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Contribution to the phytochemical study and evaluation of the biological activity of medicinal plants of the Brassicaceae

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بسم الله الرحمن الرحيم

الاهداء

الحمد لله وكفى والصلاة على الحبيب المصطفى واهله ومن وفى اما بعد:

الحمد لله الذي وفقني لتثمين هذه الخطوة في مسيرتي الدراسية وبلوغي المرحلة الاخيرة

اهدي ثمرة جهدي ونجاحي الى نفسي اولا المكافحة والمثابرة' والى من علمني حرفا والى من احمل اسمه بكل افتخار والى بسمة الحياة ومن كانت سرا في وجودي ومؤنستي في الساعات الاخيرة من الليالي ارجو الله ان يمد في عمرهما ليروا ثمارا قد حان قطافها الى اخوتي واخواني وروح فقيدي اخي رحمة الله عليه"فضيل" الى معلميني واساتذتي من الروضة الى الجامعة والى رفقاء الدرب صديقاتي كل باسمها والاقارب ومن دعا لنا بالاعانة وكل من يملكون محبة في القلب وكانوا سندا لي في الاوقات المستعصية.

نورالهدى

Summary:

The aim of this study is to estimate the antioxidant and antibacterial activity of plant extracts *Brassica. Oleracea. Var.L. capitata* From the Crusader, which was acquired from the local market of the state of Touggourt, which is located in the south-east of Algeria december2023—janury2024, the extraction process was carried out using the solvents acetone, ethanol and ethyl acetate.

Chemical detection tests for the most important active compounds showed the presence of phenols, flavonoids, Saponins, and terpenes Coumarins tannins, where the highest extraction yield was recorded in acetone extract at 27.2%, followed by ethyl acetate at 9.2% and ethanol at 3.6%, while the results of quantitative estimation of phenol showed the presence of a small amount in the extracts.

The antioxidant activity was also estimated using the root radical test method DPPH, as all extracts showed varying percentages. The highest percentage was in the ethanol extract, 65.83%, followed by the acetone extract, 41.71%, then the ethyl acetate extract, 21.12%.

It also showed results of antibacterial activity on two bacterial strainsEscherichia coliAndAeruginosaPseudomonasPositive result in ethanol extract and negative result in acetone and ethyl acetate extracts.

Key words: the Crusader family, Chemical detection, the antioxidant activity

الملخـص:

ان الهدف من هذه الدراسة هو تقدير الفعالية المضادة للأكسدة، والمضاد للبكتيريا لمستخلصات النبتة *Brassica. Oleracea.L. Var.capitata*من العائلة الصليبية والتي تم اقتنائها من السوق المحلي لولاية توقرت التي تقع في الجنوب الشرقي للجزائر تمت عملية الاستخلاص بالمذيبات الاسيتون والايثانول واسيتات الايثيل.

اختبارات الكشف الكيميائي لاهم المركبات الفعالة، أظهرت وجود الفينولات الفلافونويدات الصابونينات تربينات تانينات الكومارينات، حيث سجل مردود الاستخلاص اعلى نسبة في مستخلص الاسيتون 27.2%يليه اسيتات الايثيل بنسبة 9.2% والايثانول بنسبة 3.6%، اما نتائج التقدير الكمي للفينول أظهرت وجود كمية قليلة في المستخلصات.

كما قدرت الفعالية المضادة للأكسدة بطريقة اختبار جذر ال حر DPPHحيث ابدت جميع المستخلصات نسب متفاوتة فكانت اعلى نسبة في مستخلص الايثانول 65.83% يليه مستخلص الاسيتون 41.71% يليه مستخلص اسيتات الايثيل 21.12%.كما أظهرت نتائج الفعالية المضادة للبكتيريا على سلالتين بكتيريتينEscherichia coliوAeruginosa Pseudomonas نتيجةإيجابية في مستخلص الايثانول ونتيجة سلبية في مستخلصات الاسيتون واسيتات الايثيل

الكلمات المفتاحية: العائلة الصليبية، الكشف الكيميائي، النشاط المضاد للأكسدة,

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Introduction

Despite all the progress in synthetic chemistry and biotechnology, plants remain an indispensable source of preventive and therapeutic medicinal preparations. Hundreds of species are known to have medicinal value, many of which are commonly used for treatment and prevention of specific diseases. Medicinal properties may be present in one or all of its parts (root, stem, bark, leaf, flower, fruit, or seed). The majority of developing countries still rely on herbal remedies.[1]

It is known that the medicines currently used in the world to treat many diseases are mostly of plant origin, and the percentage of this reaches 60%. In addition, other natural sources constitute a significant percentage of use in treatment. Through his experiments, man has begun to extract these active elements, purify them, determine their atomic weight and chemical composition, and manufacture similar ones .[2]Traditional medicine, especially treatment with plants and herbs, is considered the basic system of medicine for more than two-thirds of the world's population, especially in developing countries .[3]

With the development of research and studies on the plant, its use prevailed in most fields (economic, medical, food, pharmaceutical, as well as industrial and agricultural) .[4]The interest in Nature as a source of potential chemotherapeutic agents continues, more than 50% of therapeutic medications have become natural compounds or their derivatives [5]

One of the families of plants often recommended in pharmacies is the Brassicaceae (or Cruciferae) family. This family is one of the largest families in the plant world. Brassicaceae, the mustard family of flowering plants (order Brassicales), composed of 338 genera and some 3,700 species. The family includes many plants of economic importance that have been extensively altered and domesticated by humans, especially those of the genus Brassica, which includes cabbage, broccoli, Brussels sprouts, kale, kohlrabi, napa cabbage, turnip, and rutabaga. Other important agricultural crops in the family include horseradish, radish, and white mustard. A number of species—such as basket-of-gold, candytuft, and honesty—are grown as ornamentals, and some members of the family are considered invasive species in regions outside their native range. [6]

Our study is limited specifically to the cabbage plant, or its common name, cabbage, from the same cruciferous family

We summarized the work into two parts:

The theoretical part includes:

Chapter One: A theoretical study

Chapter Two:Antioxidants andAntibacterial

The practical part:

Chapter three: Tools, materials used, and study methods

Chapter four: Results and discussion

We concluded the study with a summary that in cludes everything that was concluded from the work.

The theoretical part

Chapter One

The theoretical study of plants

I. Medicinal plants:

The scientist Dragendra knew that everything that is of plant origin can be used to treat a specific disease is a medicinal plant,and a plant is called a medicinal plant if at least one of its organs possesses therapeutic properties. In other zords,a medicinal plant is one that contains in one or more of its organs one chemical substance or more in low or high concentrations and have the physiological ability to treat a specific disease or at least reduse the symptoms of this disease.[7]

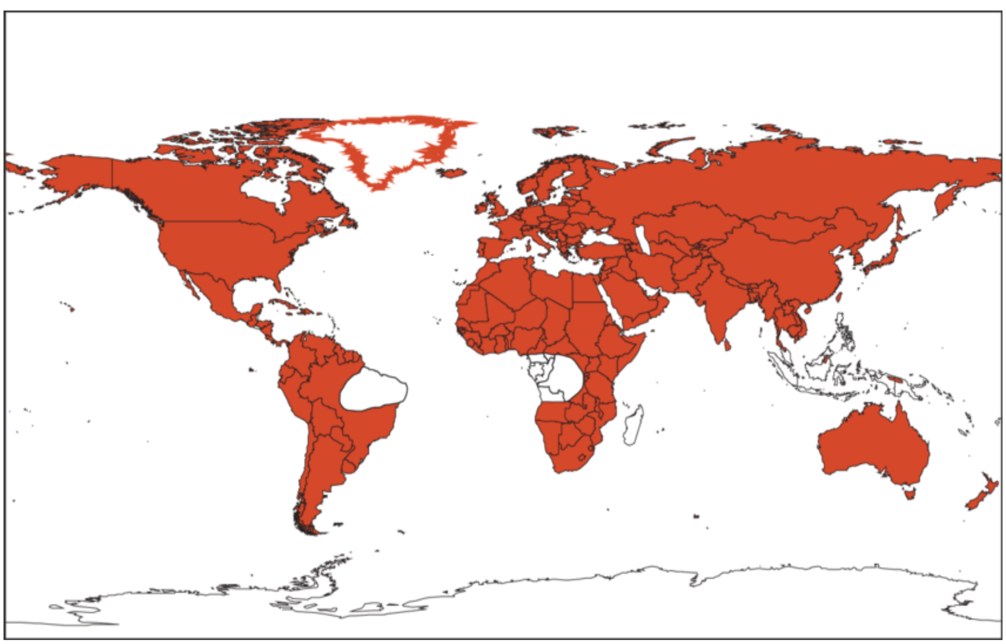
II. Family Brassicaceae:

1. Definition of family Brassicaceae:

Brassicaceae is a monophyletic family and is distributed throughout the world.[6] This family is considered one of the largest families in the plant world. Brassicaceae, the mustard family of flowering plants, consists of 338 genera and about 3,700 species. The family includes many economically important plants, which include cabbage, cauliflower, Brussels sprouts, turnips, Napa cabbage, and turnips.[6] Our study area is red cabbqge. Brassicaceae are the largest family of the Brassicales, an order characterized by the presence of glucosinolates, which contain sulfur. When these compounds react with myrosinase.[8]

2. Geographical description of the Brassicaceae:

Brassicaceae are cosmopolitan but are most abundant in temperate regions of the Northern Hemisphere and very rare in tropical regions except in mountainous areas. Many species live in arctic and alpine regions, some in deserts and brackish habitats, and some submerged. However, most species grow in open habitats, and only a few are restricted to forests (such as Caramine species).[9]



Presence of cabbage

Figure 01: Distribution of the Brassicaceae family on the world [10]

III. cabbage plant:

1. Definition of Cabbage plant:

Cabbage is an ancient and important vegetable. It is a member of the mustard family, which includes mustard, radish and many Oriental vegetables. It has been cultivated since at least 2500 B.C., with several varieties known to the Greeks and Romans .[11]

The scientific name:*Brassica Oleracea L. var capitata*[12]

Figure 02: Shows a picture of purple cabbage[13]

2, Geographical description of the Red Cabbage plant:

It developed at the beginning of the Middle Ages in northwestern Europe. It also grows in the Mediterranean regions to become an important vegetable and was introduced to all parts of the world, including the subtropical and tropical regions.[14]

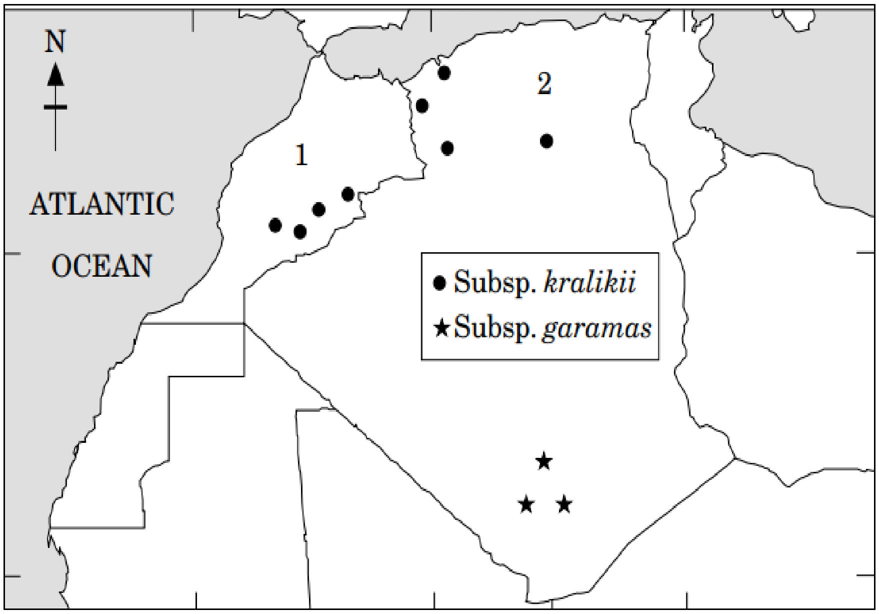


Figure 03: Its geographical northwest Africa: 1. Morocco 2. Algeria [15]

3, Botanical description of cabbage:

An erect and perennial herbaceous plant, its height ranges from 30 to 60 cm, while its flower stalk reaches a height of one to two metres. Most of its leaves are low and tufted, numbering from 7 to 15, and they form an erect rosette on a deep running pen. These leaves are oval in shape and have a wavy outline.[14]

4, Classification:

Table 01: Cabbage classification [16]

Kingdom	Plantae
Phylum	Magnoliophyta
Family	Brassicaceae
Genus	Brassica
Class	Magnoliopsida
Order	Capparales
Spieces	Legumes
The scientific name	*Brassica Oleracea L. var capitata*

5. Benefits and uses of cabbage:

Firstly: The therapeutic aspect

Cabbage has widespread use in traditional medicine, in alleviation of symptoms associated with gastrointestinal disorders (gastritis, peptic and duodenal ulcers, irritable bowel syndrome) as well as in treatment of minor cuts and wounds and mastitis. as well as alternative therapies for cancer patients , healing peptic ulcers , and facilitating the reduction of serum LDL levels .[17]

Secondly: The aesthetic aspect

- ✓ Red cabbage helps delay the appearance of signs of skin aging because it fights harmul radicals in the body and is rich in vitamin C and A.
- ✓ Red cabbage helps treat burned skin and helps heal wounds in a short time.
- ✓ Red cabbage helps eliminate blackheads and acne.[17]

Chapter Two

Antioxidants and antibacterial

I. Antioxidants

1. Definition of Oxidative stress:

Oxidation is a chemical reaction during which electrons are transferred from a substance to an oxidizing agent. Oxidation reactions lead to the production of free radicals that initiate a chain of reactions that ultimately lead to cell damage. alsoIt is the lack of balance between free radicals and their scavengers, meaning there is an increase in the production of free radicals and a decrease in their scavengers, such as antioxidants .[18]

2. Definition of antioxidants:

An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation reactions can produce free radicals, which start chain reactions that damage cells.[9]Antioxidants are used in many ways It is widely used as a nutritional supplement and to help protect health and prevent diseases such as Cancer and heart disease . [19]

All antioxidants work together Individually against free radicals.[20]

It is important for the presence of antioxidants within the organism to reduce .The danger of free radicals due to eating and consuming a wide range of foods rich in antioxidants Oxidation.[19]

Purple cabbage also contains anthocyanin, which is responsible for the pigment and is a powerful antioxidant that is 50 times stronger than vitamin E.[21]

3. Types of antioxidants:

There are two types of antioxidants, some of which are manufactured by the body Humans such as enzymes, proteins, and some of them obtained from the foodstuffs that we eat, such as vitamins, Carotenoids and flavonoids .[22]

a) Natural antioxidants:

They are natural compounds [18].It is obtained from the food we eat[22]Like Vitamin C(Ascorbic acid) and vitamin E(Tocopherols) and vitamin A (Carotenoids) and many polyphenolic compounds such as. (Coenzyme Q10) and (Lycopene) and (Anthocyanins) and (Flavonoids)[18]

b) Synthetic antioxidants:

They are synthetic chemical compounds that are not found in natural food sources.[18] Recently, interest in synthetic antioxidants has increased because these nutrients are considered therapeutic and preventive and are used in the food industry,medicines And cosmetics To prevent oxidation Fats..There are four synthetic antioxidants widely used in foods: TBHQ, PG, and BHT and BHA.[22]

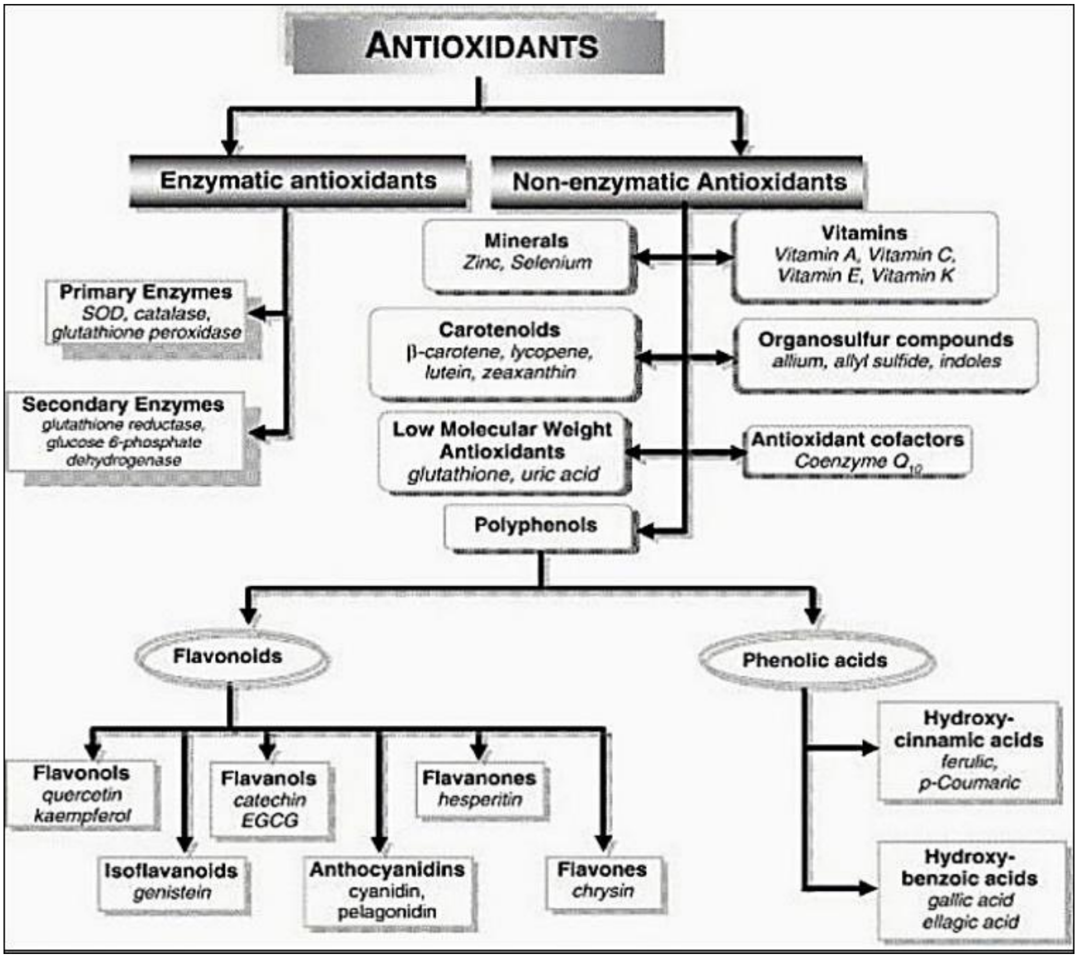


Figure 04: Classification of antioxidants [18]

4. Mechanism of action of antioxidants:

Antioxidants work by binding to free radicals and preventing their harmful effects on the body. They are also considered a defense system against stress resulting from stray oxygen atoms to protect the body's cells, or prevent the formation of free radicals in addition to repairing damage. Resulting from it.[19] Primary antioxidants also work to hinder and disable diffusion reactions, which leads to slowing down the oxidation process and returns to accelerating when it is exhausted.[20]

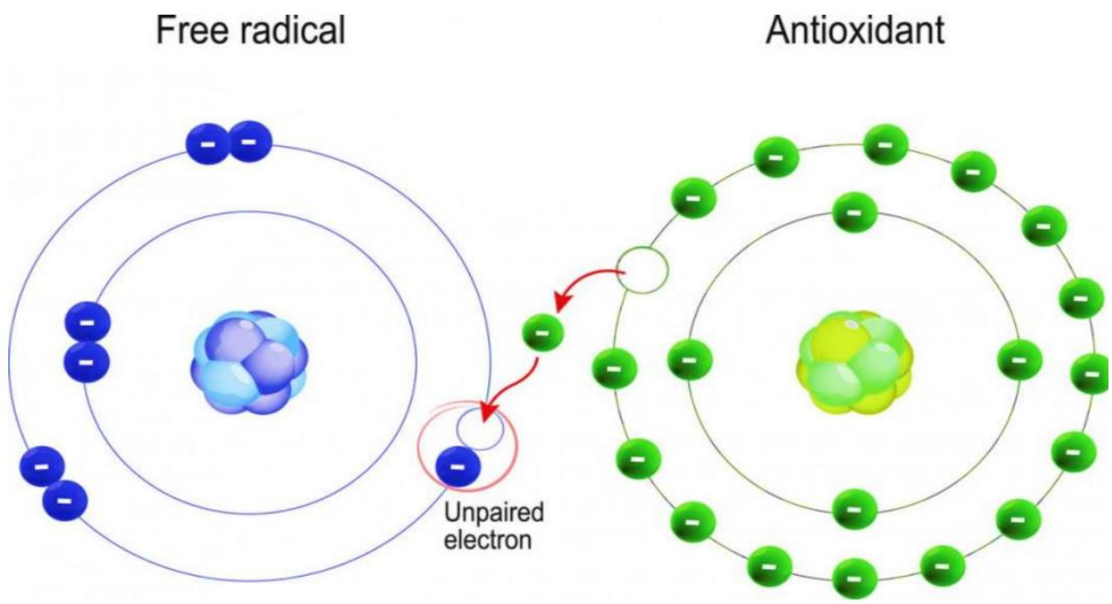


Figure 05: shows the mechanism of action of antioxidants.[24]

5. Methods for studying antioxidant activity:

Several methods are used to measure the activity of biological antioxidant compounds, and they are widely used due to their ease, speed, and sensitivity. The most widely used ones are the ABTS and DPPH methods, in addition For other tests such as FRAP test, ORAC test, etc.[23]which are known as follows:

5.1. ABTS test or TEAC (Trolox Equivalent Antioxidant Capacity):

This test is based on inhibition by antibodies Oxidation to absorbance radical positive (ABTS) Which is characterized by an absorption spectrum in long waves that appears at 660, 734, and 820 nanometers.
This test is widely used in many recent studies related to revealing the antioxidant properties of plants.[23]

5.2. DPPH test (diphenyl-2- picrylhydrazyle):

This test is considered one of the most common methods used to test activity Antioxidant for plant samples. This method is based on root displacement 1,1-dyphenyl-2-picrylhydrazyl (DPPH)By antioxidants, which leads to a decrease in absorbance at the 515 nm wavelength. When a DPPH solution is mixed with a substance capable of giving off a hydrogen atom, the degraded form of this radical is accompanied by a contrast of color [23]

5.3. FRAP test (Ferric Reducing Antioxidant Power):

The FRAP test is a technique that allows the determination of the total antioxidant capacity of compounds, which translates...with the ability to return. Test the antioxidant activity of various types of plants.[23]

5.4. ORAC test (Oxygen Radical Absorbance Capacity) :

The ORAC test uses beta-2,2'-azobis (2-amidoinopropane) dihydrochloride and an oxidized protein such as phycoerythrin (PE) 2O2H - as a hydroxyl radical generator. Use this test at +2 (AAPH) as a peroxyl radical generator, or Cu recent studies related to plants [23]

5.5. TRAP test (Total Radical Trapping Parameter):

The TRAP test is the most widely used method to measure the antioxidant properties of plasma or blood serum over the past decade, to estimate the antioxidant properties of tea, various fruits and juices. This test uses peroxyl radicals produced by AAPH. Substances found in plasma or other biological fluids. After AAPH is added to plasma, its oxidation is monitored. Substances that can be oxidized by measuring the amount of oxygen consumed during the reaction, but the oxidation process is inhibited by some antioxidants during the incubation period.[23]

Our study waz carried out using the DPPH method.

6. The importance of antioxidants for human health:

6.1. The importance of antioxidants for the skin

- ✓ Protects the skin from damage resulting from exposure to radiation the sun
- ✓ It protects skin cells from the inside, unlike sunscreen creams
- ✓ Helps fight wrinkles, such as vitamin C and E
- ✓ It works to renew skin cells and maintain its vitality
- ✓ Reducing the size of skin pores
- ✓ The importance of antioxidants for the body[21]

6.2. The importance of antioxidants for the body

- ✓ It prevents mutations in cells, and protects against carcinogens, clots and infections. It also works as an antidepressant
- ✓ It stimulates blood circulation, increases the supply of oxygen to the brain and all parts of the body.
- ✓ Protects the immune system by preventing the formation of free radicals that can Cause damage and destructive effects on the body.
- ✓ Protect the nervous system.
- ✓ Maintaining skin and blood vessel health and preventing cancer.[21]

7. Definition of free radicals:

Free radicals are molecules containing one or more unpaired electrons in their outmost atomic or molecular orbital have emerged as double edged swords.[25]

The more free radicals there are, the greater their ability to penetrate the cell membrane and their penetration inside. Here, the damage caused by these radicals is greater and the ability of antioxidants is weakened [24]

Figure 06: shows a microscopic image of free radicals [21]

8. Factors causing free radical damage:

- ✓ Increasing the consumption of food additives, such as artificial colorings, preservatives, and flavorings, contributes to increasing the production of free radicals.
- ✓ The food sterilization process, which is carried out at temperatures higher than those used in pasteurization, leads to significant changes in the nutritional value of the product.
- ✓ The nutritional value of the product, as essential vitamins are lost in canned fruits and vegetables, especially ascorbic acid
- ✓ The antioxidant activity of proteins is affected during the boiling process due to their changing nature, and the release of antioxidants is observed Partially during boiling, the boiling water is lost, and vitamin C is also lost
- ✓ Increasing oxygen consumption during vigorous exercise will increase production . Free radicals, so it is recommended to eat foods high in antioxidants, such as vegetables and fruits
- ✓ The use of pesticides and chemical fertilizers has a direct effect on the production of free radicals .Therefore, it is recommended to consume organic foods.
- ✓ Free radicals are generated within the body as a result of burning stored fat in the body .[26]

II. Generalities about bacteria:

1. Definition of bacteria:

Bacteria, also known as Bacterium, are prokaryotic organisms that are widespread in nature Found in air, soil, fresh and salt water. Bacteria are also found in the large intestines of humans and animals The external surfaces of the body, and the reason for their widespread occurrence in nature is due to their high ability to adapt to Different environmental conditions.[27] It is one of the living things that cannot be seen with the naked eye due to its precise size, which ranges between 0.2-2. A micron and its length is (2-3) microns, and a micron equals 1000 nanometers.[28]

2. General characteristics of bacteria:

- ✓ Prokaryotic microorganisms.
- ✓ It is characterized by simplicity of installation.
- ✓ Bacterial cells vary in size and shape.
- ✓ It feeds on organic and inorganic materials.
- ✓ They reproduce by simple binary fission.[27]

It shows the difference in the composition of the cell wal through staining. It is called the Gram technique, named after the Gram factor 1884

- Gram positive: When stained, it absorbs color and appears purple.
- Gram negative: releases a dye and appears red[22]

3.Bacterial cell shapes:

First:spherical bacteria(cocci)

It is spherical cocci singular coccus they appear under the microscope in different concentrations depending on the levels .Its division may be pairwise (binary), quadruple, or in the form of a chain or cluster,Example (Diplococci, Streptococci, Staphylococci,…).[27]

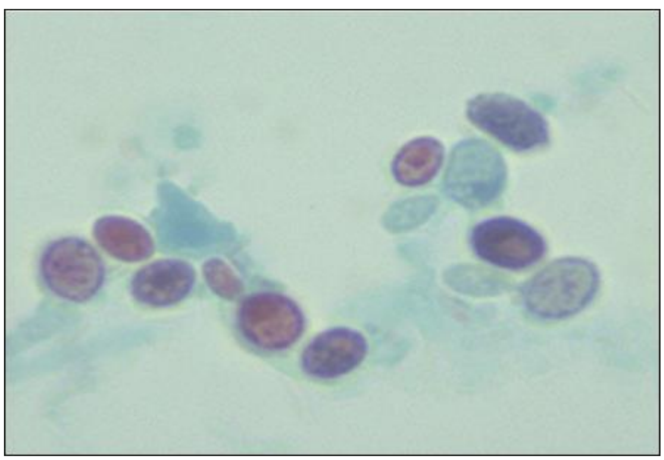


Figure 07:shows a picture of spherical bacteria [28]

Second: Bacillus bacteria

It's called Bacilli singular Bacillus they are rod-shaped or cylindrical bacteria, short or long, and are its edges are round or pointed, Example(Bacillus subtilis, Clostridium tetani, Shigella dysenteria,…).[28]

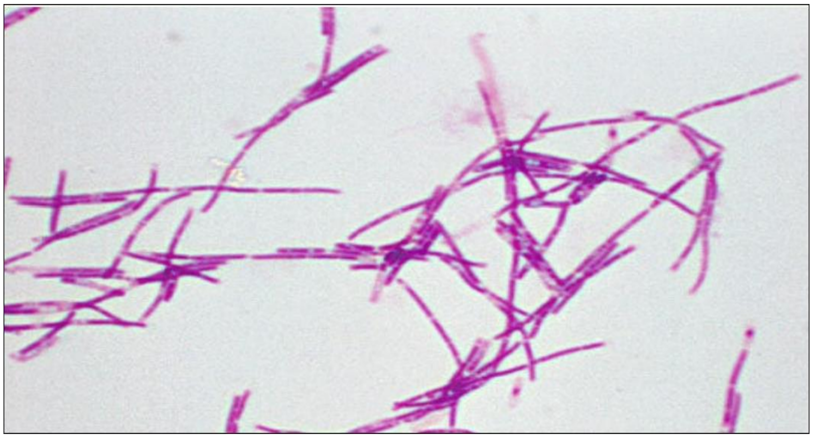


Figure 08: shows a picture of bacillus bacteria [28]

Third: filamentous bacteria (actinomycetes)

It is a group of bacteria known as actinomycetes, which are prokaryotic and their cells they are larger in size than the cells of Bacillus bacteria and have the ability to branch and form mycelium filamentous, fungi-like, not divided by transverse walls. Example of the genus Actinomycetes.[28]

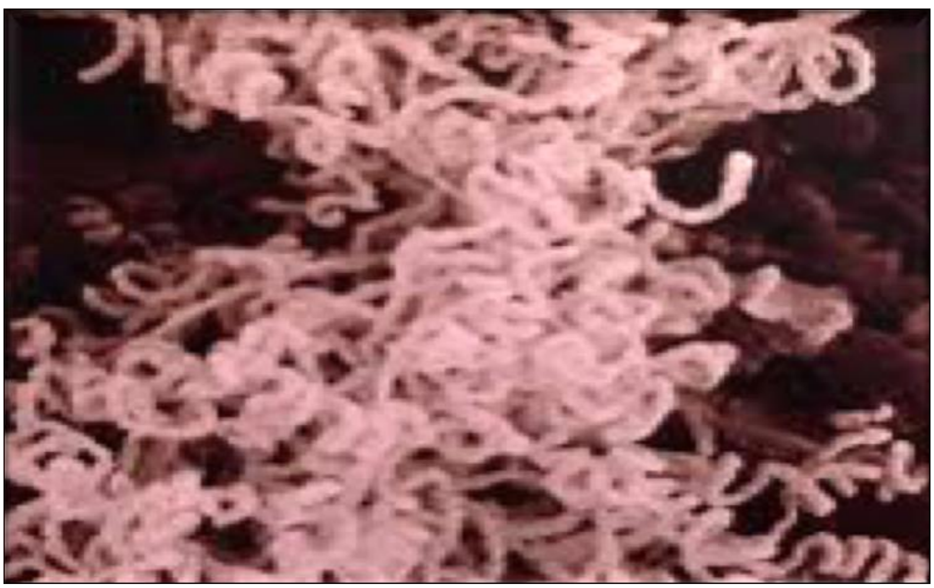


Figure 09: shows a picture of filamentous bacteria [28]

Fourth: Bacteria with different shapes

1- Rhizobium genus: These are root nodule bacteria and are in the form of the letters Z-V-X-Y.

2- Genus Corynebacterium: It takes the form of rods and sometimes has rod-like swellings.

3-Mycobacteria: rod-shaped cells, sometimes slightly curved and tend to pseudo branch, forming a threadlike shape. it quickly breaks down into sticky units of different lengths, due to the flexibility of the wall due to the lack of acid meuramic in its composition

4- Myxobacteria, individuals of which are distinguished by their ability to aggregate and form gelatinous masses. A group movement that moves .[28]

Our study waz carried out using two types of bacteria : E.coli and Pseudomonas aeruginosa

Previous studies of cabbage plants in previous years:

Cabbage (*Brassica Oleracea L. var. capitata*) is one of the most important vegetables grown worldwide. It belongs to the family Cruciferae, which includes broccoli, cauliflower, and kale. The different cultivated types of cabbage show great variation in respect of size, shape and color of leaves as well as the texture of the head

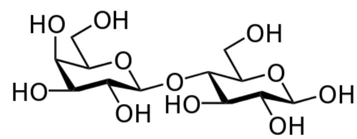
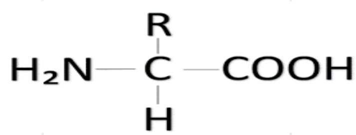
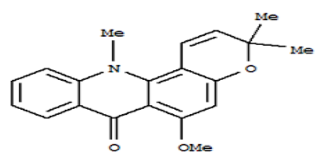
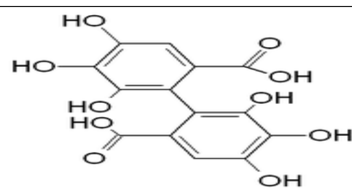
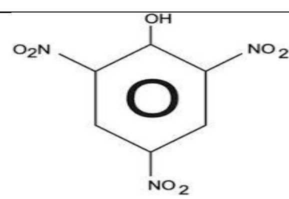
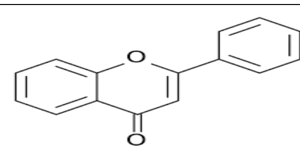
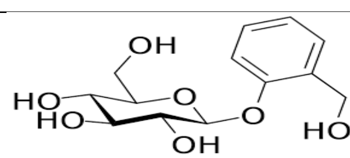
A table 02: showing the results of tests on leaf extracts*Brassica Oleracea L. var capitata*

Article	Test	Results
Article in International Journal of Pharmacy and Pharmaceutical Sciences · April 2012[12]	1.DPPH 2.Phenolic Estimation 3.IC50 value(µg/ml)	1.shows the results of the free radical (DPPH) scavenging activity in % inhibition. The result revealed that the ethanol fraction exhibited the highest radical scavenging activity highest scavenging activity. 2.The highest concentration was for total phenol (556 mg/g) is present in the ethanolic extract. 3.The IC50 value appeared lower, but the ethanolic extract was the lowest. Ethanol extract It showed significant activity with a low IC50 value.high antioxidant activity in extracts with a high percentage of polyphenols in the extract.
Article in Asian Pacific Journal of Cancer Prevention · November 2013[29]	1.Determination of phenol content. 2.Determination of flavonoid content. 3.Determination of antioxidant activity	1.Total Phenol mM (TEAC)= 32.7±0.66a 2.Total Flavonid mg Qe/g =74.2±6.77a 3.Total Antioxidantµg AAE/mg= 10.6±0.61a

Int. J. Adv. Res. Biol. Sci. (2017)[30]	1.DPPH radical scavenging activity 2.Determination of Total Phenol Content 3.Determination of Total Flavonoids Content	1.IC50Values = 26 µ g/ml 2.Total Phenols (mg GAE/g) =35.64±0.56 3.Total Flavonoids (mg QE/g) =13.98±0.23

Isolated compounds and their form in Article in International Journal of Pharmacy and Pharmaceutical Sciences · April 2012 :

A table 03: Showing the most important isolated compounds

Compound Name	Its Shape
Carbohydrates	 A
Amino acids	 B
Alkaloids	 C
Tannins	 D
Phenols	 E
Flavonoids	 F
Glycosides	 G

The practical part

Chapter three: Tools, materials used, and study methods

I Plant sample:

The plant sample was obtained cabbage violet (Brassica Oleracea. Var. capitata) from the local market in the state of TougourtLate December2023 and early January2024

It was identified at the Scientific Research Center for Dry Areas in the state of Touggourt CRISTRA by Professor Youssef Hallis

The drying process was carried out for the leaves(To reduce the percentage of water in the plant and reduce...InteractionsVitality) In the shade and in a well-ventilated place... Then grind it and store the powder in glass containers until used.

1- Preparing study samples:

These tests were carried out in the analytical chemistry laboratory at the Faculty of Mathematics and Material Sciences, Kasdi-Merbah-Ouargla University.

A. Used materials, tools and devices:

1- When extracting:

Devices	Solutions	Materials
-Electronic scale -Heating device -Rotary evaporator	- Distilled water - Ethanol - Acetone - Ethyl acetate	- Plant powder

Table 04: Showing the most important isolated compounds

2- study antioxidant activity:

Table 05: Materials, tools and devices used in studying antioxidant activity

Devices	Solutions	Materials
- Balance analysis - Spectrophotometer	- Methanol - Ascorbic Acid DPPH - (2,2-diphenyl-1 picrylhydrazy)	- Plant extracts

3- Study for quantitative determination of phenol:

Table 06: Materials, tools and devices used in studying the quantitative determination of phenol

Devices	Solutions	Materials
- Balance analysis - Spectrophotometer	- Distilled water – Methanol Methanol Folin ciocalteau - (10%) - Carbonate of sodium Na Co3 (2-7%) - Acid gallique	- Plant extracts

4- Study of antibacterial effectiveness:

Table 07: Materials, tools and devices used in studying antibacterial activity

Devices	Solutions	Materials
- Bunsen burner - Etuve Autoclave Incubator -	- Methanol – Activation medium -Planting medium Muller Hinton - Sterile physiological water - Antibiotics - Tested bacterial strains	Plant extracts The plant extracts

B. Soaking process:

We soak in 3solvents: ethanol - acetone - ethyl acetate.

We weigh 50 grams of the plant powder, put it in a bottle, put acetone on it until the entire amount is submerged, close it tightly, leave it for 24 hours, then filter it.

We weigh 50 grams of the plant powder, put it in a bottle, add ethanol to it until the entire amount is submerged, close it tightly, leave it for 24 hours, then filter it.

We weigh 50 grams of the plant powder, put it in a bottle, add ethyl acetate to it until the entire amount is submerged, close it tightly, leave it for 24 hours, then filter it.

*Repeat the soaking process 3 times

1 2

4 3

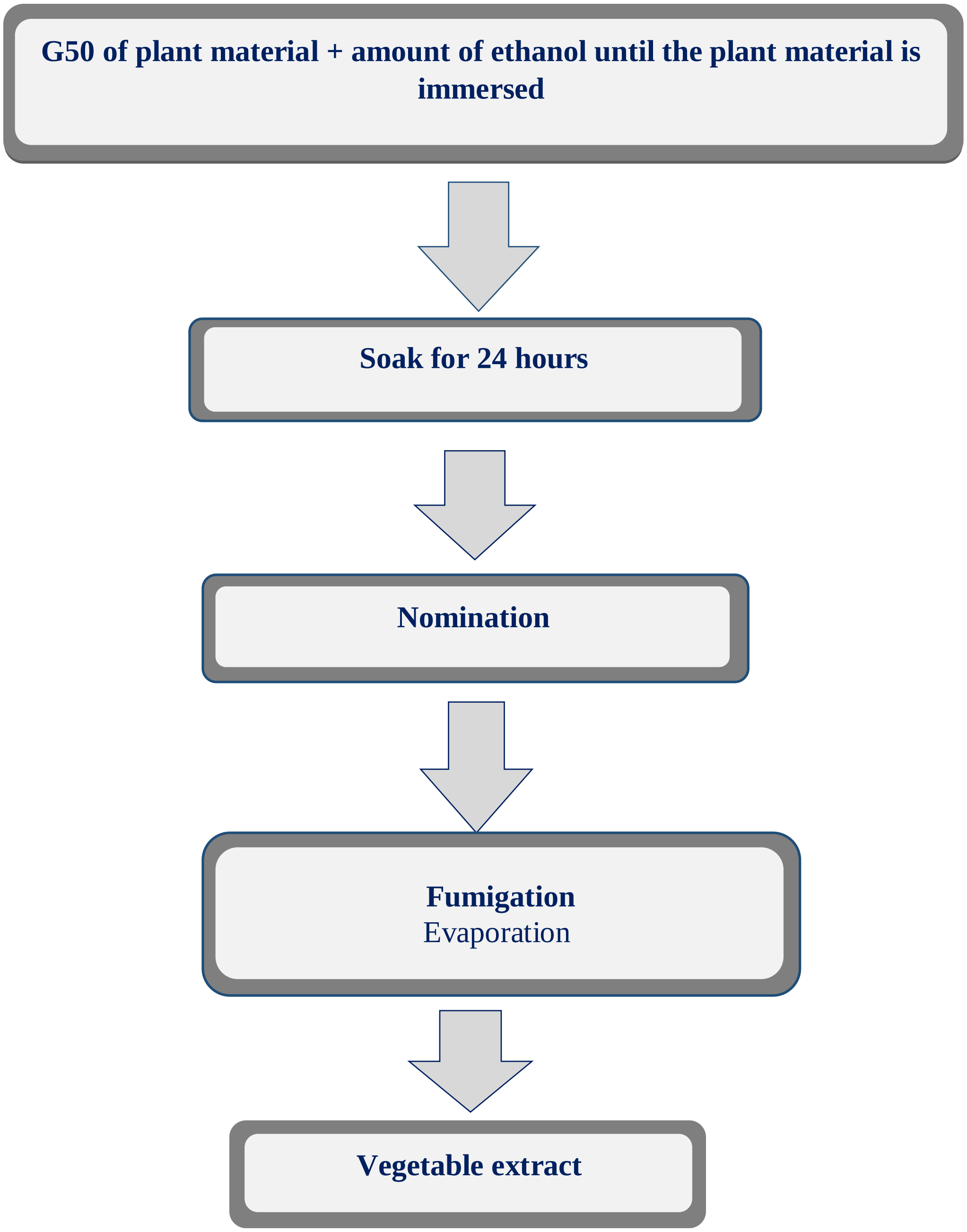
Figure (10):Extraction steps

C. Solvent recovery process:

After completing the soaking and filtration process and obtaining the extracts, we evaporate each extract separately in the rotary evaporator, so we obtain an extract that contains a small amount of solvent, and on the other hand, we obtain the recovered solvent.

3. Extraction:

Ethanol extraction:


G50 of plant material + amount of ethanol until the plant material is immersed
Soak for 24 hours
Nomination
Fumigation
Evaporation
Vegetable extract

Acetone extraction:

G50 of plant material + amount of acetone until the plant material is immersed

↓

Soak for 24 hours

↓

Nomination

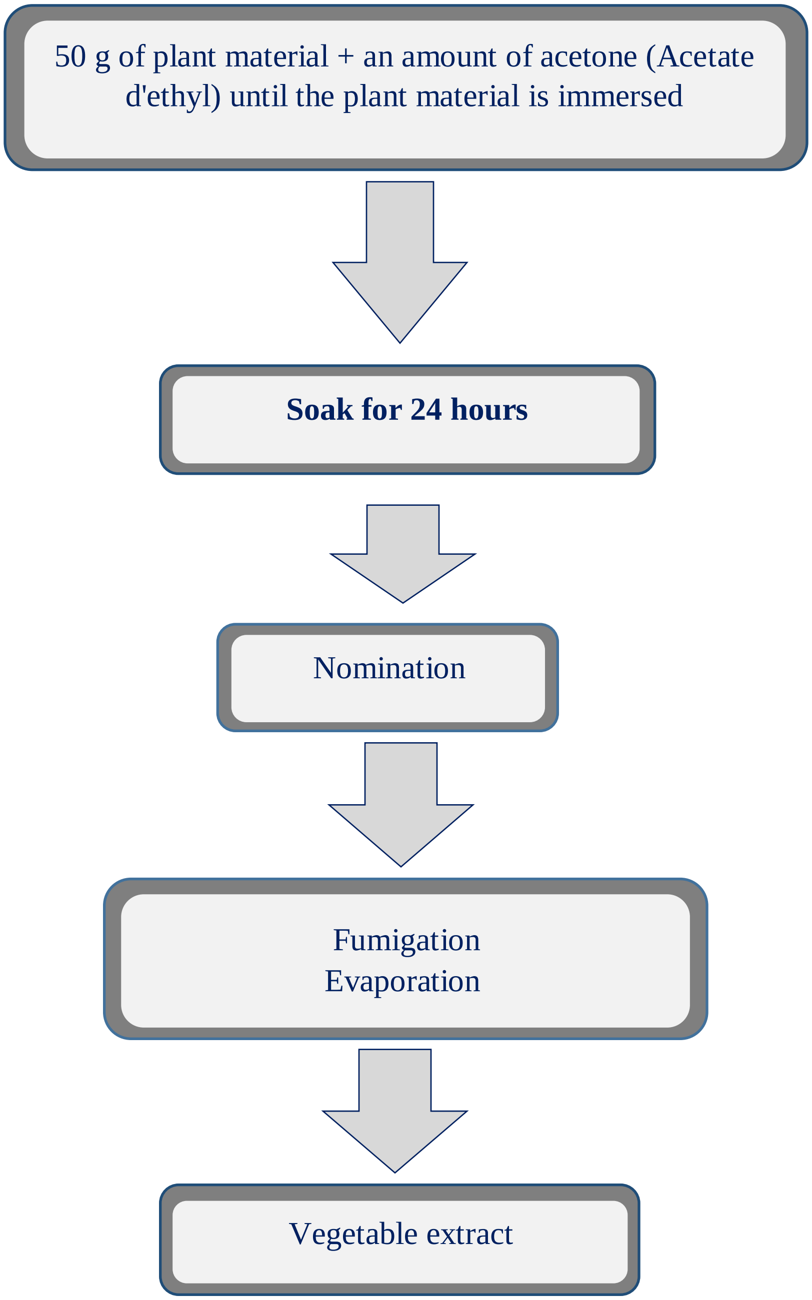
↓

Fumigation
Evaporation

↓

Vegetable extract

Extraction of ethyl acetate:



4. Estimating the return rate:

The yield is the result of dividing the mass of the plant extract by the mass of the dry plant material used in the extract and is estimated according to (Gettaf, et al 2016) with the following relationship:

$$R\ \% = (\text{mass of extract} / \text{mass of dry starting plant material}) \times 100 \qquad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

(1)Extraction yield calculation relationship

5. Chemical detection of some active substances:

5.1 Detection of phenols(Phenols):

The method of work:According to the method followed byMuhammad Qasim Samejo, boil 0.5 g of the plant part in 10 ml of distilled water, filter the mixture, and add to the filtrate after some drops of 3FeCl at a concentration of 0.1%.

Note:The appearance of a blackish-blue color indicates the presence of phenols.[22]

5.2 Detection of flavonoids:

The method of work:According to the method followed byMuhammad Qasim Samejo, 0.5 g of the plant part is boiled in 10 ml of ethanol, the mixture is filtered, and we add to the filtrate some pieces of Mg and drops of concentrated HCl.

. Note: The appearance of a red color indicates the presence of flavonoids. [22]

5.3 Detection of Saponins:

The method of work:According to the method followed byKardong, we soak 1 g of the plant part in 10 ml of distilled water, filter the mixture, put the filtrate in a test tube, add 3 ml of distilled water and shake for min5.

Note:Foam formation indicates the presence of saponin. [22]

5.4 Detection of terpenes:

The method of work:According to the method followed by, Chowdhury, Kardong, and Ganatra Bijekar from the aqueous extract in a test tube, we add 2 ml of 3CHCl and 3 ml of 4SO2H.

Note: A reddish-brown layer forms, indicating the presence of terpenes[22]

5.5 Detection of tannins:

The method of work:According to the method followed byCouchary, Djaafar, Karthishwaran Boil 0.5 g of the plant part in 20 ml distilled water, filter the mixture; add to the filtrate drops of 3FeCl at a concentration of 0.1%.

Note:A blackish blue color appears[22]

5.6 Detection of Coumarins:

The method of work:According to the method-followed byYadav, Sawant, Godghate, we put 2 ml of the aqueous extract in a test tube to which we add 3 ml of NaOH at a concentration of 10%.

Note: The appearance of a yellow color indicates the presence of Coumarins. [22]

6. Quantitative determination of polyphenols (PPT):

Phenolic compounds were estimated according to (1999Singleton V et al; this method relies on returning the components of the Folin-Ciocalteu reagent through phenolic compounds to give a Quinone and ketone to the oxides of tungsten (23 (W8 O3) and molybdenum (MO8 O3) which is characterized by the blue color DIF. M. M et al; 2015).) The method is summarized below:

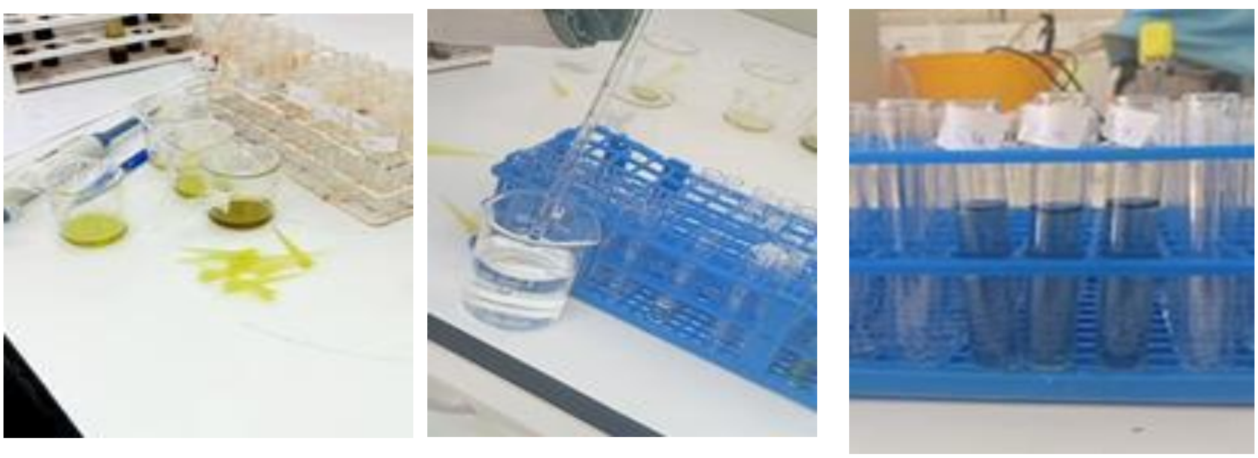
Place in a test tube125 µl of plant extract solution (1 mg of plant extract and 1 ml of methanol), 500 µl of distilled water, 125 µl of Folin -Ciocalteu. Shake the mixture and leave for 3 minutes, then add 1250 µl of sodium carbonate (3Co2Na), 1 ml of distilled water, shake well and leave. The mixture is in the dark and at laboratory temperature for 30 minutes, and the absorbance is read in the spectroscopic analysisdevice at the wavelength λ=760 nm.

. (Slinkard K et al; 1977)

We prepare several increasing concentrations of Gallique Acid, by dissolving 2 mg of Gallic acid with 2 ml of methanol to obtain the concentrations (ml / 500 µl, 400 µl/ml, 300µl/ml, 200µl/ml, 100µl/ml, 50µl/ml, µl/ml25). For the quantitative determination of polyphenols in the methanolic extract, we repeat the process with the other extracts.

We use Gallic acidGallique Acid To determine the equation of the linear curve, the results are expressed as the number of equivalent milligrams of Gallic acid per mg of weight of the plant extract.[38]

1 2 3



Figure(11): Polyphenol dosing steps

7. Biological study:

These tests were carried out at the Agricultural Experiment StationINRAA in the laboratory specialized in biological studies

7.1 Antioxidant activity test:

a) Trapping the free radicals DPPH (2,2-diphenyl-1-picrylhydrazyl)

Enjoy the methodDPPH has many advantages because it is independent, simple and fast.

The test consists of placing a rootDPPH (purple) in the presence of molecules called an “antioxidant” to measure their ability to reduce the DPPH radical.

The reduced form (yellow in color) is no longer absorbed, resulting in low absorption at this wavelength.[38]

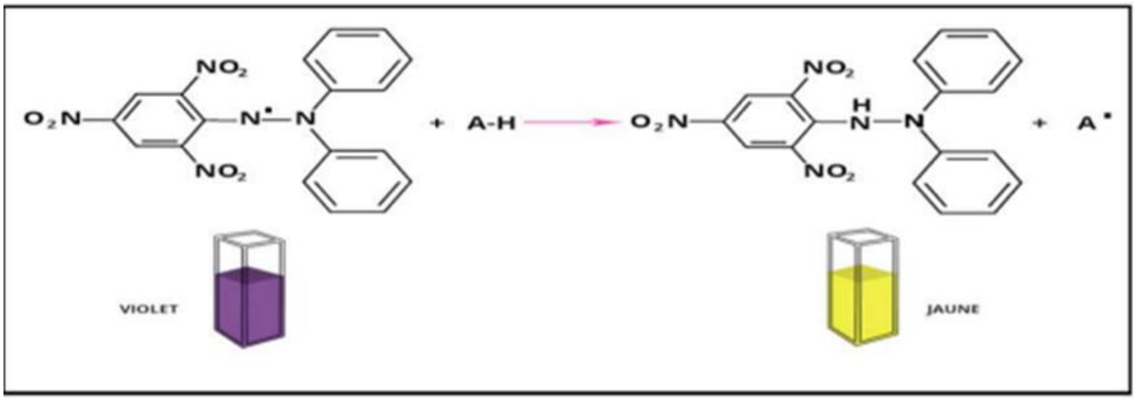


Figure 12: Free radical inhibition equationDPPH

b) Protocol

This method is based on measuring the ability of antioxidants to trap radicalsDPPH. The effect of each extract on DPPH is measured by the procedure described by Colak et al. (2017).

A volume of 100 µm was mixedliters of different concentrations ofEOs with 1 mL of methanoic solution of DPPH (0.33 mmol), 10 mg DPPH with 300 mL of fresh methanol. The reaction mixture was shaken vigorously and incubated for 30 min at room temperature and in the dark. Absorbance was measured at 520 nm. The negative control consisted of 100 µL of methanol and 1 mL of DPPH solution. The positive control is represented by a standard antioxidant solution, and the uptake of ascorbic acid (the reference antioxidant) is measured under the same conditions as the essential oils. [38]

Calculating ratiosInhibition:

The damping ratios are calculated by the following formula:

I%:- Inhibition percentage

AC: -Absorbing control

AT:- Absorption test was performed

$$I\ \% = ((AC - AT)/AC) * 100 \ldots\ldots(2)$$

(2) Calculating ratios Inhibition

Account IC_{50}:

IC_{50} or 50% inhibitory concentration (also called EC50 for effective concentration 50), is the concentration of the tested sample necessary for 50% reduction of the DPPH radical.

Iscalculated IC_{50} graphed by linear regressions of plotted graphs; This is the inhibition percentage depending on the different concentrations of the parts tested. (Bertoncelj et al. 2007; Marxen et al. 2007; Scherer and Goboy, 2009 ; Fabri et al., 2009).

$$IC_{50}=((50-1.1984)/5.2272)=9.33mg/ml \ldots (3)$$

(3) IC_{50} calculation relationship

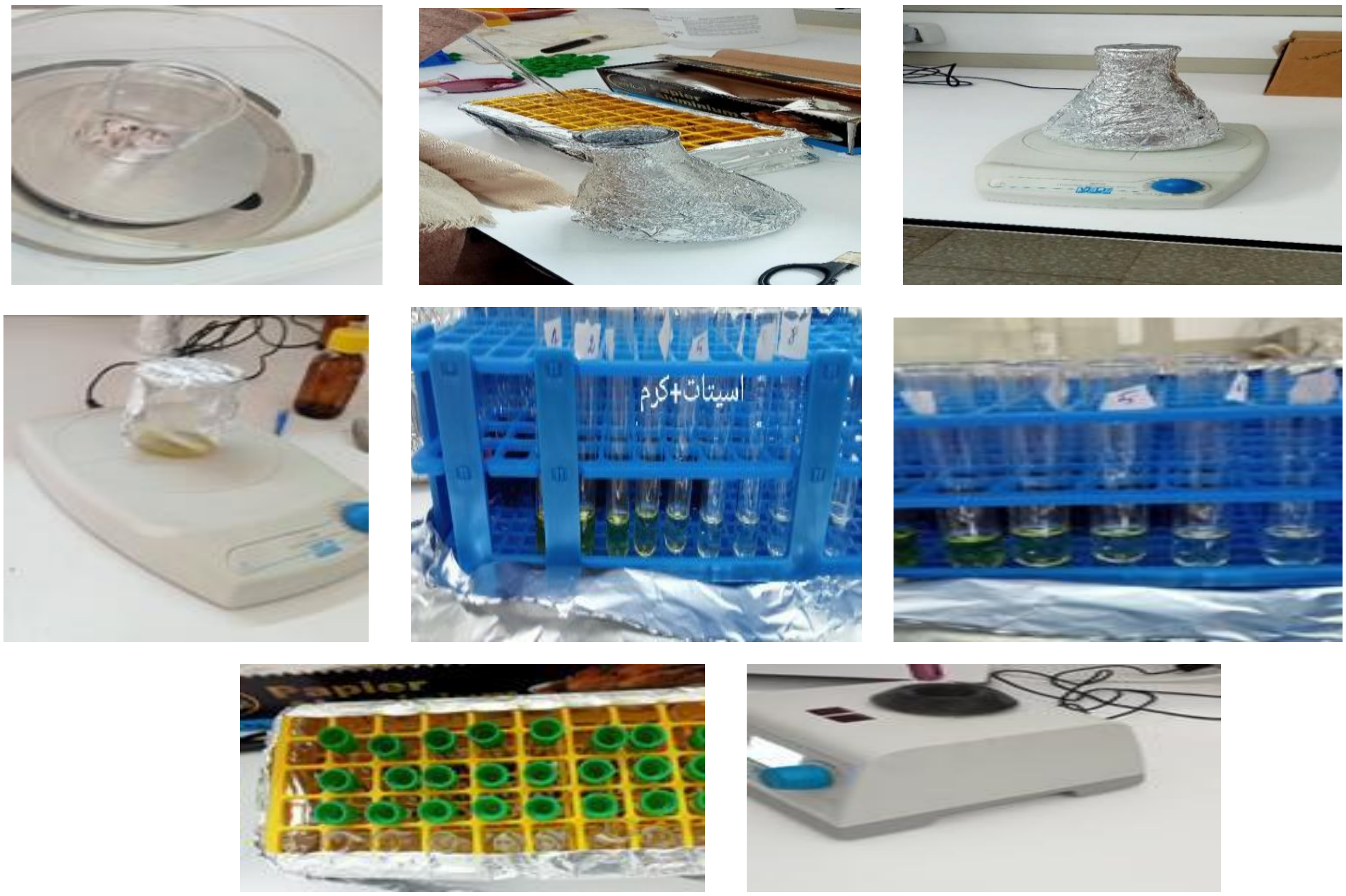


Figure 13: Antioxidant activity steps

7.2 Antibacterial activity test:

This study was carried out at the Suleiman Amirat Hospital Institution in Tougourt in the Water and Epidemiology Laboratory

In this study, we selected the sensitivity of bacteria to the previous plant extracts, and for this we applied them to 2 types of pathogenic bacterial strains, which were obtained from the laboratory of the hospital institution:

a) Generalities about the types of bacteria tested:

Escherichia coli•

It is a bacteria belonging to the familyEnterobacteriaceae live in the human body, animals, plants, and in the soil. They are aerobic, Gram-negative, rod-shaped, with dimensions from 0 to 1 micron. They grow quickly in normal environments and move in the form of disease-causing bacilli. Among the diseases they cause are: intestinal infections, genital infections. Or urinary tract meningitis and septicemia (2004M Silivia;2003;SinglentonP;). [22]

Pseudomonas aeruginosa•

It is a bacteria belonging to the familyEnterobacteriaceae is considered the most widespread in soil and water. It is a Gram-negative, aerobic, rod-shaped organism. It grows in a wide temperature range between 4°C and 43°C. It is a pathogenic agent for humans, animals and plants. It is characterized by its resistance to antibiotics and disinfectants and is responsible for serious sepsis after surgical operations. It is also pathogenic for the digestive and urinary tract and bacteremia (Hidron A et al; 2008). [22]

b) Protocol:

b.1) Preparing the culture media

First, sterilize the workplace (,jut and after preparing the culture medium and sterilizing it in a deviceAutoclave at a temperature of 121 °C. We prepare a set of Petri dishes with equal diameters of 9cm, into which we pour the Miller Hinton culture medium that was previously melted in the Autoclave. All work steps are carried out near a Benzene de Bec burner in order to obtain a sterile medium and the dishes are left on the table. Laboratory to cool and freeze (2008 Chakraborty M et Mitra A;).

b.2) Bacteria cultivation

Bacteria are cultured by dipping a sterile cotton swab in the bacterial suspension and wiping the entire previously prepared culture media with it in the form of converging lines, repeating the process 1 time and rotating the dish at an angle of 60° each time (2008(Chakraborty M et Mitra;.

b.3) Placing the discs

After preparing the culture media, we select 4 parts from each Petri dish, where we allocate 3 parts to the three studied and prepared plant extracts of high concentration.2 mg/ml. Discs prepared from Wathman paper with equal diameters of 6 mm were used, sterilized. Using sterile forceps, we took the discs saturated with the third plant extracts and placed them inside the petri cans in the section designated for them, while we placed the antibiotic (HLG Gentamicin) in the fourth part, and placed A disc saturated with ethanol as a control is placed in the center of a Petri dish for the purpose of negative comparison

-Petri boxes are placed upside down in the incubator at room temperature°C37 for 24 minutes.

- At the end of the incubation period, we take out Petri cans to measure the inhibitory diameters with mm)) for extracts, antibiotic and control (2008 Chakraborty M et Mitra ;).

Chapter four:

Results and discussion

I **Results**:

1-Detection of active ingredients:

1.1- Detection of phenols

We notice the appearance of a blue-black color in the aqueous extract of the plantbrassica Oleracea.var. capitata, which proves the presence of phenols.

Figure 14: Showing the presence of phenols

1.2- Detection of flavonoids

We notice the appearance of a red color in the ethanolic extract of the plant brassica Oleracea.var. Capitata, which proves the presence of flavonoids.

Figure 15: Showing the presence of flavonoids

1-3-Detection of Saponins

After shaking, we notice the formation of foam in the aqueous plant extractbrassica Oleracea.var. Capitata proves the presence of Saponins.

Figure 16: Showing the presence of Saponins

1.4.Detection of terpenes:

We notice the formation of a reddish-brown layer in the aqueous extract of a plantbrassica Oleracea.var. Capitata proving the presence of terpenes.

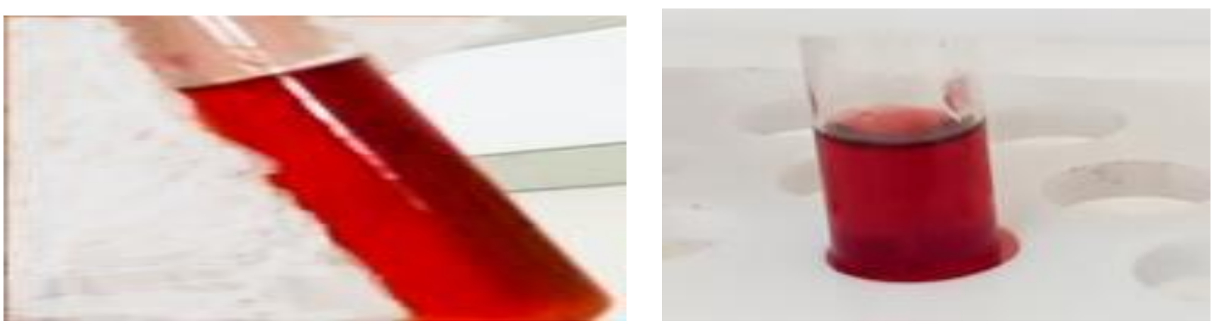


Figure 17: Showing the presence of Terpenes

1.5.Detection of tannins:

We notice the appearance of a blue-black color in the aqueous extract of the plantbrassica Oleracea.var. Capitata, which proves the presence of tannins .

Figure 18 Showing the presence of Tanins

1.6.Detection of Coumarins

We notice the appearance of a yellow color in the aqueous extract of the plantbrassica Oleracea.var. Capitata Evidence of the existence of Coumarins.

Figure 19 Showing the presence of Comarins

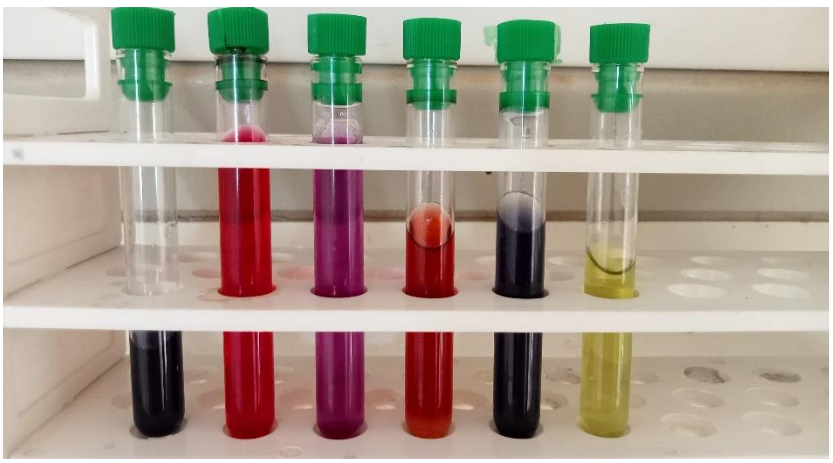


Figure 20: result obtained from detection tests

1. Yield of plant extractsR %:

We obtained plant extracts after the cold soaking process using acetone, ethyl acetate, and ethanol as solvents, where the yield of each extract was estimated based on the relationship mentioned at (Gettaf,et al 2016), where the results were as shown in the following table:

Table 08: Types of bacteria studied

R%	Raw output mass	Solvents	Mass of plant matter
3.6%	1.8 g	Ethanol	50 g
9.2%	4.6 g	Ethyl acetate	
27.2%	13.6 g	Acetone	

From the results shown in the table above, we note that it was the highest yield for a plantbrassica Oleracea. Var.capitata in the acetone extract (27.2%), followed by ethyl acetate (9.2%), and finally comes the ethanol extract (3.6%).

2. Quantitation of polyphenols:

Polyphenols were quantitatively determined using the method(1965) (Singleton et Rossi; using the Ciocalteau-Folin reagent, where the total polyphenol content of the studied extracts is expressed quantitatively using the linear equation of the standard curve for Gallic acid), where the values of polyphenols for plant extracts are estimated as mg equivalent of Gallic acid per gram of plant extract. (mgEAG/g EP) as listed in the table

Table 09: Quantity of polyphenols for the studied plant extracts

Brassica Oleracea Var. capitata +Acetate d'ethyl	Brassica Oleracea Var. capitata +Ethanol	the sample
0.14	0.688	ABS1
0.145	0.688	ABS2
0.142	0.696	ABS3
0.0262147	0.04811252	Quantity of polyphenols

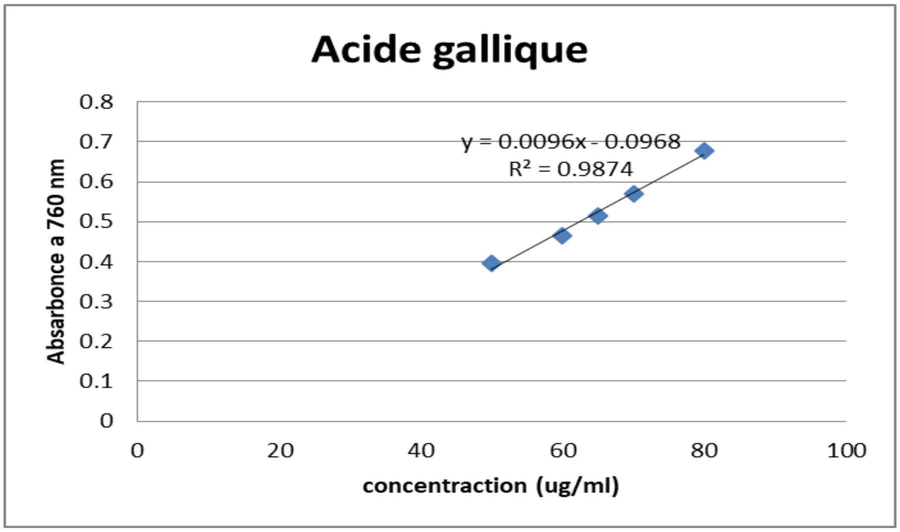


Figure 21: Standard curve of Gallic acid

Through the results obtained and with the aim of estimating the total amount of phenolic compounds in the pure extracts of the studied plant, we found that the results are close, as the ethanolic extract recorded the highest percentage, estimated at (mg/g0.04811252) followed by acetate extract (mg/g0.0262147

3. Antioxidant activity study:

In order to estimate the antioxidant activity of plant extracts, a test was usedDPPH (2, 2-Diphényle-1 picrylhydrazyle) as the most commonly used test for this purpose. Ascorbic acid was used for positive comparison. The absorbance is read and the antioxidant activity of the various concentrations is calculated for the studied extracts. To determine the extract’s inhibition of the free radical DPPH, this is done using the ascorbic standard curve.

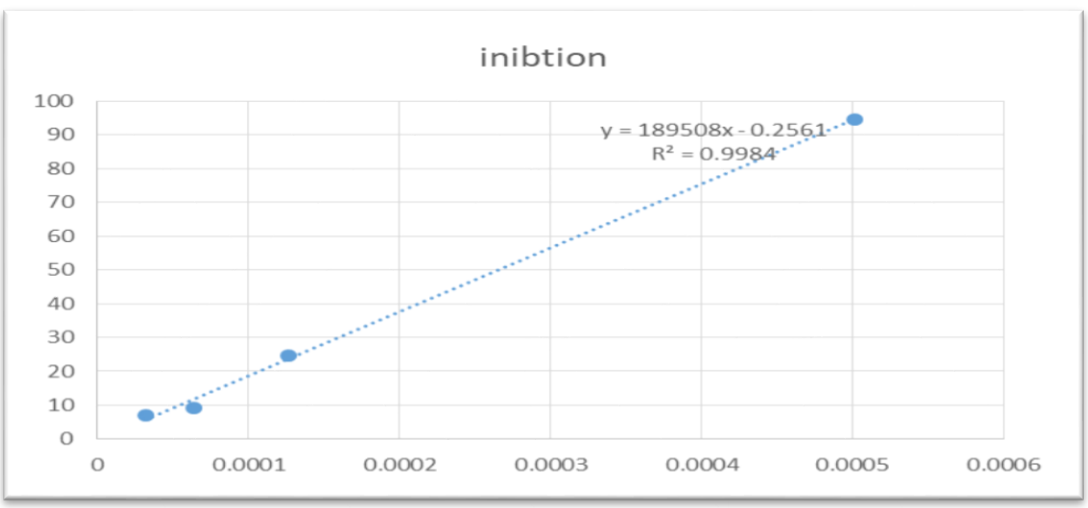


Figure 22: The curveYesStandard for ascorbic acid

Table 10: Results C Free radical inhibition test DPPH

Thefocus Concentration (mg/ml)	Brassica Oleracea Var. capitata +Ethanol	Brassica Oleracea Var. capitata +Acétate d'ethyl	Brassica Oleracea Var. capitata +Acétone
0.093	61.2485811	10.9578544	76.6666666
0.187	53.3673855	1.72413793	8.52380952
0.375	94.1997729	62.7298850	58.0476190
0.75	66.3942489	3.87931034	9.57142857
1.5	8.7400681	26.1781609	14.2380952
3	515.51267	28.7643678	523.476190
6	29.2470677	215.1340996	36.5238095
12	65.8342792	721.120689	41.7142857

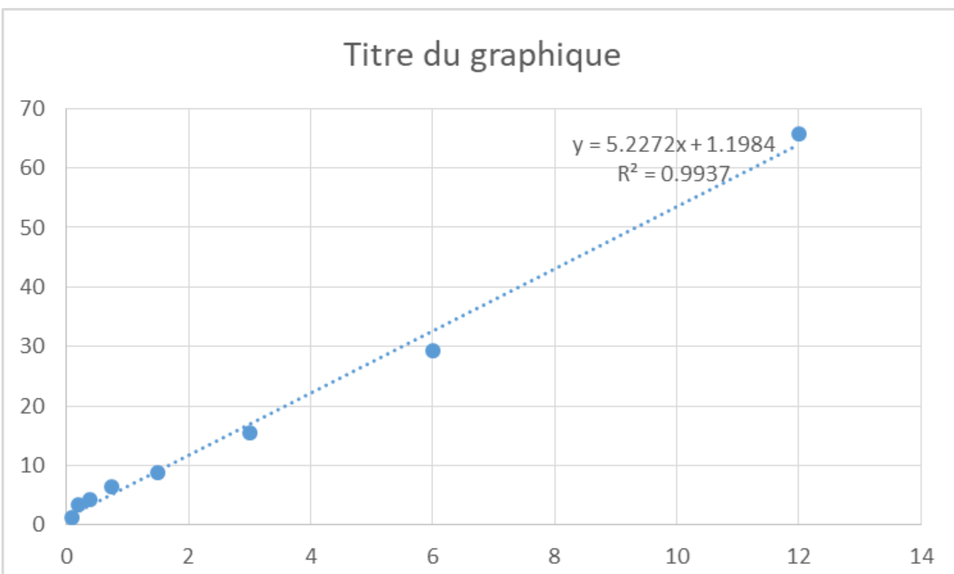


Figure 23: DPPH free radical inhibition curve of ethanolic extract

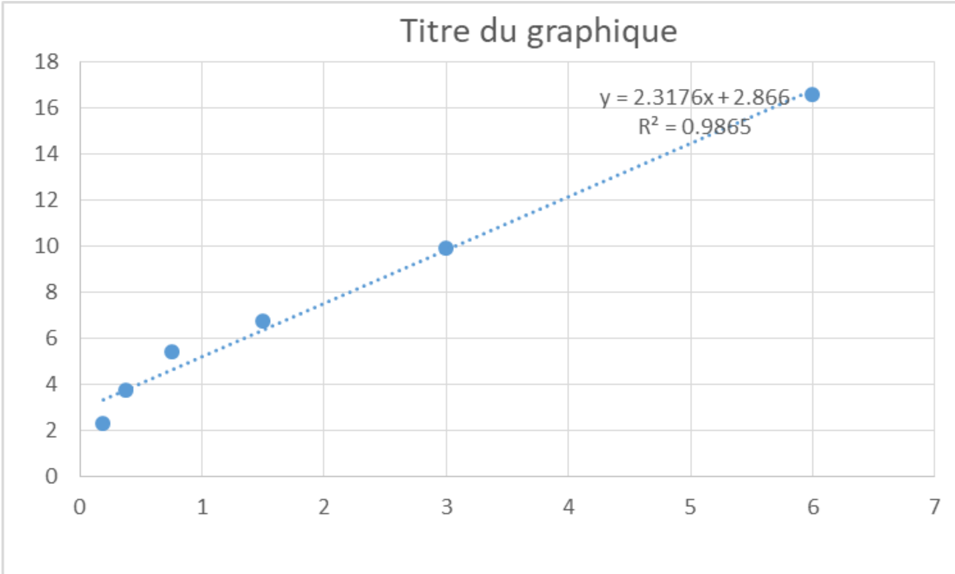


Figure 24: DPPH free radical inhibition curve of ethyl acetate extract

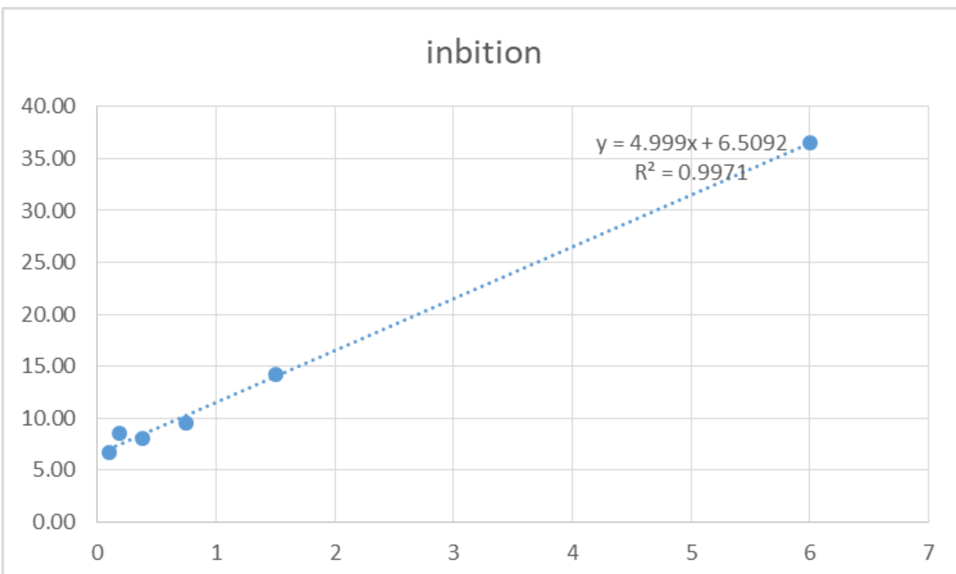


Figure 25: DPPH free radical inhibition curve for acetone extract

Based on the results obtained in the table and the graphical curves above to test the inhibition of DPPH free radicals for the plant extracts under study.It is clear that the higher the concentration of the plant extract, the greater the antioxidant activity, as the largest percentage of DPPH free radical scavenging was seen at a concentration (mg/ml) of 12 for the extracts: ethanol, ethyl acetate, and acetone in varying percentages, respectively, 65.83%, 21.12%, 41.71%, while the reference control recorded 94.34% at the same concentration In general, the inhibition rate of plant extracts was far compared to ascorbic acid.

Determine the amount of IC50:

We can calculate DPPH from the linear equation for both the inhibition curves for plant extracts and ascorbic acid as shown in Table (9)
Asa reminder:Whenever the value is 50The lower the IC, the better the anti-free radical effect.

Table 11: Result of IC50

Brassica Oleracea Var. capitata +Acétone	Brassica Oleracea Var. capitata +Acétate d'ethyl	Brassica Oleracea Var. capitata +Ethanol	Plant extracts
8.6	20.3	9.3	IC50%

- The values of the 50IC estimator, which expresses the inhibitory concentration of 50% of the free radical DPPH, were determined through linear equations of the inhibition curves (I %) for plant extracts and ascorbic acid. It is known that the antioxidant activity is inversely proportional to the 50IC values. The weaker the 50IC values, the greater the activity. Better free radical suppression

. Through the results shown in the table, which show the evaluation of the antifungal activity, where the IC 50 values obtained from the studied plant extracts showed that the acetone and ethanol extracts have greater effectiveness in inhibiting the free radical DPPH by 8.69% and 9.33%, respectively, while they were less effective in the acetate extract. Ethyl, its percentage was estimated at 20.33%.
As these values are ineffective compared to ascorbic acid, estimated at: 0.00026519%.

4. Study of antibacterial activity:

By studying the biological activity of the studied plant extracts and the antibiotic activity of some pathogenic bacterial species, we obtained the results attached in the pictures and tables (10)

Table 12: Result of antibacterial

Brassica Oleracea Var. capitata +Acétone	Brassica Oleracea Var. capitata +Acétate d'ethyl	Brassica Oleracea Var. capitata +Ethanol	*Extracts.* Vegetarianism / Bacteria
-	-	+	Escherichia coli
-	-	+	Pseudomonas aeruginosa

(+): It has antibacterial activity
(-): It has no antibacterial activity

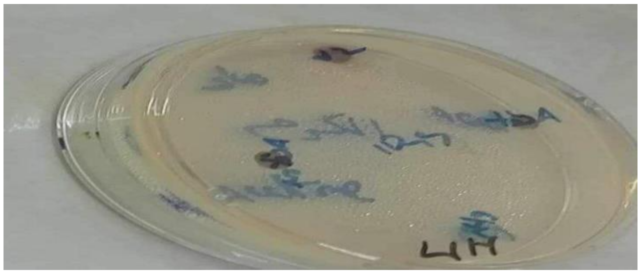


Figure 26: Effectiveness of antibacterial extractsin Escherichia coli

Figure 27: Antibacterial effectiveness of extracts InAeruginosa Pseudomonas

After conducting this study, we found that there is antibacterial activity in the ethanolic extract against both pathogenic bacteria and the absence of antibacterial activity in the acetone ethyl acetate extract.

I.Discussion:

1.Detection of active ingredients:

The results of the detection of the active substances of the plant showedBrassica Oleracea Var. capitata about the presence of phenols, flavonoids, Saponins, terpenes, tannins, Coumarins.The results are shown in Table (11)

Table 13: Result the detection of active ingredients

Active ingredients	The result
Phenols	+++
Flavonoids	+++
Saponins	+++
Terpenes	+++
Tannins	+++
Coumarins	+++

The results obtained are consistent with some previous studies from the same family as the study he conducted MOHAMMED FAZIL AHMED (2012)Which has proven the existence of these active substances

2- Extraction yield:

According to the results obtained for the process of estimating the total yield, which areshown in Table (6), the greatest yield was for a plant brassicaOleracea. Var.capitata in the acetone extract (27.2%), followed by ethyl acetate (9.2%), and finally comes the ethanol extract (3.6%). The reason for the fluctuation in the yield percentage is likely to be due to the extraction method and conditions (YEO SOUNTA et al; 2014), as MADI (2010) stated that the frequency of the extraction process and the amount of solvent for the plant material, in addition to the duration of the extraction process, determine the yield value (Gedel, 2015)

Where he explains ((Rajaei et al; 2010)This is due to the degree of saturation of the solvent, i.e. the inefficiency of the volume used to extract all the molecules of the sample and not taking enough time to do so.

3-Quantitative determination of phenol:

Through this study, the phenolic substances of the studied plant extracts were estimated. It was noted from the above table (7) that the results of quantitative estimation of phenols are weak compared to Gallic acid, and the reason for these differences is likely to be:

- This may be due to the purity of the extract, as the solvent may extract non-phenolic compounds such as sugars and proteins.

-The Folin detector is distinguished by its sensitivity to hydroxyl groups not only in phenolic compounds, but also in all sugar and protein compounds. The difference in the values of polyphenols can be attributed to this reason (Grossi et al 2015; Gmez-Caravaca et al 2006).

4- Antioxidant activity study:

Through the results obtained and shown in Tables (8) and (9), in which we noticed that, the rate of inhibitionDPPH and IC50 were far off compared to ascorbic acid and the reason for these results is likely due to:

- The estimation process and its steps, as this can be due to the solvent used during it, as chemists have proven that water is the best for this, because other organic solvents are able to bind 3NaCl is responsible for providing the most suitable medium for its determination (SCALBERT et al; 1989)

5- Antibacterial activity study:

Through the results of studying the antioxidant, activity of the studied planextracts obtained and shown in Table (10) and Figures (19) and (20)

Conclusion

Despite the developments taking place in medical science in its various specialties, interest has increased today in medicinal plants, with the aim of discovering effective substances used to treat many diseases, such as polyphenols, which are considered more widespread in the plant kingdom, where they play an important role as antioxidants.

Our interest in this study focused on the study of chemical phytochemicals and the biology of a medicinal plant from the Cruciferous family *Brassica Oleracea L.var. Capitata*

We first detected the active substances present in the plant, and the result was the presence of (flavonoids, Saponins, terpenes, tannins, Coumarins .Followed by the process of soaking in the three solvents: acetone, ethyl acetate, and ethanol, then evaporating it in a rotary evaporator, and we obtain 3 extracts: ethanolic extract, ethyl acetate extract, and acetone extract.

The results of the extraction yield showed that the three extracts varied in percentage, as we recorded the highest percentage in the acetone extract, followed by the ethyl acetate extract, followed by the ethanol extract.

In light of the results of the antioxidant activity test by free radical stabilization DPPH, several different concentrations of the third extracts were prepared, treated with DPPH solution, and the anti-ascorbic acid activity was calculated as a reference control, where the IC value of 50 was calculated for these extracts.

We also studied the quantitative determination of polyphenols, and the results were mixed compared to Gallic acid and antibacterial activity, and the result showed positive in the ethanolic extract.

We hope that this study will be completed in the future from a biological perspective, and its active ingredients will be extracted and used instead of manufactured medicines.

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