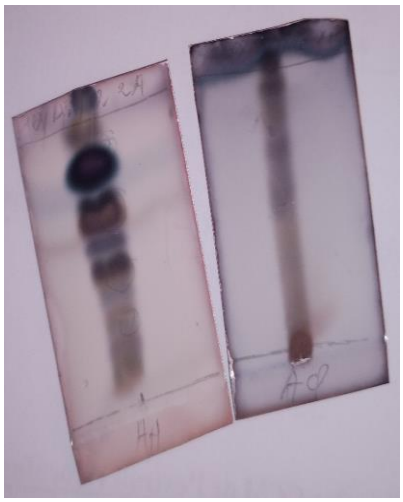
Figure (III-6) The TLC of hexane and dichloromethane extracts of the dandruff of the fruit system 01.

<u>-System 02-</u> Hexane/ CH_2Cl_2/ Ethyl acetate (2/2/1):



Before showing(λ=245 nm)

Before showing(λ =365nm)



After showing

Figure (III-7) TLC of the two extracts of hexane and dichloromethane for the dandruff. System 02.

III.2.4.2 Paper Chromatography:

We also did a preliminary analysis of the two extracts of n-butanol (B_B and A_B) for the dandruff and the pulps of the fruits of the studied plant using paper (CP Whatman N°1) paper chromatography 1D and two-dimensional 2D, using the following systems as mobile phases:

<u>1D Dimension System</u>:

- BAW: BuOH / AcOOH / H_2O (4/1/5)
- AcOOH 15% : Acetic Acid 15%

Photographs of the CP layer were taken under UV irradiation as shown in the figure (III.8):

AcOOH 15% BAW

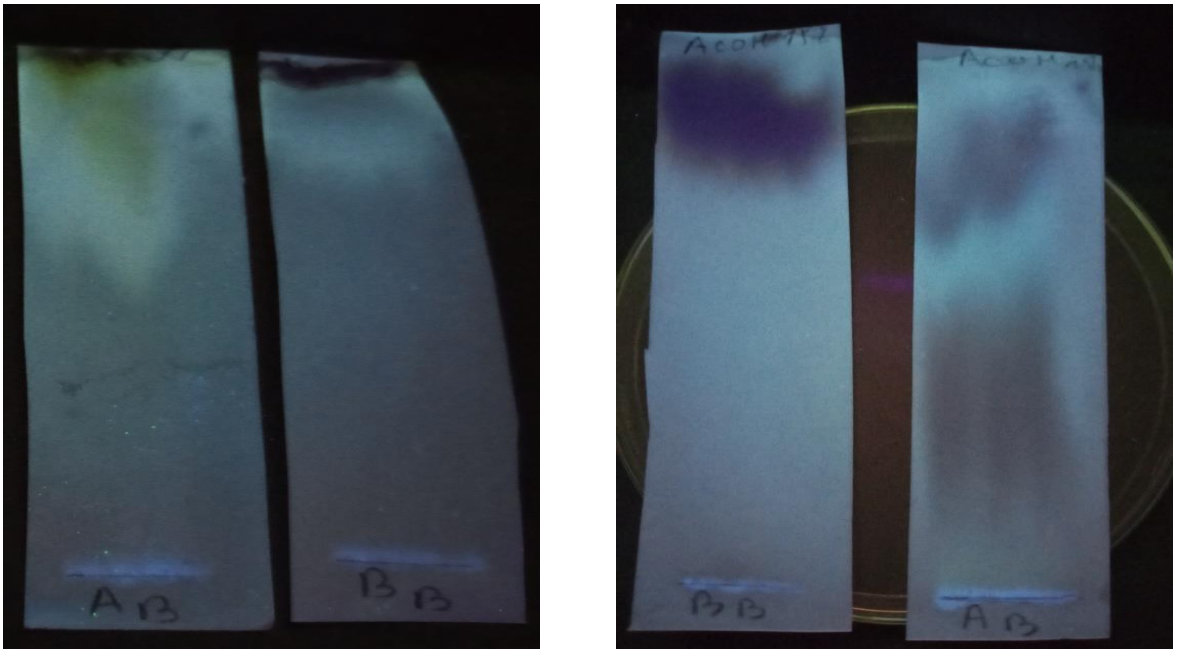


Figure (III-8) One-dimensional paper chromatography of the two butanol extracts (dandruff and pulps).

<u>Dimension 2D:</u>

We also dealt with conducting a comparative study of ethyl acetate and n-butanol extracts using (Whatman N°1) paper for the dandruff and pulps and applying the two systems BAW for the first dimension and AcOOH 15% for the second dimension as two mobile phases.

where:

- BAW: BuOH / Acetic acid / H_2O(50/10/40)
- AcOOH 15% : Acetic Acid 15%

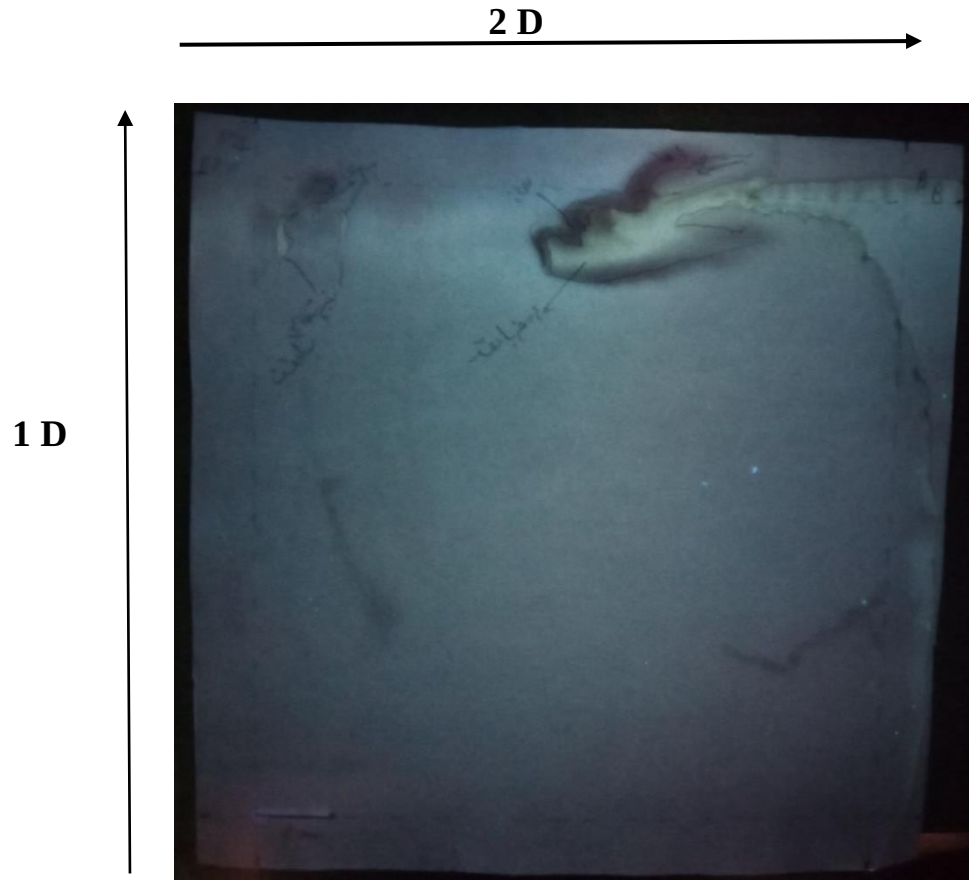


Figure (III-9) Two-dimensional paper chromatography of butanol extract (pulps).

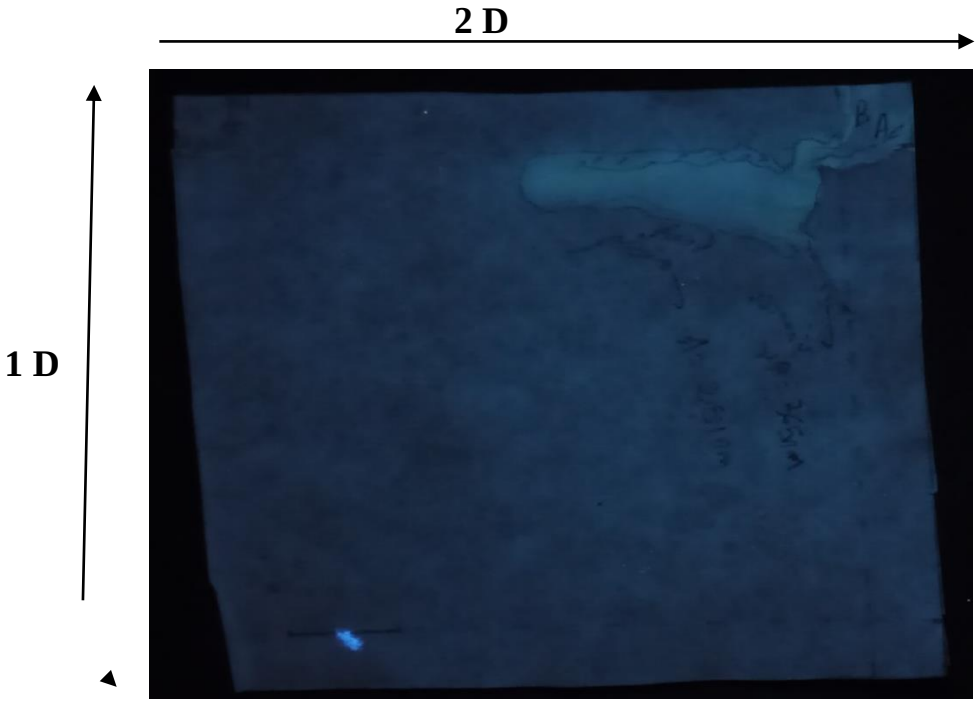


Figure (III-10) Two-dimensional paper chromatography of ethyl acetate extract (pulps).

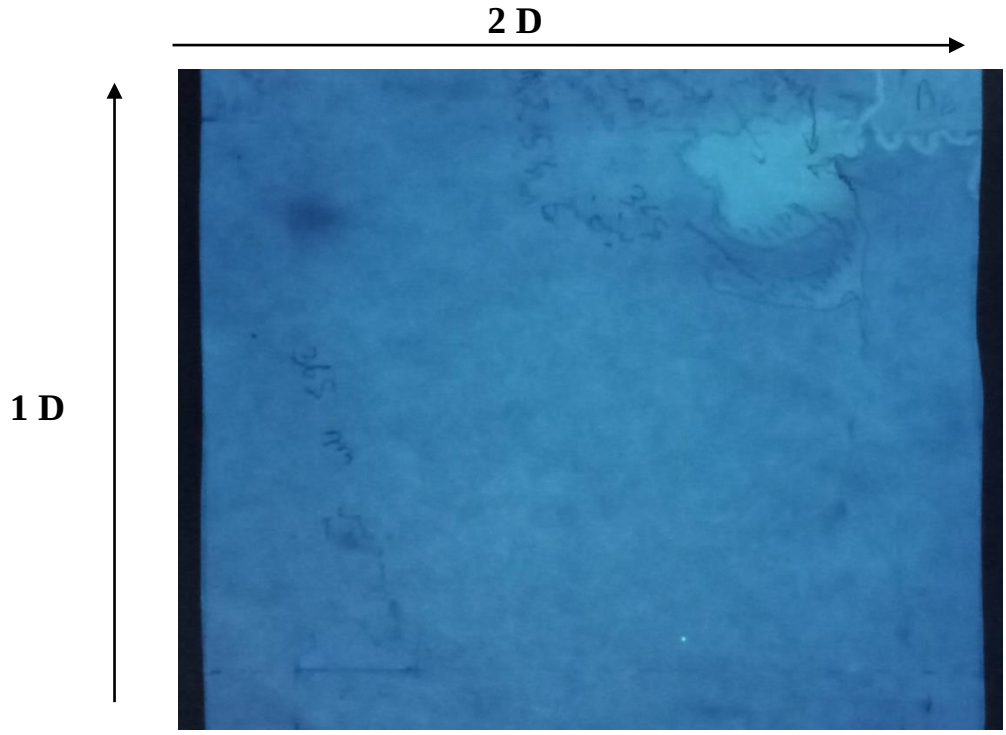


Figure (III-11) two-dimensional paper chromatography of the butanol extract (dandruff).

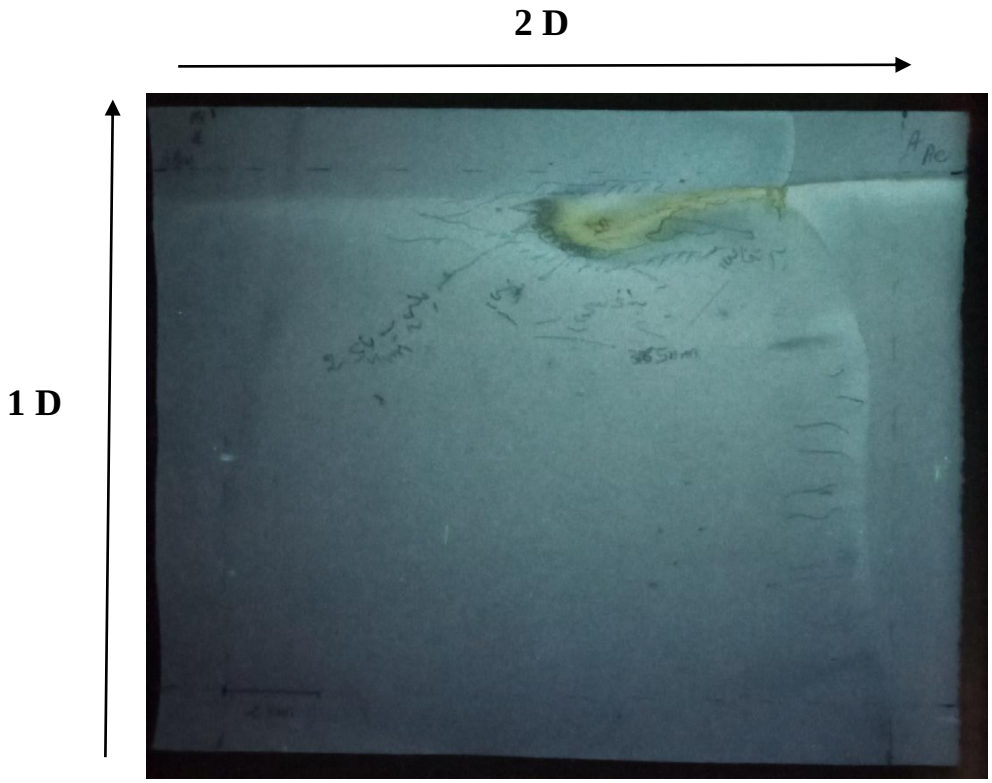


Figure (III-12) Two-dimensional paper chromatography of ethyl acetate extract (dandruff).

III. 2.5 - UV-Visible Spectroscopy Analysis of Extracts:

Ultraviolet-visible spectroscopy is considered one of the most widely used methods in the field of chemical analysis. Ultraviolet spectroscopy is regarded as one of the most important and best methods used in determining the structure of compounds, due to its ease. It also requires a small amount of the compound, as it is based on the principle that each type of compound has Its own absorption spectrum, as two absorption bands in alcoholic media characterize this spectrum. The absorption of the two bands varies with the compound.

We passed the extracts of *Azadirachta indica* A. Juss studied, after extending the extracts by EtOH and passing them in a UV-Visible spectroscopy device to know the different bands of those extracts and to detect different ones.

III.2.6 Column chromatography CC:

Accordingly, in order to separate the largest possible number of components of one of the two extracts of hexane A_H and dichloromethane A_{Cl}, we separate the components of the hexane extract from the dandruff of the fruit of the studied plant and perform a preliminary separation by using isocratic column chromatography and by selecting the appropriate mobile system:

(Hexane / DCM/ ACOOH) (4/ 2 /1) and using 5.6432 g silica gel as the stationary phase for m = 197.6 mg of hexane extract A_H.

Figure (III-14) The Isocratic column chromatography.

Isocratic column chromatography was selected in the separation of secondary metabolite compounds using silica gel, we used the isometric system each time, it was displayed using an ultraviolet lamp, and we got 38 primary fractions.
Random fractions were selected and separated by TLC chromatography and detected by UV radiation. We used a system to prefer these mixtures:

(Hexane /DCM /Ethyl acetate /Acetic acid): (4 /2 /0.5 /0.1) .

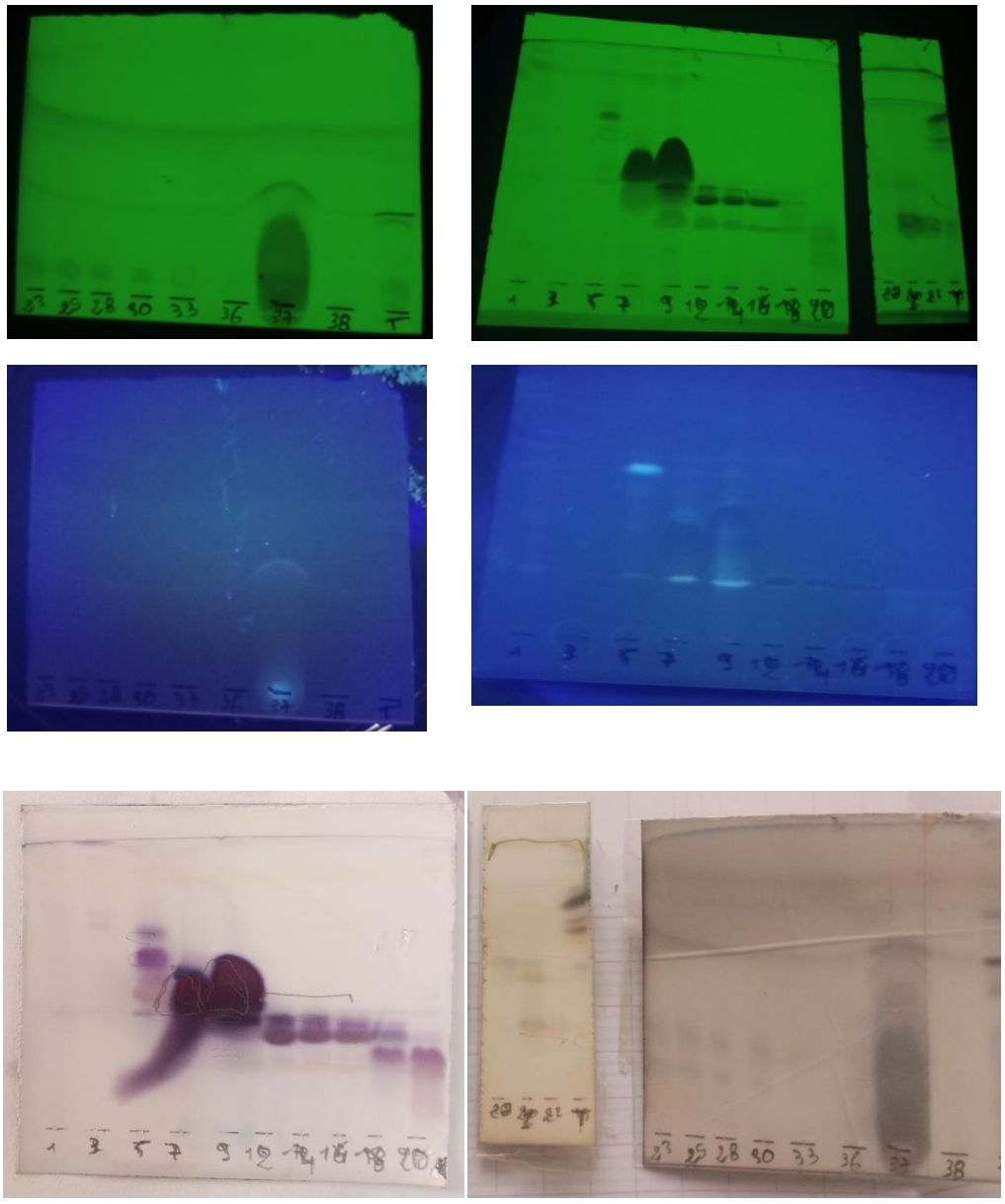


Figure (III-15) The TLC of column fractions.

We notice that some fractions, after their separation, contain the same spots, and therefore we collect them together because they contain maybe the same compounds.

Collect the fractions:

We add fractions together based on the similarity of the compounds between each fraction and on the basis of their masses as well.

- ***First assembly : (*The first grouping of fractions)**

After performing the separation technique by isocratic column chromatography, the obtained fractions were passed on TLC chromatography and the previous system was used as a mobile phase. We grouped the similar fractions, and we obtained the following grouping:

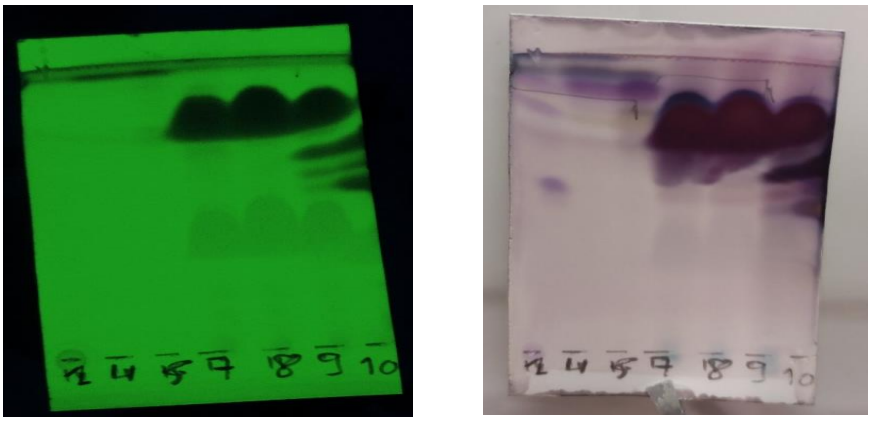


Figure (III-16) The TLC for fractions after the first grouping of fractions.

After collecting the similar fractions by thin layer chromatography, we obtained 8. We collect a second collection of the fractions bearing the same compounds.

- ***The second grouping of fractions:***

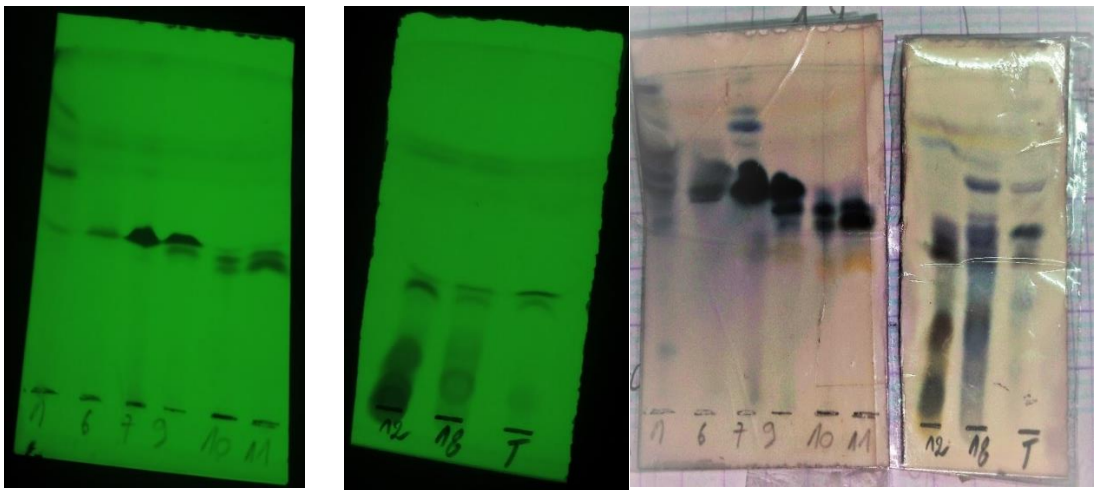


Figure (III-17) The TLC of the second grouping of fractions.

- ***The third grouping of fractions:***

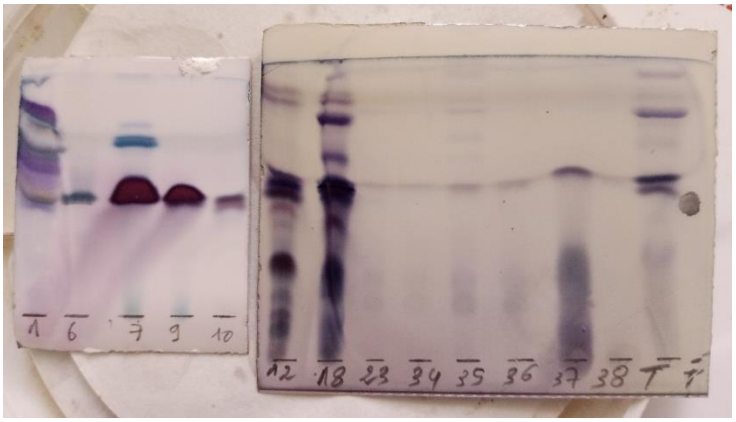


Figure (III-18) The Thin layer chromatography chromatograms for the third assembly of fractions after showing.

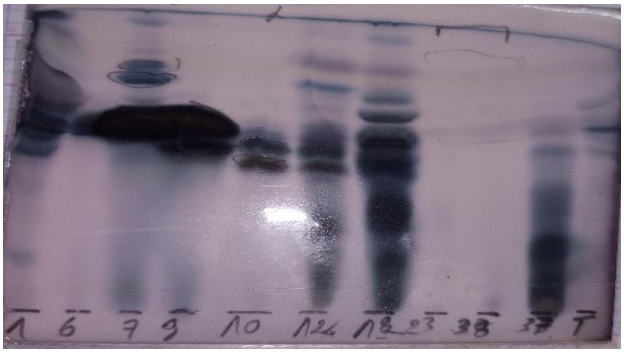


Figure (III-19) The Thin layer chromatography chromatograms for the final assembly of fractions.

After collecting the fractions and joining them to each other, we get a final collection of them, and passing them through two systems to separate them well and clearly in thin layer chromatography and passing them in the UV device.

***The first system*: (Hexane / DCM / Ethyl acetate) (4 /2 /1)**

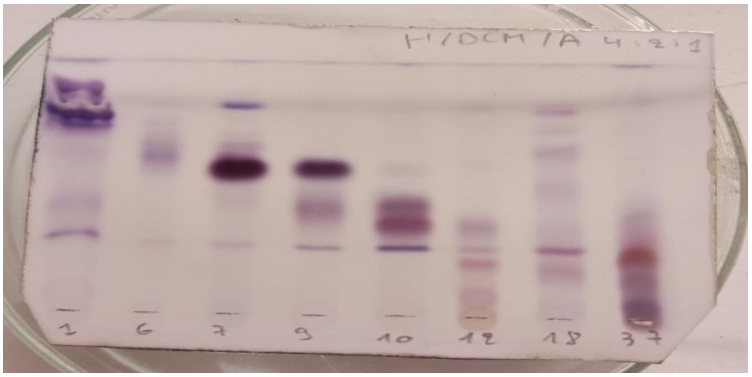


Figure (III-20) The Thin layer chromatography chromatograms for the last assembly of fractions before showing .

The second system: **(Toluene / Ethyl acetate / Acetic acid) (4 / 1/ 0.1)**

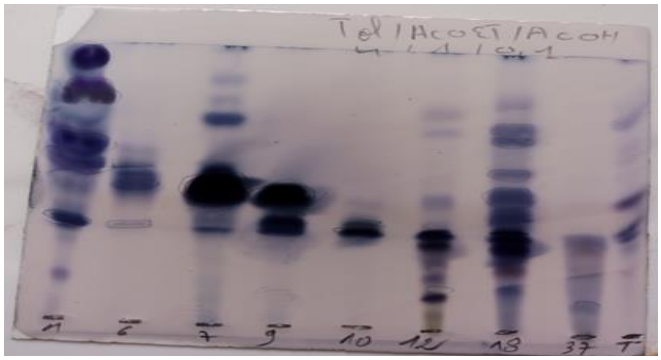


Figure (III-21) The Thin layer chromatography chromatograms for the last assembly of fractions after showing.

By comparison between the two systems, we notice the appearance of many compounds in the separation of the second system of fractions, most of which are non-polar compounds. We will study fractions 7 and 9 because they contain approximately the same major compound, in addition to fraction 10 because it contains approximately three compounds separated from each other.

The results of calcinations and collection of isocratic column fractions using the system "Hexane/DCM/Ethyl acetate (4/2/1)" were presented in the following table:

Table (III-4) The masses of fractions collected.

Fraction's number	mass (mg)
01 02 03 04 05	58.3
06	01
07 08	22.0
09	8.9
10	9.9
11 12	19.7
13 14 15 16 17 18	15

19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38	18.5

Study of fractions:

To obtain pure compounds, we study fractions 09, 07 and 10 and try to separate them using one of the separation and detection techniques.
We first detect fractions 09, 07 and 10 by thin layer chromatography using the separation system **(chloroform / ethyl acetate): (1/10)**.

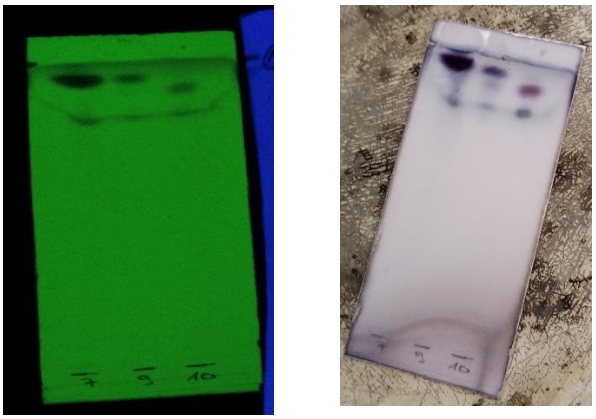


Figure (III-22) The Thin layer chromatography before showing .

We separate fractions 07 and 09 by thin layer chromatography using **the system (Toluene / Ethyl acetate / Methanol)** with **volumes (10/1/1)** to check their structural contents if they are the same compounds or not.

Figure (III-23) The Thin layer chromatography after showing for the two fractions .

We notice that fractions 07 and 09 contain the same compounds, but in different proportions, that is, the compounds present in fraction 07 are present in greater values than those present in fraction 09.

As for fraction 10, it contains compounds that different from those in fractions 07 and 09.

We used this technique to see what kind of techniques can be used to separate the compounds of the fractions to be studied. We study each part separately using different separation techniques.

Since fractions 07 and 09 contain almost the same compounds, we study only fraction 07. As for fraction 10, it is also studied separately by one of the methods of separation and detection.

Study of Fraction 07:

Compounds are detected by passing sample fraction 07 on column chromatography; we separate its compounds by thin layer chromatography and pass it on a UV-VIS spectroscopy device to find out the wavelength of each fraction resulting from the column.

After separating fraction 07 by thin layer chromatography and passing it on a UV lamp previously, three different polar spots were detected, with the appearance of a spot present in the greatest way representing one or two compounds or a number of compounds that we will seek to separate and detect.

More precisely, we pass sample fraction 07 on TLC using several systems in order to find a suitable system for column chromatography.

- **Fr-7 : ($CHCl_3$ /Ethyl acetate) (6 /1)**

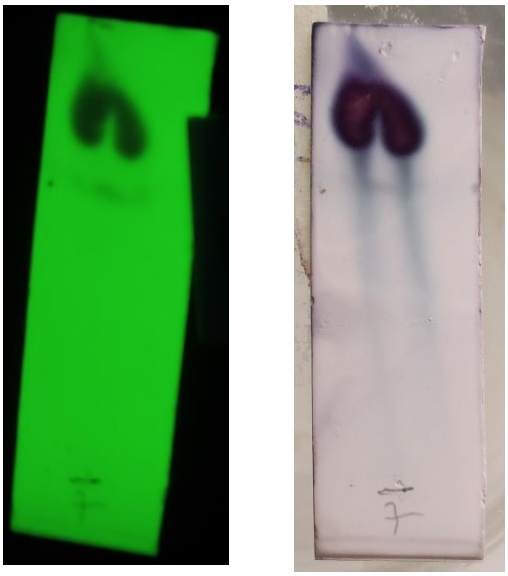


Figure (III-24) The Thin layer chromatography of fraction 7.

- **Fr-7 : (Hexane/CH_2Cl_2 /Ethyl acetate /MeOH /Acetic acid) (7/1/0.1/0.1/0.1)**

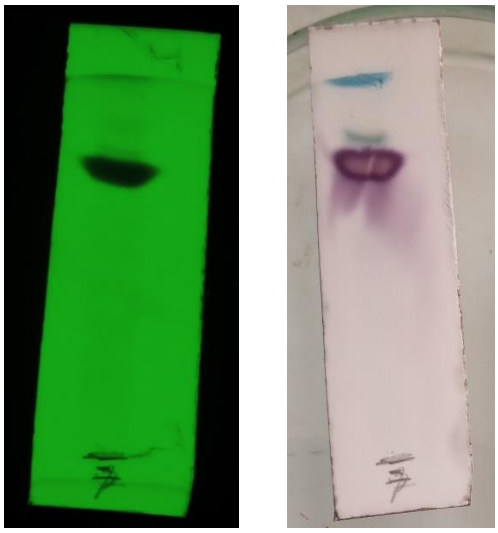


Figure (III-25) The Thin layer chromatography of fraction 7 .

After comparing the migration of the two spots in the two previous systems, we conclude that the appropriate system for separation by column chromatography for sample fraction 07 is the system: ($CHCl_3$/ Ethyl acetate) (6/1).

Through the initial separation on the compounds of the 7 fraction and in order to obtain pure compounds, we carried out the column separation process in the following

proportions, The result of grinding the column for fraction 07 is shown in the table below ,We devised a small column to separate and identify the compounds of fraction 07, which gave 08 fractions:

Table (III-11) The results of CC of fraction 07**.**

The number of fraction	**$CHCl_3$ %**	**Ethyl acetate %**	**p.s Notes**
1	100	0	Singl compound
2	80	20	Single compound
3	80	20	Cured mixture
4	50	50	
5	50	50	
6	50	50	Cured mixture
7	50	50	
8	0	100	No trace of chemical compounds

Figure (III-26) Small chromatography column of fraction separation 07.

After separating fraction 07, whose contents were similar to those and compounds of fraction 09, but in lower proportions, we separated its compounds by column chromatography. The same previous methods of separation and detection were carried out.
Separation of these compounds by TLC with appropriate systems to ensure the presence of compounds in fraction 07:

System (Toluene / Ethyl acetate / Methanol): (20/1/1)

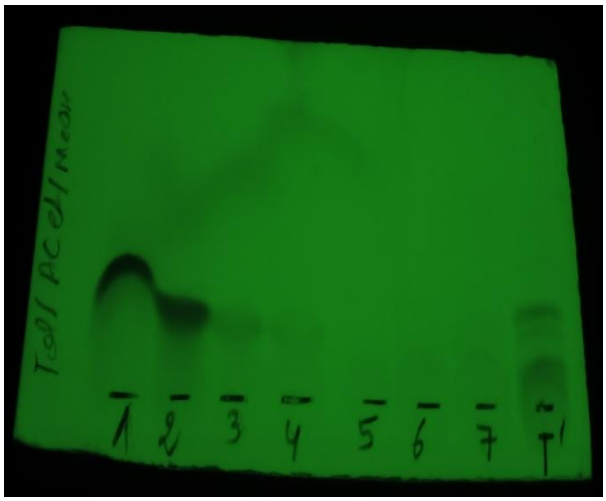


Figure (III-27) The Thin layer chromatography before showing for sub-fractions of fraction 07.

We pass it on TLC using **the system (Hexane / DCM / Methanol / Acetic acid): (10 /1/ 0.1/0.01) as a mobile phase**, so three pure spots appeared. Here we conclude the discovery of three pure compounds:

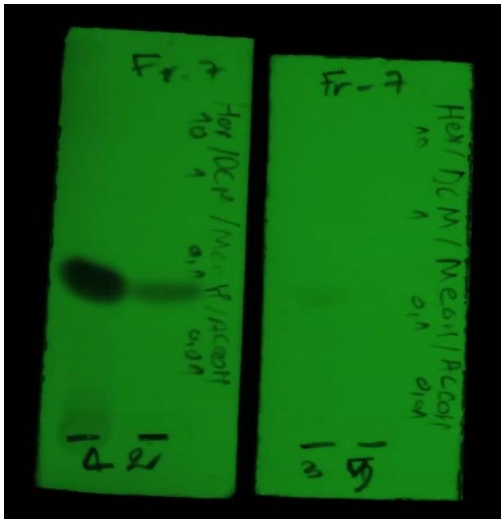


Figure (III-28) The Thin layer chromatography of pure compounds of fraction 07.

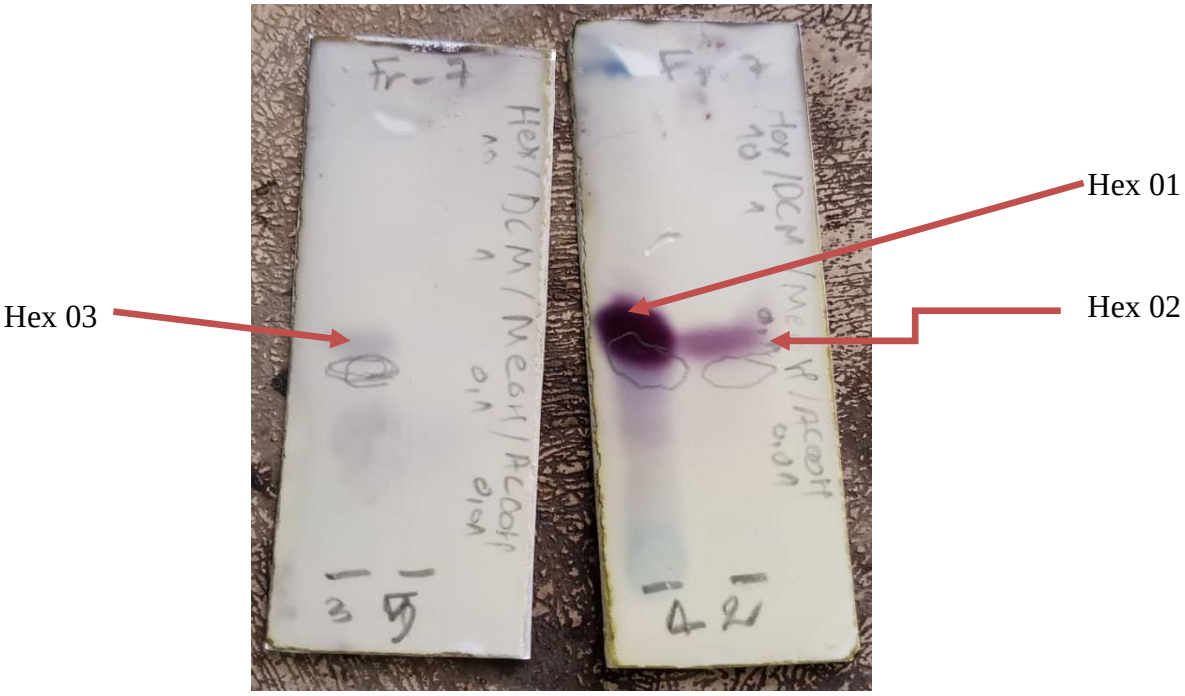


Figure (III-29) The Thin layer chromatography of isolated compounds.

The chromatogram figure (III-29) represents the pure products obtained from fraction No. 7, so we get three pure compounds Hex01, Hex02 and Hex03 In addition to product No. 5, it contains a group of compounds that need to be separated and purified more after using a lot of different systems of TLC thin-layer chromatography.

Study of Fraction 10:

We use the TLC technique for sample fraction 10 to find a suitable system in order to pass it on the Preparative chromatography, among these systems we find:

- **Fr-10 : (Hexane / CH_2Cl_2 / Ethyl acetate/Acetic acid) (10 /0.5/0.5/0.1)**

Figure (III-30) The Thin layer chromatography of fraction 10.

Among the previous systems that were conducted on Fraction 10 to find out which compounds it contains, we use the system **(Hexane / CH_2Cl_2 / Ethyl acetate/Acetic acid) (10 /0.5/0.5/0.1)** as a suitable mobile phase in the preparative TLC chromatography technique to separate and detect the compounds of Fraction 10.

Figure (III-31) The Protocol of Preparative TLC .

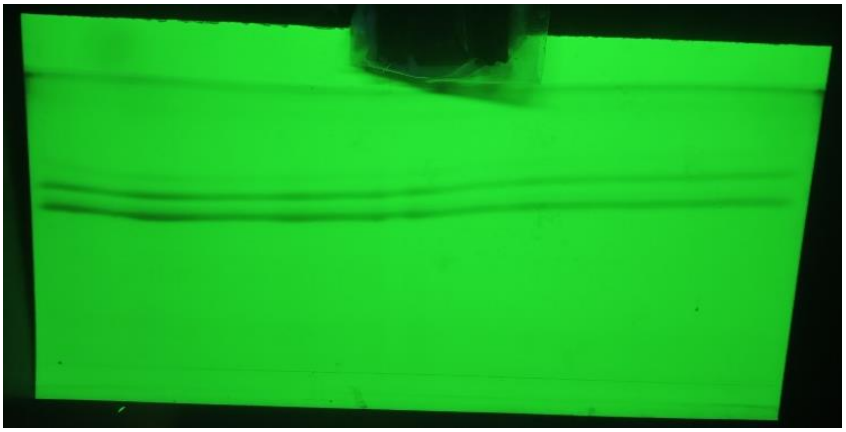


Figure (III-32) The Preparative TLC.

- We notice the appearance of three spots in the form of bands along the two plates.
- We remove the part that contains the stains with silica gel, each similar spot between the two plates in Becher separately, so we get three samples, we add a little methanol to the three samples and leave it for a period of time.
- After filtration and washing, we leave the three samples until the solvents evaporate, then pass the samples on thin layer chromatography using the system: **(Toluene /Acetate ethyl / Acetic**

acid) (20/4/0.5) ,we pass the paper over UV light to detect the compounds present in fraction 10 (figure III.34).

Figure (III-34) The Thin layer chromatography chromatogram after showing for parts of fraction 10.

- Through the resulting chromatogram of fractions 1, 2, and 3 of fraction 10 using the phase**: (Toluene /Acetate ethyl / Acetic acid)(20/4/0.5)** as a mobile phase, we see that each fraction contains impure compounds that are less complex than the fraction 10 sample, and from it more purification technology is needed to separate and identify the compounds of the fractions.

III.3 Quantitative analysis and antioxidant activity:

III.3.1 Determination of total phenols (TPC):

The phenolic content of the fruit extracts of the *Azadirachta indica* A. Juss plant was determined by using Folin-Ciocalteu reagent, the absorbance of the extracts was measured using a UV spectrophotometer at a wavelength λ = 760 nm (gallic acid was used as a reference standard with different concentrations of (0.05 to 0.3 mg /ml) [06].

The determination of total phenol contents was determined by the method of Singleton and Rossi, using the reagent Folin-ciocalteu this reagent consists of a mixture of phosphotungstic acid $H_3PW_{12}O_4$ and phosphomolybdic acid $H_3PMo_{12}O_4$ yellow color. Which is reduced by the oxidation of phenols to blue oxides of tungsten W_8O_{23} and molybdenum Mo_8O_3[06].

The Protocol [06]:

- Place 0.1 ml of the extracts in a separate test tubes
- Add 0.5 ml of Folin-ciocalteu reagent to each tube (diluted 10 times with distilled water).
- Leave the mixture for 5 minutes
- Add sodium bicarbonate Na_2CO_3 (20 %) to the tubes, after stirring slightly, leave the tubes in the dark for 30 minutes.
- Measurement of the absorbance of the extracts and the standard at 760 nm

(It is taken as a gallic acid standard with concentrations from to construct an absorbance titration curve).

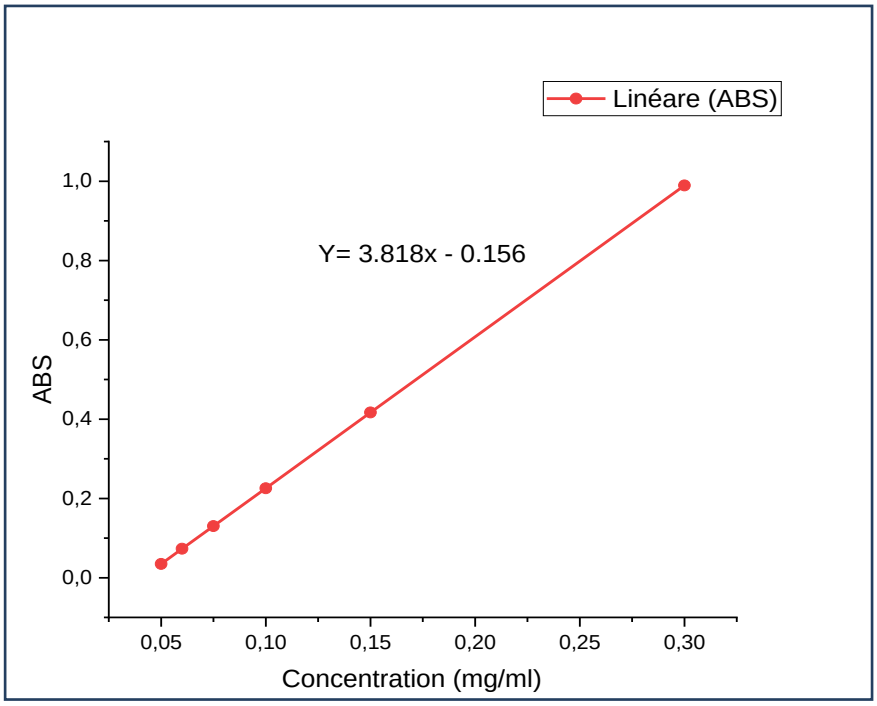


Figure (III-35) Gallic acid titration curve: concentration in terms of absorbance at λ=760 nm.

- ***III.3.2 Antioxidant activity :***

DPPH Test:

This test depends on knowing and measuring the ability of antioxidants to inhibit and sequester the stable **DPPH˙** radical with a violet color and convert it into a non-radical form **DPPH-H** yellow color, antioxidant activity is measured by measuring the decrease in violet color due to **DPPH˙** radical recombination. The effectiveness is measured by spectrophotometry at **λ =517 nm.**

The protocol:

- Mixing 0.1 ml of extracts of different concentrations with 1.9 ml of DPPH methanolic solution 0.1 mmol/l
- Leave the mixture in the dark for 30 minutes and measure the absorbance directly at 517 nm
- Ascorbic acid is used as a positive control

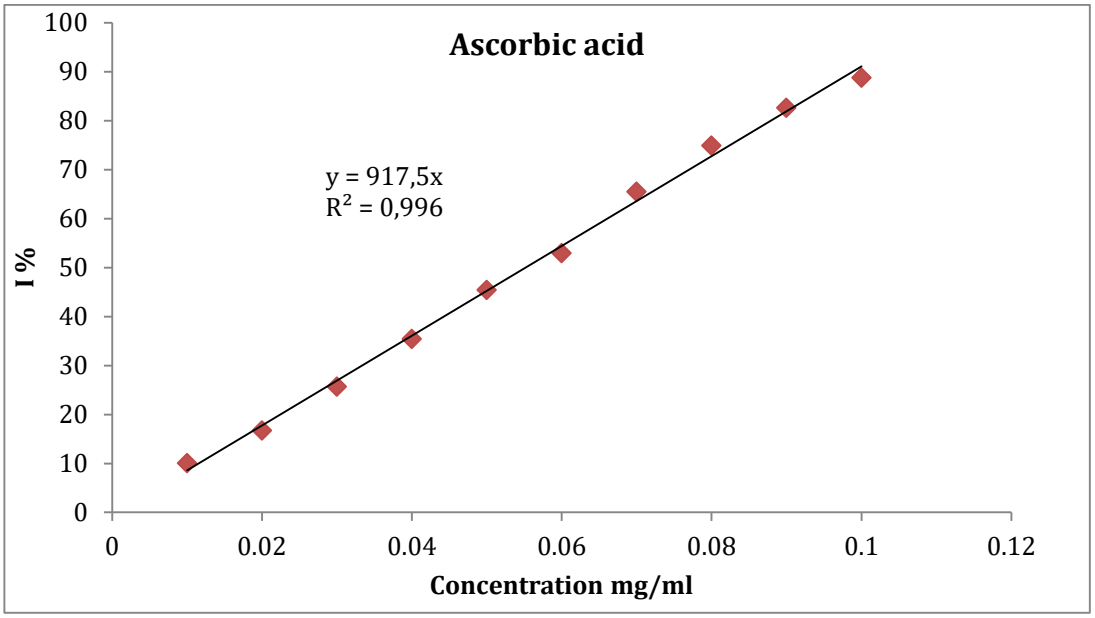


Figure (IV-7) Curve represented the scavenging effect of the radical DPPH˙ by the antioxidant Ascorbic acid

Calculation of **DPPH˙** radical inhibition percentage with the following relationship (*):

$$\mathbf{I\ \%} = \left(\frac{\mathbf{A0 - A1}}{\mathbf{A0}}\right) \times \mathbf{100} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(*)$$

where:

- ✓ A_0: the absorbance of the control at 517 nm
- ✓ A_1: The absorbance of the sample at 517nm
- ✓ I: anti-rooting effectiveness "root inhibition ratio"

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Chapter VI

Results and Discussion

IV. Results and Discussion

IV. 1 yield of extraction:

The previous filtrate is evaporated in the Rota vaporizer, in order to get rid of the solvent (Hexane), then weigh the two extracts (Hexane) and calculate the yield of the two extracts as follows:

$$\mathbf{R\ \%= 100\times\frac{To\ extract\ the\ process\ after\ \ the\ resulting\ mass}{The\ total\ mass\ of\ the\ plant}}$$

The results of hexane for the dandruff and core are recorded in the following table:

Table (IV-1) Primary extraction yield values.

Sample weight	**The dandruff 8.0725 g**		**The pulps 8.0628 g**	
	Wight (g)	**yield(%)**	**Wight(g)**	**yield(%)**
Hexane	0,2050	2,539	1.57889	**21.8148**

We notice from the values obtained from table (IV-04) that the pulps yield value is greater than the dandruff yield value for the hexane extract.

Table (IV-2) yield values of extracts.

	the dandruff 8.0725 g		**The pulps 8.0628 g**	
	Wight(g)	**yield (%)**	**Wight(g)**	**yield(%)**
Hexane	0,2050	2,539	1.57889	21.8148
CH_2Cl_2	0.2305	2.8553	0.1323	1.6408
Ethyl acetate	0.023	0.28491	2.011	24.9417
n-Butanol	0.667	8.2620	0.4821	5.9793

We note that the yield values of the pulps of the fruit are greater than the yield values of the dandruff of the fruit for the hexane and ethyl acetate extracts, while for the dichloromethane and n-butanol extracts, the yield of the dandruff of the fruit is higher than the yield of the pulps.

IV.2 Results of preliminary phytochemical tests:

The results of the preliminary phytochemical tests are summarized in the following table:

Table (IV-3) Schedule of preliminary phytochemical tests.

		The dandruff of the fruit	**The pulps of the shell**
The quality of chemical compounds	**Plant extract**	**Note**	**Note**
Reduction compounds	Methanol	/	/
	Aqueous methanol	/	/
	Distilled water	++++	++
	Acetone	/	/
	Hexane	/	/
	Ethyle acetate	/	/
Alkaloids	Methanol	+++	++
	Methanol aqueous	+++	++
	Distilled water	++	++
	Acetone	+	-
	Hexane	-	+
	Ethyle acetate	-	-
Cardiac glycosides (carbohydrates and glycosides)	Methanol	+++	+++
	Methanol Aqueous	+++	+++
	Distilled water	+++	+++
	Acetone	+	+
	Hexane	-	-
	Ethyle acetate	+	+
Tannins	Méthanol	-	-
	Methanol aqueous	/	/
	Distilled water	+++	++
	Acetone	/	/
	Hexane	/	/
	Ethyle acetate	/	/
Mucilage	Methanol	-	-
	Methanol aqueous	/	/
	Distilled water	+++	++++
	Acetone	/	/
	Hexane	/	/
	Ethyle acetate	/	/

Anthracene derivatives	$CHCl_3$	-	-
Saponins	Methanol	++++	++++
	Methanol aqueous	/	/
	Distilled water	+	+++
	Acetone	/	/
	Hexane	/	/
	Ethyle acetate	/	/
Cyanogenic compounds	Powder	++++	-
Ninhydrin (amino acids)	Methanol	-	++++
	Methanol aqueous	-	+++
	Distilled water	/	/
	Acetone	-	-
	Hexane	-	-
	Ethyle acetate	-	-
Phenolic compounds	Methanol	++++	++++
	Methanol aqueous	+++	+++
	Distilled water	/	/
	Acetone	+++	++
	Hexane	-	-
	Ethyle acetate	-	-
Terpenoids (Salouski test)	Methanol	-	-
	Methanol aqueous	+	-
	Distilled water	/	/
	Acetone	+++	+++
	Hexane	++	+++
	Ethyle acetate	++	+++
Phytosterols (Libermann-Burchards test)	Methanol	++++	+++
	Methanol aqueous	+++	+++
	Distilled water	/	/
	Acetone	+++	-
	Hexane	+++	-
	Ethyle acetate	+++	-

Phlobatannins	Methanol	++++	++++
	Methanol aqueous	++++	++
	Distilled water	/	/
	Acetone	+	-
	Hexane	-	-
	Ethyle acetate	+	-
Spot test (Fixed oils and fets)	Methanol	-	-
	Methanol aqueous	+	-
	Distilled water	/	/
	Acetone	-	+
	Hexane	-	++
	Ethyle acetate	-	+

- **Present : + , Absent : -**
- **Present in abundance:++++**
- **Present: +++**
- **Present in small quantities: +**
- **Almost present: +**
- **absence : -**

The phytochemical study of the dandruff of the fruit and the pulps of the neem plant revealed the presence of: Phenols, saponins, gums, mucilage, phytosterols, alkaloids, terpenoids, fats, oils, floptanins, and tannins. Preliminary phytochemical tests help to find chemical constituents in plant materials that may lead to their quantification and also to identify the source of a pharmacologically active chemical compound.

IV.3 Separation and purification :

IV.3.1 TLC screening:

After performing many systems on the previous extracts, the aim of this study is to detect one of the secondary metabolite products, all the extracts of *Azadirachta indica* A. Juss were processed using analytical thin layer chromatography (TLC) to select the rich extract and good systems of the separation system to give a good separation system suitable and give the best results. We chose extracts of hexane A_H and dichloromethane A_{Cl} for the dandruff to isolate their content.

IV.3.2 The paper Chromatography:

- ***1D :***

The results of one-dimensional 1D chromatography of the two butanol extracts (dandruff and pulps) of *Azadirachta indica* A. Juss in 15% AcOOH and BAW systems are summarized in the following table**:**

Table (IV-4) The results of one-dimensional 1D chromatography.

Vegetable part	**Used system**	**plant extract**	**Radiant colour λ =365 nm**	**Possible Structures**
The dandruff of the fruit	BAW	n-Butanol	Pale yellow - yellow	• Flavones (3-OH,5-OR) • Flavonols (3-OH,5-OH)
			Brilliant blue	• Flavones (5-OR) • Flavonols (3-OR, 5-OR) • Chalkon (4-OH,2-OH)
			yellowish green	• Flavones (without 5-OH) • Flavonols (Without 5-OH) • Isoflavons (without 5-OH)
			Orange	• Isoflavons
	Acetic acid 15%		Yellow	• Flavones (3-OH,5-OR) • Flavonols (3-OH,5-OH)
			Orange	• Isoflavons
			Brilliant blue - blue	• Flavones (without 5-OH) • Flavonols (Without 5-OH) • Isoflavonols (without 5-OH) • Chalkon (4-OH,2-OH)
			Light purple	• Flavones (5-OH) • Flavonols (4'-OH, 3-OR)

The pulps of the fruit	BAW	n-Butanol	Bright green	• Flavones (without 5-OH) • Flavonols (Without 5-OH) • Isoflavons (without 5-OH)
			Bright blue	• Flavones (without 5-OH) • Flavonols (Without 5-OH) • Isoflavonols (without 5-OH) • Chalkons (4-OH,2-OH)
			Light purple	• Flavones (5-OH) • Flavonols (4'-OH, 3-OR) • Chalkons (4-OH) • Flavonols (3-OR ,without 5-OH)
	Acetic acid 15%		Dark purple	• Flavones (5-OH) • Flavonols (4'-OH, 3-OR) • Chalkons (4-OH) • Flavonols (3-OR ,without 5-OH)
			Brilliant blue - blue	• Flavones (without 5-OH) • Flavonols (Without 5-OH) • Isoflavonols (without 5-OH) • Chalkons (4-OH,2-OH)

From our results of first-dimension chromatograms of the two n-butanol extracts of the outer shell and inner pulp in the two systems, and using known reagents, some spots appear that give the possibility of the presence of different flavonoid compounds in both extracts. The chromatograms performed using BAW and AcOOH 15 % are considered as a guide for the distribution of types of flavonoids.

- ***2D:***

Table (IV.5) The results of Two-dimensional 2D chromatography.

Vegetable part	Plant extract	The system	Radiant colour λ =365 nm	Possible chemical compositions
The dandruff of the fruit	Ethyl acetate	BAW	Orange - Brown	• Isoflavons
			Purple	• Flavones (5-OH) • Flavonols (4'-OH, 3-OR) • Chalkons (4-OH) • Flavonols (3-OR ,without 5-OH)
	n-Butanol		Light purple - purple	• Flavones (5-OH) • Flavonols (4'-OH, 3-OR) • Chalkons (4-OH) • Flavonols (3-OR ,without 5-OH)
			Bright green	• Flavones (without 5-OH) • Flavonols (Without 5-OH) • Isoflavons (without 5-OH)
The pulps of the fruit	Ethyl acetate	Acetic acid 15%	Bright green	• Flavones (without 5-OH) • Flavonols (Without 5-OH) • Isoflavons (without 5-OH)
			Light purple	• Flavones (5-OH) • Flavonols (4'-OH, 3-OR) • Chalkons (4-

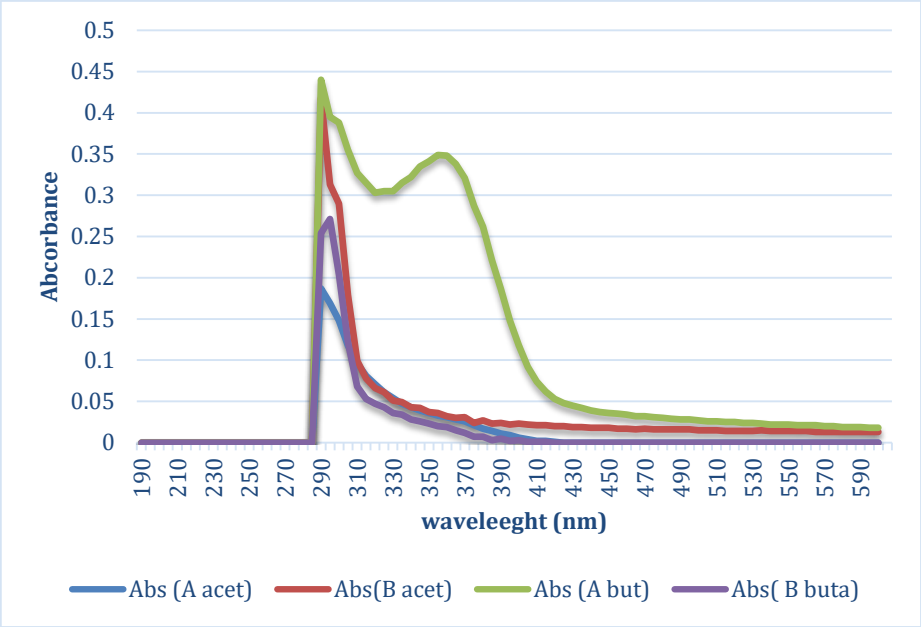
				OH) • Flavonols (3-OR ,without 5-OH)
			Blue	• Flavones (without 5-OH) • Flavonols (Without 5-OH) • Isoflavonols (without 5-OH) • Chalkons (4-OH,2-OH)
	n-Butanol		yellow	• Flavones (3-OH,5-OR) • Flavonols (3-OH,5-OH)
			Bright green	• Flavones (without 5-OH) • Flavonols (Without 5-OH) • Isoflavons (without 5-OH)
			Brown	• Isoflavons

By examining the various second-dimensional chromatograms of the extracts of n-butanol and Ethyl acetate for the pulps and dandruff in the two systems and using UV lamp detection accompanied by known reagents, some spots appear that give colored maps as evidence of the possible presence of various flavonoid compounds in the aforementioned extracts. The chromatograms made using BAW are considered and AcOOH as an index for the presence and distribution of different types of flavonoids.

IV.4 UV- Vis analysis:

IV.4.1 Uv-Vis analysis of extracts:

This absorption spectrum can be changed by adding a specific reagent to the alcoholic solution of the compound, giving distinct colors. This results from the formation of complexes between the studied compounds and the used reagent.



Figure(IV-1) Absorbance curve of extracts of Butanol and Ethyl acetate.

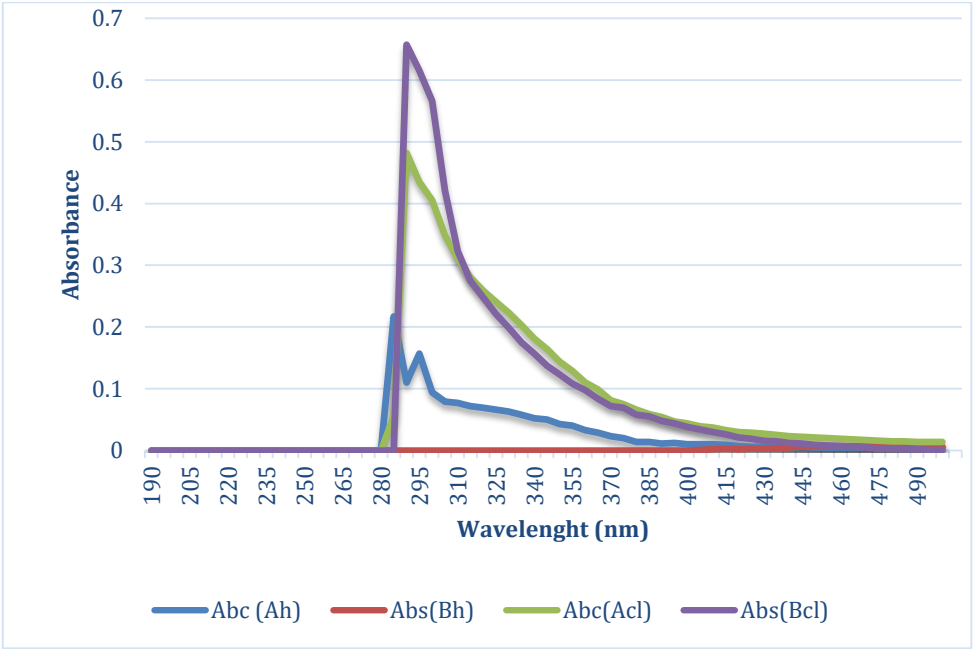


Figure (IV-2) Absorbance curve of extracts of Hexane and CH_2Cl_2.

The results can be summarized in the following table:

Table (IV.6) the Uv-Vis Bands of extracts analysis

Plant extract	Chemical detector	Bond (I)	Bond (II)	ε(mol.cm.l^{-1})
A_{Hexane}	Ethanol	285 nm	295 nm	10.70
B_{Hexane}		480 nm	/	0.023
A_{Cl2H2}		290 nm	/	3.266
B_{Cl2H2}		290 nm	/	1.575
$A_{Ethyl\ acetate}$		290 nm	/	0.108
$B_{Ethyl\ acetate}$		290 nm	/	4.835
$A_{Butanol}$		290 nm	370 nm	0.261
$B_{butanol}$		295 nm	/	0.355

And by using Pere Lambert's law $A=\varepsilon \times l \times C$ in calculating ε different in values, in addition to the bands belonging to the wavelengths that range from 200 to 400 the possibility of the presence of flavonoids or terpenes.

IV.4.2 Uv-Vis analysis of fractions

IV.4.2.1 Uv-Vis Spectroscopy of Fraction 07

We initially pass fraction 07 and detect its wavelength by a Uv-Vis spectrophotometer. The curve at the bottom shows two bands resulting from the fracture.

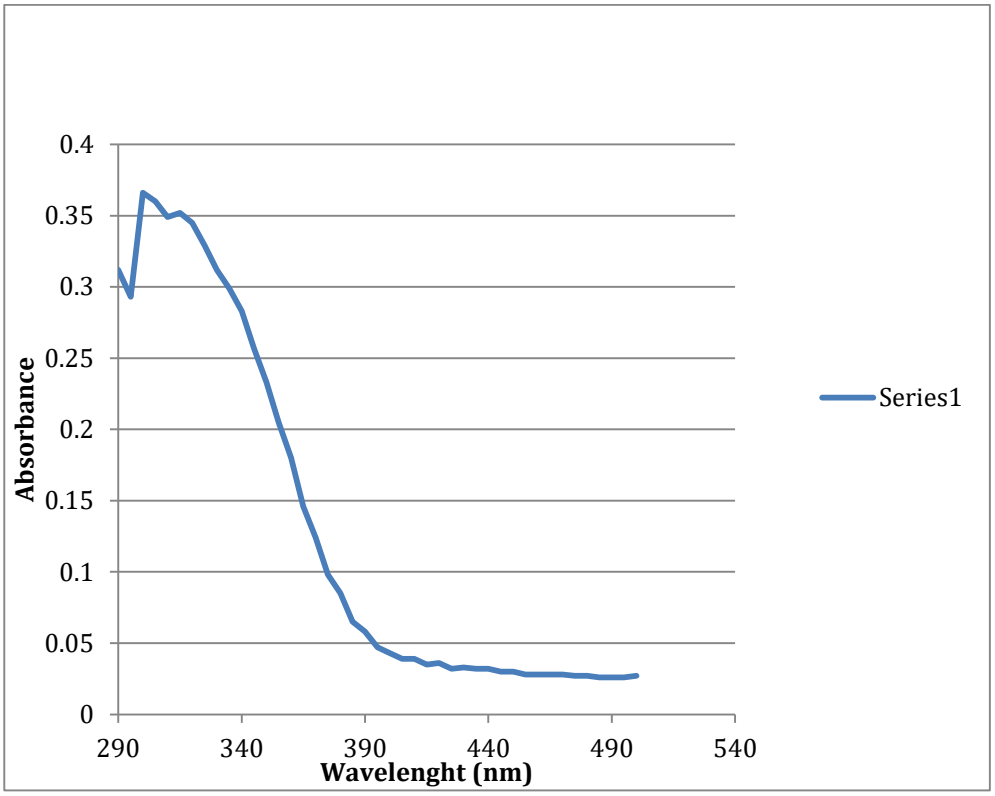


Figure (IV-3) Absorbance curve of fraction 07's uv-vis chromatogram.

The curve represents the absorbance (Absorbance) in terms of wavelength (λ). The curve contains two bands where:

- **Band (I)** with coordinates (λ , Abs): (300,0.366)
  It gave Absorption Abs=0,366 at wavelength λ = 300 nm,

From the absorption spectrum of ultra violet ,not that :

- The transition **n** ⟶ **Π***with values of wavelength from 270 nm to 280 nm represents near ultraviolet.
- While the transition Ϭ ⟶ Ϭ* represents the rang from 165 to 200 nm (the far ultraviolet).
- The transition n ⟶ Ϭ* represents the rang from 183 to 210 nm .

As for the absorption spectrum of fraction 07, it gives a band with a wavelength of 300 nm it belongs to 270 nm to 400 nm .It requires that the compounds containted in fraction 7 in the near UV indicate either the presence of terpenes.

In order to prove the type of compounds and reveal what they are, we establish them by other chromatographic techniques .

To be sure, we use the column chromatography separation of fraction 07 to obtain compounds that are less simplistic and more pure than the first fractionation, in order to know which family of compounds it belongs too.

The Visible and ultraviolet spectroscopy of isolated compounds of fraction 07:

An analysis of aggregate fractions **Hex01**, **Hex02**, **Hex03** and **compound 05** resulting from fraction 07 was performed in a Uv-Vis spectrophotometer in order to find out the wavelength of each compound produced by this fraction.

The four curves at the bottom represent the bands resulting from scrolling on the device, and each curve indicates:

Absorption curve in terms of wavelength (λ).

- **Curve 01: compound 01 (Hex01) of Fraction 07**

The curve contains two adjacent sharp bands:

- Band (I):

with coordinates (λ ,Abs): (310 ,0.82)

That is, Absorption (Abs) was recorded at max wavelength (λ_{max}) = 300 nm.

- Band (II) :

with coordinates (λ ,Abs): (305,0.8)

Absorption (Abs) was recorded at wavelength (λ) = 315 nm.

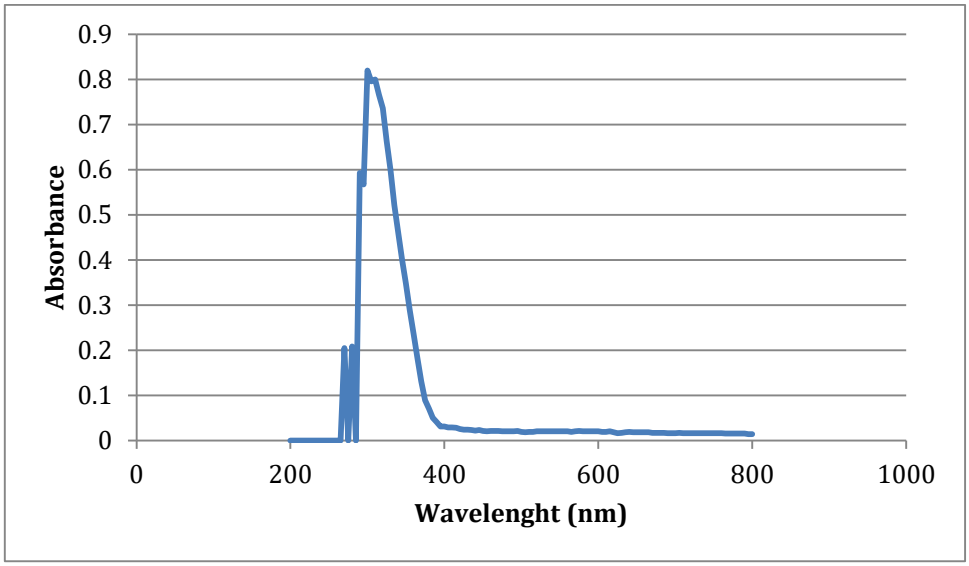


Figure (IV-4) UV chromatogram fractional compound Hex01.

- **Curve 02: compound 02(Hex02) of Fraction 07**

The curve contains one sharp band:

- Band (I):

with coordinates (λ ,Abs): (290 ,0.455)

That is, Absorption (Abs) was recorded at max wavelength (λ_{max}) = 290 nm.

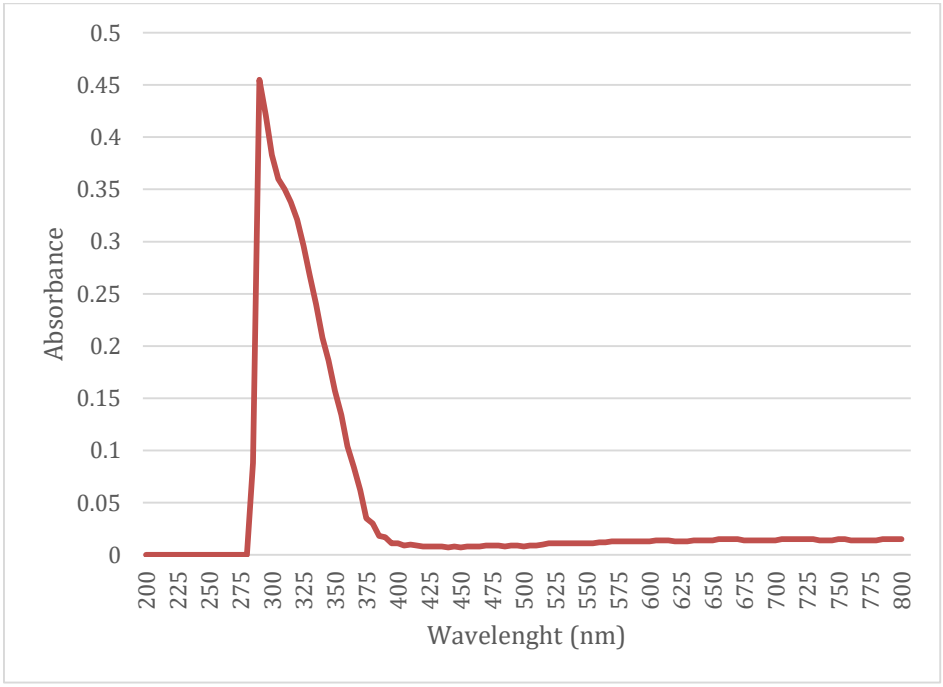


Figure (IV-5) UV chromatogram fractional compound Hex02.

- **Curve 03: compound 03 (Hex03)of Fraction 07**

The curve contains one band:

- Gang (I):

with coordinates (λ ,Abs): (300 ,0.203)

That is, Absorption (Abs) was recorded at max wavelength (λ_{max}) = 300 nm.

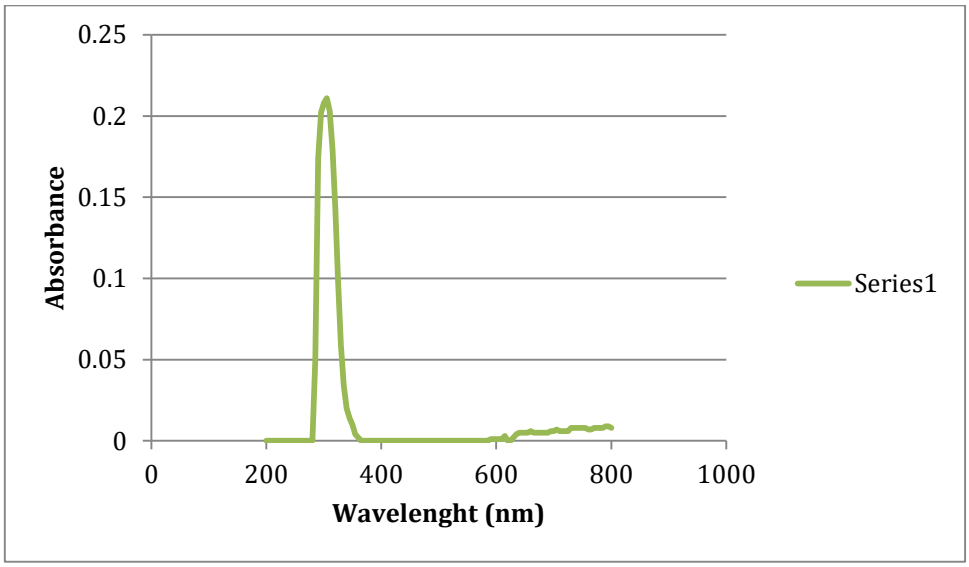


Figure (IV-6) UV chromatogram fractional compound Hex03.

- **Curve 04: compound 05 of Fraction 07**

The curve contains one band:

- Gang (I):

with coordinates (λ ,Abs): (285 ,0.507)

That is, Absorption (Abs) was recorded at max wavelength(λ_{max}) = 285 nm.

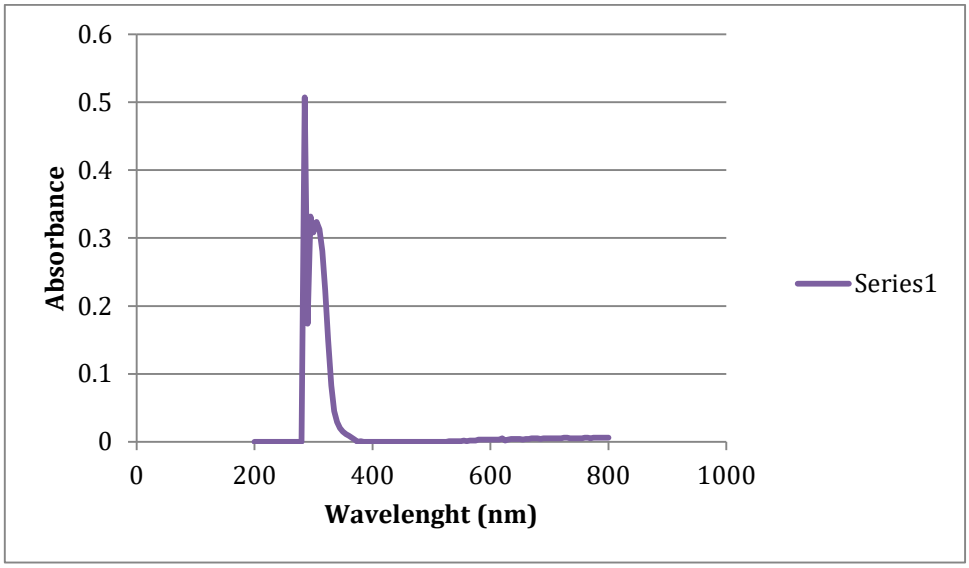


Figure (IV-7) UV chromatogram fractional compound 05.

As for the absorption spectras of **Hex01**, **Hex02**, **Hex03** and **05 compounds**, they also give difference bands with wavelengths belonging to the range 200 - 400 nm in near-ultraviolet spectroscopy, The probability indicates the presence of terpenes.

In order to prove the type and nature of the compounds and reveal what they are, they need more purification and other spectroscopy analyzes to identify their chemical structures.

IV.5 Quantitative analysis and antioxidant activity :

IV.5.1 Total phenolic content determination :

The absorbances of pulps and dandruff :

Table (IV.7) The absorbances of pupls and dandruff **.**

Extract	Vegetabl part	C (mg/ml)
CH_2Cl_2	**the pulps of the fruit**	*0.417*
	the dandruff of the fruit	*0.124*
Ethyl acetate	**The pulps of the fruit**	*0.091*
	the dandruff of the fruit	*2.347*
n-Butanol	**The pulps of the fruit**	*0.818*
	the dandruff of the fruit	*1.232*

Through the previous results, we estimate the total content of phenols in the extracts.
Through the previous table, we say that:

The phenolic content in the fruit dandruff extracts was found to be higher than the phenolic content found in the fruit pulps extracts.

By comparison between the results of **Ethyl acetate** and **n-butanol** extracts, we see that phenolic compounds in the dandruff of the neem fruit are present in greater quantity than in the pulps of the fruit .

IV.5.2 Antioxidant activity

The DPPH:

Studying the variability of the anti-radical activity as a function of the concentration of the extracts makes it possible to determine the concentration that corresponds to the percentage of 50% of the inhibition (IC_{50}). The lower the value of (IC_{50}), the more powerful the extract is in inhibiting activity. The IC_{50} is calculated from a curve of its linear regression, by replacing the value of the inhibitor with 50.

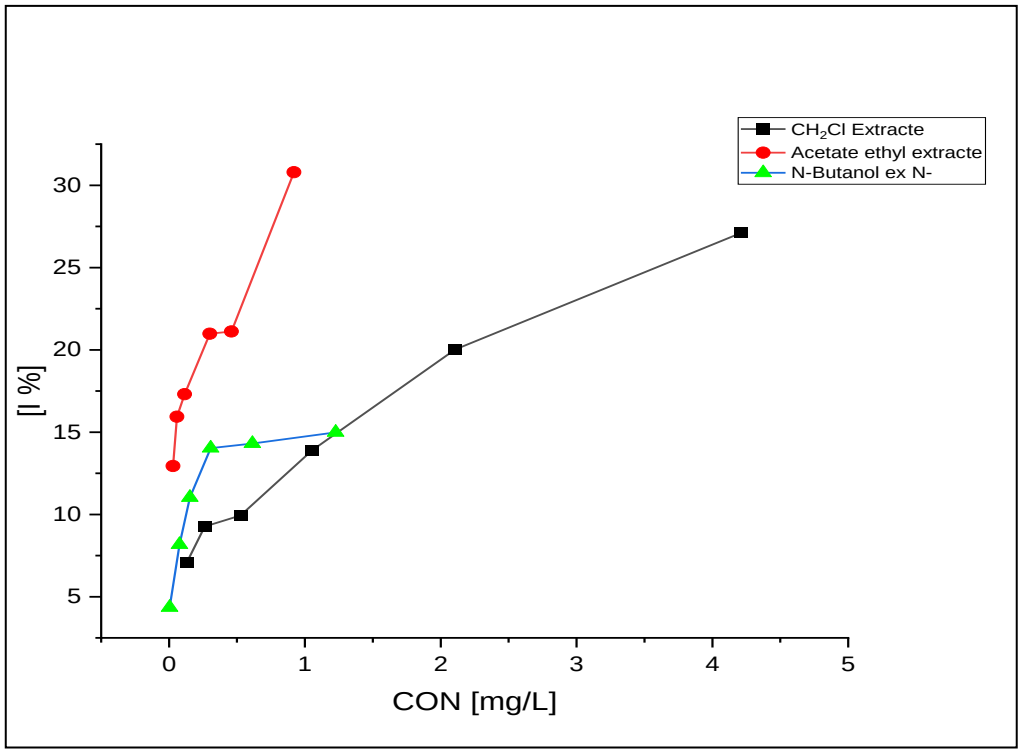


Figure (IV-8) The pulps extracts curve: Inhibition ratios of DPPH radical pulps extracts are represented.

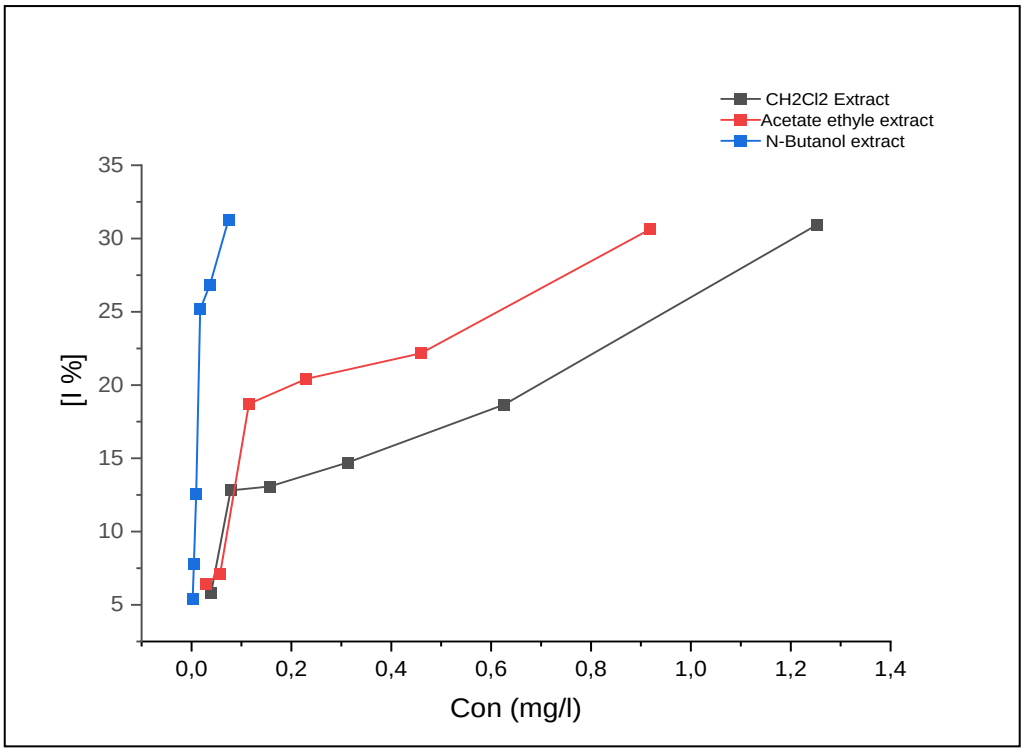


Figure (IV-9) The dandruff extracts curve: Inhibition ratios of DPPH radical dandruff extracts are represented.

The free radical scavenging activity and inhibition of the extracts of the dandruff of the fruit and the pulps of the fruit were carried out along with the reference standard of ascorbic acid, so that the method is based on the reduction of stable free radicals of **DPPH**· The violet color, by donating hydrogen to the radical to turn into a non-radical compound DPPH-H with a yellow color. The inhibition values for the extracts were given in the following tables (IV-8) From the positive control curve or reference Ascorbic acid :

(Table IV-8) Inhibition values of DPPH root extracts of pulps and dandruff of fruit.

	IC_{50}(mg/l) Values	
	The pulps of the fruit	**The dandruff of the fruit**
CH_2Cl_2 extract	10.345	3.024
Ethyl acetate extract	1.947	8.504
n-butanol extract	1.617	0.094
Ascorbic acid	0.09	

In comparison with the curves of **DPPH** root inhibition ratios between all dandruff and pulps extracts of *Azadirachta indica* A. Juss fruit, the ability of the fruit's dandruff extracts to inhibit **DPPH**· rooting was higher than that of the pulps extracts.

More comparisons between extracts and Ascorbic acid :

• In comparison between the IC_{50} of the extracts and the IC_{50} of ascorbic acid, we note that:

the IC_{50} of the extracts is much lower than the IC_{50} of the ascorbic acid, so it can be said that the extracts have effectiveness and activity in inhibiting and curbing the radicals of **DPPH**· , For the two dichloromethane extracts, and compared between the IC_{50} of the two samples, the dandruff sample extract had a stronger role in inhibiting and curbing the root.

As for the two ethyl acetate extracts, there is significant difference between the IC_{50} ratios, the pulps extract has an IC_{50} lower than the dandruff extract, so it is more inhibiting..

However, the n-butanol extract of the dandruff of the fruit had another role and a greater role in inhibiting the root and more effective in inhibiting the root **DPPH**· compared to the butanolic extract of the pulps of the fruit, despite its good ability in inhibition as well.

And from the foregoing: we conclude that the highest ability and effectiveness in inhibition are the extracts of the dandruff of the fruit (dichloromethane and n-butanol extracts) in comparison with the two extracts of the pulps of the tree *Azadirachta indica* A. Juss.

From which we can conclude that since the extract, the dandruff of the fruit is more inhibiting and effective for free radicals, that is, it contains more effective compounds than the pulps of the fruit.

Conclusion

Conclusion :

The main objective of the work we have done is to promote botanicals from north of the Sahara used in traditional medicine. It was selected among the least studied species in Algeria. The plant chosen is *Azadirachta indica* A.Juss. (Neem).

Firstly, the phytochemical examination, as a qualitative analysis, showed the richness of this species in secondary metabolites because it contains many active ingredients and the main component is alkaloids.

We first quantitatively determined the total phenol content in the extracts obtained by simple maceration. The plant has a phenolic content of 1.232 mg/ml. Next, we were interested in evaluating the antioxidant activity by DPPH, as extracts and isolated products of this type showed better antioxidant activities as an example of butanol extract IC_{50} =0.094 mg /l.

Second, the four extracts obtained by maceration were subjected to chromatography by TLC (we used several systems), silica gel columns (we used an equal system), on paper (1D and 2D) and through a preparative plate. We obtained 3 pure compounds from the hexane extract (Hex01, Hex02, and Hex03). Analysis was performed by UV-VIS on the extracts and isolated products, the absorbance of compounds and extracts in the region of 300–400 nm.

Finally, further complementary work is then necessary to isolate the majority compounds from the other extracts as well as to identify the isolated compounds with the usual spectroscopic techniques (IR, 1D and 2D NMR, mass spectrometry)