PEOPLE'S DEMOCRATIC REPUBLIC OF ALGERIA MINISTRY OF HIGHER EDUCATION AND SCIENTIFIC RESEARCH

UNIVERSITY OF MSILA

FACUALTY OF SCIENCES
DEPARTMENT OF CHEMISTRY
N°:…..…/……../……

DOMAIN: SCIENCES OF MATTER
STREAM: CHEMISTRY
OPTION: PHARMACEUTICAL

Thesis submitted to the department of chemistry for obtaining a master's degree in pharmaceutical chemistry.

Theme:

Phytochemical & biological study of medicinal plant *"Thapsia garganica"*

Submitted by
Ms BENSEDDIK Hesna

Supervised by
Dr MERATATE Faiza

Publically defended before the following jury

M^{m}BELHADDAD Oumelkheir	MCB-University of Msila	Chairperson
M^{m}MERATATE Faiza	MCB-University of Msila	Supervisor
M^{m}MOHAMADI Sabrina	MCB-University of Msila	Examiner

2020-2021

Acknowledgments

Above all, all praise and gratitude to God who guided me throughout my journey and gave me strength, patience and faith to complete this work well, which is my beginning to a new start, May ***Allah*** gives me the steadfastness to through it.

In the beginning, I would like to express my sincere thanks and appreciation to my supervisor Dr.Meratate faiza, for the great help she gave me during the realization of my work, and her constant guidance and encouragement throughout the work.

My sincere thanks to the members of the jury: Ms. Mohammadi. S, Ms. Belhaddad.O. Each on his way to accept the discussion of the work and put their touch on it, which will increase its scientific value.

Likewise, I would like to express my grateful thanks to all the teachers of the Faculty of Material Sciences, especially the Pharmaceutical Chemistry major.

Also, my sincere thanks to the head department and the administrative staff of Chemistry faculty for their permanent help and support, Without forgetting the engineers of the chemistry and biology laboratory at the University of M'Sila and the University of Constantine for their welcome, sympathy and constructive assistance.

Best Regards to all my fellows in promotion Master's Degree 2020 major in Pharmaceutical Chemistry for the beautiful moments we had together.

The best for the last, special thanks and respects to all my honorable professors: Dr. Ladgham Chikosh.D, Dr. Belhaddad. O, Dr. Cheriet. Th, Dr. Khenish. H, for offering me all the help and support during my studies, as well as their patience and constant encouragement, also for providing me with all the knowledge that will accompany me for the rest of my academic and professional journey.

Without forgetting everyone who participated from near or far in carrying out this work.

H. BENSEDDIK

Dedications

First, I would like to dedicate this work, to thank and express my deepest gratitude to my parents; the deeper words for the treasure of my life "Mum" thank you for all the sacrifices you've made, for giving me the strength and lighting my soul in the darkest night.

Also for the first hand, I ever held "Dad" thank you for always being there for me and taught me how to be hard worker to reaching my goals.

A special dedicate for my brother "Ahmed" and my two sisters "Asma and Souad" thank you for not ceased to support and believe in me and without you, I will not achieve my goals.

Without forgetting, tiny dedicate for all my nephews thank you for brightening my life and give it a beautiful color.

The last dedicate for pretty amazing people who stood right by my side: Imane, Houda, Karima, Dounia, Kader, Hamid, Hako, I'm extremely grateful and blessed to have you all in my life, all I can say thank you from the bottom of my heart.

H. BENSEDDIK

Table of Contents

FIRST PART
BIBLIOGRAPHIC PART

ChapterI

Study of medicinal plants

Chapter II

Secondary metabolites

Chapter III

Biological activity

SECOND PART
EXPERIMENTAL PART

Chapter IV

Materials and methods

Chapter V

Results and discussions

List of Abreviations

Unities

°C	Degree Celsius
h	Hours
M	Mole per litre
Mm	Millimetre
mg	Milligram
mL	Millilitre
min	Minute
nm	Nanometre
µL	Microlitre
µm	Micromitre
µg	Microgram
g	Gram

Other abbreviations:

H.E	Essential oil
MeOH	Methanol
H2SO4	Sulfuric acid
Fecl3	Ferric chloride
I2	Iodine
AlCl3	Aluminum chloride
DPPH	2, 2-Diphenyl-1-picrylhydrazyl
AA	Antioxidant activity
ATCC	American Type Culture Collection
DMSO	Dimethyl sulfoxide
NB	n-butanol
AC	Ethyl acetate
CH	Chloroform
PE	Petroleum ether
IC50	Half maximal inhibitory concentration
H2O	Water
H	Hydrogene
HCLO	Hypochlorous acid
TFC	Total flavonoid content
UV	Ultraviolet
ROS	Oxygen reactive species

SOD	Antioxidant enzymatic system
ERO	Reactive oxygen species
NOS	Nitric oxide synthase
STL	Sesquiterpene lactones

List of Figures

Chapter I

Chapter II

Chapter IV

Chapter V

List of Table

Chapter I

Chapter II

Chapter III

Chapter IV

Chapter V

General Introduction

Introduction

Type the word 'phytochemical' into any online search engine and it will return literally thousands of hits. This is a reflection of the role plant derive chemicals have played medicine and other areas since humans have looked to nature to provide cures for various ailments and diseases **(Tiwari, B & al .2013).**

Medicinal plants are a rich source of bioactive phytochemicals or bionutrients. Studies carried out during the past 2–3 decades have shown that these phytochemicals have an important role in preventing chronic diseases like cancer, diabetes and coronary heart disease. The major classes of phytochemicals with disease-preventing functions are dietary fibre, antioxidants, anticancer, detoxifying agents, immunity-potentiating agents and neuropharmacological agents. Each class of these functional agents consists of a wide range of chemicals with differing potency **(Gelaw, H & al 2012).** This constituents known as plant secondary metabolites are a diverse group of molecules that are involved in the adaptation of plants to their environment but are not part of the primary biochemical pathways of cell growth and reproduction. In general, the terms plant secondary compounds, phytochemicals, antinutritional factors, and plant xenobiotics have been used in the literature to refer to this group of compounds **(Makkar, H. P. & al 2007).**

Plant produces a high diversity of secondary metabolites not during photosynthesis, but result from subsequent chemical reactions among which terpenoids, alkaloids and phenolic compounds are distinguished. With their remarkable structural diversity, the latter, also called polyphenols, constitute a wealth already widely exploited by the agri-food, cosmetic and pharmaceutical industries.

Polyphenols (mainly, flavonoids, phenolic acids, tannins) are present in all parts of the plant. They are found in the composition of the most common consumer products,

especially fruits and vegetables but also processed products such as chocolate and tea (Muanda, F. N.2010).

Secondary metabolites of plant origin are the subject of much research in the fight against oxidative stress, in particular in the search for new molecules such as phenolic compounds, essential oils and alkaloids to which various biological properties are attributed.

From this perspective, The present work focused on certain antioxidant and biological activities of extracts of *Thapsia garganica.*

This medicinal plant has been known since ancient times for its curative and preventive efficacy, it occupies a privileged place in the treatment of certain pathologies, and it belongs to the apiaceae family. The latter are a very homogeneous family of dicotyledonous plants, one of the easiest in the flora to recognize, thanks to their umbellate inflorescences **(Guignard, 1998).**

The genus "*Thapsia*" includes about 41 species, native to Africa, Asia and Europe.

Thapsia garganica is a widespread species in Algeria, best known for the use of its roots in cooking and traditional medicine **(Gómez, 2007).**

Fig: a faithful photography of "*Thapsia gracanica*" (F. Bauer.1787).

Our work will be divided into five chapters:

- The first chapter is devoted to a bibliographical synthesis on the studied plant.
- In the second chapter we talk about secondary metabolites from plants and their different extraction and separation methods.
- The third chapter talks about the biological activities including antioxidant activity and antibacterial activity.

- The fourth chapter is devoted to materials and working methods.
- The final chapter brings together all the results which will be followed by a discussion. Finally we ended our work with a conclusion which is a set of reflections completes this work.

Reference list:

*Tiwari, B. K., Brunton, N. P., & Brennan, C. (Eds.). (2013). Handbook of plant food phytochemicals: sources, stability and extraction. John Wiley & Sons.

* Gelaw, H., Adane, L., & Tariku, Y. (2012). Journal of Pharmacognosy and Phytochemistry Vol. 1 No. 4 2012 www.phytojournal. com Page| 15 Isolation Of Crotepoxide From Berries of Croton Macrostachyus and Evaluation of Its Anti-Leishmanial Activity

* Makkar, H. P., Siddhuraju, P., & Becker, K. (2007). Plant secondary metabolites (pp. 101-106). Totowa, NJ, USA:: Humana Press.

* Muanda, F. N. (2010). Identification de polyphénols, évaluation de leur activité antioxydante et étude de leurs propriétés biologiques. Thčse de doctorat en Chimie organique. Ecole doctorale SESAMES Université Paul Verlaine-Metz, 294

* Guignard J.-L. (1998). Abrégés botaniques. 11ème Ed. Masson. 166-171. ISBN : 2-

225-83519-5.

*Gómez F. L. M. (2007). Síntesis de análogos de las tapsigarginas. Mémoire en vue d'obtention du grade de doctorat en chimie. Département de chimie organique. Faculté des sciences. Université de Cádiz. puerto real. Espagne.

FIRST PART

BIBLIOGRAPHIC PART

Chapter I:

Study of medicinal plants

The primitive man's consideration of plants as a basic source of nutrition allowed him to recognize many plants that grow around him, some of which cause him disease and some others that can cure him. Therefore, man-made plants a basic source in the treatment and prevention of various diseases since ancient times because they contain different effective compounds that have a curative and preventive effect, and with the development of man, he was able to extract these chemical compounds and get to know them and study the possibility of using them as medicinal plants.

I.1 General information on medicinal plants:

Plants produce hundreds of chemical compounds with functions that include defense against insects, fungi, disease, and herbivorous mammals. Several phytochemicals known as secondary metabolites of potential or stable biological activity have been identified. However, since a single plant contains widely diverse phytochemicals, the effects of using an entire plant as a drug are uncertain. Moreover, the phytochemical content and pharmacological procedures, if any, of many plants with medicinal potential remain unevaluated through rigorous scientific research to determine efficacy and safety **(Ahn, K. 2017).**

I.1.1 Medicinal plants:

Medicinal plant is defined as "everything that is of botanical origin and is used medicinally. It is a medicinal plant". **(هيكل محمد السيد.1993).**

Medicinal plants have discovered and used in traditional medicine since prehistoric. they are a rich source of bioactive phytochemicals or vital nutrients, and it clearly known that they have a role in protecting human health because they contain one or more chemicals in low or high concentrations, have the physiological ability to treat a specific disease or reduce symptoms of this disease if they are given to the patient in its pure form or in fresh

form of plant herb or partially extracted **(العابدإبراهيم,2009).** Thus, not all substances produced by plants are effective. Rather, they can produce ineffective substances and have no medicinal effect **(أبو زيد،ش.ن.1992).**

A definition of medicinal plants should include the following **(Sofowora.A.2008; Evans,W.C. 2008):**

a. Plants or plant parts used medicinally in galenical preparations (e.g. decoctions, infusions, etc.) e.g. Cascara bark;
b. Plants used for extraction of pure substances either for direct medicinal use or for the hemi-synthesis of medicinal compounds (e.g. hemi-synthesis of sex hormones from diosgenin obtained from *Dioscorea* yams);
c. Food, spice, and perfumery plants used medicinally, e.g. ginger;
d. Microscopic plants, e.g. fungi, actinomycetes, used for isolation of drugs, especially antibiotics. Examples are ergot (*Claviceps purpurea* growing on rye) or *Streptomyces griseus;* and
e. Fibre plants, e.g. cotton, flax, jute, used for the preparation of surgical dressings.

I.1.2.The importance of medicinal plants:

Modern science has concluded that manufactured drugs often pose a great danger to human health. Since all medicines can do harm as well as can aid in healing, the issue of safety is relative **(Donna, J. & al. 1994).** Since numerous experiments have shown that industrial pharmaceutical chemicalsmost often have harmful side effects in addition to the main therapeutic effect for which they are used (Omar and Haikal 1993).Hence, the importance of medicinal plants in pharmaceutical research and drug development, both directly as therapeutic agents but also as raw materials for drug synthesis or as a model for pharmaceutically active compounds **(Decaux,I. 2002).**

I.2.The family of Apiaceae:

The Apiaceae known by man since the most ancient times. Many local plants of this family used in primitive cultures because people soon noticed their odour, flavour, esculence or toxicity. Several Apiaceae were known in the early languages of China (Materia medica) and in Sanskrit (Constance, 1971). An utilitarian basic classification was developed by the Native Americans of Mexico, long before the discovery of the New World **(Rodriguez, R.L. 1957).**

I.2.1. Distribution and morphology:

I.2.1.1. Distribution:

The Apiaceae (or Umbelliferae) is a plant family comprising at the present time 466 genera and about 3800 species **(Plunkett,G.M. & al . 2018).** Commonly known as the **celery**, **carrot** or **parsley family** consists of dicotyledonous plants characterized in particular by their typical inflorescence **(Magee,A.R& al.2010).**

It is distributed nearly worldwide **(Table.1.1).** but is most diverse in temperate climatic areas, such as Eurasia and North America. The largest number of genera, 289, and the largest generic endemism 177, is found in Asia. There are 126 genera in Europe, but only 17 are endemic.

Africa has about the same total with 121 genera, where North Africa encompasses the largest occurrence of 82 genera, 13 of which are endemic**(Plunkett,G.M. & al . 2018).**

In Algeria, according to **Quezel and Santa (1962)**, it represented by 55 genera, 130 species and 27 subspecies.

Table.1.1: Distribution of "Apiaceae" family in the word**.**

Continent	Genera	Endemic
Asia	289	177
Europe	126	17
Africa	121	50
North Africa	82	13

I.2.1.2. Morphology:

In the Apiaceae family many species are similar and difficult to identify which make it a complexe family. even the species that is identifited, because of many existing variations it is often still quite complex at the infraspecific level , (habit, leaf, umbel organization, sexuality of flowers).

The plants belonging to the family Apiaceae, sometimes merely designated as 'Umbellifers', are mainly herbs. Although some woody subshrubs, shrubs or rarely trees are extant in this plant group.

Among these plants, hairiness is quite often absent (glabrous) but can also be scabrous to densely hispid, and sometimes glandular.

Most of the Apiaceae are aromatic, due to a network of secretory ducts throughout the plant.

Plant heights vary considerably, from very dwarf plants (few centi-metres) to giant plants reaching 3–5 m (Heracleum, Ferula). Stems are often erect, but they can also be creeping (Helosciadium repens, Centella asiatica), prostrate, decumbent or ascending; they can be hollow or solid, and the pres-ence of pith is quite frequent. Apiaceous plants are generally branched, with rare unbranched examples.**(Geoffriau, E. & al. 2020).**

Leaf:

Leaf arrangements are usually alternate **(Figure.1.1),** rarely opposite or in a whorl around the stem (verticillate).

The petioles are gen-erally present and typically sheathing at the base, with the sheaths quite often inflated (Ferula).

Leaf blades are very frequently compound, usually much in-cised or divided, giving the classical aspect of 'Umbel' leaf (Geoffriau, E. & al. 2020).

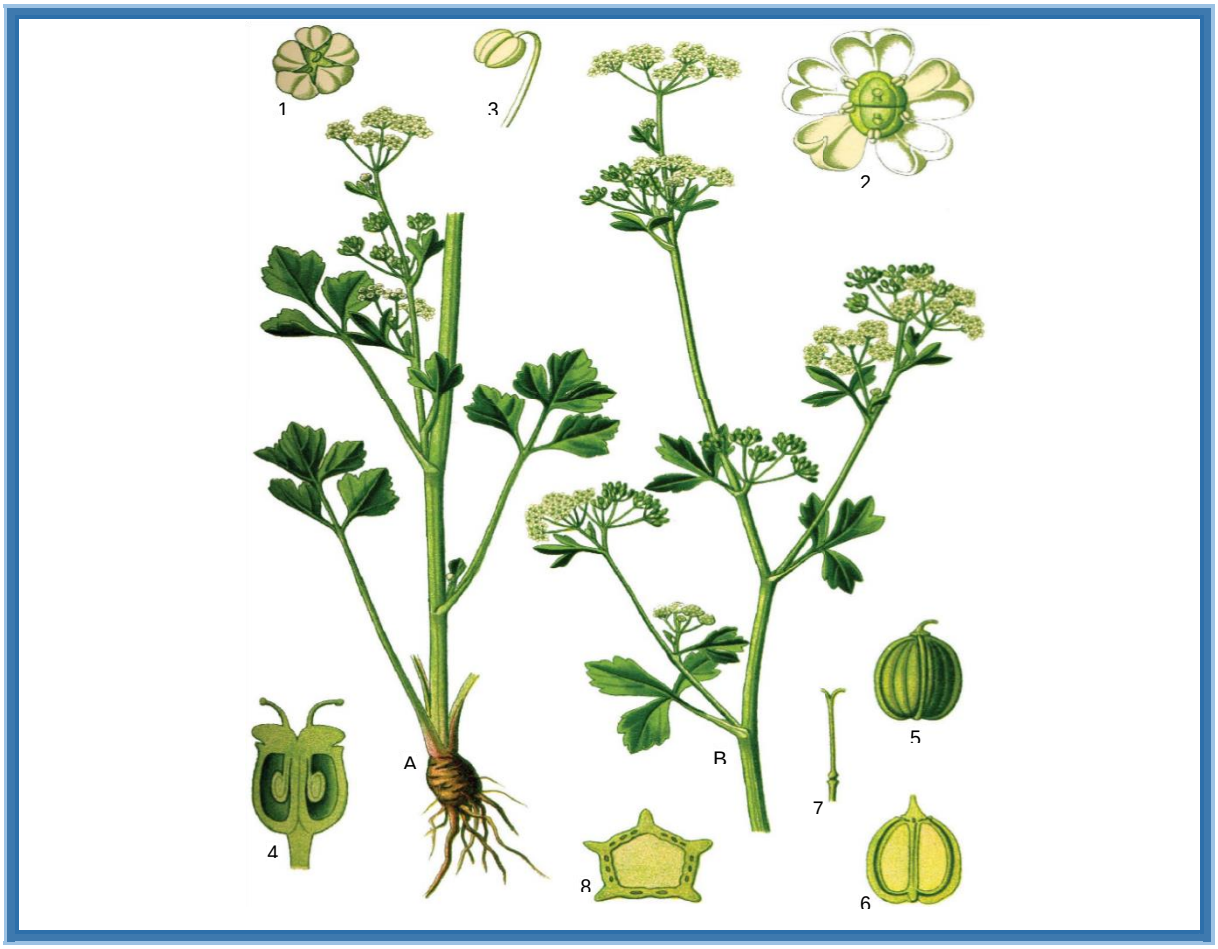


Fig.1.1: Apium graveolens, the type species of the family Apiaceae (Adapted from Thomé, 1885)**(Geoffriau, E. & al. 2020).**

The inflorescences:

The inflorescences of most Apiaceae are compound umbels, what inspired the early name of the family, 'Umbelliferon'. The umbels are typically subtended by bracts inserted at the base of the rays, forming an involucre; the bracts are often en-tire but can be toothed or dissected **(Daucus carota, Figure.1.2),** or sometimes ab-sent which is a useful feature for identification **(Geoffriau, E. & al. 2020).**

Fig.1.2: Flowering umbel of Daucus carota var.**(Geoffriau, E. & al. 2020).**

The flowers:

The flowers **(Figure.1.3)** of the Apiaceae are perfect to staminate, epigynous, 5-merous and actinomorphic (but sometimes zygomorphic for the outer flowers of the umbel or umbellule). Calyx lobes are typically short, and sometimes very small or obscure.

(Geoffriau, E. & al. 2020)

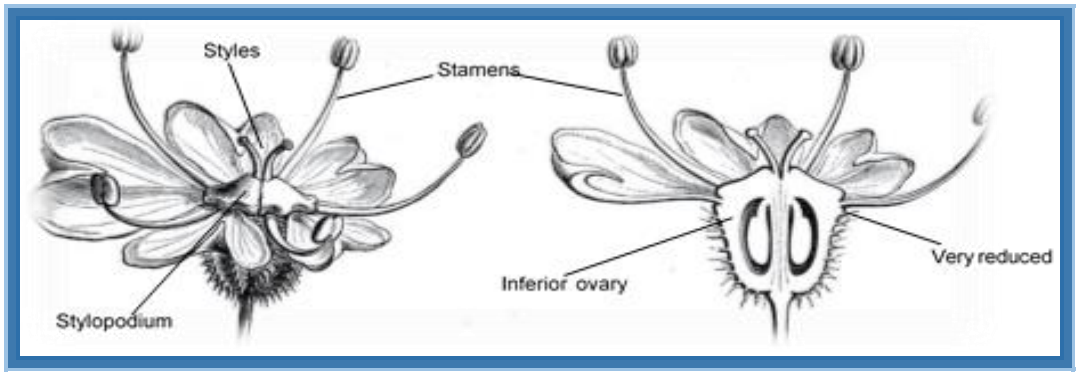


Fig.1.3: Flower morphology of Daucus carota **(Geoffriau, E. & al. 2020).**

The fruits

The fruit **(Figure.1.4)** of Apiaceae is dry (very rarely fleshy, Apiopetalum, Mackinlaya), splitting when ripe into two usually equal mericarps attached to a thin axis called a carpophore, bifurcated or entire**(Geoffriau, E. & al. 2020).**

It's a schizocarp with two cylindrical or flattened mericarps with a very thin seed coat, a fleshy, oily endosperm and a very small upright embryo with unequal cotyledons**. (Chaker el kalamouni, 2010).**

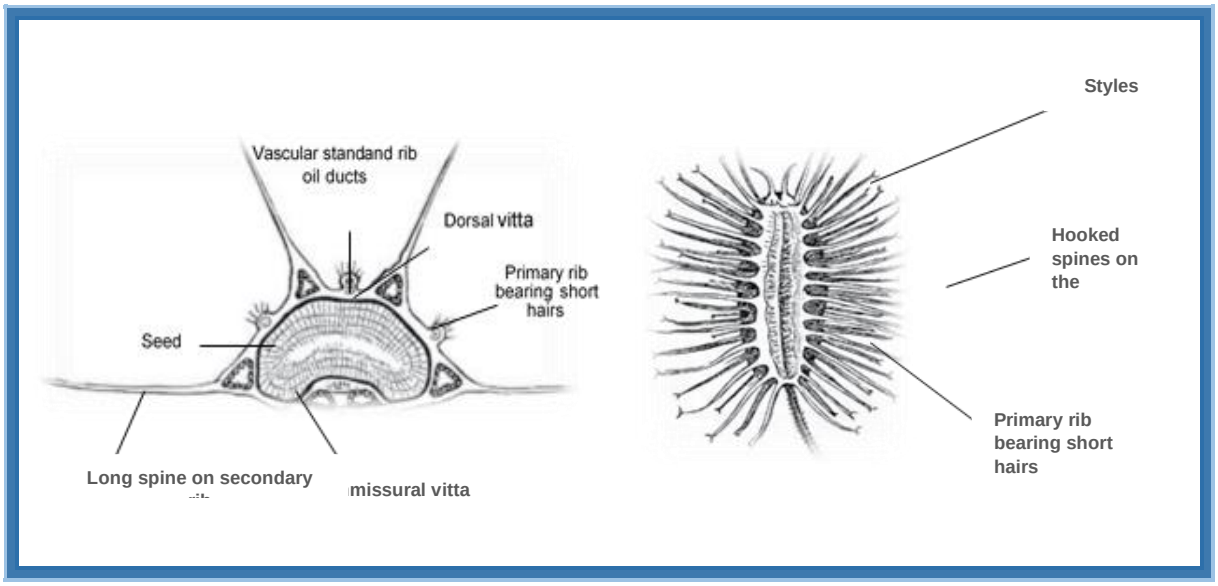


Fig.1.4: Fruit morphology of (A) Daucus involucratus and (B) Daucus carota **(Geoffriau, E. & al. 2020).**

I .2.2.Properties and benefits of the family:

The Apiaceae family possesses many economically important species, which makes it have many uses, some of which are food plants (carrots, fennel, celery ...), and others are spices that have been used for a long time in cooking due to the essential oils produced by their secretory channels (parsley, coriander). Caraway ...). In herbal medicine (drias,...) they are mainly attributed to the properties of the digestive system**(Daroui-Mokaddem, H. 2012).**

This family has economic and medical interests due to its richness in secondary metabolites, including coumarins, flavonoids, acetylene compounds, sesquiterpene lactones. This family of plants known for having a large amount of essential oil in almost

all of its anatomical organs. So far, 760 essential oils have been isolated from them, which makes it a wealth of Apiaceae essential oils**(Chaker el kalamouni, 2010).**

I.2.3. Classification:

According to the Cronquist Classification **(Gómez, F.L.M. 2007)**the systematic position of the Apiaceae family is as follows:

Table.1.2: classification of the "Apiaceae" family

Reign:	Plantae
Sub-reign:	Tracheophyta
Division	Angiosperm
Class	Dicotyledon
Subclass	Archychlamideae
Ordre:	Umbelifloral
Family	Umbelliferae = Apiaceae

I.3. Genre *Thapsia*:

Thapsia is a genus belonging to the Apiaceae family. Are flowering plants with 41 species native to Africa, Asia and Europe.

Thapsia garganica.L is a widespread species in the Mediterranean basin, which inhabits roadsides and fields **(Gómez, F.L.M. 2007).**

I.3.1. Vernacular names:

Scientific name: *Thapsia garganica.*

Common Name: *Tapisia*, بونافع ,الدرياس

I.3.2. Description of the species:

Thapsia garganica it is a perennial plant, with an erect, branching flowering stem, reaching a height of about 1.50 meters. It has large leaves in tufts, very indented, linearly divided with peduncles sheathed at the base, the upper leaves are reduced to a thick, green-gray sheath like the stem.

The flowers are small, yellowish, arranged in large, nearly spherical umblas.

The fruits are oval, reach more than 2 cm in length, wide-winged. The roots are rhizome-shaped **(Figure.1.5).**Flowering occurs between April and July.

Fig.1.5: Photographs of *Thapsia garganica.*

I .3.3.Classificationgenre *Thapsia garganica*:

Table.1.3: Classification genre *Thapsia garganica.*

Reign	Plantae
Family	**Umbelliferae = Apiaceae**
Genus	**Thapsia**
Species	**Thapsia garganica**

I .3.4.Pharmacological properties:

Thapsia garganica (L) is a perennial plant that belongs to the family Apiaceae and is widely used in Algeria because of its important bioactive properties, is an ancient medicinal plant that mainly contains terpenes, which are sesquiterpene lactones. The roots mainly contain volatile constituents as sesquiterpene lactones (STL), including thapsigargin. **(Drew, D.P. 2012).**

The sesquitepene lactones of the species *Thapsia garganica* belong to the group of guaianolides.

The thapsigargins are the dominant components of this plant and two major compounds are characterized in it are thapsigargine and thapsigargicine, which have important

biological properties, and have been used medicinally. Cellular toxicity is one of the biological properties of these substances **(Drew D.P. & al. 2009).**

The flagship molecule of thapsigargines (Tg), it is a sesquiterpene lactone whose pro-drug derivatives are used in phase I for the treatment of prostate cancer **(Christensen.S.B. & al. 2009),** it is extracted roots and leaves of Thapsia Garganica.

Thapsigargine is able to induce the release of histamines from various cells in the body.

This property is at the origin of the vesicant character but also of human intoxication if it is consumed**(H. Ali, S. and al, 1985).**

Sesquiterpene lactones (STL) are mainly in plants of the Asteraceae families, and more marginally in Apiaceae and Magnoliaciae. Their toxicity has long been demonstrated (illustration of their strong biological activities) and they often appear in the form of colorless substances with a bitter taste. To date, there are more than five thousand isolated STLs biological activities are often marked (in particular, anti-inflammatory and anti-tumor drugs are found). They are present in traditional medicine (in particular for their anti-inflammatory properties) **(Macé, F. 2012).**

I.3.5. Uses in traditional medicine:

Since ancient times, the roots and seeds of *Thapsia garganica* been used in traditional medicine in Europe and in some Arab countries on the Mediterranean coast.

Extracts from the roots of *Thapsia garganica* used for the treatment of lung diseases, colds and relief of rheumatic pain **(Gómez F. L. M. 2007).**in French, 1971. They contain strong irritants to the skin, constituting an important cause of contact dermatitis, which manifests itself by erythema, itching and the formation of small vesicles.

The main active compounds in the majority of root extracts responsible for these effects identified **by (Christensen,S.B.& al. 1982)** as sesquiterpenes known as thapsigargine and thapsigargicine.**(figure.1.6).**

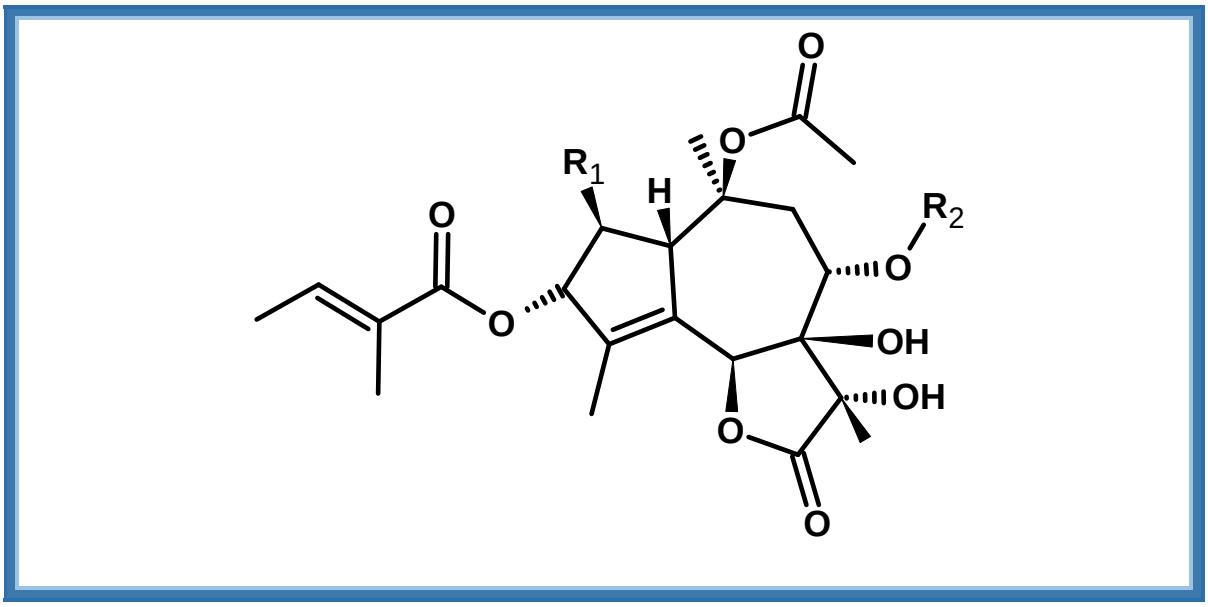


Fig.1.6: Structure of the thapsigargine skeleton of the genus *Thapsia* **(Andrews,S.P. & al. 2007; Christensen,S.B.1997)** in (**Drew,D.P. & al. 2009).**

I.3.6. Cytotoxicity:

A study on the cytotoxicity of tapsygargin and phenylpropanoid esters isolated from *Thapsia garganica* flowers conducted by **(Liu,H. & al. 2006).** The tests done on leukemia, and breast cancer cell. IC50s revealed that thapsigargine has higher cytotoxic potentials compared to phenylpropanoid esters

Reference list:

هيكل محمد السيد. (1993). النباتات الطبية و العطرية كيمياؤها إنتاجها فوائدها.

يوسفي محمد, غراف نور الدين, & العابد إبراهيم.(2009). دراسة الفعالية المضادة للبكتريا والمضادة للأكسدة لمستخلص القلويدات الخام لنبات الضمران.Traganum nudatum

أبو زيد،ش.ن. (1992).النباتات العطرية ومنتجاهتا الزراعية والدوائية. الدار العربية للنشر والتوزيع.

*Ahn, K. (2017). The worldwide trend of using botanical drugs and strategies for developing global drugs. BMB reports, 50(3), 111.

*Sofowora, A. (2008). Medicinal Plants and Traditional Medicine in Africa. 3rd. Ibadan, Nigeria: Spectrum Books.

*Evans, WC. (2008). Trease and Evans' Pharmacognosy. 16th Edition. London: WB Saunders Company Ltd; 2008n: Spectrum Books.

*Donna J. Schroeder, Shalini S. Lynch Annals. (1994) of Pharmacotherapy, vol. 28, 9: pp. 1038-1044. First Published Sep 1.

*Decaux I. (2002). Phytothérapie: mode d'emploi.Ed Le Bien Public:p 6-7

*Rodriguez, R.L. (1957) Systematic anatomical studies on Myrrhidendron and other woody Umbellales. University of California, Publications in Botany 29(2), 145–318.

*Plunkett, G.M., Pimenov, M.G., Reduron, J.-P., Kljuykov, E.V., Lee, B.-Y., van Wyk, B.-E., Tilney, P.M., Watson, M.F., Ostroumova, T.A., Spalik, K., et al. (2018) Apiaceae. In: Kadereit, J. and Bittrich, V. (eds) The Families and Genera of Vascular Plants. Vol. 15. Flowering Plants. Eudicots. Springer, New York, pp. 9–20

*Magee, A. R., Calviño, C. I., Liu, M., Downie, S. R., Tilney, P. M., & Wyk, B. E. V. (2010). New tribal delimitations for the early diverging lineages of Apiaceae subfamily Apioideae. Taxon, 59(2), 567-580.

*Pierre Quézel et Sébastien Santa (préf. Louis Emberger), Nouvelle flore de l'Algérie et des régions désertiques méridionales, Paris, CNRS, 1962-1963, 1170 p.

*Geoffriau, E., & Simon, P. W. (Eds.). (2020). Carrots and Related Apiaceae Crops (Vol. 33). CABI.

*Chaker El Kalamouni . (2010). Caractérisations chimiques et biologiques d'extraits de plantes aromatiques oubliées de Midi-Pyrénées (Doctoral dissertation).

*Daroui-Mokaddem, H. (2012). Etude phytochimique et biologique des especes: Eucalyptus globulus (Myrtaceae), Smyrnium olusatrum (Apiaceae), Asteriscus maritimus et Chrysanthemum trifurcatum (Asterarceae) (Doctoral dissertation, Doctoral Thesis of Biochemistry, Badji Mokhtar-Annaba University. www. biblio. univ-annaba. Dz.

* Gómez F. L. M. (2007). Síntesis de análogos de las tapsigarginas. Mémoire en vue d'obtention du grade de doctorat en chimie. Département de chimie organique. Faculté des sciences. Université de Cádiz. puerto real. Espagne.

*Drew, D. P., Rasmussen, S. K., Avato, P., & Simonsen, H. T. (2012). A comparison of headspace solid-phase microextraction and classic hydrodistillation for the identification of volatile constituents from Thapsia spp. provides insights into guaianolide biosynthesis in Apiaceae. Phytochemical Analysis, 23(1), 44-51.

*Drew D.P., Krichau N., Reichwald K. & Simonsen H.T. (2009). Guaianolides in apiaceae: perspectives on pharmacology and biosynthesis. Phytochem.Rev. 8:581– 599.

*Christensen S. B., Skytte D. M., Denmeade S. R., Dionne C., Møller J. V., Nissen P. & John T. Isaacs J. T. (2009). A Trojan Horse in Drug Development: Targeting of Thapsigargins Towards Prostate Cancer Cells. Anti-Cancer Agents in Medicinal Chemistry. 9, 3, 276-294.

*H. Ali, S. Brogger Christensen, J.C. Foreman, F.L. Pearce, W. Piotrowski et O. Thastrup, (1985). « The ability of thapsigargin and thapsigargicin to activate cells involved in the inflammatory response ». British Journal of Pharmacology, 85, 705-712.

Macé, F. (2012). Approche efficace des thapsigargines (guaianolides) et synthèse d'azulènes rouges via un intermédiaire commun de type bicyclo [5.3. 0] décane (Doctoral dissertation, Grenoble)

*Christensen S. B., Larsen I. K. & Rasmussen U. (1982). Thapsigargin and Thapsigargicin : Two Histamine Liberating Sesquiterpene Lactones from Thapsia garganica. X-ray Analysis of the 7, 11-Epoxide of Thapsigargin. J. Org. Chem. 47 : 4 : 649-652.

*Christensen, S. B., Andersen, A., & Smitt, U. W. (1997). Sesquiterpenoids from Thapsia species and medicinal chemistry of the thapsigargins. Fortschritte der Chemie organischer Naturstoffe/Progress in the Chemistry of Organic Natural Products, 129-167

*Andrews, S. P., Ball, M., Wierschem, F., Cleator, E., Oliver, S., Högenauer, K., ... & Ley, S. V. (2007). Total Synthesis of Five Thapsigargins: Guaianolide Natural Products Exhibiting Sub-Nanomolar SERCA Inhibition. Chemistry–A European Journal, 13(20), 5688-5712.

*Liu H., Jensen K. G., Linh My Tran L. M., Chen M., Zhai L., Olsen C. E., Sohoel H., Denmeade S. R., Isaacs J.T. & Christensen S.B. (2006). Cytotoxic phenylpropanoids and an additional thapsigargin analogue isolated from Thapsia garganica. Phytochemistry. 67 : 2651–2658.

Chapter II:

Secondary metabolites

Plant secondary metabolites are a diverse group of molecules that are involved in the adaptation of plants to their environment but are not part of the primary biochemical pathways of cell growth and reproduction **(Makkar, H.2007)**, whereas plants do interact with the environment using such molecules either to resist pests or to promote pollination.

Secondary metabolites showed a range of biological activity **(Table.2.1)** such as antioxidant, anti-proliferative, immunosuppressant, anti-infective, cholesterol lowering and growth promoting also termed as bioactive molecules. Such molecules have a wide range of commercial and pharmaceutical utility, for example, shikonins as dyes/therapeutic, vanillin, and capsaicin as flavors, nicotine as stimulants, taxol, artemisinin, and atropine and quinine as therapeutic agents.**(Walton, N. & al 2003; Siddiqui, M. & al .2017**).

Table.2.1: Biological properties of secondary plant metabolites (Gelaw, H.2013).

Classification	Main groups of compound	Biological function
Antibacterial & Antifungal	Terpenoids, alkaloids, phenolics	Inhibitors of micro-organisms, reduce the risk of fungal infection
Antioxidants	Polyphenolic compounds, flavonoids, carotenoids, tocopherols, ascorbic acid	Oxygen free radical quenching, inhibition of lipid peroxidation
Anticancer	Carotenoids, polyphenols, curcumine, Flavonoids	Inhibitors of tumor, inhibited development of lung cancer, anti-metastatic activity
Detoxifying Agents	Reductive acids, tocopherols, phenols, indoles, aromatic isothiocyanates, coumarins, flavones, carotenoids, retinoids, cyanates, phytosterols	Inhibitors of procarcinogen activation, inducers of drug binding of carcinogens, inhibitors of tumourogenesis
Other	saccharides Alkaloids, terpenoids, volatile flavor compounds, biogenic amines	Neuropharmacological agents, anti-oxidants, cancer chemoprevention

II.1. Classification of secondary plant metabolites:

The exact classification of secondary metabolites could have not been performed so far, because of the wide variety of them.

Secondary constituents are the remaining plant chemicals such as alkaloids, terpenes, flavonoids, lignans, plant steroids, curcumines, saponins, phenolics, flavonoids and glucosides **(Hahn.N.I. 1998) (figure2.1).**

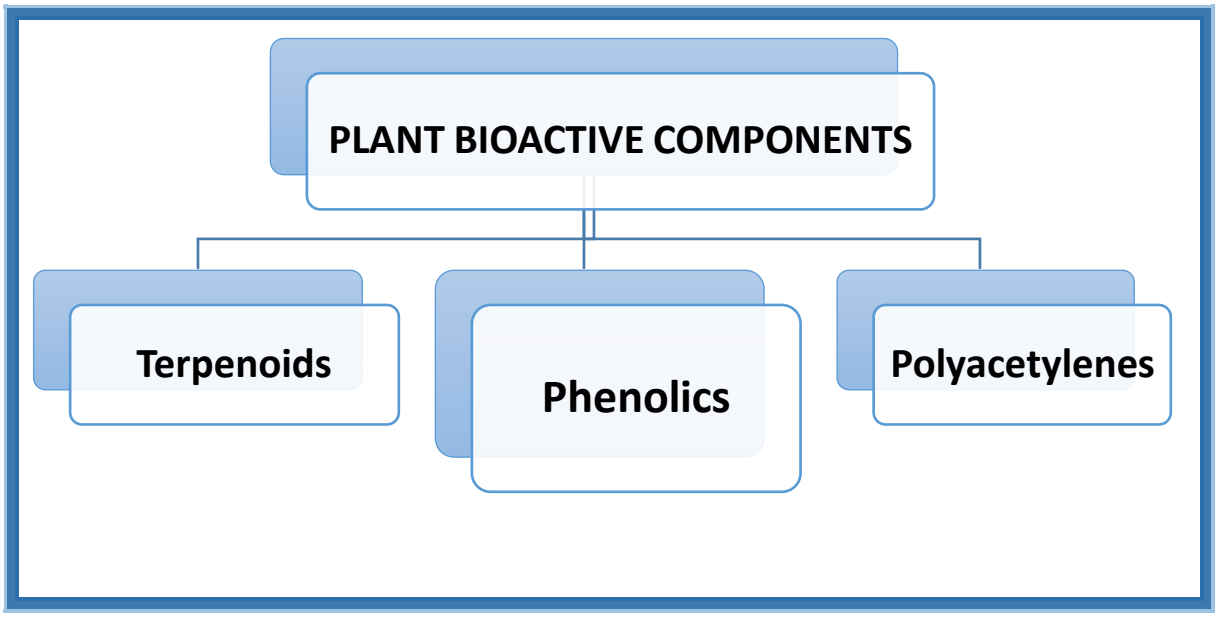


Fig.2.1: Classification of the plant secondary metabolites**(Siddiqui, M. & al .2017).**

Literature survey indicate that phenolics are the most numerous and structurally diverse plant phytocontituents **(figure2.2)**

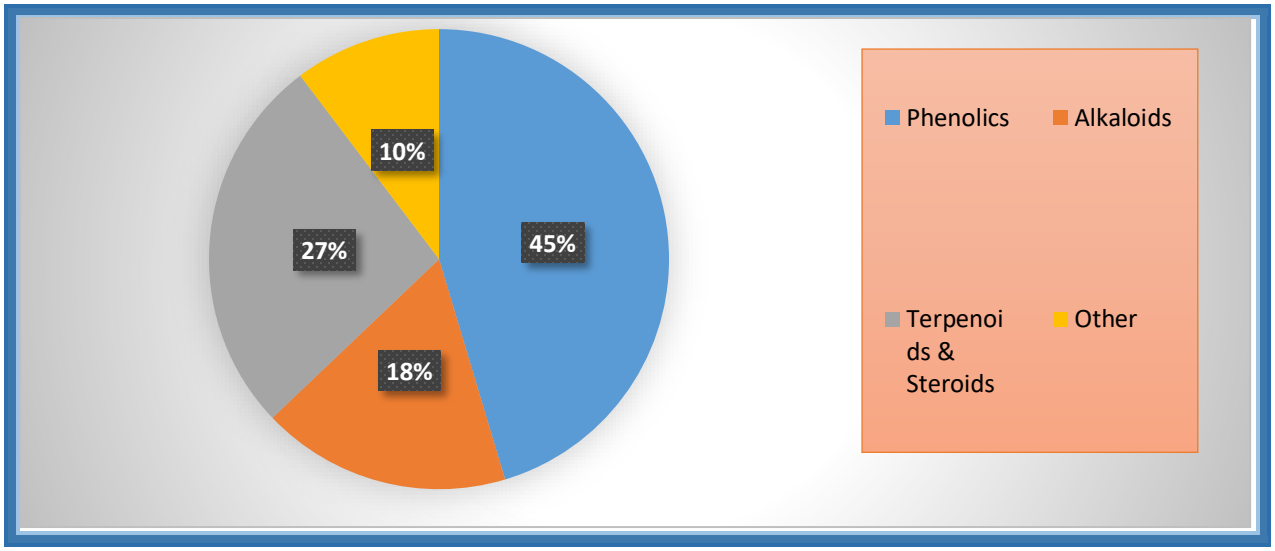


Fig.2.2: Pie chart representing the major groups of plant **(Gelaw, H.2013).**

II.1.1.Phenols and polyphenols:

Phenolic compounds are a large and complex group of chemical constituents found in plants. The three most important groups of dietary phenolics are flavonoids, phenolic acids, and polyphenols **(Gelaw, H.2013).**

Polyphenols are the largest and most widespread group of secondary metabolites in the plant kingdom. They are an integral part of human and animal nutrition. More than 8000 phenolic structures are currently known **(figure.2.3),**ranging from simple low molecular weight phenolic molecules such as phenolic acids to highly polymerized compounds such as tannins **(Martin.S. & al . 2002)**.

Phenolic compounds are considered to be good antioxidants due to their high redox potential and their relative stability to radicals **(Jovanovic,S.V. & al 2000**).They have antioxidant properties and are able to scavenge free radicals **(Middleton,E. & al., 2000).**

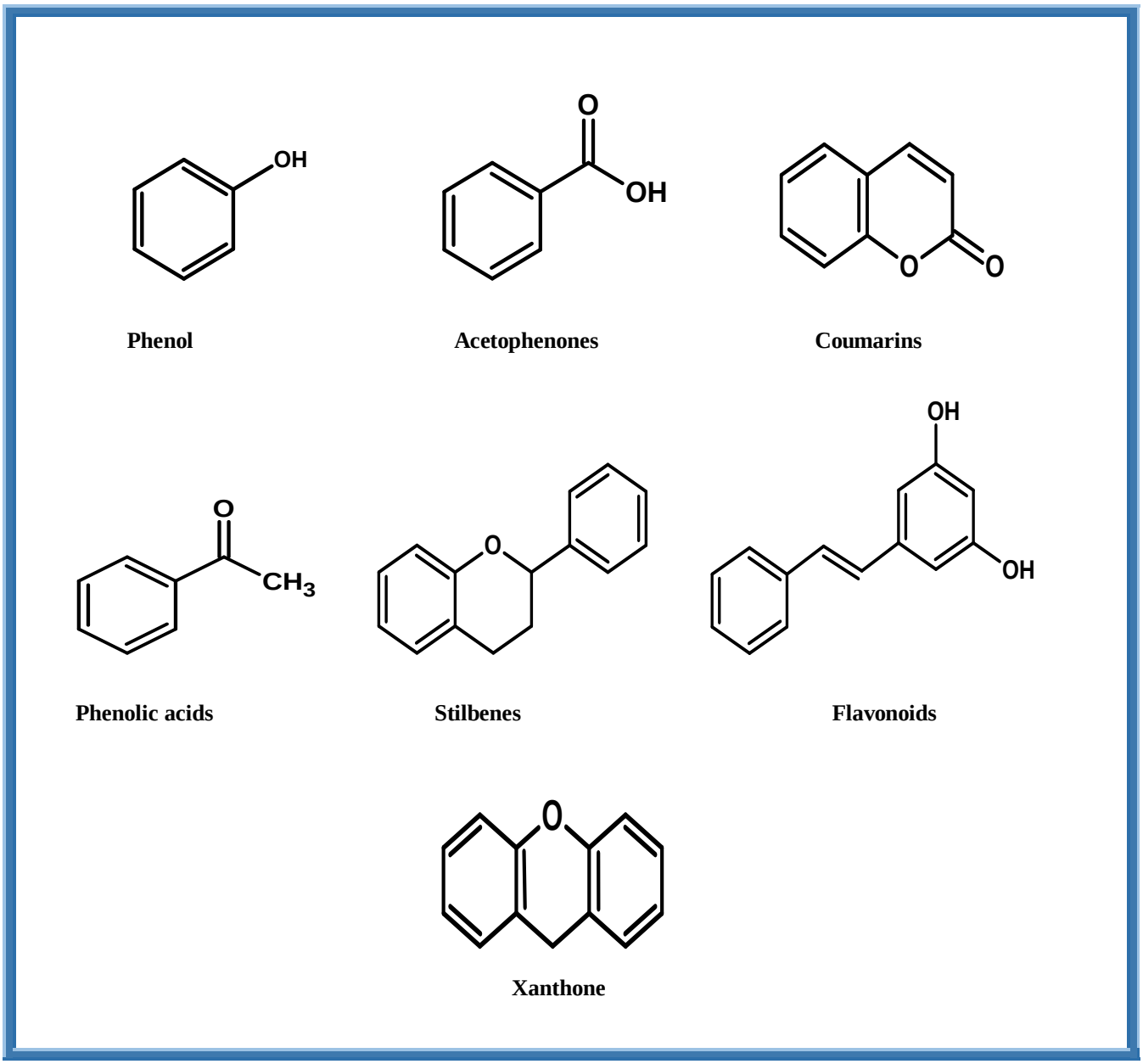


Fig.2.3: Basic skeleton of phenolic compounds.

II .1.2.Polyphenols classification:

Phenolic are hydroxyl group (-OH) containing class of chemical compounds where the (-OH) bonded directly to an aromatic hydrocarbon group. They can be grouped into many classes **(figure.2.4).**

Which are differentiated first by the complexity of the basic skeleton (ranging from a simple C6 to very palymé-rized forms), then by the degree of modifications of this skeleton (degree of oxidation, hydroxylation, methylation …), finally by the possible bonds of these molecules. **(Sarni-Manchado, P. & al.2006) (Table. 2.2).**

Phenol (C6H5OH) considered the simplest class of this group of natural compounds.

Flavonoids are the largest group of plant phenols and the most studied. Phenolic acids form a diverse group that includes the widely distributed hydroxybenzoic and hydroxycinnamic acids.

Phenolic polymers, commonly known as tannins, are compounds of high molecular weight that are divided into two classes: hydrolyzable and condensed tannins (Mamta Saxena and al 2013).

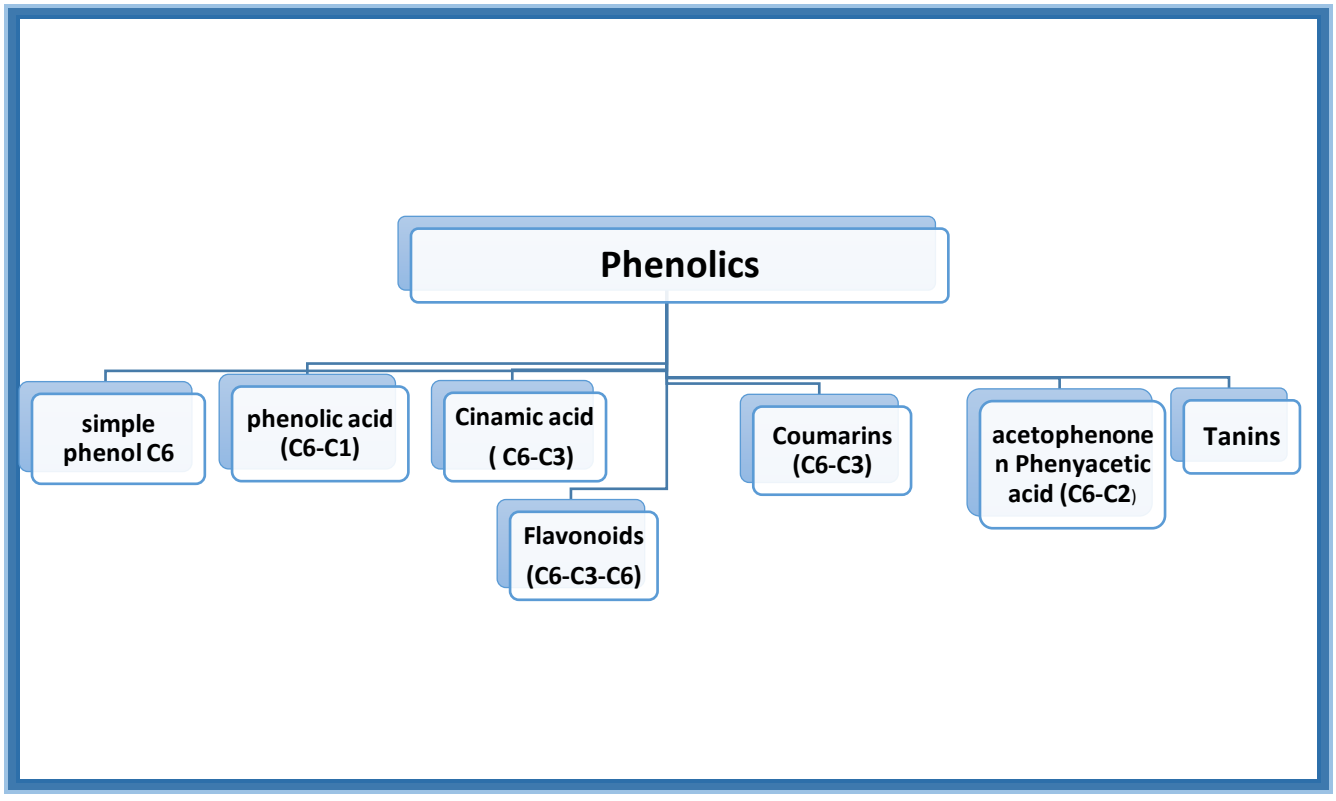


Fig.2.4: Classification of the plant secondary metabolites (Siddiqui, M. & al .2017).

Table.2.2: Main classes of phenolic compounds (Bruneton, J.1999; Hennebelle, T.2006).

Number of carbon atom	Basic skeleton	Class	Examples
6	**C6**	Simple phenols Benzoquinones	Cathecol, hydroquinone
7	**C6-C1**	Phenolic acids	Salicylic acid, Vailinilic acid, Gallic acid.
8	**C6-C2**	Acetophenones Tyrosine derivatives	3-acetyl6- methoxybenzaldehyde
9	**C6-C3**	Coumarins	Coumaric acid, Caffeic acid
10	**C6-C4**	Naphthoquinones	Shikonin
13	**C6-C1-C6**	Xanthones	Bellidifolin, mangoctin
14	**C6-C2-C2**	Stilbenes	Hydrangenol, Pinosylvin
15	**C6-C3-C6**	Flavonoids	Quercetin, Rotenoid
18	**$(C6-C3)_2$**	Lignans	matairesinol
30	**$(C6- C3-C6)_2$**	Bioflavonoids	Amentoflavone, Hinokiflavone
N	**$(C6-C3-C6)_n$**	Condensed tannins	Aesculitannins

II.1.3. Flavonoids:

Flavonoids are polyphenolic compounds that are ubiquitous in nature. More than 4,000 flavonoids have been recognised, many of which occur in vegetables, fruits and beverages like tea, coffee and fruit drinks**(Pridham, J. B. 1960).**The name flavonoids come from the term flavido for the outer layer of orange peels, but other authors have assumed that the term flavonoid is taken from flavus (flavus = yellow) instead **(Ghedira, K.2005).**

The flavonoids appear to have played a major role in successful medical treatments of ancient times, and their use has persisted up to now. **(Harborne,J.B. & al.1999).**

- Chemical structure and classification:

The flavonoids are benzo-γ-pyran derivatives **(Škerget, M. & al.2005).** They all sharing the same basic 15-atom structure formed by two aromatic rings linked by three carbons (C6-C3-C6) which can bind together forming an oxygen cycle **(figure.2.5).**

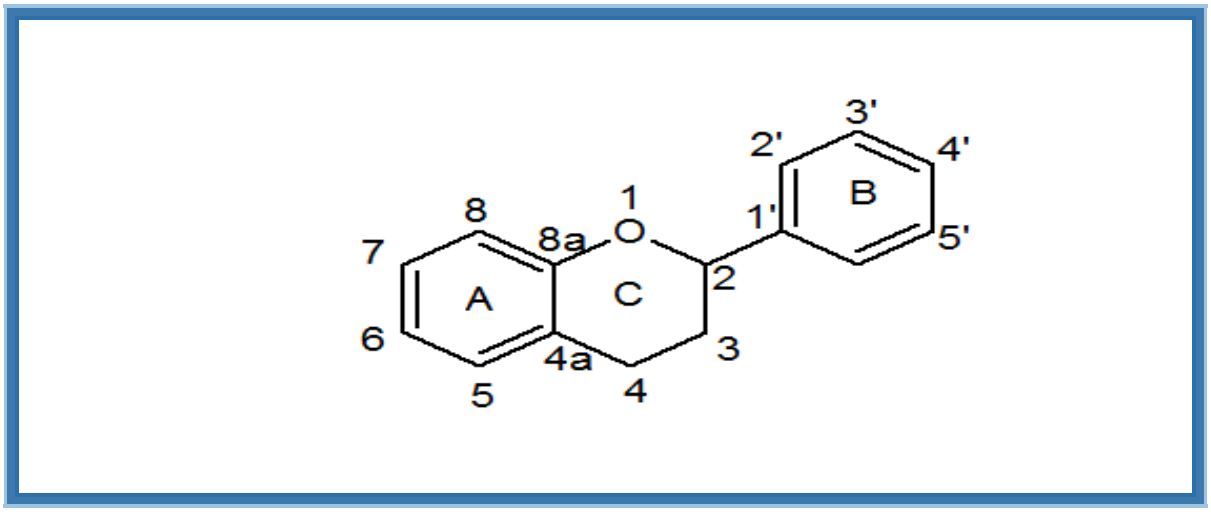
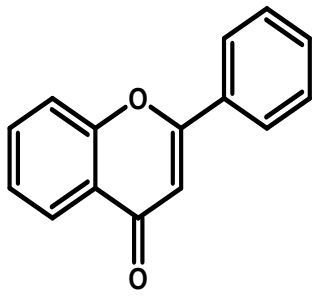
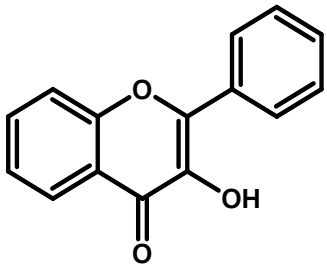


Fig.2.5: basic structure of flavonoids.

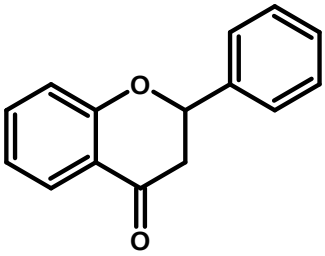
According to the degree of oxidation, flavonoids are classified into six classes: flavonols, flavones, flavanones, flavan-3-ols, isoflavones and anthocyanins **(Ghedira, K. 2005).(figure.2.6).**



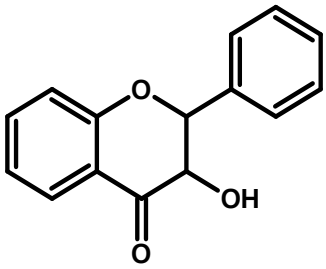
Flavone



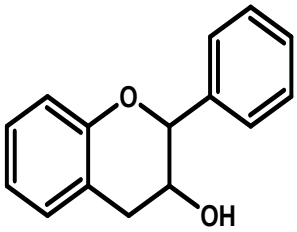
Flavonol



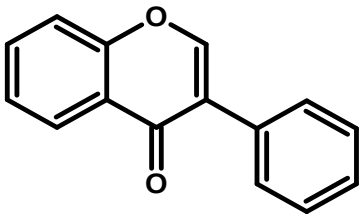
Flavanone



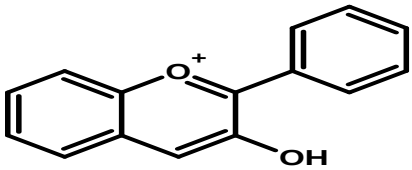
Flavanonol



Flavan-3-ols



Isoflavone



Anthocyne

Fig.2.6: Classification of flavonoids.

- Activity of Flavonoids:

Flavonoids have gained recent attention because of their broad biological and pharmacological activities. In these order, they have been reported to exert multiple biological property including antimicrobial, cytotoxicity, anti-inflammatory as well as antitumor activities but the best-described property of almost every group of flavonoids is their capacity to act as powerful antioxidants which can protect the human body from free radicals and reactive oxygen species.

The capacity of flavonoids to act as antioxidants depends upon their molecular structure. The position of hydroxyl groups and other features in the chemical structure of flavonoids are important for their antioxidant and free radical scavenging activities **(Tapas, A. R .& al.2008).**

II.1.4. Terpenoids:

The terpenoids are a class of natural products, which have derived from five-carbon isoprene units. Most of the terpenoids have multi cyclic structures that differ from one another by their functional groups and basic carbon skeletons. These types of natural lipids can be found in every class of living things, and therefore considered as the largest group of natural products **(Barton, D. & al.1999).**

- Classification and structure of Terpenoids:

Terpenes are widespread in nature, mainly in plants as constituents of essential oils (Table.2.3). Their building block is the hydrocarbon isoprene, CH2=C(CH3)-CH=CH2. Terpene hydrocarbons therefore have molecular formula (C5H8) n and they are classified according to the number of isoprene units **(figure.2.7),(Langenheim,J.H.1994).**

Table.2.3: Classification of Terpenoids **(Langenheim, J.H.1994).**

1.Hemiterpenoids:	**2.Monoterpenoids:**	**3.Sesquiterpenes:**
Consist of a single isoprene unit. The only hemiterpene is the Isoprene itself, but oxygen-containing derivatives of isoprene such as isovaleric acid and prenol is classify as hemiterpenoids.	Biochemical modifications of monoterpenes such as oxidation or rearrangement produce the related monoterpenoids. Monoterpenoids have two isoprene units. Monoterpenes may be of two types i.e linear (acyclic) or contain rings e.g. Geranyl pyrophosphate, Eucalyptol, Limonene, Citral, Camphor and Pinene.	Sesquiterpenes have three isoprene units e.g. Artemisinin, Bisabolol and Fernesol, oil of flowers, or as cyclic compounds, such as Eudesmol, found in Eucalyptus oil.
4 .Diterpenes:	**5 .Triterpenes:**	**6 .Tetraterpenoids:**
It composed for four isoprene units. They derive from geranylgeranyl pyrophosphate. There are some examples of diterpenes such as cembrene, kahweol, taxadiene and cafestol. Retinol, retinal, and phytol are the biologically important compounds while using diterpenes as the base.	It consists of six isoprene units e.g. Lanosterol and squalene found in wheat germ, and olives.	It contains eight isoprene units which may be acyclic like lycopene, monocyclic like gamma-carotene, and bicyclic like alpha- and betacarotenes.

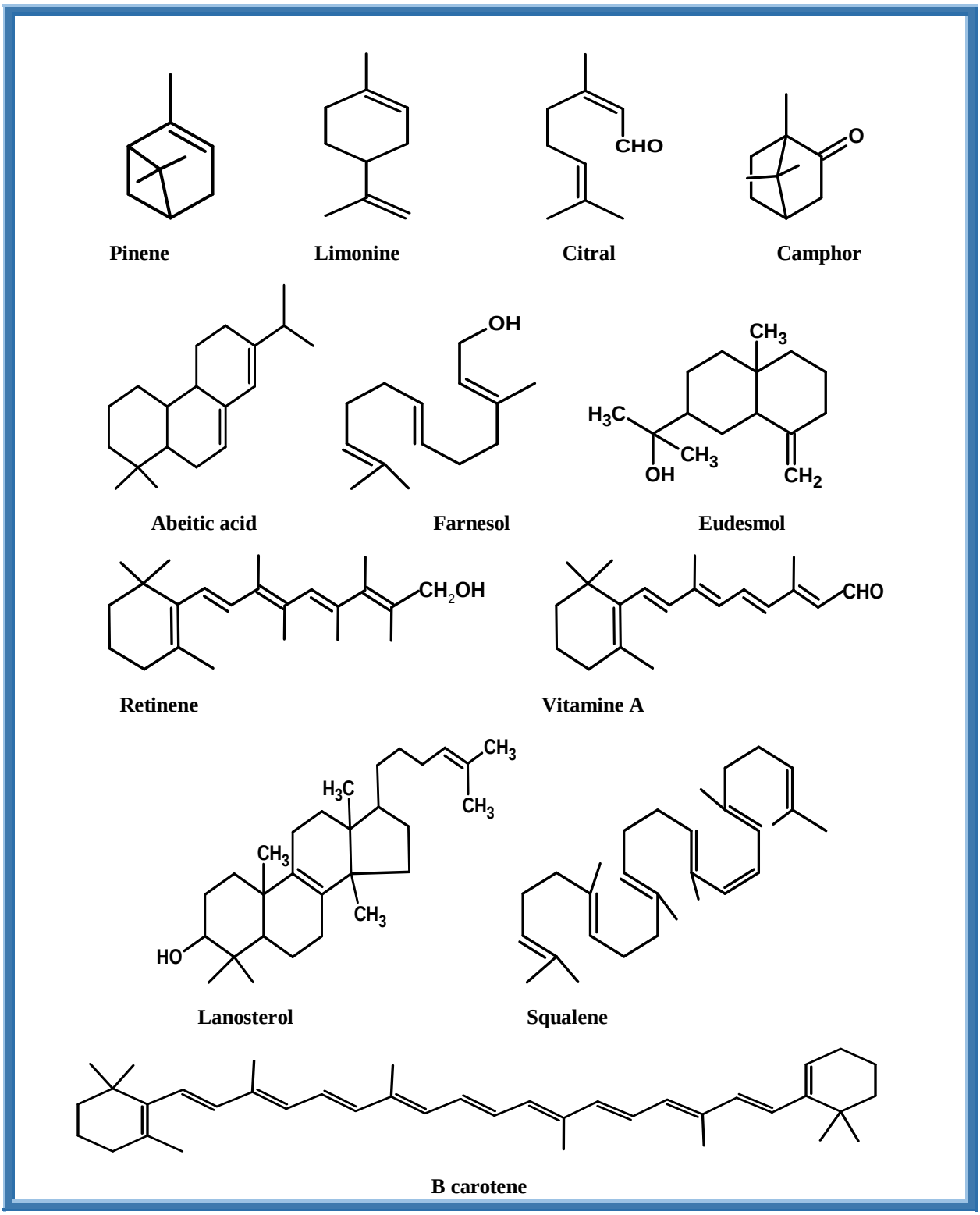


Fig.2.7: Structures of the important terpenes of each class.

- Activity of Terpenes :

Among plant secondary metabolites terpenoids are a structurally most diverse group; they function as phytoalexins in plant direct defense, or as signals in indirect defense responses, which involves herbivores and their natural enemies **(McCaskill,D. & al .1998).** Many plants produce volatile terpenes in order to attract specific insects for pollination or otherwise to expel certain animals using these plants as food **(Degenhardt,J. & al. 2003).**

Last, but not least, terpenes play an important role as signal compounds and growth regulators (phytohormones) of plants, as shown by preliminary investigations. In addition, terpenoids can have medicinal properties such as anticarcinogenic (e.g. perilla alcohol), antimalarial (e.g. artemisinin), anti-ulcer, hepaticidal, antimicrobial or diuretic (e.g. glycyrrhizin) activity and the sesquiterpenoid antimalarial drug artimisinin and the diterpenoid anticancer drug taxol **(Dudareva,N. & al.2004).**

II.1.5.Coumarins:

The name coumarin comes from "cumaru", which in the Amazonian language represents the tree of "Tonka". These compounds are very important and diverse and many of them occur naturally. Indeed, today, nearly a thousand coumarins have described in more than 800 plant species and in microorganisms **(Bouderdara, N.2013).**

Coumarins are formed in different parts of plants and mainly accumulate in fruits and roots, such as fluvial roots, followed by leaves and bark, as well as aged or damaged tissue **(Lacy, A.& al.2004).**

- Classification and structure of coumarins:

Coumarins, which are also derivatives of C6-C3 **(figure.2.8),** belong to the group of compounds known by benzo-α-pyrone, all of which are substituted at C7 by a hydroxy (Murray, R. D.2002). Coumarin is the starting point of a family of compounds, which are formed by substitution of its aromatic ring (Boutaoui, N.2012).

Therefore, according to the nature of the substituents, Coumarins can be classified into: simple coumarins, furocoumarins and pyranocoumarins.

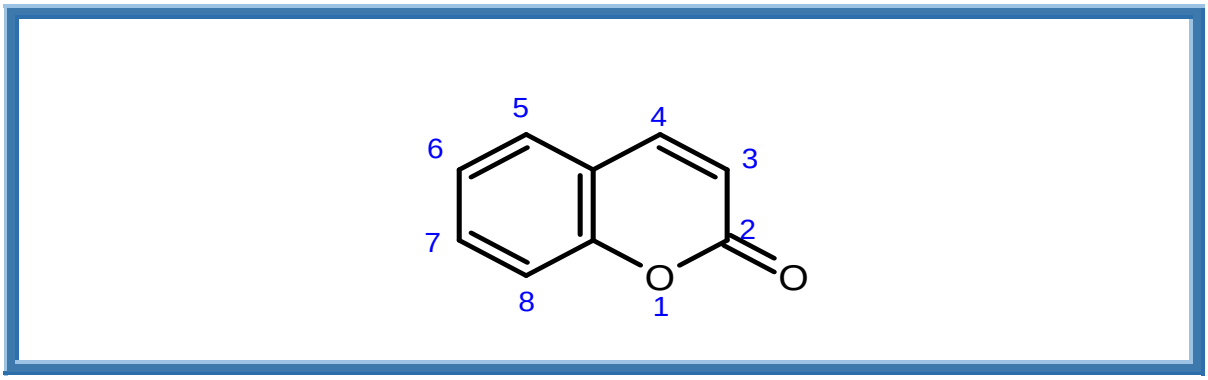


Fig.2.8: The mother structure of coumarin.

- Activity of coumarins :

Both natural and synthetic coumarin derivatives are considered to have a wide range of biological activity, such as anti-inflammatory, anti-cancer, anti-coagulant, anti-oxidant, as well as anti-HIV and anti-bacterial. Biological activity of coumarins has becoming an appealing point of studies owing to its different effects to diseases and less damage to normal cells. Previous studies demonstrated that coumarin chalcone fibrates can down-regulate the total cholesterol (TC), phospholipids (PL), and triglycerides (TG), and regulate the levels of VLDL, LDL and HDL **(Xu, L. & al.2015).**

II .1.6. Alkaloids:

Alkaloids are natural product that contains heterocyclic nitrogen atoms, are basic in character fig. The name of alkaloids derives from the “alkaline” and it was used to describe any nitrogen-containing base **(Mueller-Harvey,I. & al.1992).**They are naturally synthesis by a large numbers of organisms, including animals, plants, bacteria and fungi **(figure.2.9).** Some of the fires natural products to be isolated from medicinal plants were alkaloids when they first obtained from the plants materials in the early years of 19th century, it was found that they were nitrogen containing bases which formed salts with acid **(Rao,R. & al.1978).**

Due to their potent biological activity, most of the known alkaloids, around 12,000, have been exploited as drugs, stimulants, narcotics and as poisons. Unlike most other types of secondary metabolites, the many classes of alkaloids have unique biosynthetic origins **(Ziegler, J. & al.2008).**

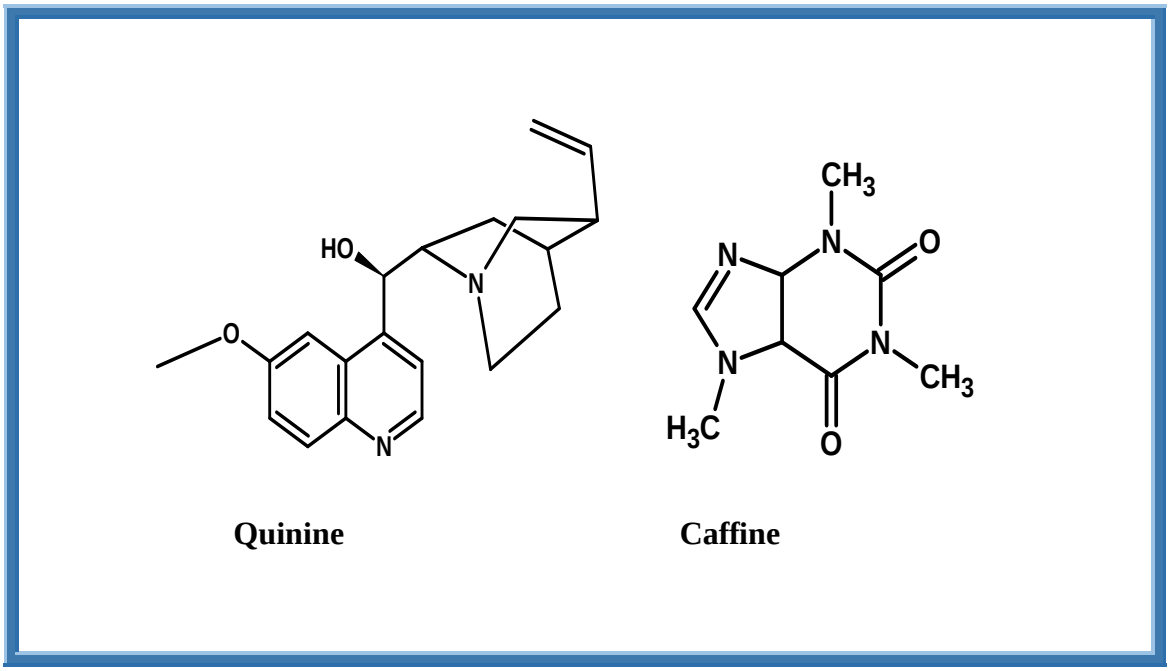


Fig.2.9: Structure of some alkaloids.

- Classification and structure alkaloids:

Alkaloids are so numerous and involve such a variety of molecular structure that their rational classification is difficult. However, the best approach to the problem is to group them into families, depending on the type of heterocyclic ring system present in the molecule **(Krishnan, R. & al.1983**). For historical reasons as also because of their structural complexities **(figure.2.10),** the nomenclature of alkaloids has not been systematized. The names of individual members are, therefore, generally derived from the name of the plant in which they occur, or from their characteristic physiological activity. The various classes of alkaloids according to the heterocyclic ring system they contain are listed below **(Table.2.4), (Gelaw, H. & al. 2012).**

Table.2.4: Different classes of alkaloids. **(Gelaw, H. & al. 2012).**

Pyrrolidine alkaloids:	**Pyridine alkaloids:**	**Pyrrolidine-pyridine alkaloids:**
They contain pyrrolidine (tetrahydropyrrole) ring system. E.g Hygrine found in Erythroxylum coca leaves.	They have piperidine (hexahydropyridine) ring system. E.g Coniine, piperine and isopelletierine.	The heterocyclic ring system present in there alkaloids is Pyrrolidinepyridine.E.g Myosmine, Nicotine alkaloid found in tobacco (Nicotiana tabacum) plant.
Pyridine-piperidine alkaloids:	**Quinoline Alkaloids:**	**Isoquinoline alkaloids:**
This family of alkaloids contains a pyridine ring system join to a piperidine ring system the simplest member is Anabasine alkaloid isolated from poisonous Asiatic plant anabasis aphyllan.	These have the basic heterocyclic ring system quinoline .E.g Quinine occurs in the bark of cinchona tree. It has been used for centuries for treatment of malaria.Synthetic drugs such as primaquinine have largely replace quinine as an anti-malarial.	They contain heterocyclic rig system isoquinoline. E.g Opium alkaloids like narcotine, papaverine, morphine, codeine, and heroine.

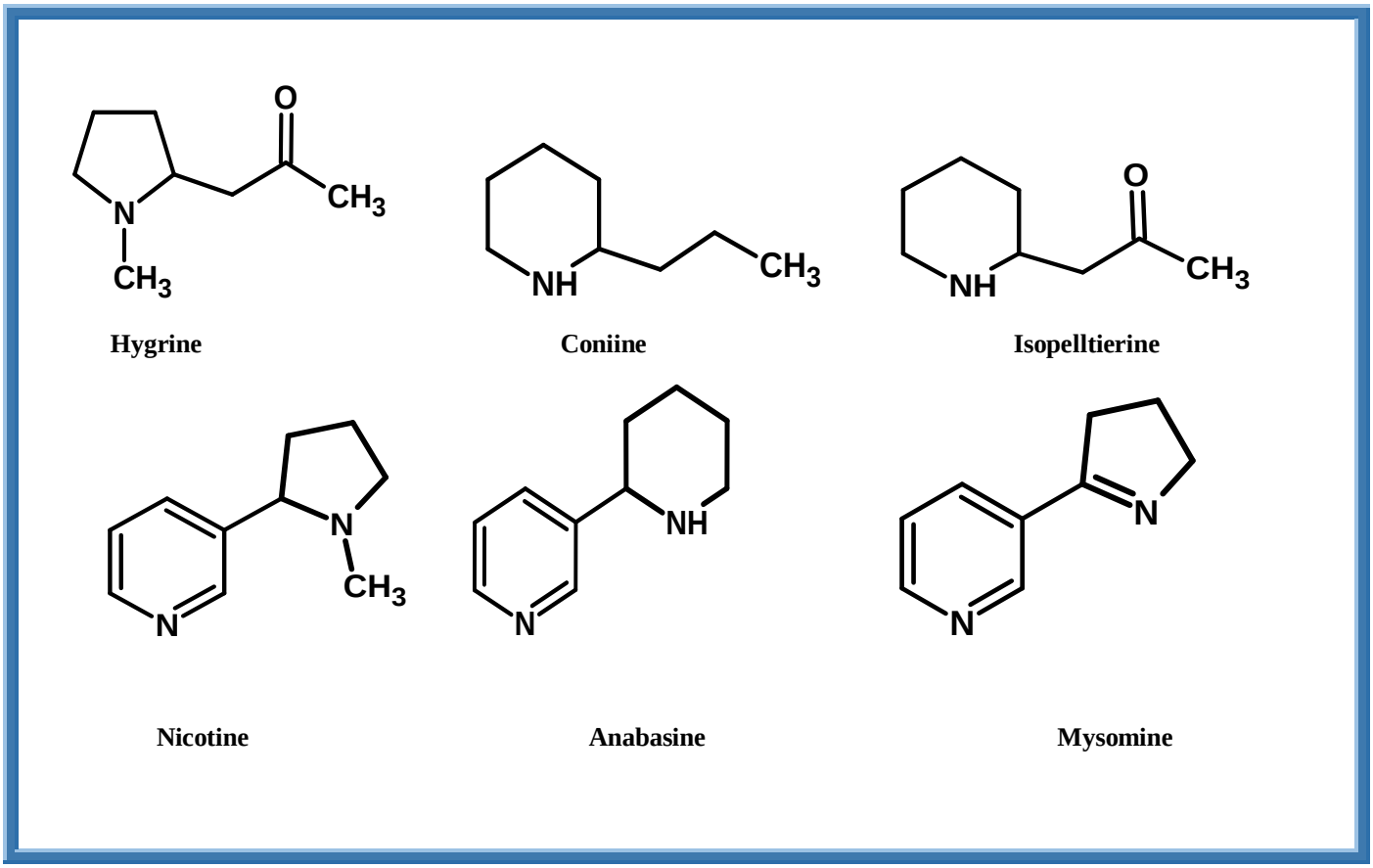


Fig.2.10: Structures of the important naturally occurring alkaloids.

- Activity of Alkaloids :

Alkaloids are significant for the protecting and survival of plant because they ensure their survival against micro-organisms (antibacterial and antifungal activities), insects and herbivores (feeding deterrens) and also against other plants by means of allelopathically active chemicals **(Molyneu,R. & al 1996).**

Also, they are particularly interesting substances for their pharmacological activities which are exerted in the most varied fields:

♦ At the level of the central nervous system as antidepressants (morphine, scopolamine) or stimulants (strychnine, caffeine),

♦ At the level of the autonomic nervous system: sympathomimetics (ephedrine) or sympatholytics, parasympathomimetics, anti-cholinergics and ganglioplegics. We should also note the existence of curariants, local anesthetics, anti-fibrillants, anti-tumor drugs, and anti-malarial drugs **(Bruneton,J. 1999).**

II .1.7.Essential oils:

The term 'essential oil' was used for the first time in the 16th century by the founder of the discipline of toxicology, Paracelsus von Hohenheim. Paracelsus named the active component of a drug, 'Quinta essential'**(Bouchaale, Z .2015).**Essential oils are oily, volatile and fragrant substances that are secreted by aromatic plants that are extracted by various processes including water vapor entrainment and hydro-distillation (Iserin and al., 2007). An essential oil can be a set of molecules for a chemist, an aroma for a perfumer or the quintessence or spirit of a plant for an alchemist **(Moro Buronza, A. 2008).**

Essential oils are used in perfumes and make-up products, as food preservers and additives, in sanitary products, in agriculture, and as natural remedies.

They are natural multi-component systems, they consist largely in small molecole, such as terpenes, usually formed from only carbon and hydrogen, but often also oxygen containing **(Masotti,V. & al. 2003; Angioni,A. & al. 2006).**

- **Location of essential oils in the plant:**

Essential oils are only found in higher plants. They are produced in the cytoplasm of secretory cells and usually accumulate in specialized glandular cells, often located on or near the surface of plant tissues. and covered with a cuticle. Then, they are stored in cells called essential oil cells (Lauraceae or Zingiberaceae), in secreting hairs (Lamiaceae), in secretory pockets (Myrtaceae or Rutaceae) or in secretory channels (Apiacieae or Asteraceae).

They can be stored in various plant organs: flowers (ylang-ylang, bergamot tree, rose, etc.), flowering tops (marigold, lavender, etc.), leaves (lemongrass, eucalyptus, bay leaf, etc.), roots (vetiver), rhizomes (ginger, turmeric, etc.), fruits (star anise, etc.), wood

(rosewood, sandalwood, etc.) or seeds (ambrette, nutmeg, etc.) **(Oussala,M.2006; Bruneton,J.1999; Teuscher, E, 2005).**

They differ in size, wall or content, sometimes characterizing a given family. They play an important role in determining the conditions of extraction of the volatile products they contain **(Bruneton,j.1999).**

- **Chemical composition of essential oils:**

Essential oils are complex blends that can contain over 300 different compounds. These compounds are volatile molecules belonging for the most part to the terpene family **(Bakkali,F. & al. 2008).**

Only the most volatile terpenes, that is to say those whose molecular mass is not too high are found there (i.e. mono-terpenes and sesquiterpenes of general formula (C5H8) n, oxygenated compounds derived fromthese hydrocarbons including alcohols, aldehydes, esters, ethers, ketones, phenols and oxides. It is estimated that there are over 1000 mono-terpenes of which 3000 are sesquiterpene structures. Other compounds include phenylpropanesand specific compounds containing sulfur or nitrogen **(Svoboda,k. & al. 1999).**

The figure shows the structure of some components found in essential oil.

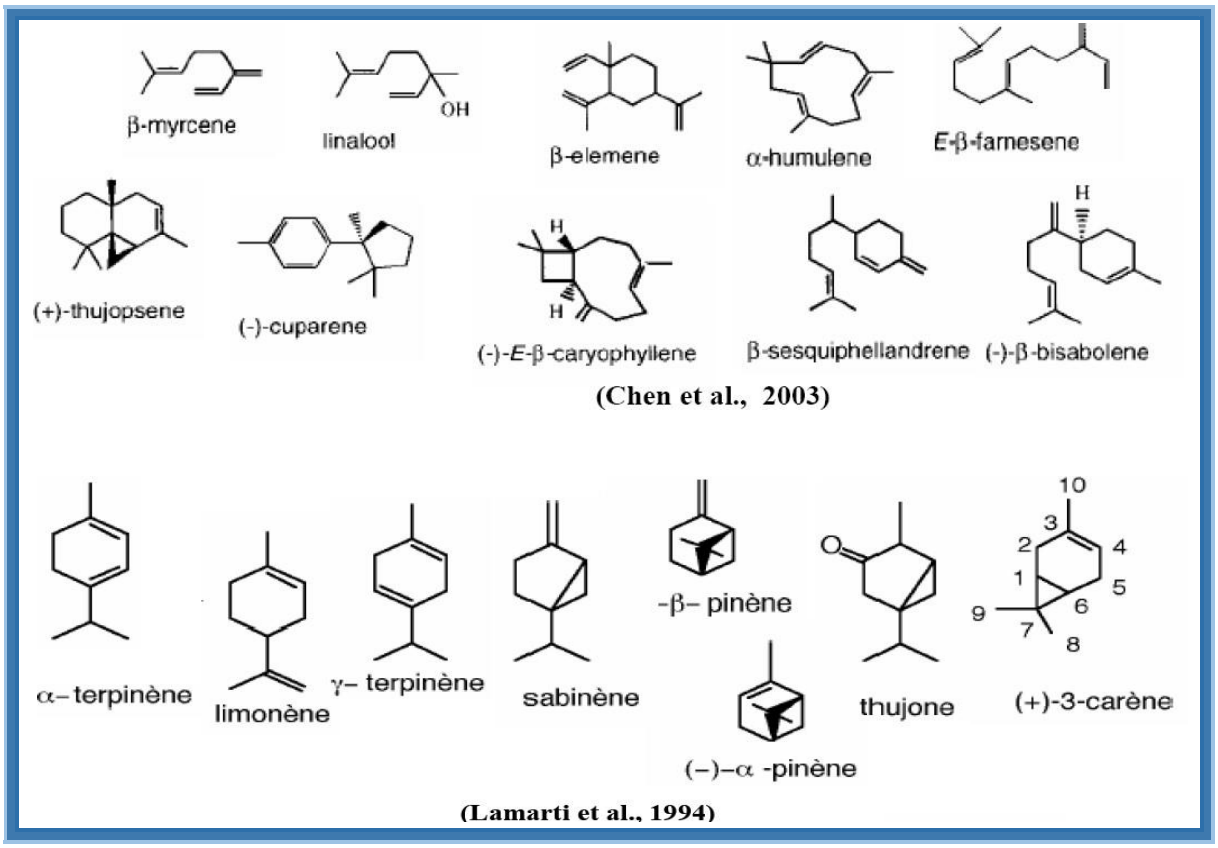


Fig.2.11: Structure found in essential oils.

- **Activity of essential oils:**

Do to their antibacterial, antifungal and insecticidal activities, essential oils have been largely employed for their properties already observed in natural environment. Nowdays more than 3000 essential oils are known, 300 of which are commercially important, especially for industries. Some essential oils have particular medicinal properties that have been praised to cure certain organ dysfunction or systemic disorder **(Simic, A.2004; Perry,N.S. & al.2003).**

- **Essential oils as antibacterial agents:**

The Ancient Egyptians used aromatic plants (and the essential oils content in them) in embalming, in that manner, bacteria stop to growth and decay was prevent. This was confirmed from strong in vitro evidence. In fact, essential oils can act as antibacterial agents against a wide spectrum of pathogenic bacterial strains, including: Listeria monocytogenes, L. innocua, Salmonella typhimurium, Escherichia

coliO157:H7, Shigella dysenteria, Bacillus cereus, Staphylococcus aureusand Salmonella typhimurium and many more. Also, Commiphora africana (A.Rich.) Endl. Essential oil can inhibit some pathogenic bacterial strains, such as Staphylococcus aureus, Escherichia coli, Candida albicans and Helicobacter pylori **(Properzi,A. & al.2013)**

- **Essential oils as antioxidant agents:**

Free radicals and other reactive oxygen species produce oxidation of proteins, amino acids, unsaturated lipids and DNA. Reactive oxygen species produce molecular alterations related to aging, arteriosclerosis and cancer, Alzheimer's disease, Parkinson's disease, diabetes and asthma. The human body has defense mechanisms against free radicals present in almost all cells. Is possible that occur an imbalance between free radical production and their removal by the body's antioxidant system; this imbalance bring to a phenomena known as 'oxidative stress'. Balance between free radicals and antioxidants can be recovered from an external supply of antioxidants. Essential oils are rich in phenolic compounds, and for this reason, attract investigators to evaluate their activity as antioxidants or free radical scavengers

(Properzi,A. & al.2013).

II.2.The different methods of extraction and separation of secondary metabolites:

The secondary metabolites of the medicinal plant are the components naturally present in this plant; They give it its therapeutic activity. Often these components are in very small quantities in the plant: they make up only a small percentage of the total weight of the plant, but these are the main ones. Therefore, it is necessary to carry out an extraction process which consists of removing (extracting) one or more chemical species from a solid or liquid medium and separating them from each other. Several scientific techniques are available to extract and separate the active ingredients from medicinal plants.

II.2.1. Extraction methods:

Different methods are used for the extraction of plant essences. In general, the choice of the extraction method will depend on the nature of the plant material to be treated (seeds, leaves, twigs), the nature of the compounds (for example, flavonoids, essential oils, tannins), the yieldin oil and the brittleness of certain constituents of oils at high temperatures. We divide the methods into two types, Conventional and non-Conventional extraction **(figure.2.12).**

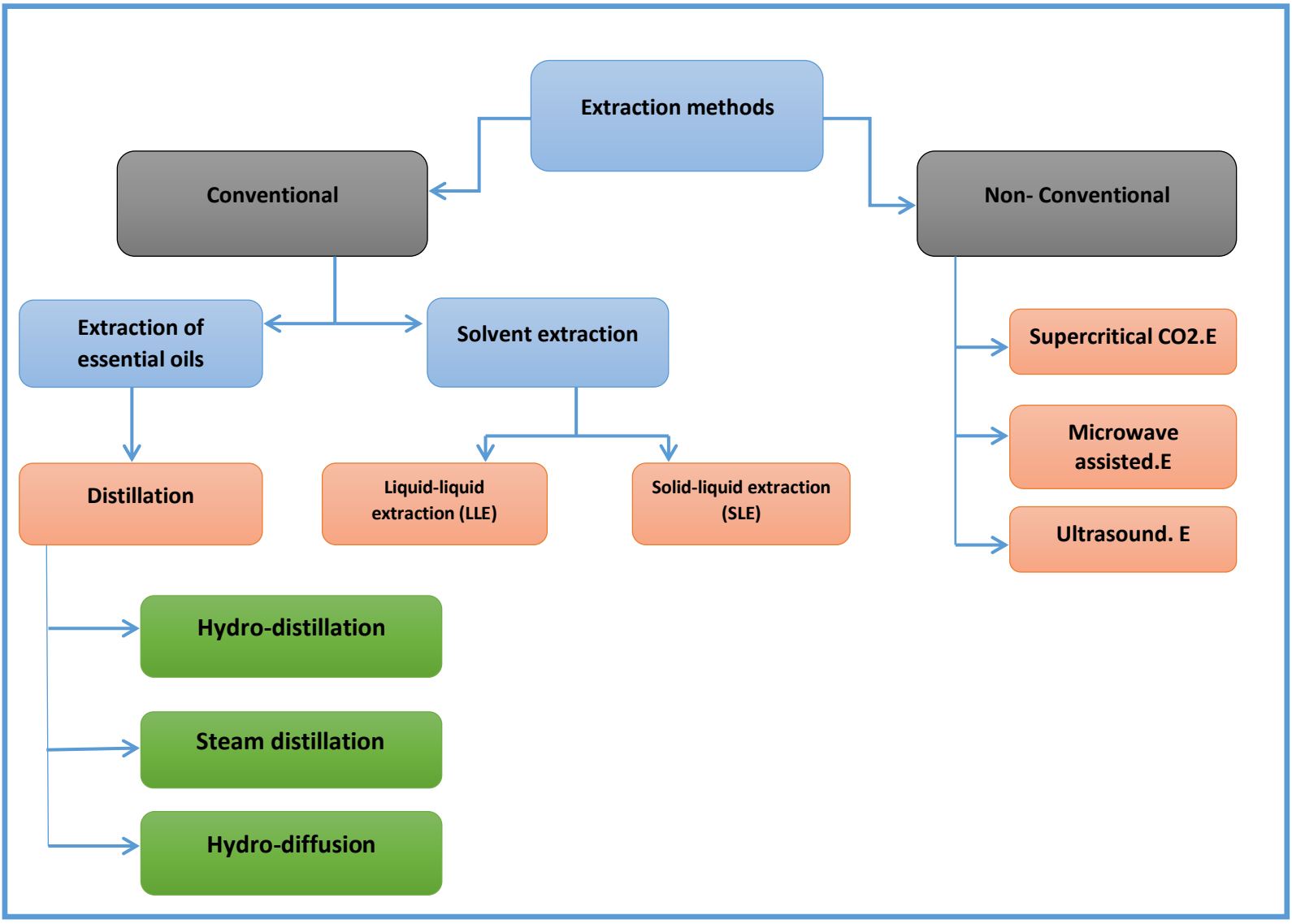


Fig.2.12: Extraction methods.

II.2.1. 1.Conventional extraction techniques:

Conventional extraction is usually performed using reflux, cold maceration, soxhlet and simple distillation techniques.

These methods which have been used for many decades are very time consuming and require relatively large quantities of solvents. **(Bandar, H. & al.2013**).

II.2.1.1.1.Solvent extraction:

- **General information on solvents:**

A solvent is the component of a solution that is present in the greatest amount. It is the substance in which the solute is dissolved. Usually, a solvent is a liquid. However, it can be a gas, solid, or supercritical fluid. The amount of solvent required to dissolve a solute depends on temperature and the presence of other substances in a sample. The word "solvent" comes from the Latin *solvō*, which means to loosen or untie **(Tinoco, I. & al.2002).**

Whatever the extraction technique, the choice of an appropriate solvent is very important for obtaining optimal extraction yields. Thus, the solvent composition but also the solvent to feed ratio must be carefully selected to optimize extraction yield and extraction time **(Rostagno, M. & al 2013).**

- **Choice of Solvents:**

Secondary metabolites have different degrees of polarity so the solvent(s) should be chosen for the extraction should be considered carefully to ensure dissolution of secondary metabolites under study (**Table.2.5**) **(Roopashree, K. M. & al.2019).**

Table.2.5: Solvent employed **(Roopashree, K. M. & al.2019).**

Polar Solvents	Non-polar Solvent	Semi-polar Solvents	Azeotropic Mixtures
The polar compounds like polysaccharides, phenols, aldehydes, ketones, amines, and other oxygen containing compounds dissolve in water due to formation of hydrogen bonding.	Non-polar solvents have low dielectric constants and dissolve non-polar solutes with similar internal pressures through induced dipole interactions. Ionic and polar solutes are insoluble or slightly soluble in non-polar solvents. Weak Van-Der-Waals and London type of forces are responsible for the solubility of molecules.	Semi-polar solvents like ketones and alcohols can induce a certain degree of polarity in non-polar solvent like benzene is readily polarizable, becomes soluble in alcohol. Semi-polar compounds act as intermediate solvents which bring about miscibility of polar and non-polar liquids.	Azeotropes are mixture of different solvent with varying polarity which has near boiling points. They affect the dissolution properties and degree of extraction of extractable matters.

A. Solid-liquid extraction (SLE):

It is a process of separating one or more of the liquid or solid components present in a solid body by dissolution with a solvent (water, alcohol, chloroform, etc.) **(Brown, K.1997).**

The solid–liquid extraction processes can be batch, continuous, or quasi-continuous. Continuous plants can be percolation, immersion, or direct extraction plants…etc.

- **Maceration:**

This method is suitable for certain plants whose active ingredients take a long time to extract or are sensitive to heat: the plant is then left in contact with water, at room temperature, for a certain time, more or less depending on the'species or part **(figure.2.13)(Bellakhdar, J.2006).**

Fig.2.13: Extraction by maceration method.

- **Infusion**:

Infusions are prepared by soaking a drug in water for a specialized period of time. The

process can be either hot or cold, depending upon the type of the ingredients present as decomposition may occur at higher temperatures. Infusions are generally prepared for immediate use, as preservatives are absent. In some cases preservatives like alcohol are used and the infusions concentrated by boiling **(figure.2.14),(Roopashree, K. M.2019).**

Fig.2.14: Extraction by infusion.

- **Decoction**:

Decoctions are prepared in a similar manner to that of infusions but the ingredients

areboiled with that of water for a specified period of time or till a definite volume is attained. The term decoction is used when the preparation is prepared using hard plant parts like root, bark, wood etc.**(figure.2.15).** Decoctions are usually the method of choice when working with tougher and more fibrous plants, barks and roots (and which have water soluble chemicals) **(Mukherjee, P. K.2002)**

Fig.2.15: Extraction by decoction.

- **Percolation (Exhaustive Extraction):**

Percolation in usually one of the most widespread methods for plant extraction since it does not require much manipulation or time. It is a continuous process in which the saturated solvent is constantly being displaced by fresh menstruum **(figure.2.16).**Normally, percolation is not used as a continuous method because sample is steeled in solvent in the percolator for 24hrs (for up to three times), and then the extracted materials are collected and pooled (Roopashree, K. M. & al. 2019).

Fig.2.16: Extraction by percolation.

- **Hot continuous extraction (Soxhlet extraction):**

Soxhlet extraction method described by soxhlet in 1837.In this methods fat and oil from solid material is extracted by repeated washing with organic solvent under reflux**(figure.2.17).** Organic solvent commonly used are hexane and petroleum ether. Disadvantage of this process are-polar lipid, long time involved large volumes of solvents, hazards of boiling solvents **(ATTAURRAHMAN. 1989).**

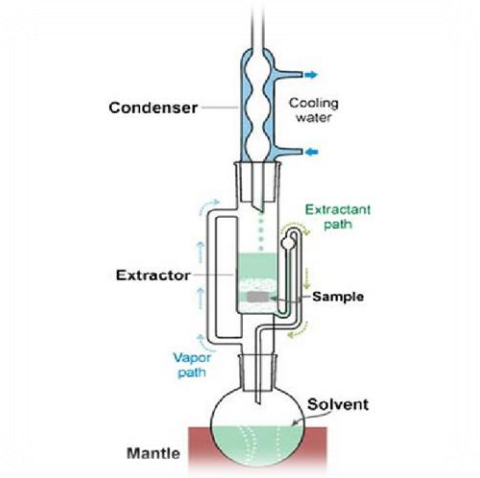
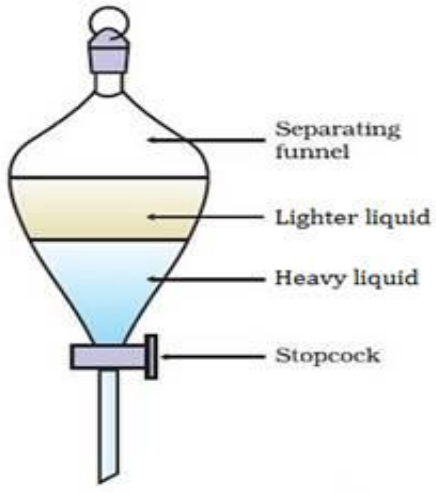


Fig.2.17: Soxhlet extraction device**.**

B. Liquid-liquid extraction (LLE) :

Liquid-liquid extraction, also known as partitioning, is a separation process consisting of the transfer of a solute from one solvent to another, the two solvents being immiscible or partially miscible with each other **(figure.2.18).**



Frequently, one of the solvents is water or an aqueous mixture and the other is a nonpolar organic liquid. As in all extraction processes, liquid-liquid extraction comprises a step of mixing (contacting), followed by a step of phase separation.

Fig.2.18: Separating funnel device.

It is important to consider both steps in the selection of solvents and modes of operation. Thus, while vigorous mixing is favorable to the transfer of the extractable from one solvent to the other, it may also impair the ease of phase separation by forming emulsions **(Berk, Z. 2018).**

Two laboratory devices can be used to perform liquid-liquid extraction: separating funnel and continuous extractor.

II.2.1.1.2. Extraction of essential oils:

There are several methods of obtaining essential oils, namely steam training, hydro-distillation, etc. In our case, we used the hydro-distillation technique with "Clevenger" device, it is an extraction technique in which the solvent is water.

It can be used to extract species that are insoluble in water.

1. Distillation:

According to **(Piochon,M. 2008),**there are three different processes using the principle of distillation: hydro-distillation, hydro-diffusion and steam entrainment.

- **Hydro-distillation**

This is the simplest method and therefore the oldest used. The plant material is immersed directly in a still filled with water, placed on a heat source; everything is then brought to a boil. The vapors are condensed in acoolant and the essential oil separates from the hydrolate by a simple difference in density.

The essential oil being lighter than water floats above the hydrosol **(figure.2.19).** However, hydro-distillation has its limits. Indeed, a prolonged and too powerful heating generates the degradation of certain aromatic molecules **(Lucchesi, M. E. 2005).**

Fig.2.19: "Clevenger" hydrodistillation device.

- **Steam distillation:**

In this type of distillation, the plant material does not macerate directly in water. It is placed on a perforated grid through which the water vapor passes **(figure.2.20).**Steam damages the structure of plant cells and thus releases volatile molecules which are then entrained towards the refrigerant.This method improves the quality of the essential oil by minimizing hydrolytic alterations**(KHOLKHAL, F.2014).**

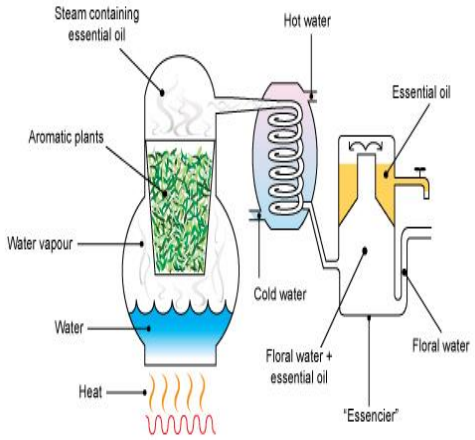


Fig.2.20: The steam extraction device.

- **Hydro-diffusion:**

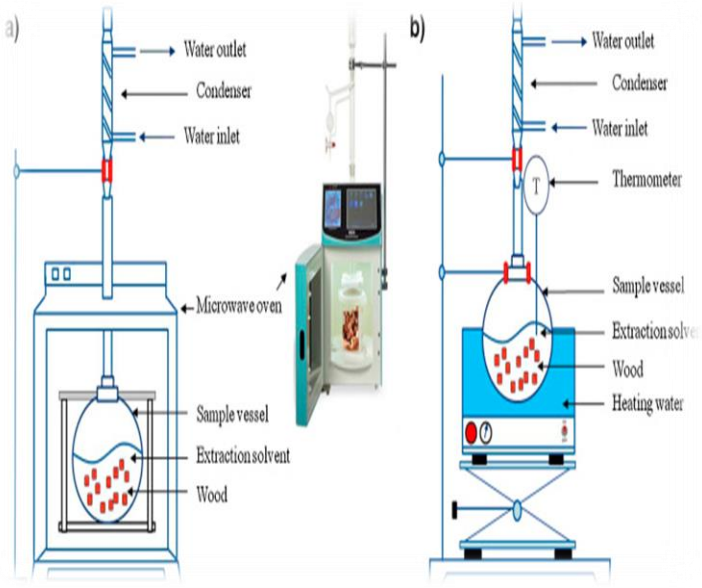
This technique is relatively recent. It involves passing water vapor from top to bottom at reduced pressure through the plant material. The advantage of this method is that it is faster and therefore less damaging to volatile compounds **(KHOLKHAL, F.2014).**

2. Non-Conventional techniques of extraction:

Extraction using non-conventional methods (microwave assisted extraction and ultrasound assisted extraction, Supercritical CO2 extraction) can result in a yield increase in shorter time using less solvent. they has many advantages compared with other methods due to its reduced extraction time, higher extraction efficiency, less labor and high extraction selectivity which makes it a favorable method in extraction of bioactive compounds from leaves and stems **(Bandar, H. & al.2013).**

- **Microwave assisted extraction:**

Microwave assisted extraction is a new technique that combines the use of microwaves and other traditional methods. In this process, the plant material is heated by microwave in a closed chamber in which the pressure is reduced sequentially. The volatile compounds are carried away by the water vapor formed from the water specific to the plant **(figure.2.21).**



They are then recovered using conventional methods, namely condensation, cooling, and settling. Studies show that this technique has several advantages such as saving extraction time, use small amounts of solvent and obtaining a high extraction yield **(Hemwimon, S. & al.2007).**

Fig.2.21: Microwave assisted extraction device .

- **Supercritical CO2 extraction:**

Extraction by supercritical fluids has gained a lot in recent years concerning the extraction of plant extracts.

The main advantage of this technique is that it combines the characteristics of gases and liquids during the extraction process **(figure.2.22).**

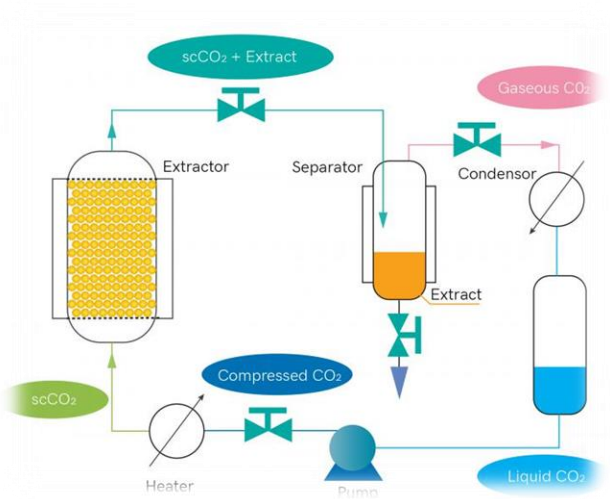


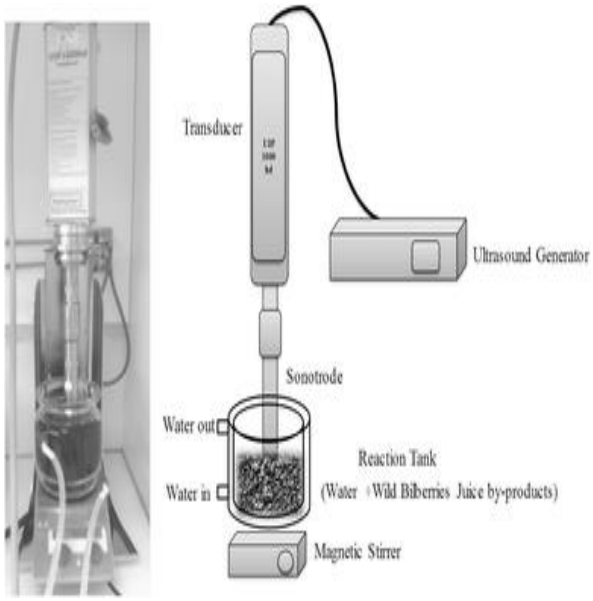
Fig.2.22 : Supercritical CO2 extraction device.

In addition, all possible degradation processes such as oxidation or isomerization are minimiz because the extraction time is reduced there. Beyond the critical point (P = 73.8 bars, T^{o} = 31.1 ° C), CO2 has properties intermediate between those of liquids and those of gases, which gives it good extraction power **(Piochon,M.2008).**

- **Ultrasound Extraction :**

Extraction of intracellular compounds by cell-lysis is done by using ultrasound.

When sound wave propagate in liquid media results in high-pressure and low-pressure cycle (figure.2.23).



The main effects of ultrasound extraction can be summarizing as:

- To increase the permeability of the cell walls
- To produce cavitation i.e. the spontaneous formation of bubbles in a liquid below its boiling point resulting from strong dynamic stressing
- To increase mechanical stressing of the cells so called interface friction **(Roopashree, K. M.& al.2019).**

Fig.2.23: Ultrasound Extraction device.

II.2.2. Chromatographic separation methods:

The wide chemical variety of plant constituents requires suitable quali-quantitative methods to evaluate one or more marker compounds after the extraction step . It is generally accepted that a single analytical technique will not provide sufficient detection of the plant metabolomic profile which is generally a compromise between speed, selectivity and sensitivity of the analytical method **(Exarchou, V. & al.2006).**

- **Paper chromatography (PC):**

This method is very old but interesting because it allows the separation of a large quantity of product and detaches the maximum of phenolic acids **(Herborne, J. B.1973).**This is the best method for separating flavonoids due to their adsorption capacity as in the case

of Whatman 3 paper, using several eluent systems that contain acetic acid to improve the separation of the compounds **(Bruneton, J.1999).**

- **Thin Layer Chromatography (TLC) and High Performance-Thin Layer Chromatography (HP-TLC):**

TLC is the oldest analytical chromatographic technique for the screening of plant extracts. The separation process involves a suitable adsorbent (stationary phase) and a solvent or solvent mixture (mobile phase) **(Seidemann, J. & al.1970).**

By TLC methods, a broad range of substances dissolved in all solvents even aggressive reagents can be tested. However, the separation efficiency of HPLC and capillary GC are considerably higher than TLC. On the other hand, analytical TLC techniques require a very simple and low cost-equipment as well as reduced amounts of samples.

The UV detection and densitometry also allow quantitative determination. Recently HP-TLC technique, allows faster and authomatic development **(Pothier, J. & al. 2001; Galand, N. & al. 2002).**

- **Column chromatography (CC):**

It is a separation technique based on the difference in affinity existing between the compounds in which a stationary phase used as silica gel; cellulose or polyamide and another mobile phase consisting of various solvent systems as eluent **(Markham, K. R.1982).**

- **High-Performance Liquid Chromatography (HPLC) :**

It is a proven technique that has been used in laboratories worldwide over the past 30-plus years**(Baltimore,R. & al.2001;Łuczkiewicz, M. & al.2005**). Chromatograms of plant extracts are used as fingerprints and compared with standard compounds in order to identify the plant material and its constituents. HPLC is thus one of the best-suited technique for an efficient separation of the crude plant extracts, as shown by**(Ismail, Y. & al.2003)**who claim to have found a method capable of quantifying every polyphenol in vegetables, fruits and teas.

The reversed-phase columns may be considered the most popular columns used in the analytical separation of plant secondary metabolites, even if new stationary phases have been exploited **(Tanaka, N. & al.2002.2004).**

HPLC chromatography is a qualitative and quantitative analysis technique that allows the identification, separation and determination of chemical compounds in a liquid mixture. The sample to be analyzed containing one or more species is entrained by a mobile phase current in contact with a stationary phase. Each species present migrates at a speed, which depends on its characteristics, and those of the two phases present **(Malviya, R. & al.2010).**

It uses a solid or liquid stationary phase, immobilized in a column, and a liquid mobile phase, which moves through the stationary phase. The compounds are introduced (we say injected), in mixture, at the head of the column, they then interact with the stationary and mobile phases according to a series of successive equilibria **(Hainque, B.2008).**

- **Gas-Chromatography Mass Spectrometry (GC-MS):**

It is the most popular and useful analytical tool in the research field of volatile plant secondary metabolites **(Adams, R. P.2007).**

The advantages of GC clearly lie in its high sensitivity of detection for almost all the volatile chemical compounds present in the apolar plant extracts or essential oils. Furthermore, the high selectivity of capillary columns guarantees high resolution of many volatile compounds simultaneously within comparatively short times. This fact is extremely important in the case of essential oils, generally contain a hundred of terpenes and derivatives.

In addition, the unambiguous identification of plant metabolite is possible by computer matching with commercial and experimental databases of mass spectra and retention indices. In fact, the method of ionization generally used in conjunction with gas chromatography is Electron Impact, a hard-ionization method that provides reproducible mass spectra and allows library searching. In the case of gas chromatography; it is well known that the volatility and thermal stability of the analytes represent sometimes a great limit.

Reference list:

*Siddiqui, M. W., Bansal, V., & Prasad, K. (Eds.). (2017). Plant Secondary Metabolites, Volume Two: Sti .

*Makkar, H. P., Siddhuraju, P., & Becker, K. (2007). Plant secondary metabolites (pp. 101-106). Totowa, NJ, USA:: Humana Press.

*Gelaw, H., Adane, L., & Tariku, Y. (2012). Journal of Pharmacognosy and Phytochemistry Vol. 1 No. 4 2012 www.phytojournal. com Page| 15 Isolation Of Crotepoxide From Berries of Croton Macrostachyus and Evaluation of Its Anti-Leishmanial Activity

*Hahn, N. I. (1998). Are phytoestrogens nature's cure for what ails us? A look at the research. Journal of the American Dietetic Association, 98(9), 974-976

*Walton, N. J., Mayer, M. J., & Narbad, A. (2003). Vanillin. Phytochemistry, 63(5), 505-515.

*Martin, S., & Andriantsitohaina, R. (2002, December). Mécanismes de la protection cardiaque et vasculaire des polyphénols au niveau de l'endothélium. In Annales de Cardiologie et d'Angéiologie (Vol. 51, No. 6, pp. 304-315). Elsevier Masson.

*Jovanovic, S. V., & Simic, M. G. (2000). Antioxidants in nutrition. Annals of the New York Academy of Sciences, 899(1), 326-334.

*Middleton, E., Kandaswami, C., & Theoharides, T. C. (2000). The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacological reviews, 52(4), 673-751.

*Sarni-Manchado, P., & Cheynier, V. (Eds.). (2006). Les polyphénols en agroalimentaire. Techniques & documentation.

*Bruneton, J.(1999). Pharmacognosie, Phytochimie, Plantes médecinales, 3e edition, Edition Lavoisier TEC et DOC.

*Hennebelle, T. (2006). Investigation chimique, chimiotaxonomique et pharmacologique de Lamiales productrices d'antioxydants: Marrubium peregrinum, Ballota larendana, *Ballota pseudodictamnus (Lamiacées) et Lippia alba (Verbénacées) (Doctoral dissertation, Lille 1).

*Pridham, J. B. (1960). Phenolics in plants in health and disease. Proceedings of a Plant Phenolics Group Symposium held at Bristol, April 1959. In Phenolics in plants in health and disease.Proceedings of a Plant Phenolics Group Symposium held at Bristol, April 1959..Pergamon Press, Oxford & London.

*Ghedira, K. (2005). Les flavonoïdes: structure, propriétés biologiques, rôle prophylactique et emplois en thérapeutique. Phytothérapie, 3(4), 162-169.

*Harborne, J. B., & Baxter, H. (1999). The handbook of natural flavonoids.Volume 1 and Volume 2.John Wiley and Sons.

*Škerget, M., Kotnik, P., Hadolin, M., Hraš, A. R., Simonič, M., & Knez, Ž. (2005). Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. Food chemistry, 89(2), 191-198.

*Tapas, A. R., Sakarkar, D. M., & Kakde, R. B. (2008). Flavonoids as nutraceuticals: a review. Tropical journal of Pharmaceutical research, 7(3), 1089-1099.

*Barton, D., & Meth-Cohn, O. (Eds.). (1999). Comprehensive natural products chemistry.Newnes.

*Langenheim, J. H. (1994). Higher plant terpenoids: a phytocentric overview of their ecological roles. Journal of chemical ecology, 20(6), 1223-1280.

*McCaskill, D., & Croteau, R. (1998). Some caveats for bioengineering terpenoid metabolism in plants. Trends in Biotechnology, 16(8), 349-355.

*Degenhardt, J., Gershenzon, J., Baldwin, I. T., & Kessler, A. (2003). Attracting friends to feast on foes: engineering terpene emission to make crop plants more attractive to herbivore enemies.Current opinion in biotechnology, 14(2), 169-176.

*Dudareva, N., Pichersky, E., & Gershenzon, J. (2004). Biochemistry of plant volatiles.Plant physiology, 135(4), 1893-1902.

*Bouderdara, N. (2013). Séparation et détermination de structures des métabolites secondaires de Cachrys libanotis L.

*Lacy, A., & O'Kennedy, R. (2004). Studies on coumarins and coumarin-related compounds to determine their therapeutic role in the treatment of cancer.Current pharmaceutical design, 10(30), 3797-3811.

*Murray, R. D. (2002). The naturally occurring coumarins.Fortschritte der Chemie organischer Naturstoffe/Progress in the Chemistry of Organic Natural Products, 1-619.

*Boutaoui, N.(2012). Recherche et détermination structurale de métabolites secondaires de Matricaria Chamomilla (Asteraceae) Etude de la phase acétate d'éthyl.

*Xu, L., Zhao, X. Y., Wu, Y. L., & Zhang, W. (2015, June). The study on biological and pharmacological activity of coumarins.In 2015 Asia-Pacific Energy Equipment Engineering Research Conference.Atlantis Press.

*Mueller-Harvey, I., & Reed, J. D. (1992). Identification of phenolic compounds and their relationships to in-vitro digestibility of sorghum leaves from bird-resistant and non-bird-resistant varieties. Journal of the Science of Food and Agriculture, 60(2), 179-196.

*Rao, R. K., Rao, J. S., & Vimaladevi, M. (1979). Phytochemical investigation of Cassia absus (roots and leaves). Journal of Natural Products, 42(3), 299-300.

*Ziegler, J., & Facchini, P. J. (2008). Alkaloid biosynthesis: metabolism and trafficking. Annu. Rev. Plant Biol., 59, 735-769.

*Krishnan, R., Chandravadana, M. V., Ramachander, P. R., & Bharathkumar, H. (1983). Inter-relationships between growth and alkaloid production in Catharanthus roseus G. Don. Herba Hungarica, 22, 47-54.

*Molyneux, R. J., Nash, R. J., & Asano, N. (1996). Alkaloids: Chemical and Biological Perspectives, Vol. 11, Pelletier SW, ed.

*Bouchaale Ikram Kahalerras Amira, Z. S. (2015). Etude comparative de l'activité antibactérienne des huiles essentielles de Laurus nobilis de deux régions (Algérie et Tunisie).

*Moro Buronzo, A. (2008). Le Grand Guide des huiles essentielles: Santé. Beauté, Bien être, 14-43.

*Masotti V, Juteau F, Bessière JM, Viano J. (2003) Seasonal and phenological variations of the essential oil from the narrow endemic species Artemisia molinieri and its biological activities. J Agric Food Chem 51: 7115-7121.

*Angioni A, Barra A, Coroneo V, Dessi S, Cabras P. (2006) Chemical composition, seasonal variability, and antifungal activity of Lavandula stoechas L. ssp. stoechas essential oils from stem/leaves and flowers. J Agric Food Chem 54: 4364-4370.

*Oussalah, M., Caillet, S., Saucier, L., & Lacroix, M. (2006). Antimicrobial effects of selected plant essential oils on the growth of a Pseudomonas putida strain isolated from meat. Meat science, 73(2), 236-244.

*Teuscher, E., Anton, R., & Lobstein, A. (2005). Plantes aromatiques: épices, aromates, condiments et huiles essentielles. Tec & Doc.

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

*Svoboda, K. P., & Hampson, J. B. (1999). Bioactivity of essential oils of selected temperate aromatic plants: antibacterial, antioxidant, antiinflammatory and other related pharmacological activities. Plant Biology Department, SAC Auchincruive, Ayr, Scotland, UK., KA6 5HW, 16, 1-7.

*Simic A, Sokovic MD, Ristic M, Grujic-Jovanovic S, Vukojevic JJ, et al. (2004) The chemical composition of some Lauraceae essential oils and their antifungal activities. Phytother Res 18: 713-717.

*Perry NS, Bollen C, Perry EK, Ballard C (2003) Salvia for dementia therapy: review of pharmacological activity and pilot tolerability clinical trial. Pharmacol. Biochem Behav 75: 651-659.

*Properzi A, Angelini P, Bertuzzi G, Venanzoni R (2013) Some Biological Activities of Essential Oils. Med Aromat Plants 2: 136. doi: 10.4172/2167-0412.1000136.

*Bandar, H., Hijazi, A., Rammal, H., Hachem, A., Saad, Z., & Badran, B. (2013).Techniques for the extraction of bioactive compounds from Lebanese Urtica Dioica. American Journal of Phytomedicine and Clinical Therapeutics, 1(6), 507-513.

*Tinoco, I.; Sauer, K.; Wang, J.C. (2002). Physical Chemistry.Prentice Hall.ISBN 978-0-13-026607-1.

*Rostagno, M. A., & Prado, J. M. (Eds.). (2013). Natural product extraction: principles and applications (No. 21). Royal Society of Chemistry.

*Roopashree, K. M., & Naik, D. (2019). Advanced method of secondary metabolite extraction and quality analysis. Journal of Pharmacognosy and Phytochemistry, 8(3), 1829-1842.

*Brown, K. (1997). Plain tales from the grasslands: extraction, value and utilization of biomass in Royal BardiaNational Park, Nepal. Biodiversity & Conservation, 6(1), 59-74.

*Bellakhdar, J. (2006). Plantes médicinales au Maghreb et soins de base: précis de phytothérapie moderne. Eds Le Fennec.

*Mukherjee, P. K. (2002). Quality control of herbal drugs: an approach to evaluation of botanicals. Business Horizons.

*Attaurrahman. (1989). Isolation and structural studies on new natural-products of potential biological importance. Pure and Applied Chemistry, 61(3), 453-456.

*Berk, Z. (2018). Food process engineering and technology.Academic press

*Piochon, M. (2008). Étude des huiles essentielles d'espèces végétales de la flore laurentienne: composition chimique, activités pharmacologiques et hémi-synthèse. Université du Québec à Chicoutimi.

*Lucchesi, M. E. (2005). Extraction Sans Solvant Assistée par Micro-ondes Conception et Application à l'extraction des huiles essentielles (Doctoral dissertation, Université de la Réunion).

*KHOLKHAL, F. (2014). Etude Phytochimique et Activité Antioxydante des extraits des composés phénoliques de Thymus ciliatus ssp coloratus et ssp euciliatus (Doctoral dissertation).

*Bandar, H., Hijazi, A., Rammal, H., Hachem, A., Saad, Z., & Badran, B. (2013).Techniques for the extraction of bioactive compounds from Lebanese Urtica Dioica. American Journal of Phytomedicine and Clinical Therapeutics, 1(6), 507-513.

*Hemwimon, S., Pavasant, P., & Shotipruk, A. (2007). Microwave-assisted extraction of antioxidative anthraquinones from roots of Morinda citrifolia.Separation and purification technology, 54(1), 44-50.

*Exarchou, V., Fiamegos, Y. C., van Beek, T. A., Nanos, C., & Vervoort, J. (2006). Hyphenated chromatographic techniques for the rapid screening and identification of antioxidants in methanolic extracts of pharmaceutically used plants. Journal of Chromatography A, 1112(1-2), 293-302.

*Herborne, J. B. (1973). Phytochemical methods.A guide to modern techniques of plant analysis, 5-11.

*Seidemann, J. (1970). Stahl, E.: Thin-Layer Chromatography, A Laboratory Handbook, 2nd. Edit., fully revised and expanded, with Contribution by 25 authors.Translated from the German by MRF Ashword.Springer-Verlag Berlin–Heidelberg–New York 1969. Distribution rights for UK and Commonwealth (excluding Canada): Allen & Unwin Ltd., London. 1041 Pages with 241 Figures and 3 plats in color, DM 128,20.

*Pothier, J., Galand, N., El Ouali, M., & Viel, C. (2001). Comparison of planar chromatographic methods (TLC, OPLC, AMD) applied to essential oils of wild thyme and seven chemotypes of thyme. Il Farmaco, 56(5-7), 505-511.

*Galand, N., Pothier, J., Dollet, J., & Viel, C. (2002). OPLC and AMD, recent techniques of planar chromatography: their interest for separation and characterization of extractive and synthetic compounds. Fitoterapia, 73(2), 121-134.

*Markham, K. R. (1982). Techniques of flavonoid identification (Vol. 36). London: Academic press.

*Baltimore, R. S., Huie, S. M., Meek, J. I., Schuchat, A., & O'Brien, K. L. (2001). Early-onset neonatal sepsis in the era of group B streptococcal prevention. Pediatrics, 108(5), 1094-1098.

*Łuczkiewicz, M., & Głód, D. (2005).Morphogenesis-dependent accumulation of phytoestrogenes in Genista tinctoria in vitro cultures.Plant science, 168(4), 967-979.

*Ismail, Y., Kemmoku, Y., Takikawa, H., & Sakakibara, T. (2003, January). Operating Strategy of a Stand-Alone Photovoltaic/Diesel/Battery Power System. In International Solar Energy Conference (Vol. 36762, pp. 387-392).

*Tanaka, N., Kimura, H., Tokuda, D., Hosoya, K., Ikegami, T., Ishizuka, N., ...& Cabrera, K. (2004). Simple and comprehensive two-dimensional reversed-phase HPLC using monolithic silica columns. Analytical chemistry, 76(5), 1273-1281.

*Tanaka N, Kobayashi H, Ishizuka N et al. Monolithic silica columns for high-efficiency chromatographic separations. J Chromatogr A.2002; 965:35-49.

*Malviya, R., Bansal, V., Pal, O. P., & Sharma, P. K. (2010). High performance liquid chromatography: a short review. Journal of global pharma technology, 2(5), 22-26.

*Hainque, B. (2008). Appareils et méthodes en biochimie et biologie moléculaire.

*Adams, R. P. (2007). Identification of essential oil components by gas chromatography/mass spectrometry (Vol. 456). Carol Stream, IL: Allured publishing corporation.

Chapter III:

Biological activity

Isolation or extraction of medicinal plants mainly produced one or several substances that are responsible for any activity and are closely related to each other. Plants are the main source of drugs in modern medicinal system, folk medicinal system, traditional medicinal system, food supplement, and for synthetic drug, they contain different types of novel and unique substances to treat infectious and chronic disease.

A recent research study shows that medicinal plants show mainly antioxidant activity. .e phenolic compound such as flavonoids, lignin, and vitamins A, C, and E, and tannins all are antioxidants and are present mainly in plants **(Hassan, A. & al. 2019).**

III.1.Antibacterial activity:

Bacterial infections are caused by different microorganisms and are the cause of the fatal diseases and the most widespread epidemics. Many antibiotics have been developed to treat them; however, their overuse is the cause of the emergence of bacterial multidrug resistance **(ABDALLAH, R., & al 2019).**

Plant biomolecules have been reported to be alternatives to antibiotics resistance of human pathogens because of their proven effectiveness and availability **(Wintola,O. & al 2015).**

III.1.1.General information on bacteria:

Bacteria are microscopic, single-celled organisms that thrive in diverse environments.

These organisms can live in soil, the ocean and inside the human gut.

Humans' relationship with bacteria is complex. Sometimes bacteria help us, such as by curdling milk into yogurt or helping with our digestion. In other cases, bacteria are destructive, causing diseases like pneumonia and methicillin-resistant Staphylococcus aureus (MRSA) **(Aparma.V. 2019).**

Bacteria generally have a diameter of less than 1μm.They can be seen under a light microscope, fresh or after staining. Their shape can be spherical (cocci), rod (bacilli), and curved (vibrios) or spiral (spirochetes). Details of their structure can only be seen under electron microscopy **(Nauciel,C. & al.2005).**

III.1.2.Classification:

A few different criteria are used to classify bacteria. The organisms can be distinguished by the nature of their cell walls, by their shape, or by differences in their genetic makeup.

There are broadly speaking two different types of cell wall in bacteria that classify bacteria into Gram-positive bacteria and Gram-negative bacteria. The names originate from the reaction of cells to the Gram stain, a long-standing test for the classification of bacterial species, named for Hans Christian Gram, who developed the technique in 1884. The test stains Gram-positive bacteria, or bacteria that do not have an outer membrane. Gram-negative bacteria don't pick up the stain. **(Gram, HC. 1884).**

In Gram-positive bacteria, the wall consists almost exclusively of the peptidoglycan layer, with which polymers of teichoic acids are associated. Gram-negative bacteria have a more complex wall; the peptidoglycan layer is thinner than that of Gram-positive, and an outer membrane made up of lipopolysaccharides and lipoproteins surrounds it. The lipopolysaccharide part of the Gram-negative wall comprises the endotoxin molecules (lipid A) which contribute to bacterial pathogenicity **(Prescott,L. & al. 2003).**

There are three basic bacterial shapes: Round bacteria called cocci (singular: coccus), cylindrical, capsule-shaped ones known as bacilli (singular: bacillus); and spiral bacteria, aptly called spirilla (singular: spirillum). The shapes and configurations of bacteria are often reflected in their names.

For example, the milk-curdling Lactobacillus acidophilus are bacilli, and pneumonia-causing S. pneumoniae are a chain of cocci. Some bacteria take other shapes, such as stalked, square or star.

III.1.3. Reproduction:

Bacteria reproduce primarily by binary fission, an asexual process whereby a single cell divides into two. Under ideal conditions some bacterial species may divide every 10–15 minutes—a doubling of the population at these time intervals. Eukaryotic microorganisms reproduce by a variety of processes, both asexual and sexual. Some require multiple hosts or carriers (vectors) to complete their life cycles. Viruses, on the other hand, are produced by the host cell that they infect but are not capable of self-reproduction **(Michael, J. &al 1993).**

III.1.4.Antibiotics:

The elimination of pathogenic microorganisms uses substances called: antibiotics (from the Greek: anti, against and bios, life). The latter are microbial products synthesized by microorganisms (most often fungi). They have the ability to either destroy bacteria (bactericidal effect), or inhibit their growth (bacteriostatic effect) **(Elghozi,J. & al .1992).**

Substances like sulfonamides are sometimes referred to as antibiotics although they are synthetic chemotherapy agents of non-microbial origin. An antibiotic has the following characteristics:

- Antibacterial activity

- In vivo activity unlike disinfectants.

- Good absorption and good diffusion in the body **(Prescott,L. & al. 2003).**

III.1.5. Classification of antibiotics:

The number and importance of antibiotics are such that many attempts at classification have been proposed: among 4000 antibiotics currently described, 70% are of microbial origin.

The classification can be based on several criteria: the spectrum of action, the target, or the chemical family. The latter is the most frequently encountered **(Meyer,A. & al.2004).**

Depending on the origin: produced by a living micro-organism (bacteria, fungus, etc.) or produced by synthesis. At present, these will be molecules, most often obtained by semisynthesis.

- Depending on the site of action: wall, membrane, nucleic acids, etc.

-According to the spectrum of activity: broad spectrum or narrow spectrum, antimycotics, antivirals, antiprotozoa.

- According to the chemical nature: this is the most widely used classification, because antibiotics are composed of chemically defined units, the number of which is always low **(Meyer,A. & al.2004).**

The main chemical families of antibiotics are: Beta-lactams: penicillin and cephalosporins; Aminoglycosides: streptomycin, gentamycin; Chloramphenicol and thiamphenicol; Cyclines: tetracyclines, doxycycline. Macrolides and related:erythromycin, oleandomycin **(Cohen, Y. & al.2001).**

III.1.6. Antimicrobial activity of plant extracts:

Many research groups have studied the antimicrobial activity of extracts from medicinal plants such as fennel (Foeniculum vulgare), peppermint (Mentha piperita), thyme (Thymus vulgaris), they have found that these extracts are active not only against bacteria that may also against fungi, yeasts and viruses. Other groups of researchers have taken a step further, they have isolated and identified the metabolites responsible for the antimicrobial activity of plant extracts, this step constitutes a platform for several implications including the pharmaceutical industry, alternative medicine, and the natural therapy**(Mourad, B. & al.2011).**

- polyphenolic compounds

Several invitro and invivo studies are focused on evaluating the antibacterial and antifungal properties of polyphenols. At present, this effect is certain and has been demonstrated by numerous experimental research **(Madi, A. 2010).**

The antimicrobial effect of polyphenols is due in part to the disruption of the lipid fractions of the plasma membrane of microorganisms, which leads to poor permeability of the membrane and loss (leakage) of its intracellular organelles, as well as the physicochemical properties of polyphenolic compounds (the solubility in water and lipids) may affect this Antibacterial effect **(Dussault, D. 2008).**

- flavonic compounds

The inhibitory action of flavonoids on bacterial growth was studied by Katarzyna et al (2007). they have shown that many flavonic compounds (apigenin, kaempferol and others) are endowed with a significant effect on various bacterial strains gram negative (Escherichia coli ..) and gram positive (Staphylococcus… ..)**(Madi, A. 2010).**

It has been shown that 5-hydroxyflavanones and 5-hydroxyisoflavanones with one, two or three hydroxyl groups in position 7,2 'and 4' would inhibit the growth of Streptococcus sp, the most important hydroxylation for activity being that in position 2 '. On the other hand, methoxylations considerably reduce the antibacterial effects**(Morel, S. 2011).**

III.2. .Antioxidant activity:

Antioxidant phytochemicals have recently become an attractive subject for scientists in many different research areas. The scope of research in phytochemicals is no longer limited to the functionality associated with plants and organoleptic properties, but has been ex-panded to include their functionality in human health, which is linked to various epidemiological diseases and micro-nutrition. Most phytochemicals in natural agricultural sources have been generally recognized as bioactive or health-promoting compounds, which play an important role in preventing cardiovascular diseases, cancers, obesity and diabetes, lowering blood cholesterol level, and reducing inflammatory action **(Halliwell,B.1996).**

III.2.1.Historic:

In the mid-1950s, R. Gerschman then D. Hartman evoked the toxicity of oxygen and the "free radical theory" to explain the aging process, since that time the terms free radicals, reactive oxygen species (ERO), oxidative stress and antioxidants are becoming more and more familiar to health professionals and even to the general public.

Americans McCord and Fridovich demonstrated for the first time in 1969 that our bodies produce reactive oxygen species from which it must protect themselves by isolating the antioxidant enzymatic system SOD from human red blood cells. This discovery will be

the starting point for extensive scientific research around the world on oxidative stress and antioxidants **(Margaritis, I. & al.2003).**

III.2.2.Oxidative stress:

Oxidative stress is a phenomenon caused by an imbalance between production and accumulation of oxygen reactive species (ROS) in cells and tissues and the ability of a biological system to detoxify these reactive products. ROS can play several physiological roles (i.e., cell signaling), and they are normally generated as by-products of oxygen metabolism **(Pizzino,G. & al. 2017).**

This imbalance can occur when the antioxidant defense system is overworked by increased oxidants or when the defenses are weakened by a deficiency in the supply and / or production of antioxidants **(Kirschvink, N.& al., 2008).**

Environmental stressors (i.e., UV, ionizing radiations, pollutants, and heavy metals) and xenobiotics (i.e., antiblastic drugs) contribute to greatly increase ROS production, therefore causing the imbalance that leads to cell and tissue damage (oxidativestress) **(Pizzino,G. & al. 2017).**

III.2.3.Free radical:

Free radicals are also known as reactive oxygen species (ROS) defined as any molecule or atom having one or more unpaired electrons, capable of existing in independent form, containing at least one free electron on its outer shell (or containing two electrons of the same spin in a cell quantum). This considerably increases its reactivity by the need to combine with another electron to achieve stability according to an oxidation phenomenon**(Finaud,J. & al.2006 ; Close,G.L. & al. 2007).** Its lifespan is very short (a few milliseconds or even a few nanoseconds) and it is symbolized by a point which indicates where the free electron is located (example: ˙OH)**(Close,G. & al. 2007; Sayre,L. & al, 2008; Goto,M. & al.2008).**

Superoxide radicals (O_2•−), hydrogen peroxide (H_2O_2), hydroxyl radicals (•OH), and singlet oxygen ($1O_2$) are com-monly defined reactive oxygen species (ROS); they are gener-ated as metabolic by-products by biological systems**(Sato,H & al, 2013;Navarro-**

Yepes, J & al, 2014). Processes, like protein phosphorylation, activation of several transcriptional factors, apoptosis, immunity, and differentia-tion, are all dependent on a proper ROS production and presence inside cells that need to be kept at a low level **(Rajendran, P. & al.2014).**

III.2.4. Free Radical Production:

ROS production basically relies on enzymatic and non-enzymatic reactions.

- Enzymatic reactions able to generate ROS are those involved in respiratory chain, prostaglandin synthesis, phagocytosis, and cytochrome P450 system**(Halliwell, B & al 2015, Bahorun, T. & al 2006).**

The mitochondrial respiratory chain is one of the major sources of ROS. This production results from the addition of an electron to molecular oxygen. Such a reaction catalyzed by mitochondrial cytochrome oxidase **(Sevanian,A. & al .1990).**

$$O_2 + 1e^- \longrightarrow O_2^{\bullet -}$$

(Marfak, A. & al. 2003)

Superoxide radical (O_2•−) is generated by NADPH oxidase, xanthine oxidasen, and peroxidases. Once formed, it is involved in several reactions that in turn generate hydrogen peroxide, hydroxyl radical (OH•), peroxynitrite (ONOO−), hypochlorous acid (HOCl), and so on. H_2O_2 (a nonradical) is produced by multiple oxidase enzymes, that is, amino acid oxidase and xanthine oxidas**(Pizzino,G. & al. 2017).**

ROS can occur during pathological processes where the production of the radical responds to stimulation and intervenes in the inflammatory process (NADPH oxidase and xanthine oxidase) **(Van Antwerpen, P.2006).**

$$Xanthine + 2O_2 + H_2O \xrightarrow{\text{Xanthine oxidase}} \text{urique acid} + (O_2^{\bullet -}) + 2H^+$$

(Marfak, A. & al. 2003)

Another reactive oxygen species produced during ignition is hydrogen peroxide (H_2O_2); in order to react directly or to produce hypochlorous acid by the intervention of

myeloperoxidase. This species (HClO) is characterized by a markedly higher oxidizing power than H_2O_2) **(Favier,A. 2003).**

$$H_3O^+ + H_2O_2 + Cl^- \xrightarrow{\text{myeloperoxidase}} HClO + 2H_2O$$

(Van Antwerpen, P.2006)

Hydroxyl radical (OH•), the most reactive among all the free radical species in vivo, is generated by reaction of O_2•− with H_2O_2, with Fe^{2+} or Cu^{2+}as a reaction catalyst (Fenton reaction)**(Pizzino,G. & al. 2017).**

Nitric oxide radical (NO•), which plays some important physiological roles, is synthesized from arginine-to-citrulline oxidation by nitric oxide synthase (NOS). **(Pizzino,G. & al. 2017).**

- Non-enzymatic reactions also can be responsible for free radical production, that is, when oxygen reacts with organic compounds or when cells are exposed to ionizing radiations. Non-enzymatic free radical production can occur as well during mitochondrial respiration **(Pizzino,G. & al. 2017).**

III.2.5.Antioxidants:

Antioxidants sometimes called "free radical scavengers" are all molecules capable of inhibiting production in low doses, limiting the proliferation or destruction of reactive oxygen species. They prevent cell damage from highly reactive and unstable molecules called "free radicals" **(Shalaby, E. & al.2019; Favier,A.2003).**

The balance between antioxidants and free radicals in our bodies is important for health. If not controlled, free radicals lead to cell damage associated with a variety of chronic diseases.

The sources of antioxidants can be natural or artificial. The body also produces some antioxidants known as endogenous antioxidants in contrast to antioxidants that come from outside the body called exogenous (Shalaby, E. & al, 2019).**(Table.3.1).**

Table.3.1: Human antioxidant defense systems (Bouayed, J., & Bohn, T. (2010).

Antioxidant defense system	
Endogenous antioxidants	**Exogenous antioxidants**
Enzymatic antioxidants • Superoxide dismutase (SOD): enzyme detoxifying superoxide radical ($\mathbf{O_2}^{-\cdot}$) • Catalase (CAT) and glutathione peroxidase (GPx): enzymes involved in the detoxification of peroxides (CAT against $\mathbf{H_2O_2}$, and GPx against both $\mathbf{H_2O_2}$ and rOOH) • Glutathione reductase: enzyme involved in the regeneration of glutathione • Thioredoxin reductase: enzyme involved in the protection against protein oxidation • Glucose-6-phosphate dehydrogenase: enzyme involved in the regeneration of NADPH. **Non-enzymatic antioxidants (principal intracellular reducing agents)** Glutathione (GSH), uric acid, lipoic acid, NADPH, coenzyme Q, albumin, bilirubin	**Prinicipal dietary antioxidants from fruits, vegetables and grains** • Vitamins: vitamin C, vitamin e • Trace elements: zinc, selenium • Carotenoids: b-carotene, lycopene, lutein, zeaxanthin • Phenolic acids: chlorogenic acids, gallic acid, cafeic acid, etc., • Flavonols: quercetin*, kaempferol*, myricetin* • Flavanols: proanthocyanidins and catechins • Anthocyanidins: cyanidin* and pelagonidin* • Isoflavones:genistein*, daidzein* and glycitein* • Flavanones:naringenin*, eriodictyol* and hesperetin* • Flavones: luteolin* and apigenin

III.2.5.1.Endogenous antioxidants:

The body has two major enzymatic and non-enzymatic defense systems that work in a complementary and synergistic manner according to a specific compartment to protect cells **(Percival,M.1998):**

- Enzymatic system:

One of the enzymatic antioxidant defense systems made up of three enzymes: Superoxide dismutase SOD (decreases the lifespan of the superoxide anion O_2^{-}).

$$2\,O_2^{-\cdot} + 2H^+ \xrightarrow{\text{Superoxide dismutase}} H_2O_2 + O_2$$

(Halliwell,B. 2006)

GSH-Px glutathione peroxidase (destroys hydrogen peroxide and lipid peroxides)

$$H_2O_2 + 2GSH \xrightarrow{\text{glutathion peroxidase}} H_2O_2 + 2GSH$$

(Halliwell,B. 2006)

Catalase (CAT): converts hydrogen peroxide H_2O_2 into a single molecule of water.

$$2\ H_2O_2 \xrightarrow{\text{Catalase}} 2\ H_2O + O_2$$

(Halliwell,B. 2006)

These enzymes have a complementary action on the radical cascade at the level of superoxide and hydrogen peroxide, ultimately leading to the formation of water and molecular oxygen **(Lehucher-Michel, M. & al., 2001).**

- Non-enzymatic antioxidants:

This group of antioxidants is made up of several compounds capable of reacting directly or indirectly with ROS. The indirect mechanism involves the chelation of transition metals which prevents the production of the highly toxic hydroxyl radical **(Kohen,R. & al. 2002).**

III.2.5.2.Exogenous antioxidants:

A large number of antioxidant substances derive from food (fruits, vegetables, spices….). Indeed, food is an important source of carotenoids, tocopherols, ascorbic acid and phenolic compounds (plants) which are essential antioxidants for humans whose contributions can prevent and even help in the treatment of diseases linked to oxidative stress **(Curtay & Robin, 2000).**

- Antioxidants of plant origin:

  Carotenoids and polyphenols constitute large families of compounds (several hundred) including β- carotene, caffeic acid and quercetin (**Table.3.2).**

Table.3.2: Antioxidants of plant origin.

Phenolic compounds	Flavonoids	Anthocyanins
Phenolic compounds are considered to be good antioxidants due to their high redox potential and their relative stability to radicals**(Jovanovic & Simic, 2000).**They have antioxidant properties and are able to scavenge free radicals **(Middleton,E. & al., 2000).**	Flavonoids effectively prevent peroxidation since they can react with most free radicals capable of removing hydrogen from the CH2 group located between the two double bonds of polyunsaturated fatty acids **(Milane,H. 2004).**	Due to their chemical structure, these pigments are capable of reacting with many active substances, which gives them several properties including antioxidant activity which is mainly due to the presence of hydroxyl groups which allow, among other things, the chelation of metal ions (free iron and copper). Anthocyanins are able to reduce the level of oxidized lipoproteins and the activity of nitric oxide synthetase **(Kowalczyk,E. & al.2003).**

Reference list:

*Hassan, A., & Ullah, H. (2019). Antibacterial and antifungal activities of the medicinal plant veronica biloba. Journal of Chemistry, 2019

*Abdallah, R , Frikha, D , & Sassi , S. M. E. S. (2019). Evaluation in vitro de l'activite antibacterienne et antifongique de quatre especes algales marines in vitro, evaluation of the antibacterial and antifungal activities pf marine algae. Journal de l'Information Médicale de Sfax, 38

*Wintola, O. A., & Afolayan, A. J. (2015). The antibacterial, phytochemicals and antioxidants evaluation of the root extracts of Hydnora africana Thunb. used as antidysenteric in Eastern Cape Province, South Africa. BMC complementary and alternative medicine, 15(1), 1-12

*Aparna Vidyasagar 2019. Live Science Contributor Article.

*Nauciel, C., & Vildé, J. L. (2005). Bactériologie médicale. Elsevier Masson.

*Gram, HC (1884). "Über die isolierte Färbung der Schizomyceten in Schnitt- und Trockenpräparaten". Fortschr. Med. 2: 185–89.

*Prescott, L., Harley, J., & Klein, D. (2003). Microbiologie, 5ème ed, eds De Boeck Université.

*Michael J. Pelczar, Jr., E.C.S. Chan, and Noel R. Krieg, Microbiology: Concepts and Applications (1993), is an introduction to the science of microbiology.

*Elghozi, J. L., & Duval, D. (1992). Pharmacologie 2ème Ed: Médecine Flammarion. Paris. 289p

*Meyer, A., Deiana, J., & Bernard, A. (2004). Cours de microbiologie avec problèmes et exercices corrigés-2e édition.

*Cohen, Y., & Jacquot, C. (2001). Pharmacologie. (DEPRECIATED).

*Mourad, B., Mihoub, Z. M., & Sétif, U. F. A. (2011). Etude de l'activité antioxydante et antimicrobienne d'extraits d'Artemisia campestris L. Mémoire pour l'obtention du diplôme de Magister, Université Ferhat Abbes-Setif.

*Madi, A. (2010). Caractérisation et comparaison du contenu polyphénolique de deux plantes médicinales (Thym et Sauge) et la mise en évidence de leurs activités biologiques. Research Master, Mentouri Constantine University, Constantine.

*Dussault, D. (2008).Étude des mécanismes de radiorésistance de la bactérie Pantoea agglomerans (Doctoral dissertation, Université du Québec, Institut National de la Recherche Scientifique).

*ELŻBIETA, R. K., Janusz, C., & Katarzyna, C. (2007). The effect of pretreatment of shredded celeriac using solutions of enzymatic browning inhibitors on the quality of minimally processed product. Journal of food quality, 30(5), 594-608.

*Morel, S. (2011). Etude phytochimique et évaluation biologique de Derris ferruginea Benth.(Fabaceae) (Doctoral dissertation, Université d'Angers).

*Halliwell, B. (1996). Antioxidants: the basics-what they are and how to evaluate them. Advances in pharmacology, 38, 3-20.

*Margaritis, I., Palazzetti, S., Rousseau, A. S., Richard, M. J., & Favier, A. (2003). Antioxidant supplementation and tapering exercise improve exercise-induced antioxidant response. Journal of the American College of Nutrition, 22(2), 147-156.

*Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., ...& Bitto, A. (2017). Oxidative stress: harms and benefits for human health. Oxidative medicine and cellular longevity, 2017.

*Kirschvink, N., de Moffarts, B., & Lekeux, P. (2008). The oxidant/antioxidant equilibrium in horses. The Veterinary Journal, 177(2), 178-191.

*Finaud, J., Lac, G., & Filaire, E. (2006). Oxidative stress. Sports medicine, 36(4), 327-358.

*Goto, M., Ueda, K., Hashimoto, T., Fujiwara, S., Matsuyama, K., Kometani, T., & Kanazawa, K. (2008). A formation mechanism for 8-hydroxy-2′-deoxyguanosine mediated by peroxidized 2′-deoxythymidine. Free Radical Biology and Medicine, 45(9), 1318-1325.

*Close, G. L., & McArdle, F. (2007). Antioxidants and free radicals. Nutrition and Sport, 153.

*Sayre, L. M., Perry, G., & Smith, M. A. (2008). Oxidative stress and neurotoxicity. Chemical research in toxicology, 21(1), 172-188.

*Sato, H., Shibata, M., Shimizu, T., Shibata, S., Toriumi, H., Ebine, T., ...& Suzuki, N. (2013). Differential cellular localization of antioxidant enzymes in the trigeminal ganglion. Neuroscience, 248, 345-358

*Navarro-Yepes, J., Zavala-Flores, L., Anandhan, A., Wang, F., Skotak, M., Chandra, N., ...& Franco, R. (2014). Antioxidant gene therapy against neuronal cell death. Pharmacology & Therapeutics, 142(2), 206-230.

*Rajendran, P., Nandakumar, N., Rengarajan, T., Palaniswami, R., Gnanadhas, E. N., Lakshminarasaiah, U., ...& Nishigaki, I. (2014). Antioxidants and human diseases. Clinica chimica acta, 436, 332-347.

*Halliwell, B., & Gutteridge, J. M. (2015). Free radicals in biology and medicine. Oxford University Press, USA.

*Bahorun, T., Soobrattee, M. A., Luximon-Ramma, V., & Aruoma, O. I. (2006). Free radicals and antioxidants in cardiovascular health and disease. Internet Journal of Medical Update, 1(2), 25-41.

*Sevanian, A., Nordenbrand, K., Kim, E., Ernster, L., & Hochstein, P. (1990). Microsomal lipid peroxidation: the role of NADPH—cytochrome P450 reductase and cytochrome P450. Free Radical Biology and Medicine, 8(2), 145-152.

*Marfak, A., Trouillas, P., Allais, D. P., Champavier, Y., Calliste, C. A., & Duroux, J. L. (2003). Radiolysis of kaempferol in water/methanol mixtures. Evaluation of antioxidant activity of kaempferol and products formed. Journal of agricultural and food chemistry, 51(5), 1270-1277

*Van Antwerpen, P. (2006). Contribution à l'étude du pouvoir antioxydant de divers agents d'intérêt thérapeutique: ciblage du système myélopéroxydase/péroxyde d'hydrogène/chlorure

*Favier, A. (2003).Le stress oxydant. Intérêt conceptuel et expérimental dans la compréhension des mécanismes des maladies et potentiel thérapeutique. Actualité en chimie, pp 108-115.

*Shalaby, E., & Catala, A. (2019). Antioxidants. BoD–Books on Demand.

*Percival M. (1998). Antioxidants. Clinical Nutrition Insights. p : 1-4.

*Halliwell, B. (2006). Free radicals in biochemistry and medicine. Reviews in Cell Biology and Molecular Medicine.

*Lehucher-Michel, M. P., Lesgards, J. F., Delubac, O., Stocker, P., & Durand, P. (2001). Stress oxydant et pathologies humaines: Bilan et perspectives préventives. La Presse médicale (1983), 30(21), 1076-1081.

*Kohen, R., & Nyska, A. (2002). Invited review: Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. Toxicologic pathology, 30(6), 620-650.

*Curtay, J. P., & Robin, J. M. (2000). Intérêt des complexes antioxydants. Nutrithérapie Info, 1-4

*Jovanovic, S. V., & Simic, M. G. (2000). Antioxidants in nutrition. Annals of the New York Academy of Sciences, 899(1), 326-334.

*Middleton, E., Kandaswami, C., & Theoharides, T. C. (2000). The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacological reviews, 52(4), 673-751.

*Milane, H. (2004). La quercétine et ses dérivés: molécules à caractère pro-oxydant ou capteurs de radicaux libres; études et applications thérapeutiques (Doctoral dissertation, Strasbourg 1.

*Kowalczyk, E., Krzesiñski, P., Kura, M., Szmigiel, B., & Blaszczyk, J. (2003). Anthocyanins in medicine. Polish journal of pharmacology, 55(5), 699-702.

*Bouayed, J., & Bohn, T. (2010). Exogenous antioxidants—double-edged swords in cellular redox state: health beneficial effects at physiologic doses versus deleterious effects at high doses. Oxidative medicine and cellular longevity, 3(4), 228-237.

SECOND PART

EXPERIMENTAL PART

Chapter IV:

❖ Botanical description and geographical distribution of *Thapsia garganica L.*

This perennial plant has a striated, glabrous stem, branched in its upper part, reaching 0.90 to 1.40 m in height. The leaves are green, hairless. The primordial leaves are small, elliptical and entire, the following ones are palmatilobed. The leaves at the base of the stem are large, 2-3 pinnately shaped, the upper ones are reduced to a wide sheath. The root is large, blackish on the outside, white on the inside.The inflorescence is a large, compound umbel with 15-20 rays, bearing yellow flowers. The involucre and involucelle are absent. Umbellules are globular in shape.

The fruit is elliptical, dorsally compressed, 10-15 by 20-25 mm, with notches more or less wide at the top and at the base. Lateral wings very developed, shiny, straw yellow, finely streaked (Quezel and Santa, 1962-1963; Pottier-Alapetite, 1979).*Thapsia garganica* is a Mediterranean plant. It is present in Morocco, Algeria, Tunisia and Libya (Dobignard and Chatelain, 2010-2013), but also in Turkey, Spain, Portugal, Italy and Greece.

Fig.4.1: Illustration of *Thapsia garganica* (top left, inflorscences, bottom left, leaves and right, plant habit), 06.15.2021, photos K. Rebbas.

IV.1. Vegetable material.

IV .1.Harvesting of plant material

The aerial parts (the umbel) of the studied plant collected in June (BEJAIA .ALGERIA) and identified by Pr REBBAS, a botanist from the University of M'sila, The choice of this plant is based on its use in traditional Algerian medicine.

IV.2. Drying and grinding:

After harvesting, the collected plant material dried at room temperature and protected from light. The resulting dry matter reduced to powder using an electric grinder. The latter kept in closed glass jars and stored away from light.

II.3. Extraction of essential oil

The aerial parts of the plant are subjected to hydrodistillation (for 3 hours) using a "clevenger" type extraction device **(figure.4.1),** the operation consists of immersing a quantity of the plant mass (100g) in a glass flask. (6 liter) containing a sufficient quantity of distilled water without completely filling the flask.

The mixture is brought to a boil using a heating mantle. The vapors charged with the essential oil pass through the refrigerant where the condensation will take place. Due to the difference in density, the oil floats on the surface of water and it is recovered, and then dried by a desiccant (sodium sulfate) to remove the little water likely to have been retained in the oil. The essential oils obtained are stored in bottles protected from light and at a temperature of 4 ° C.

Fig.4.2: Hydro-distillation extraction by "Clevenger" device.

- Yield determination of essential oil :

The essential oil yield is the ratio between the weight of the oil extracted and the weight of the plant to be treated **(Carré, P. 1951).(Table.4.1).** The yield expressed as a percentage calculated by the following formula:

$$\mathbf{Y = (PB / PA) \times 100}$$

PB: the mass of H.E obtained.

PA: the mass of dry plant matter.

IV.4.Extraction

IV.4.1. Solid-liquid extraction:

- Maceration method:

This is an extraction technique to isolate substituents from a solid (usually plant material), nine hundred grams of the aerial parts of the plant studied are macerated in a mixtre at room temperature. The mixture used is a hydroalcoholic solution (70% methanol and the rest is made up with distilled water). The operation repeated 3 times for

a period of 24 hours. After filtration of the hydroalcoholic solution, it evaporated (at 38 ° C) under reduced pressure until a concentrated extract obtained. (Crude methanolic extract).

IV.4.2. liquid-liquid extraction:

- Decontaction method :

Due to the high polarity of the hydroalcoholic extract obtained, which created difficulties in the separation phase, The obtained solution filtered, and the filtrate has undergone liquid-liquid extractions as a first stage of separation which is necessary to obtain different extracts depending on the polarity of the solvents used. Therefore, petroleum ether (to separate chlorophyll and fatty acids) was used first, and secondly chloroform (to separate non-polar components, especially terpenes and alkaloids), then ethyl acetate (to separate phenol aglycones) and finally n-butanol (to separate polar and highly polar components such as saponins and glycosylated flavonoids).

The process carried out by decontation **(figure.4.2)**for 3 nights per solvent, and it based on the principle of the sharing of substance (A) between the aqueous phase (E) and the organic phase(s), which is a very important physicochemical property of this substance (A), especially with regard to its philophilic character for fat or water.

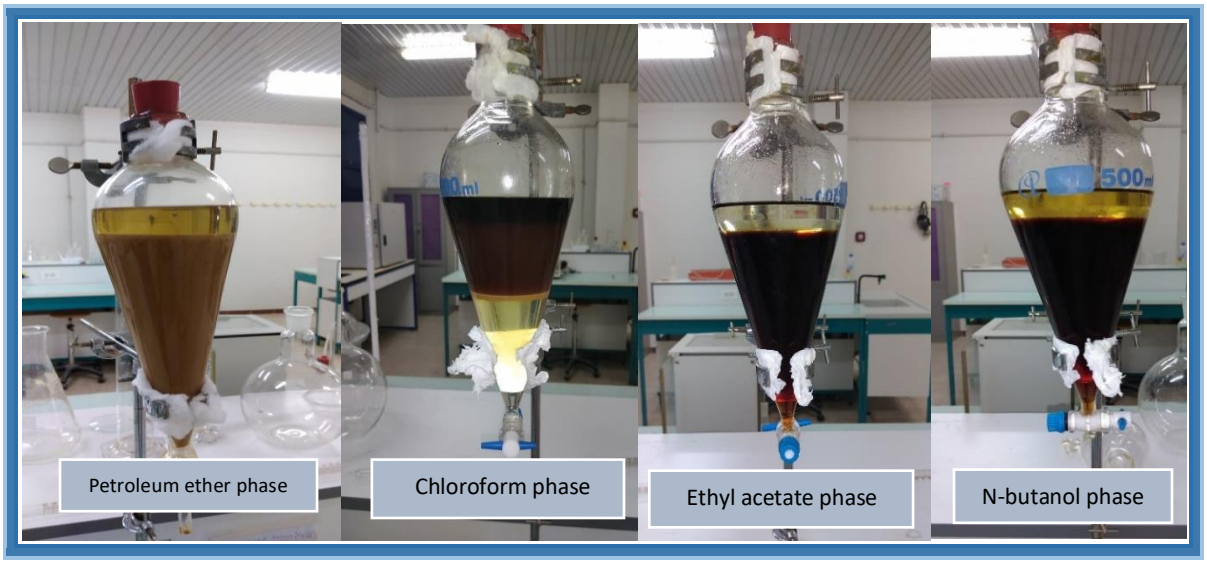


Fig.4.3: pictures of liquid-liquid extraction (Decontation) by different solvents.

The organic phases recovered are concentrated under reduced pressure to dryness and weighed using the Rota-vapor (figure.4.3).

Fig.4.4: Rota-viber device.

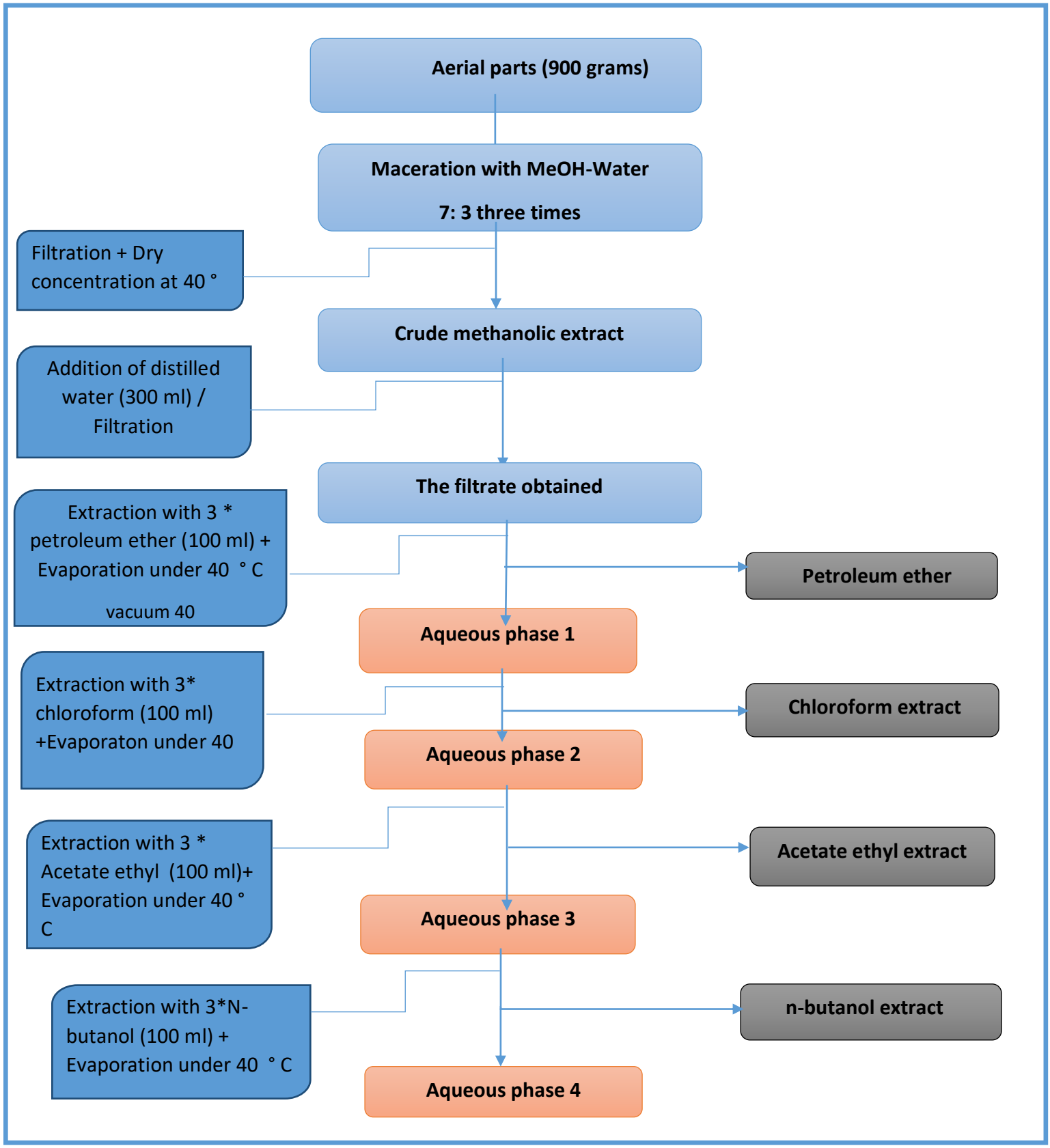


Fig.4.5: Protocol for extracting plant material (L-L).

- **Yield determination of the extracts :**

The yield refers to the mass of the extract determined after evaporation of the solvent, it expressed as a percentage relative to the initial mass of the plant subjected to extraction.

IV.2.Phytochemical and biological study of the plant

IV.2.1.Screening for secondary metabolites:

Phytochemical screening does not provide information on the structure of a well-determined molecule. It only highlights the presence of a particular chemical family that may contain in a sample **(Douhou, N.2003; Senhaji, O.2005)**.

- Detection Saponosides

A few mg of extract dissolved in distilled water in a test tube and stirred vigorously for at least 5 min.

The appearance of a column of foam of about 1cm and persisting for at least 15 minutes indicates the presence of saponosides **(Chavanne, M & al. 1991).**

- Coumarin

Confirmatory test:

1g of vegetable powder placed in a tube, in the presence of a few drops of water. The tubes covered with paper soaked in diluted NAOH and brought to the boil. Any fluorescens testifies to the presence of coumarins after examination under **UV(Douhou, N.2003).**

- Detection Tannins:

1.5 g of dry vegetable matter placed in 10 ml of 80% MeOH. After 15 minutes of stirring, the extracts filtered and placed in tubes; the addition of 1% makes it possible to detect the presence or absence of tannins. The color turns black blue in the presence of gallic tannins and greenish brown in the presence of catechetical tannins **(Douhou, N.2003).**

- Detection triterpenes

Libermann-Burshard test:

Two milliliters of infusion added to three drops of acetic anhydride then stir lightly, after that added a drop of concentrated$H_2\ SO_4$. The change in color observed for one hour, a blue-green color indicates the presence of triterpenes **(Douhou, N.2003).**

- Detection of polyphenols

Two milliliters of each extract mixed with a few drops of $FeCl_3$ solution (2%). The appearance of an intense blackish blue or green color indicates the presence of polyphenols **(Bidie, A.P.& al 2011).**

- Starch research :

A few drops of iodine (I_2) are added to the decoction in the test tube and a change in color towards blue is noted, indicating the presence of starch **(Senhaji, O.2005).**

IV.2.2.Determination of the total flavonoid content:

- Principle:

The quantification of flavonoids carried out by a method based on the formation of complexes between phenolic compounds and aluminum trichloride **(Ribéreau-Gayon. 1968).**

- Operating mode:

The content of total flavonoids in the extracts of the plant studied was determined according to the method described by (**Ayad.R, & al. 2017**). One milliliter of each extract dissolved in methanol (1mg / ml) added to 1ml of AlCl3 solution (2% in methanol). The mixture stirred vigorously and the absorbance read after 10 minutes at 430 nm.

A calibration curve produced by quercetin at different concentrations, performed under the same operating conditions as the samples.

IV.2.3.Evaluation of antioxidant activity in vitro:

- **Principle:**

Indeed, DPPH characterized by its ability to produce stable free radicals; this stability is due to the delocalization of free electrons within the molecule**(figure.4.4).** The presence of these DPPH • radicals gives rise to a dark violet color in the solution. has been proven that the reduction of DPPH • radicals by an agent antioxidant causes discoloration of the solution, and the color change can be followed spectrophotometrically at 517nm and in this way the antioxidant potential of a substance or a plant extract can be determined **(Molyneux, P.2004**).

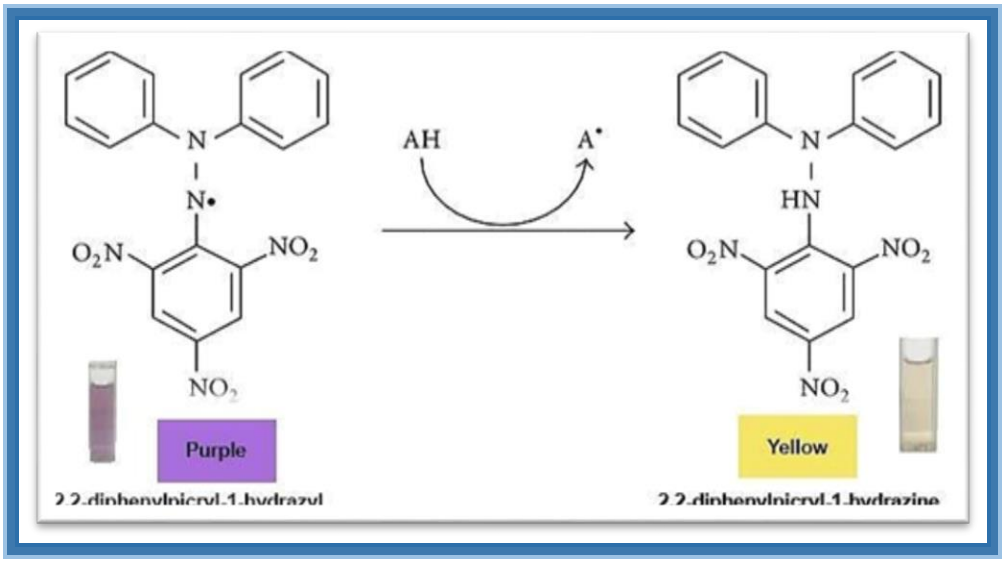


Fig.4.6: The reaction between antioxidants and the DPPH • radical**(Marjoni, M.R,2017).**

IV.2.3.1. Preparation of the DPPH solution:

DPPH 2,2-Diphenyl-1-picrylhydrazyl ($C_{18}H_{12}N_5O_6$; Mr: 394.33), is dissolved in absolute methanol (4 mg / 100ml).

IV.2.3.2.Extract solution:

For the test, the samples were prepared by dissolving in absolute methanol. For all extracts, solutions in absolute methanol were prepared. These solutions, called stock solutions, will then undergo dilutions in order to have different concentrations.

IV.2.3.3.The DPPH tests:

The protocol used for the evaluation of the scavenger effect of plant extracts against the DPPH radical is that of "Cuendet" with a small modification **(Cuendet.M, 1997).**

IV.2.3.4.Operating mode:

50 µl of the test solution are introduced into dry and sterile tubes, 1250 µl of the DPPH solution are added. After vortexing, the tubes placed in the dark at room temperature for 30 minutes. For each concentration, the test repeated 3 times. The reading taken by measuring the absorbance at 517 nm by a spectrophotometer. The negative control is composed of 1250 µl of the methanoic solution with DPPH and 50 µl of methanol.

IV.2.3.5.Expression of results:

To obtain the effective concentration, which reduces the initial concentration of DPPH by 50%, the results expressed as antioxidant activity. The antioxidant activity, which expresses the capacity to scavenge the free radical, estimated by the percentage of discoloration of the DPPH in solution in methanol. The "AA%" antioxidant activity given by the following formula:

$$\mathbf{AA\ \%} = \mathbf{100} - \{[(\mathbf{Abs_{test}} - \mathbf{Abs_{blanc}}) \times \mathbf{100}]/\mathbf{Abs_{control}}\}$$

$$\mathbf{Inhibition\ \%} = (\mathbf{Abs_{control}} - \mathbf{Abs_{test}})/\mathbf{Abs_{control}} \times \mathbf{100}$$

AA: Antioxidant activity.

Abs: Absorbance at the wavelength of 517 nm.

The results expressed as the mean of two measurements ± standard deviation. The IC 50 value was determined for each extract, is define as the concentration of the substrate, which causes the loss of 50% of the activity of DPPH (color).

IV.2.4.Evaluation of antimicrobial activity:

The aim of this work is to evaluate the antibacterial activity of each of the essential oil extract and the other four extracts (n-butanol extract, ethyl acetate extract, chloroform extract and petroleum ether extract) of thapsia garganica against bacteria.

The strains used to detect the antibacterial activity of thapisia extracts belong to four genera of microorganisms, which are reference strains of the American Type Culture Collection (ATCC), these are: *Staphylococcus aureus (ATCC 25293), Escherichia coli (ATCC 25922), Pseudomonas aeruginosa (ATCC 27853),Enterococcus(ATCC 51299).*

IV.2.4.1.Diffusion method in solid medium:

The study carried out by the diffusion method, which is initially designed for antibiotics (antibiogram), but substituting the antibiotic discs with others impregnated with (n-butanol extract, extract of acetate ethyl, chloroform extract, petroleum ether extract) of *Thapsia garganica*. This method consists of depositing sterile Wattman paper discs N°3 and 6 mm in diameter impregnated with 10 µl of each extract with different concentrations, the discs placed at the surface of the petri dish in the presence of discs soaked with an aqueous solution (negative control) placed at the agars seeded with the germ to be tested and to measure the diameters of inhibition in millimeters (mm) after incubation.

IV.2.4.2.Preparation of solutions:

We dissolved each of the essential oil extract and four other organic extracts so that:

- The different organic extracts of the studied plants were dissolved in DMSO and dilutions were made to obtain concentrations of 0.2 g/ml for each tested extract.
- The essential oil extract obtained from distillation was dissolved in DMSO in order to obtain a concentration of 0.1mg/ml.

IV.2.4.3.Microbial strains used:

Our study focused on 4 reference strains which are the following: bacteria pathogenic to Gram+ and Gram-

Table.4.1: Microbial strains used.

Gram+	*Gram-*
• *Staphylococcus aureus (ATCC 25293)* • *Enterococcus (ATCC 51299)*	• *Escherichia coli (ATCC 25922).* • *Pseudomonas aeruginosa (ATCC 27853).*

IV.2.4.4.Preparation of the inoculum:

First, a bacterial suspension is prepared from a pure and young culture (18 hours old). This inoculum used to inoculate Mueller Hinton agars poured into petri dishes to a thickness of 4 mm and then dried in an oven at 37 ° C before use **(figure.4.5).**

Inoculation carried out by swabbing, from the freshly prepared inoculum. It consists of dipping a sterile cotton swab in the suspension then rubbing it, after having wrung it inside the tube, three times over the entire agar surface so the inoculation carried out by swabbing, from the inoculum to form tight streaks, rotating the dish approximately 60 ° after each application to obtain an even distribution of the inoculum.

Pre-sterilized watman paper discs 6 mm in diameter placed on the surface of the inoculated agar after having been loaded with 10 µl of extract. After 24 hours of incubation at 37 ° C, the diameter of inhibition is measured.

Fig.4.7: Preparation of the inoculums of the four extracts and essential oil.

Reference list:

*Dobignard, A., Chatelain, C., Fischer, M., Orso, J., & Jeanmonod, D. (2010). Index synonymique de la flore d'Afrique du Nord. Conservatoire et Jardin botaniques.

*Pottier-Alapetite G. (1979). Flore de la Tunisie, vol. 1, Tunis, Ministère de l'Enseignement supérieur et de la Recherche scientifique et ministère de l'Agriculture,612 p.

*Quézel, P., & Santa, S. (1962). Nouvelle flore de l'Algérie et des régions désertiques méridionales.*Carré, P. (1951). Précis de technologie et de chimie industrielle... Baillière & Fils.

*Douhou, N.2003.Screening phytochimique dune endémique IBERO MAROCAINE THYMELAEA LYTHROIDES.Bull.Soc.Pharm.Bordeaux.61-78.

*Senhaji, O.2005.Etude de l'activité antifongique de divers extraits de gingembre.J.Mycologie.Med.220-229.962.

*Chavanne, M. Beausoin, G,J. Julien, A. Flamand, E. 1991.chimie organique expérimentale. 2é.éd. Modulo.

*Bidie, A.P.; N'guessan, B.B.;Yapo, A.F.; N'guessan, J.D.; Djaman, A.J. Antioxidant activities of ten plants used in ivorianpharmacopoe. Sci. Nat, 2011, 8(1), 1 – 11.

*Cuendet, M. Hostettmann, K. Dyatmiko, W. Potterat, O. 1997. Iridoid glucosides with free radical scavenging propreties from Fagraea blumei.Helvitica Chimica Acta. 80: 1144 -1152.

*Ayad, R.; Cakmak, Y.S.; Ozusaglam, M.A.; Medjroubi, K.; Akkal, S. In vitro antioxidant

and antimicrobial activities of aerial parts of Algerian Jurineahumilis DC (Asteraceae).

Tropical Journal of Pharmaceutical Research, 2017, 16 (12), 2903-2909.

*Ribéreau-Gayon. 1968. Composés phénoliques des végétaux.editiondunod. P : 105-133.

*Molyneux, P. The use of the stable free radical diphenylpicryl-hydrazyl (DPPH) for estimating antioxidant activity.SongklanakarinJornal of Science and Technologie, 2004, 26, 211–219.

*Marjoni, M.R.;Zulfisa A. Antioxidant Activity of Methanol Extract/Fractions of Senggani

Leaves (Melastomacandidum D. Don).Pharm Anal Acta, 2017, 8(8).

Chapter V

Results and discussions

1. Phytochemical screening results:

The results of this study indicate that (extracts) the aerial part of the *plant thapisia garganica* contains several classes of secondary metabolites including: polyphenols, tannins, saponosides.

Table.5.1: Results of phytochemical tests.

Test	Results obtained from *Thapsia garganica*
Polyphenols	++
Coumarins	-
Triterpenes	+++
Saponosides	+
Starch	-

(+): Weak presence (+++): Strong presence.

(++): Average presence; (-): Absence.

2. Yield results of essential oil:

Table.5.2: yield calculation of essential oil.

Extract	Mass (gram)	Yield %
100	0.0219	0.0219

3. Yield results of aerial parts of *Thapsia garganica*:

Table.5.3: The extraction yield of the aerial parts of the plant studied.

Extract	Mass (gram)	Yield%
Crude extract	**66.3**	**7.36**
Petroleum ether	**0.7**	**0.07**
Chloroform	12.8	**1.42**
Acetate ethyl	1.019	**0.11**
N-butanol	3.4	**0.37**

4. Total flavonoid content (TFC) results:

The determination of the flavonoids was carried out according to the trichloride method of aluminum $AlCl_3$ and the standard was quercetin,

The flavonoid content was calculated for the crude extract which is expressed in µg EQ / mg of extract.

Before proceeding to the determination of the content of flavonoids we have established a calibration curve using quercetin as a reference compound **(figure.5.1).**

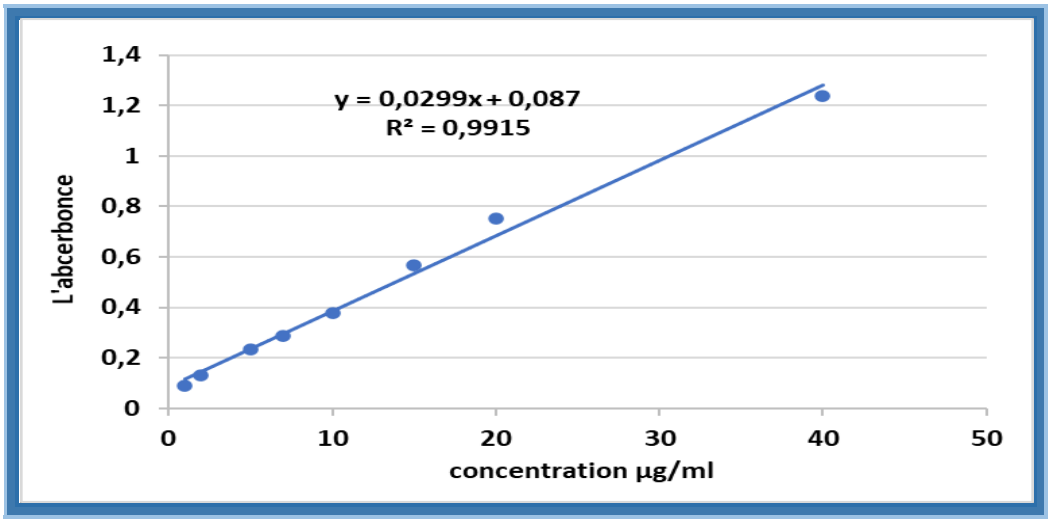


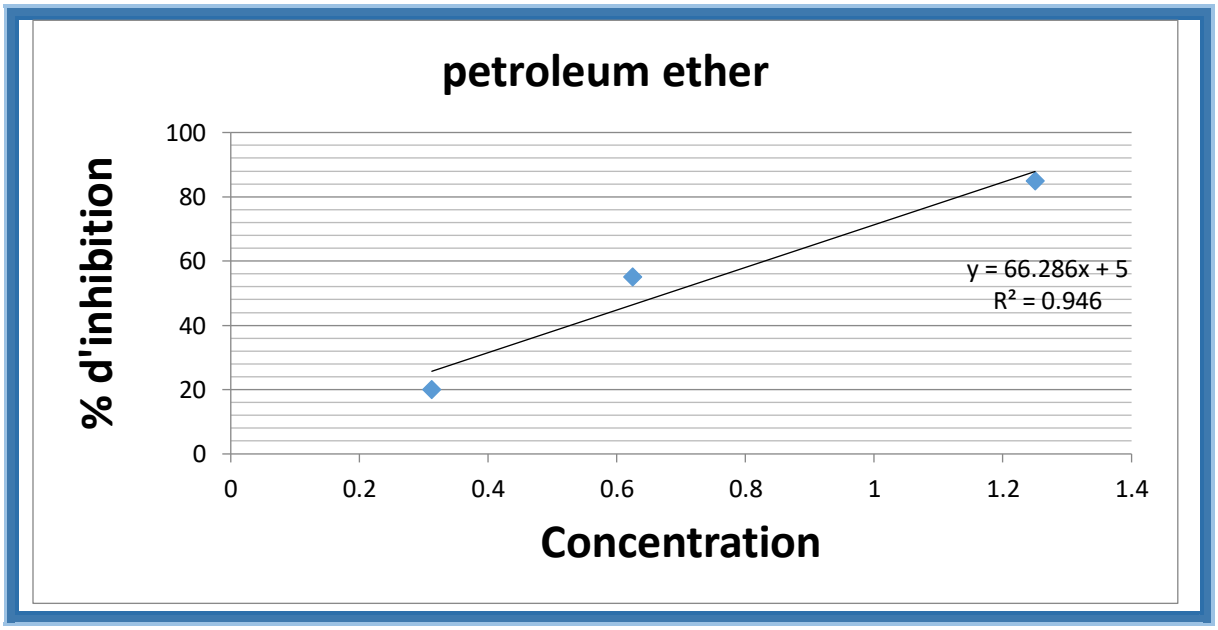
Fig.5.1: Quercetin calibration curve.

The flavonoids were evaluated by the method of aluminum chlorides $AlCl_3$, the content is estimated at 5.6009 mg EQ / g dry matter in the crude extract.

5. Result of antioxidant activity evaluation in vitro:

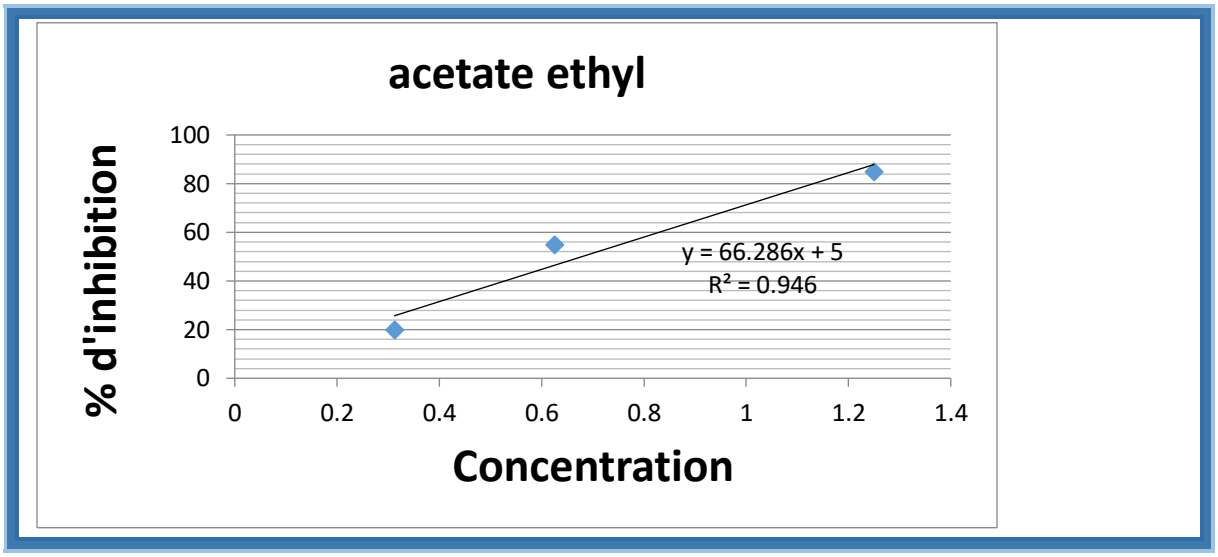
- Trapping of the 2-diphenyl-picrylhydrazyl radical (DPPH) :

The DPPH test has attracted a lot of attention due to its speed, sensitivity and reproducibility. The results obtained in this study reveal a proportional relationship between the concentration of petroleum ether / acetate ethyl extracts as well as the crude extract and the BHT standard with the percentage inhibition of the DPPH radical **(Figure.5.2-5.3).**



IC50 = 42,2938144 mg/ml

Fig.5.2: Result of the antioxidant test of petroleum ether extract



IC50 = 0,67893784 mg/ml

Fig.5.3: Result of the antioxidant test of acetate ethyl

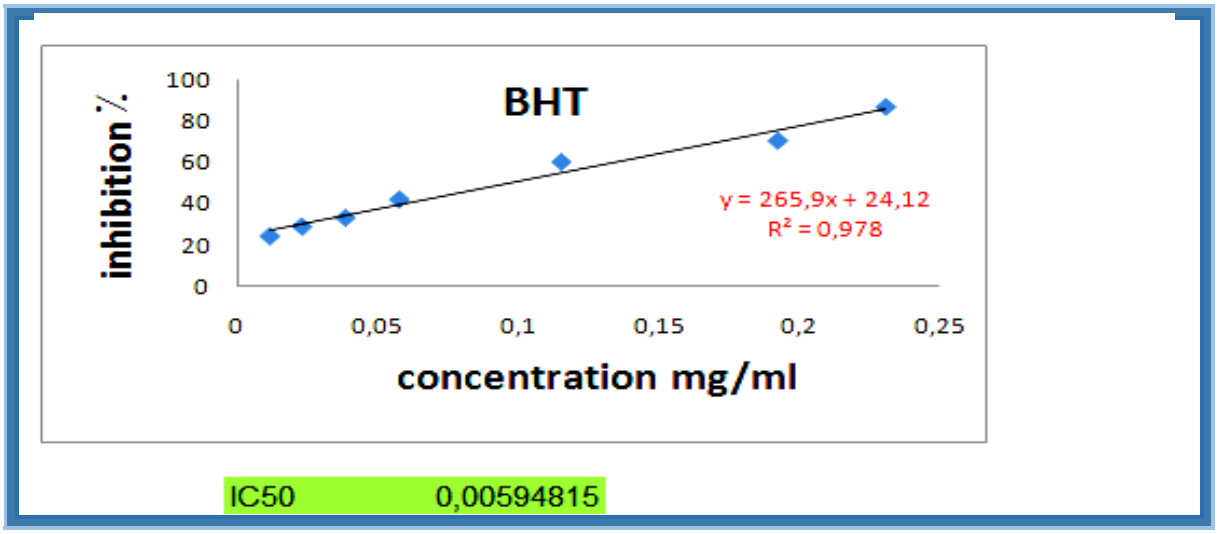


Fig.5.4: Result of standard BHT antioxidant test

The IC 50 of the different extracts are compared with the BHT standard. The IC50s in the order of antioxidant power were: petroleum ether (42,2938144 mg / ml) > acetate ethyl (0,67893784mg / ml> BHT (0.0059 mg / ml).

Depending on the results we have obtained, we can predict that flavonoids are first class antioxidant agents.

6. Antimicrobial activity:

Antibacterial activity of the four extracts and the essential oil extract are determined by measuring the diameter of the zone of inhibition produced around the discs after 24 h of incubation at 37 ° C.

The results obtained shown in the following tables:

Table.5.4: Antimicrobial activity of essential oil and the extracts of "thapsia garganica".

Bacterial strain	Diameters of inhibition zones (mm)				
	N-butanolextract (NB)	acetate ethyl extract (AC)	chloroform extract (CH)	Petroleum ether extract (PE)	Essential oil extract
Escherichia coli (ATCC 25922	6	6	6	6	6
Staphylococcus aureus (ATCC 25293),	6	6	6	6	6
Pseudomonas aeruginosa (ATCC 27853)	6	6	6	6	6
Enterococcus	6	6	6	6	6

The results regarding the antimicrobial activity of the essential oil and the four extracts petroleum ether, chloroform, acetate ethyl, n-butanol of "thapsia garganica" are indicated in **Table.5.4.** The results obtained from disc diffusion method indicated that the essential oil and the four extracts didn't show any antimicrobial activity against all microorganisms tested.

The diameters of inhibition of Pseudomonas aeruginosa (ATCC 27853), Staphylococcus aureus(ATCC 25293),Escherichia coli (ATCC 25922),Enterococcus(ATCC 51299),was 6mm and this indicates the insensitivity of the bacteria against both the essential oil and the extracts.

As the figures 5.5. below show:

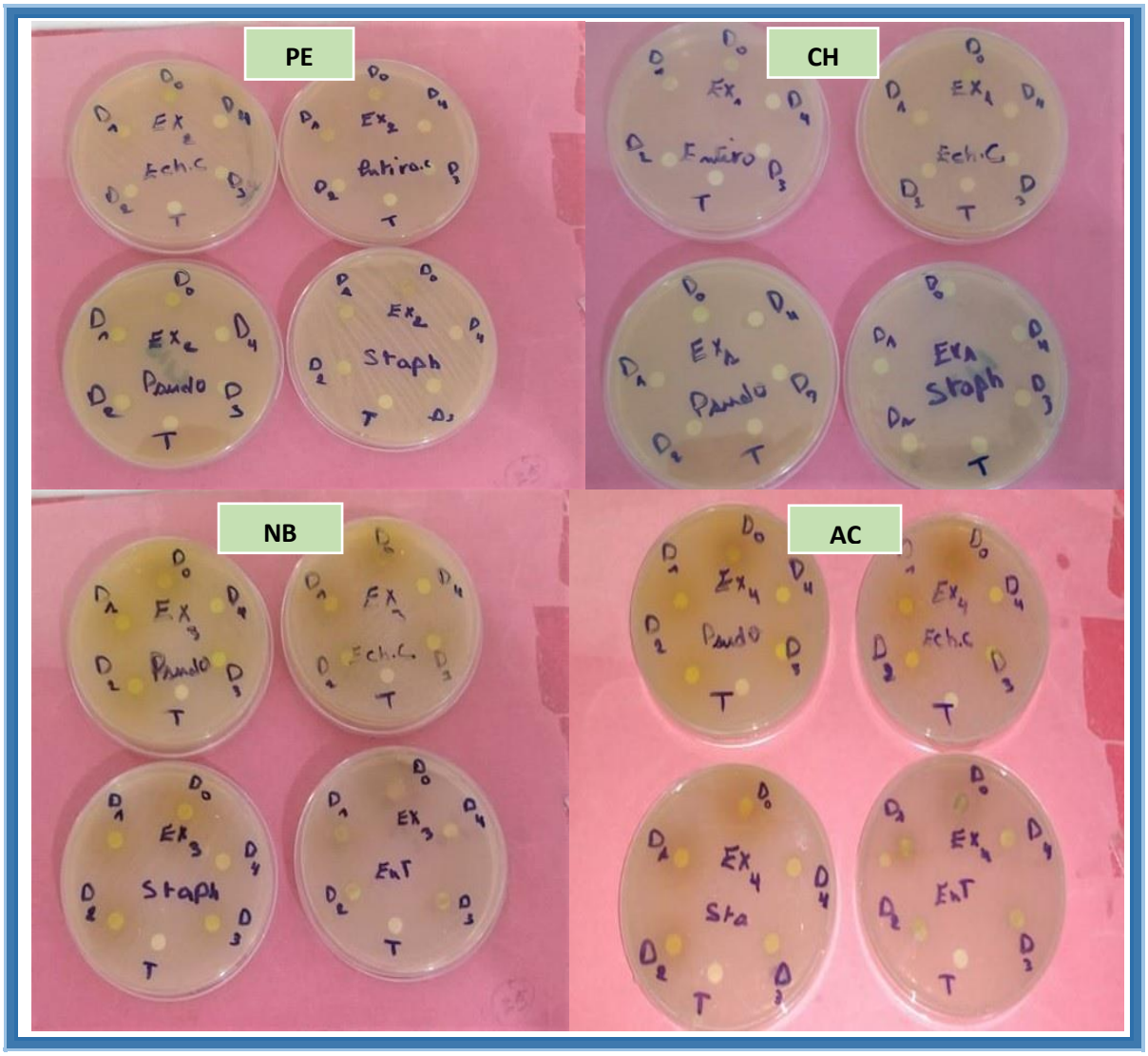


Fig.5.5: Antimicrobial activity of extracts 1-2-3-4

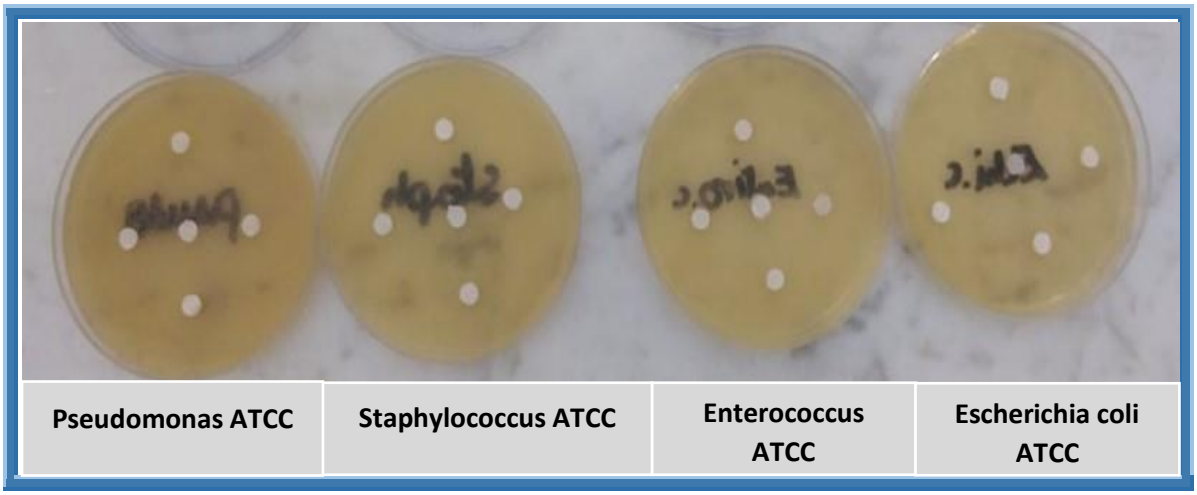


Fig.5.6: Antimicrobial activity of essential oil extract.

Conclusion

Plants are really important to our planet and all living things especially to humans; they provide us with food, fiber, shelter and medicine.

Through a process called photosynthesis, plants produce chemicals, especially secondary metabolites that are synthesized as a measure of self-defense against insects, pests, pathogens, and environment a hazards, and are also essential for drug discovery and development of new therapeutic agents against diseases, not just to reduce the side effects of synthetic drugs. These plants are called medicinal plants, also called medicinal herbs, they have been discovered and used in traditional medicine practices since prehistoric times and continued to be used to day in modern medicine.

In this work, one of the most important plants in traditional and modern medicine is addressed, which is *thapsia garganica*, belongs to Apiaceae family. It was devoted to studying the antibacterial and antioxidant properties of this specie, as well as the discover of its polyphenolic content.

Quantitative determination of total flavonoids by the alimunium chloride $AlCl_3$reagent revealed that *thapsia garganica* is rich in flavonoids.

The study of the antibacterial activity by the method of diffusion on agar medium showed that the plant did not exhibit an antibacterial activity on most of the strains used.

The study of the antioxidant activity showed that the plant have rich of antioxidant elements.

Abstract:

The plant ***Thapsia garganica*** belongs to the family Apiaceae and is widespread in the Mediterranean basin. It is considered one of the most famous medicinal plants since ancient times.

We started this work with the methanolic extraction of all the components contained in the plant in order to obtain a crude extract, which we separated with different polar solvents "petroleum ether, chloroform, and ethyl acetate and n-butanol"

The flavonoids were evaluated by the method of aluminum chlorides AlCl3, the content is estimated at 5.6009 mg EQ / g dry matter in the crude extract.

We extracted the essential oils using a Clevenger device, and then we studied the antimicrobial and antioxidant activity of all extracts and compared them among themselves.

Key words: medicinal plants, antioxidant activity, antibacterial activity.

الملخص:

ينتمي النبات ***Thapsia garganica*** إلى عائلة Apiaceae وهو منتشر في حوض البحر الأبيض المتوسط. و يعتبر من أشهر النباتات الطبية منذ القدم.

بدأنا هذا العمل بالاستخلاص الميثانولي لجميع المكونات التي يحتوي عليها النبات من أجل الحصول على مستخلص خام، والذي فصلناه بمذيبات مختلفة القطبية ، وهي إيثر البترول، والكلوروفورم،والإيثيل. أسيتات و ن-بيوتانول

وقد تم كذلك تحديد نسبة الفلافونويدات في المستخلص الخام. فكانت على التوالي 5,6009 مغ مكافئ الكارستين لكل غ من النبتة وهي جافة.

قمنا باستخلاص الزيوت الأساسية بواسطة جهاز كلافنجر، ثم قمنا بدراسة النشاطية المضادة للميكروبات و النشاطية المضادة للأكسدة لجميع المستخلصات ومقارنتها فيما بينها .

الكلمات المفتاحية: النباتات الطبية ، نشاط مضاد للأكسدة، نشاط مضاد للبكتريا.

Résumé:

La plante ***Thapsia garganica*** appartient à la famille des Apiacées , elle est répandue dans le bassin méditerranéen. Elle est considérée comme l'une des plantes médicinales les plus célèbres depuis l'Antiquité.

Nous avons commencé ce travail par l'extraction méthanolique de tous les composants contenus dans la plante afin d'obtenir un extrait brut, que nous avons séparé avec différents solvants polaires, " l'éther de pétrole, le chloroforme et l'éthyle. acétate et n-butanol"

Les flavonoïdes ont été évalués par la méthode de chlorures d'aluminium AlCl3, la teneur est estimée à 5,6009 mg EQ/ g de matière sèche dans l'extrait brute .

Nous avons extrait les huiles essentielles à l'aide d'un appareil Clavinger, puis nous avons étudié l'activité antimicrobienne et antioxydante de tous les extraits et les avons comparés entre eux.

Mots clés : plantes médicinales, activité antioxydante, activité antibactérienne.