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Phytochemical study of desert plant *SalsolaVermiculata* L.

Discuss on:30 /06 /2019

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University year: 2018/2019


بسم الله الرحمن الرحيم
إنه من سليمان وإنه

DEDICATION:

We dedicate this work modest to:

Muhammadpeace be upon him.

Our Mothers and our Fathers …

Our teachers…

Our family small, and the family large_Algeria _

To youth of February 22nd.

The spirit of the martyr Mohammad MURSI

The family of University.

OULED SALEM Hasna

NECIB Asma

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List of abbreviation:

Abs: Absorbance

AEAC: Ascorbic acid equivalent antioxidant capacity

ATP: Adenosine_triphosphate

BHA: Butylatedhydroxyanisole

BHT: Dibutylhydroxytoluene

CC: Column chromatography

CHI: Chalconeisomerase

CI_{50}: Concentration at inhibition of 50%

COSY: Correlation spectroscopy

DAHP: 3_deoxy_D_arabino_heptulosonate 7_phosphate

DPPH: α, α-diphenyl-β-picrylhydrazyl

EPSP: 5_enolpyruvylshikimate_3_phosphate

F3H: 2_Flavanone_3hydroxylase

FLS: 5_Flavonol synthase

HMBC: Heteronuclearmultiple_bond correlation spectroscopy

HSQC: Heteronuclear single_ quantum correlation spectroscopy

IFS:3_Isoflavone synthas, 4_flavone synthase

IL-10: Interleukine

MIC: Methyl isocyanate

NAD^+: Nicotinamide adenine dinucleotide

$NADP^+$: Nicotinamide adenine dinucleotide phosphate

NMR: Nuclear magnetic resonance

NOESY: Nuclear Overhauser effectspectroscopy

PEP: Phosphoenolpyruvate

PGE2: Prostaglandin E2

RNS: Reactive nitrogen species

ROESY: Rotating frame nuclear Overhauser effect spectroscopy

ROS: Reactive oxygen species

TAC: Total phenolic content

TFC: Total flavonoids content

TLC: Thin_ layer chromatography

TNFα: Tumor necrosis factor alpha

UV: Ultraviolet

General introduction

The natural product is chemical substance produced by a living organism; a term used commonly in reference to chemical substances found in nature that have distinctive pharmacological effects. [1]

The World Health Organization estimates that plant extracts or their active ingredients are used in traditional medicine by more than 80% of the world's population. [2] Over 50% of all modern clinical drugs are products of natural origin and natural products play an important role in drug development programs in the pharmaceutical industry. [3] Many researchers around the world have studied the effects of herbal extracts in microorganisms. [4-5]

Algeria is characterized by a climatic diversity which is favorable for growth and development of a flora rich in medicinal and aromatic plants; Mediterranean climate in the north (collectively known as the Tell), semi-arid climate in the HautsPlateaux (high steppin plains) and arid across the Sahara, In Algeria, many patients use medicinal plants a streatment for many ailments and serious diseases, such as diabetes and arterial hypertension.[6]

The objective of our contribution to this research work is to promote the flora of the southern region of Algeria through the search for compounds possessing therapeutic properties and biological activity. Therefore, a plant from the *Chenopodiaseae* family, *Salsolavemiculata L.*, is the subject of our study.

- ✓ Chapter one composes a bibliographical synthesis in which is presented the botanical and phytochemical elements of the plant and their traditional and medicinal use as well as some previous studies on this plant such as biological activities.
- ✓ The second chapter will study a bibliographic review of secondary metabolites in general and Super family of plant.
- ✓ The third chapter will study a bibliographic review of biological activity such as (antibacterial and antioxidant).
- ✓ The fourth chapter of our work relates to the experimental part so that it contains the materials and the method of work of extraction and antioxidant activity and separation of phenolic compounds.
- ✓ Finally, the fifth chapter concerns the results of the phytochemical study and the antioxidant tests on the extracts as well as the study of the relation between the phenolic content, the flavonoids content, the antioxidant activity of different extracts. At the end of this chapter, we describe the identification of isolated compounds.

- ✓ A general conclusion that contains the most important research results and prospects for the future work of this.

THEORETICAL REVIEW

Chapter (I):

Phytochemistry of plant

I. Phytochemistry of plant:

*Salsolavermiculata*L. (Mediterranean saltwort), a member of the Chenopodiaceae family, is one of the dominant perennial species in the Mediterranean arid zone. This species is distributed throughout the Middle East and North Africa, including Algeria, Egypt, Iran, Jordan, Lebanon, Libya, Morocco, the Mediterranean islands of Sardinia and Sicily, Spain, Syria, and Tunisia .[7]

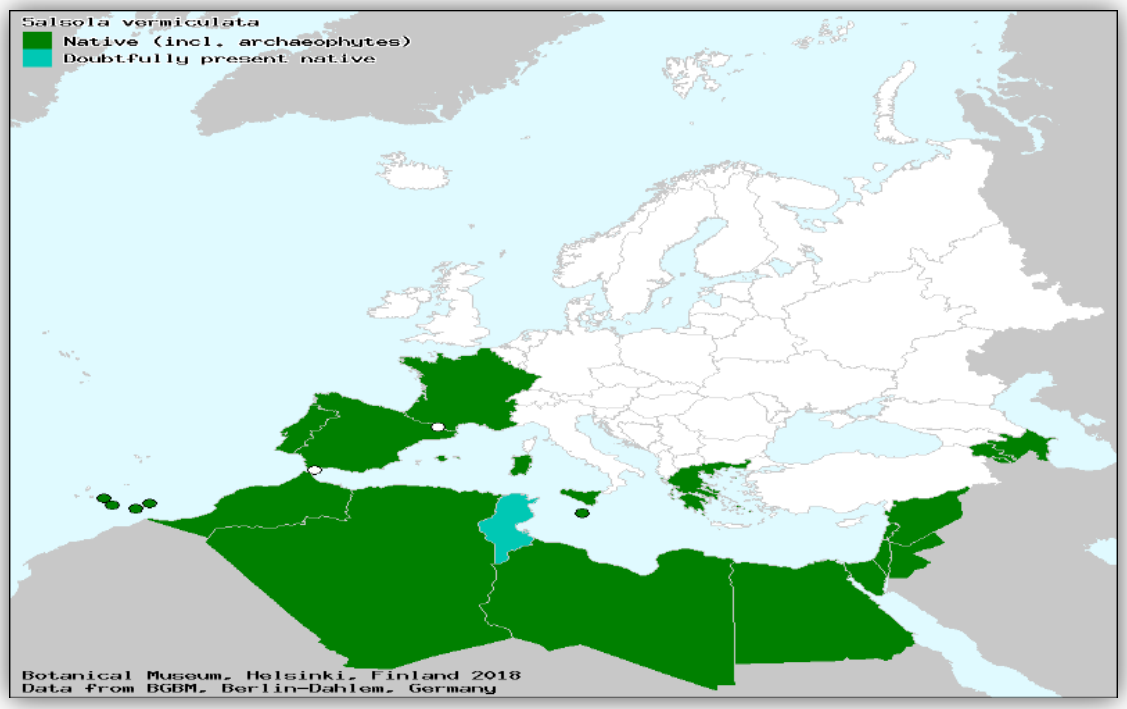


Figure1: Mapof*salsolavermiculata*[8]

I.1. Plant classification and systematic:

- **Synonym (Scientific):**

Caroxylonvermiculatum(L.) AkhaniandRoalson

SalsoladamascenaBotsch.

- **Life form (Raunkiaer):**

Chamaephyte.

- **Flowering and Reproduction :**

Petal or tepal color: green.

Sexuality and Reproductive Morphology:Flowers hermaphrodite only.

Sporangia or Seed Homogeneity: Homogeneous seeds-fruits.

Flowering Time: August, July, June, May, September.

- **Leaf Arrangement** :

Alternate (one leaf per node).

Leaf Type: Scale.

- **Habitat:**

Habitat: Desert, Shrub-steppes, Salty habitats.

Chorotype: Irano-Turanian - Saharo-Arabian.

halophyte .

- **Synanthrop:**

obligate natural.

Figure 2:*salsolavermiculata*.

I.1.a.Botanical classification:

Kingdom: plantae.

Phylum:Spermaphyte.

Subphylum : Angiosperms.

Class: Dicotyledonae.

Ordre : EudicotsCaryophyllales.

Family : Amaranthaceae (chenopodiaceae).

Subfamily:salsoloideae.

Genus : Salsola.

Species : Salsola vermiculate. L.

I.2. Botanical description:

Shrubby perennial with ascending to erect stem. Much branched, up to 60 cm, grey, yellowish-villous, or pubescent, with long denticulate hairs; sometimes branches becoming ± spinescent at tips. Lower leaves alternate. Filiform, terete or semiterete, half-clasping at base, 3-15 mm long, villous: upper leaves or those of the shorter branches ovate. Scale-like, ± densely imbricated, alternate, obtuse. Bracts ovate to short-cuspidate, concave, scarious margined; bracteoles *c.* as long as theBract, suborbicular, concave, ± keeled beneath. Scarious margined. Flowers as long as or exceeding the bract, solitary, forming ± dense spikes. Perianth segments usually 5, almost free, ± connivent, ovate-triangular, ± hairy, broadly scarious-margined; usually developing obovate to semi-orbicular. imbricated wings at back, (7-) 8·12 mm across, pink, red, white or yellowish in color; seed horizontal, c. 2.5 mm in diam, with membranous testa. [9]

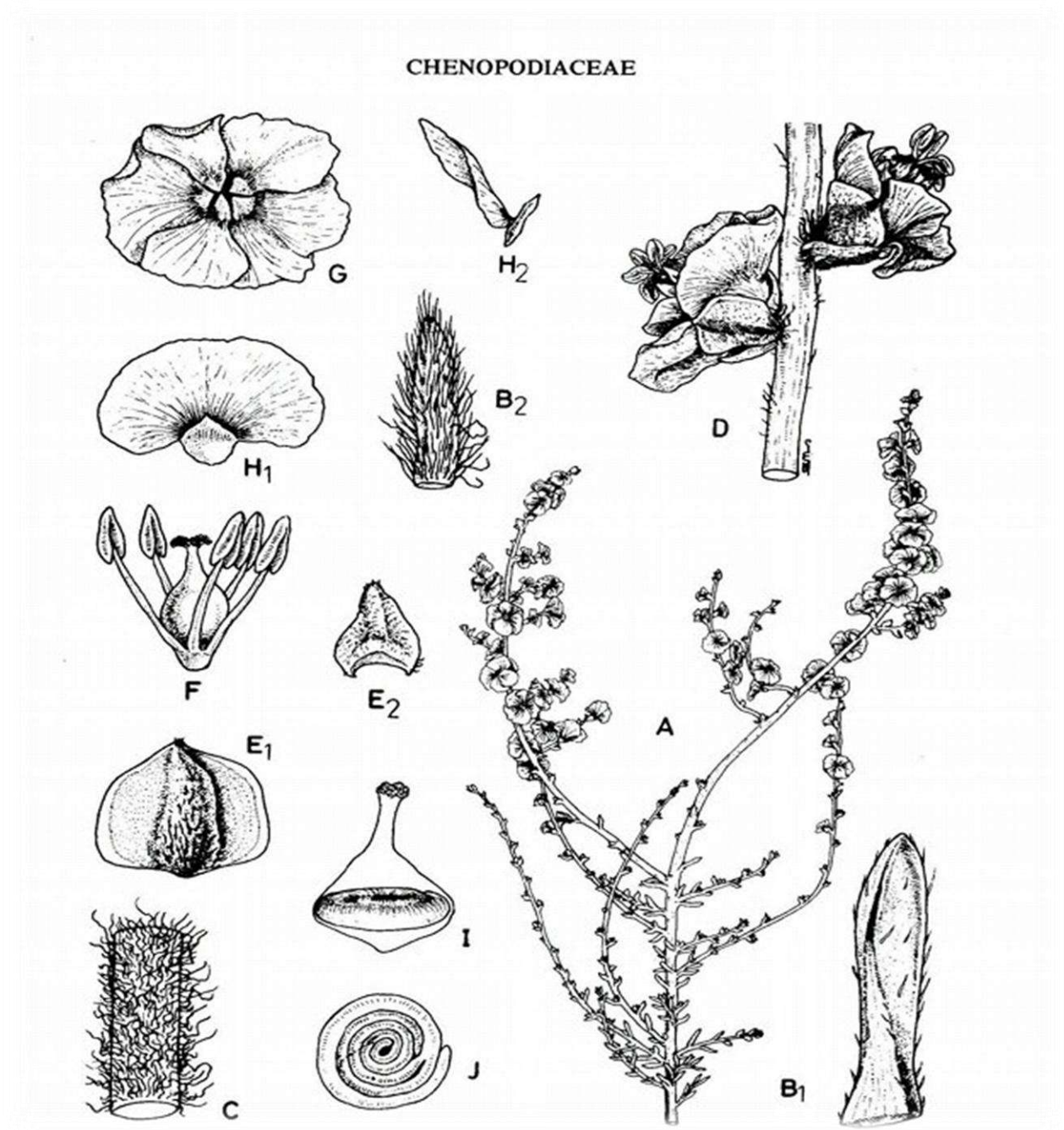


Figure 3: ***Salsolavermiculata:*****A .Portion of fruiting stem, B_1-B_2. Ieaf variation, C. Portion of branch showing hairs, D. Portion of inflarescence, E_1.Bract, E_2. Bracteole, F. Flower with perianth removed showing stamens and gynoecium, G. Fruitingperianth segments on a flower (top view), H_1. Fruiting perianth segment (front view), H_2. the same (side view), I. fruit with horizontal seed, J. seed (embryo) (dorsal view).**

I.3. Pharmacological properties:

*Salsolavermiculata*had been reported to cause autolysis of membranous structures, induce significant aortic dilation, inhibit phagocytic activity and nitric oxide production of certain cells, and reduce levels of tumor necrosis factor-alpha (TNFα), prostaglandin E2 (PGE2), and interleukin-10 (IL-10) without affecting ATP levels. Overall, the unique properties of Salsolavermiculata and Suaedavermiculatamethanolic extracts may be potentially applied as a promising source of antioxidant and antimicrobial agents and may be used for the management of drug-resistant pathogens of burn infections.[10]

I.4. Use in traditional medicine:

Our search for uses the*Salsolavermiculata*pant in medicine, Biskra and Ouargla use it in traditional medicine in the treatment of diabetes**.**

I.5. Previous chemical studies of *Salsolavermiculata*:

I.5.1. Antibacterial activity and Antifungal activity of *S. vermiculate L*. extracts:

The present study indicated that *Salsola vermiculate* L. leaves, stems and roots volatile fractions are rich in carvone (52.2%, 53.0% and 49.9% respectively). *S. vermiculate* extracts are active in particular on the positive gram bacteria (*E. faecalis*and *S. aureus*). However, they have low antifungal activity against the three tested fungal species (*C. krusei*, *C. parapsilosis*and *C. glabrata*).[11]

The richness of *S. vermiculate* in carvone could explains its antifungal activity. Carvone has both antibacterial and antifungal potential. It showed antimicrobial activity against *Aspergillusniger*, *Saccharomyces cerevisiae*, *Candida albicans*, *Campylobacter jejuni*, *Listeria monocytogenes*, *Enterococcus faecium*, *Escherichia coli*, *Salmonella typhimurium*and *Photobacteriumleiognathi*.[12]

I.5.2. Essential oils:

I.5.2.1. Analytical Gas chromatography:

The chromatographic analysis of *S. vermiculate* volatile fractions permitted us to identify forty-four compounds belonging to several chemical classes as shown in Table 1. [13]

Table 1: Chemical composition of *S.Vermiculate* volatile fractions.

Compounds	L.r.i.	*S. Vermiculate L.* volatile fractions		
		Leaves	Stems	Roots
α-pinene	941	1.3	-	1.1
5-mehtyl-3-heptanone	945	-	1.8	-
Camphene	955	-	-	0.4
Sabinene	977	-	0.2	0.3
β-pinene	982	0.6	0.9	0.5
Myrcene	992	-	0.3	-
2-pentyl furan	993	0.7	-	0.6
Limonene	1032	0.7	17.4	0.8
1,8-cineole	1034	1.0	0.7	0.7
Linalool	1101	7.1	11.3	8.2
Nonanol	1103	0.6	-	0.4
Camphor	1145	-	0.7	0.4
Isoborneol	1158	-	1.2	-
4-terpineol	1179	-	0.3	-
α-terpineol	1191	-	1.0	-
Decanal	1206	0.8	-	0.6
Verbenone	1206	-	-	-
Cumin aldehyde	1241	6.0	1.2	4.4
Carvone	1243	52.2	53.0	49.9
Perilla aldehyde	1272	-	-	0.3
α-terpinen-7-ol	1284	0.9	-	0.7
(E)-anethole	1285	1.3	-	0.7
Carvacrol	1301	0.6	-	-

P-vinyl guaiacol	1308	0.6	-	-
α-copaene	1377	0.6	0.6	0.9
β-caryophyllene	1419	5.8	7.5	8.5
α-humulene	1455	-	0.4	0.6
Germacrene D	1482	-	-	0.5
β-selinene	1486	0.6	-	0.5
Valencene	1493	0.7	-	0.5
α-selinene	1496	-	-	-
Bicyclogermacrene	1496	-	-	0.4
β-bisabolene	1509	0.7	-	0.6
Trans-γ-cadinene	1514	0.7	-	-
δ-cadinene	1524	1.3	-	1.2
Caryophyllene oxide	1582	1.2	-	1.1
Carotol	1595	0.5	-	-
α-acorenol	1632	-	-	0.4
T-cadinol	1641	1.0	-	0.5
T-muurolol	1642	1.2	-	1.1
α-muurolol	1646	0.9	-	0.5
Ar-turmerone	1666	2.8	-	3.1
n-octadecane	1800	-	-	0.5
hexahydrofarnesylacetone	1845	3.5	-	3.3
monoterpene hydrocarbons		2.6	18.4	3.1
Oxygenated monoterpenes		67.8	69.4	64.6
Sesquiterpene hydrocarbons		10.4	8.5	13.7
Oxygenated Sesquiterpene		7.6	0.0	6.7
Phenylpropanoids		1.3	0.0	0.7
Apocarotenes		3.5	0.0	3.3
Non-terpene derivatives		2.7	1.8	2.1
Total identified		95.9	98.5	94.2

I.5.3. Enhancement of the adsorptive properties of a desert *SalsolaVermiculata* species:

This study showed that the low-cost natural *SalsolaVermiculata* was capable of adsorbing acetic acid, nickel (II) and copper (II) ions from synthetic aqueous solutions.[14]

I.5.4. Methylene blue and iodine adsorption onto an activated of *S.Vermiculata*:

The ability of *S.Vermiculata*plant to adsorb methylene blue and iodine has been investigated. This study showed that the chemically activated plant has a high adsorptive capacity for dyes in comparison with well-known commercial activated carbon.The iodine number values pointed out that significant additional surface area can be achieved through zinc chloride activation and that microporosity contributes considerably to the total surface area of the prepared material making it a very good adsorbent for small compounds. Nonetheless, the significant methylene blue adsorption also showed that this remarkable powdered species is suitable for adsorbing large compounds such as dyes, which may make this desert plant a successful biosorbent in the treatment of colored effluent waters. [15]

Chapter (II):

Secondary metabolites

II. Secondary metabolites:

The plants are one of the most important sources of medicines in the world, it was apparent in their use in alternative medicine. Natural products have been exploited by humans for thousands of years, used as foods, drugs, antioxidants, flavors, fragrances, dyes, insecticides, and pheromones, improving our health, enhancing crop production, unraveling complex ecological interactions, and shaping our way of life. [16] Natural products have been the mainstay of cancer chemotherapy for the past 30 years. [17]

We will focus here in this study on some of the products of secondary metabolism, what is the secondary metabolism? Where are the products of secondary metabolism?

II.1. Secondary Metabolites of plant:

Humanity has been exploiting plant chemicals in the form of potions and poisons for thousands of years. The attitude toward the physiological significance of this plethora of small molecules is reflected in the terminology that was assigned to them: secondary metabolites, it is a line of defense the first to plant, which describes it into further demonstrate function; the research group of *Hans Grisebach* showed that secondary metabolites have a role in plant defense.[18]

Secondary metabolites are the most active fraction of the chemical compounds found in plants and it is estimated today that about 1/3 currently on the market drugs contain at least one such plant substance.[19]Consists your Secondary metabolites of flavonoid, Alkaloid, Comarines… .

II.2.Super family of plant:

II.2.1. Flavonoids:

Flavonoids are a group of plant secondary metabolites characterized by a diphenyl propane structure. [20] Flavonoids are a group of natural compounds with variable phenolic structures and are found in plants. In 1930, a new substance was isolated from oranges. At that time it was believed to be a member of a new class of vitamins and wasdesignated asvitamin P. [21] to date, about 6000 flavonoid compounds have been isolated and identified, and many are common in higher plants. [22]

II.2.1.1. Definition:

Flavonoids contain C15 atoms in their basic nucleus and C15 atoms composed of two aromatic rings linked through a heterocyclic pyrane ring,all flavonoids share the basic C6-C3-C6 Structural skeleton,[23_24] consisting of two aromatic C6 rings (A and B) and a heterocyclic ring (C) that contains one oxygen atom. [23_21]

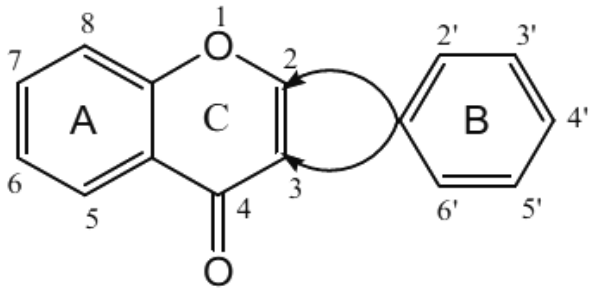


Figure 4: Basic flavonoid structure.

II.2.1.2. Biosynthesis of flavonoids:

Like other flavonoids, kaempferol has a diphenylpropane structure (C6-C3-C6) and is synthesized by condensation of 4-coumaroyl-CoA (C6-C3) with three molecules of malonylCoA (C6). [20]

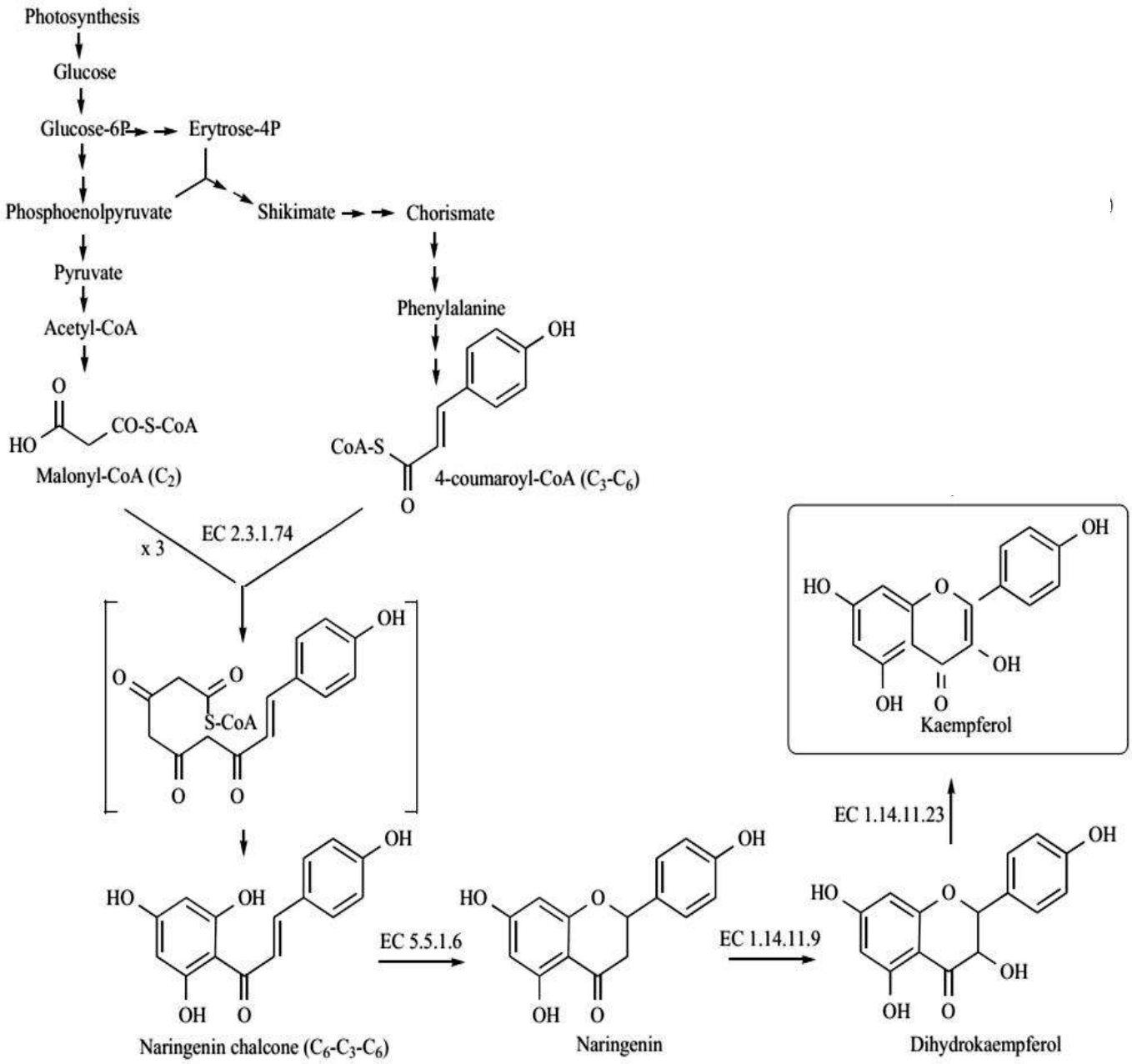


Figure 5: Biosynthesis of kaempferol and some glycosides of kaempferol (see text for further details). EC 2.3.1.74: chalcone synthase; EC 5.5.1.6: chalconeisomerase; EC 1.14.11.9: flavanone 3-dioxygenase; EC 1.14.11.23: flavonol synthase. [20]

Chalcone is the starting point for the manufacture of other varieties of Flavonoids using special enzymes.

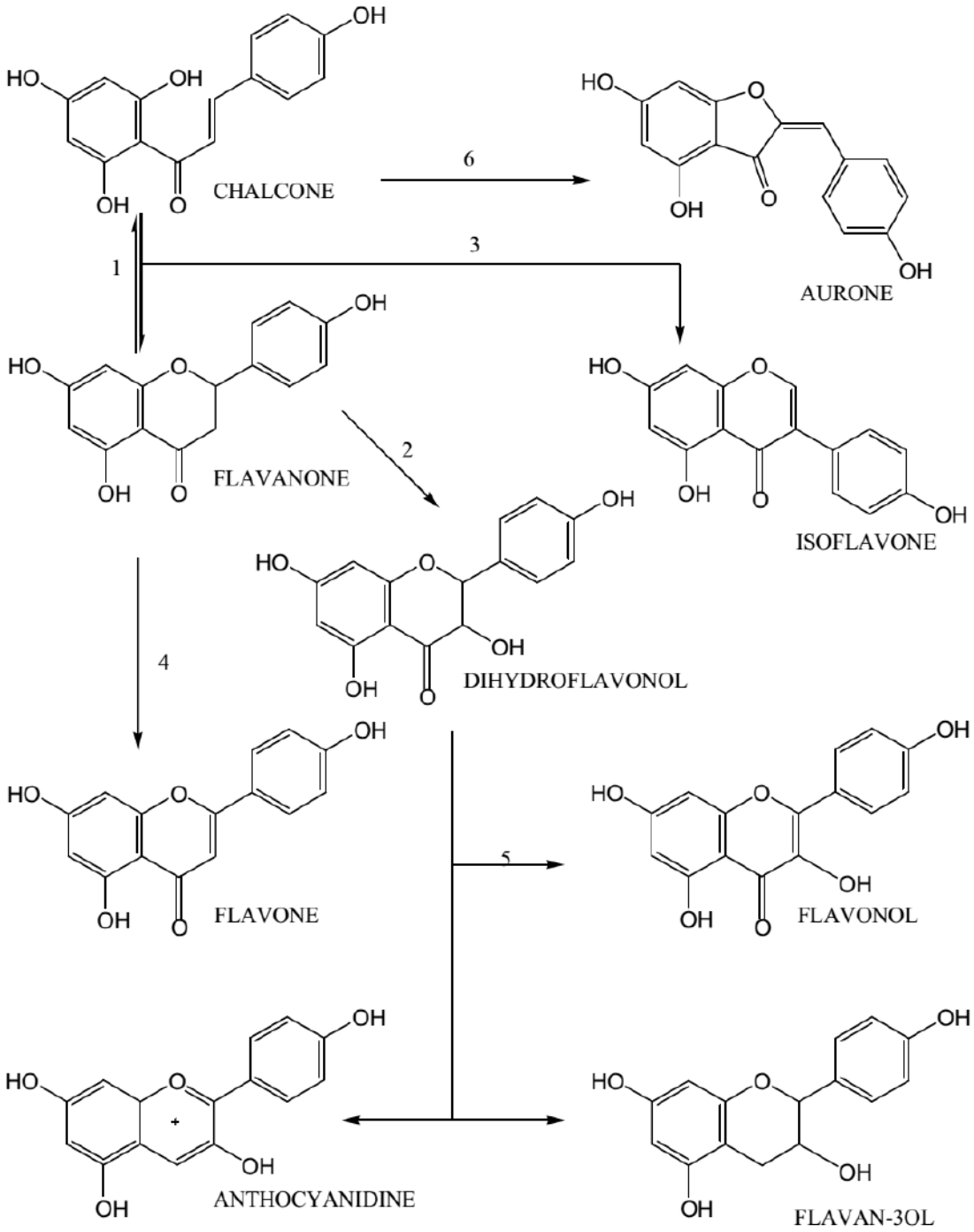


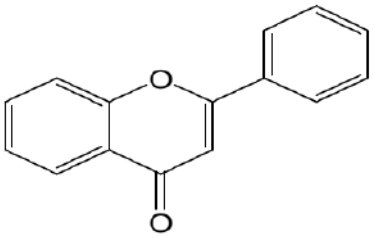
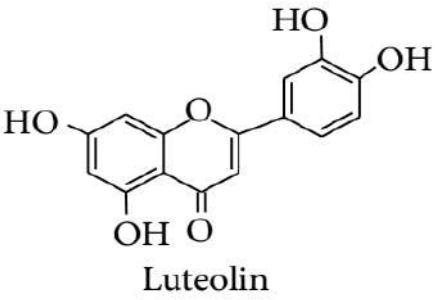
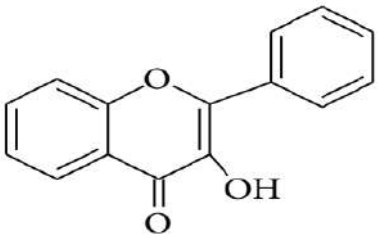
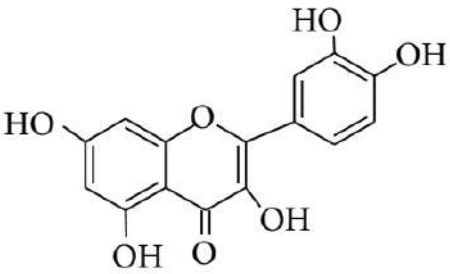
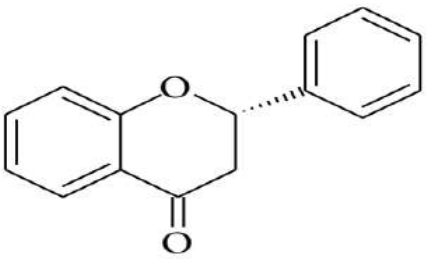
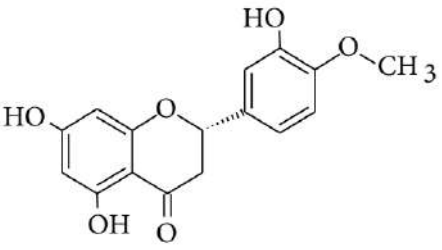
Figure 5: plan showing the rest of flavonoids from Chalcone(1 -chalconeisomérase (CHI), 2-flavanone -3hydroxylase (F3H), 3-isoflavone synthas,4-flavone synthase(IFS), 5-flavonol syntase (FLS), 6-withont catalyzes). [25]

II.2.1.3. Classification (distribution):

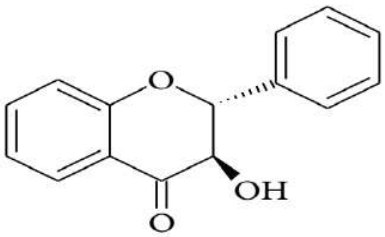
They can be divided into a variety of classes such as flavones (e.g., flvone, apigenin, and luteolin), flavonols (e.g, quercetin, kaempferol, myricetin, and fietin), flavanones (e.g, flvanone, hesperetin, and naringenin), and others. Their general structures are shown in Table 2.The

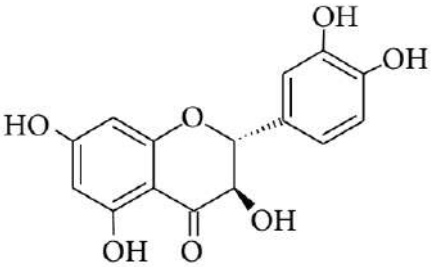
various classes of flvonoids differ in the level of oxidation and patternof substitution of the C ring.[21]

Table 2: flvonoids Structures.

Group of flvanoid	Structure backbone	Examples
Flavones		 Luteolin
Flavonols		 Quercetin
Flavanones		 Hesperetin

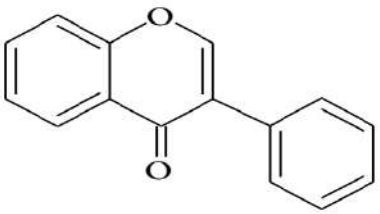
Flavanonol

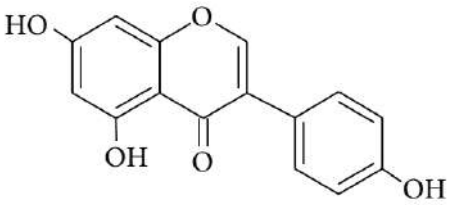




Taxifolin

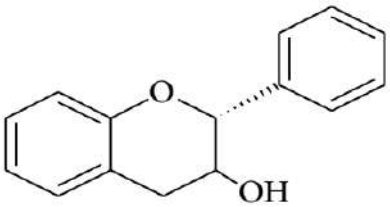
Isoflvones

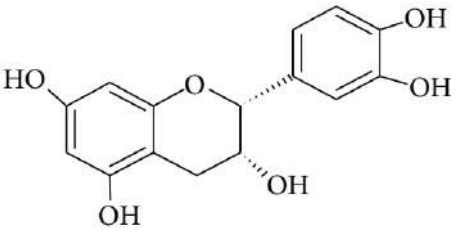




Genistein

Flavan-3-ols





Epicatechin

II.2.1.4. Application in Industrial Chimistry:

Many studies and research have shown that flavonoids are important for the treatment of many diseases.[26_27_28]A link between the chemical composition of flavonoids and their therapeutic properties has been observed, the increase in hydroxyl groups results in increase tumor activity, the increase in the metoxyl group results in increased antibody activity Cancer,[28_29_30] and some are effective against infections.[31]As that for same flavonoids effects anti-infalammatory[32_33] Allergies,[34_35]Microbes[36_37] and Viruses.[38_39]

II.2.2.Comarines:

Coumarins owe their class name to ‘Coumarou’, the vernacular name of the tonkabean , from which coumarin, it was isolated in 1820[40] and more than 1300 coumarins have been identified as secondary metabolism from plants, bacteria and fungi .[41]The Coumarin of vehicles used in the industry, cosmetics, as well as industries pharmaceuticl.

II.2.2.1. Definition:

Coumarin is a fragrant chemical compound of benzopyrone found in many plants. It has a distinctive odor, which has led to its use as a food additive and ingredient in perfume. [42]It is natural aromatic substance;Coumarin (Figure 7) is used in perfumery. Its smell is similar to vanillin and freshly cut hay.

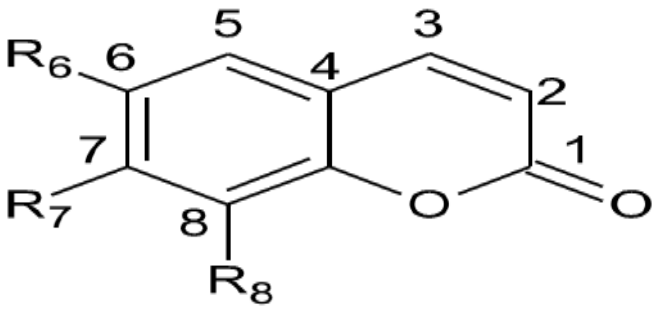


Figure 7: Basic Coumarin structure.

II.2.2.2. Classification (Distribution):

The coumarin is the point of a family of compounds, which are formed by substitution of its aromatic cycle. [43] Because of this according to the nature of the substituent. We can classify the coumaren in: coumarines simples, furocoumarines and pyranocoumarines .[44]

A. Coumarines simple:

Depends on structure for infrastructure of coumarins, is associated with the minds of (H, OH, OCH3...).

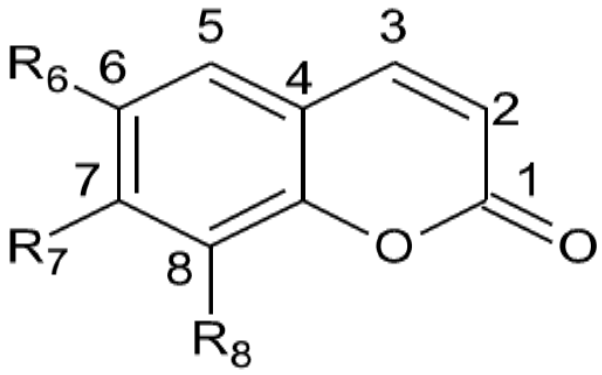


Figure 8: Basic Coumarin simples.

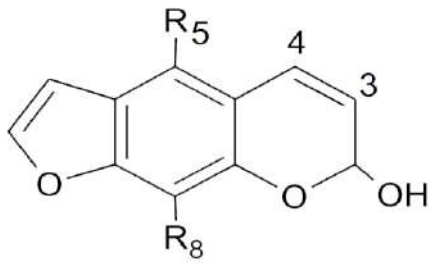
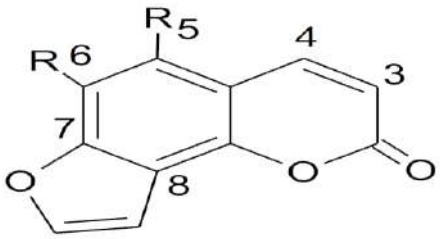
Table 3:Examples form composite Coumarin simples and sites correlation in atoms C_6, C7 and C8.

Coumarines simples			
Composite	**R6**	**R7**	**R8**
Ombehferone	H	OH	H
Dqphnetine	H	H	OH
Esculitetine	OH	H	OH
Esculetol	OH	OH	H
Coumarin is linked to OCH_3			
Scopoletine	OCH_3	H	OH
fraxetine	**OCH_3**	**OH**	**OH**

B.Furocoumarines:

Furecoumarinare tricyclic molecules produced by the combination of two heterocycles (coumarin and furan). [42]

Table 4: Examples form compositeFurocoumarines.

linear			angular		
					
Composite	R5	R8	**Composite**	R5	R6
Psoralene	H	H	Angelicine	H	H
Bergaptene	OCH_3	H	Pimpinelline	OCH_3	OCH_3
Isopimpenelline	OCH_3	OCH_3	Sphondine	H	OCH_3

C. Pyranocoumarines simple:

There is no formula essential for Pyranocoumarines, which is a coumarin linked ring hétérocyclepyrane. [42]

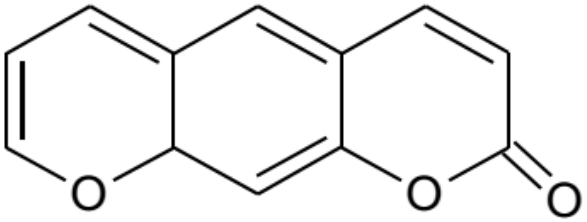
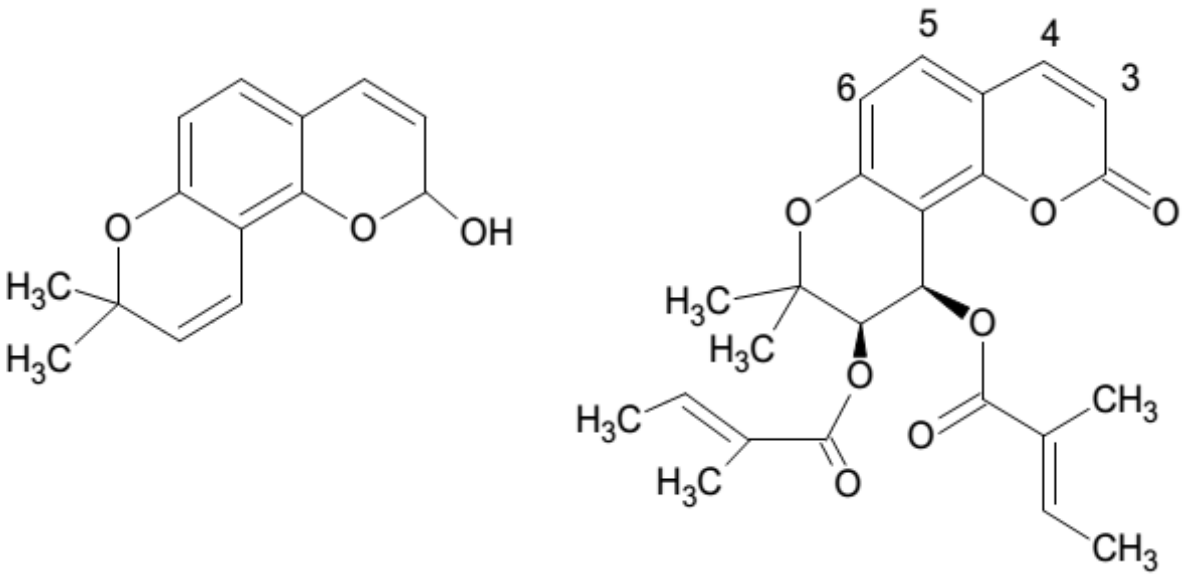


Figure 9: Xanthyltine.



(a) **(b)**

Figure 10: Composite (a) is a Seseline and (b) is aanomaline

II.2.3.PhenolicAcid:

Phenolic compounds are secondary metabolites, which are produced in the shikimic acid of plants and pentose phosphate through phenylpropanoidmetabolization ,[45] and despite their origins, these molecules present antioxidant, antimutagenic, antiviral, antibacterial algicidal, antifungal, insecticidal, estrogenic and keratolyticactivities that may serve to protect the organism from competing ones in their biological environment.[46]

II.2.3.1. Definition:

The organic compounds at least one carboxylic function and one phenolic hydroxyl are referred to as phenol acid, [47] They contain benzene rings, with one or more hydroxyl substituents, and range from simple phenolic molecules to highly polymerized compounds. [46]

Table 5: Structures of the important naturally occurring phenolic acids.

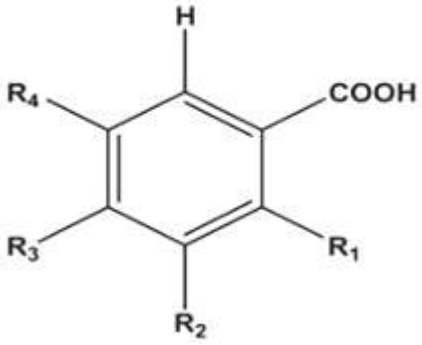


Figure 11: Hydroxybenzoic acid.

Position	**R_1**	**R_2**	**R_3**	**R_4**
Benzoic acid	H	H	H	H
Gallic acid	H	OH	OH	OH
Vaillinic acid	H	OCH_3	OH	H
Salicylic acid	**OH**	**H**	**H**	**H**

II.2.3.2.Biosynthesis:

The C6-C3 compounds often collectively referred to as phenylprepanes, are the most numerous of the metabolites of shikimic acid. Whatever the degree of oxidation of their side chain, they come from cinnamic acids. [48]

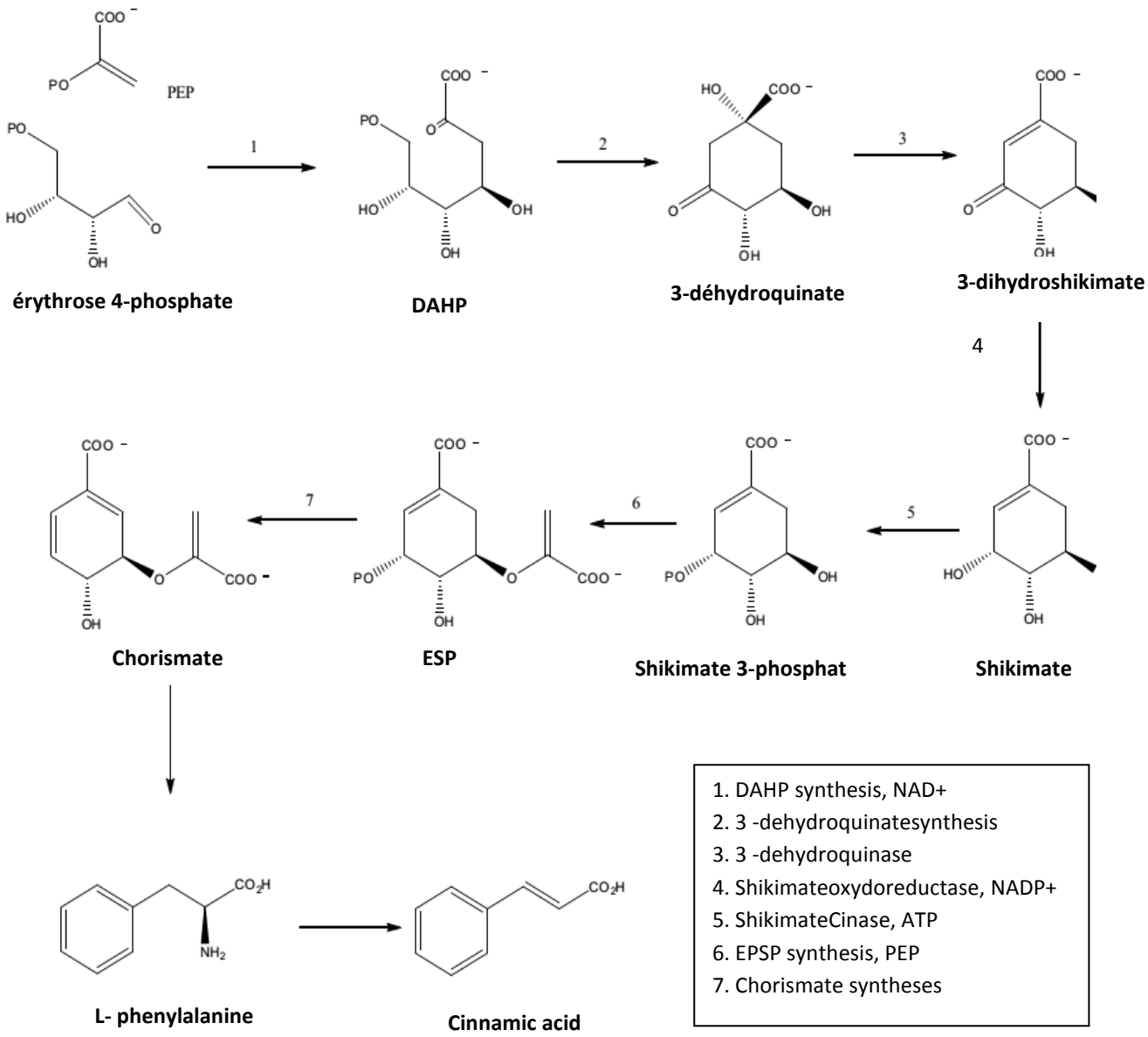


Figure 12: Biosynthesis of cinnamic acid. [46]

II.2.4. Alkaloids:

Alkaloids are among the largest groups of secondary metabolites being extremely diverse in terms of structure and biosynthetic pathways, including more than 20000 different molecules distributed throughout approximately 20 % of known vascular plants**.** [48]

II.2.4.1. Definition:

Alkaloids are a group of secondary metabolites that contain in structure one or more atoms (containing at least one Nitrogen atom), alkaloids with a different form and participate in the structure of one.

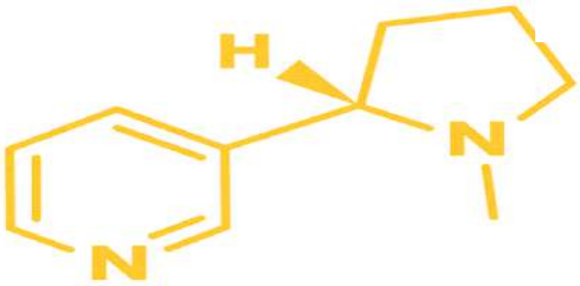
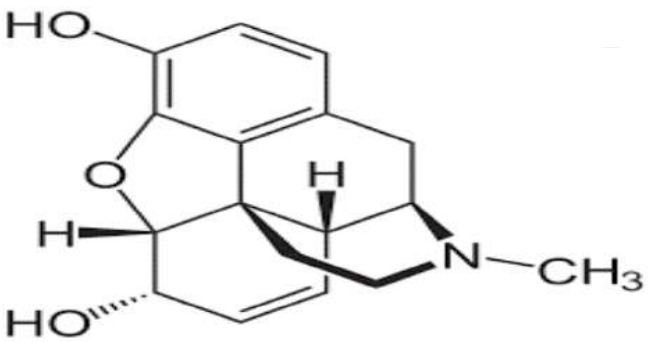
II.2.4.2. Classification (Distribution):

When compared with other class of naturally occurring compounds, there is no uniform structure classification for alkaloids. Present days alkaloids classified based on the carbon skeleton that present in the alkaloids. [49]

A. True alkaloids:

It contains heterocyclic ring with Nitrogen and originated from amino acids. [50]

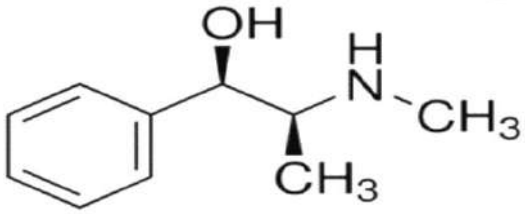
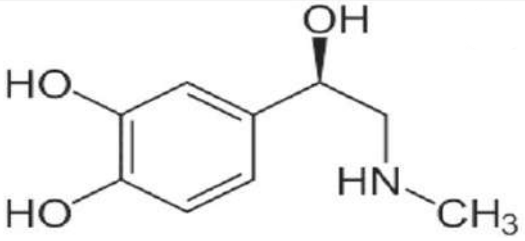
Table6:Examples form composite True alkaloids.

Composite	Chemistry formula
• Nicotine	
• Morphine	

B. Proto alkaloids:

No heterocyclic ring with Nitrogen and derived from amino acids.[50]

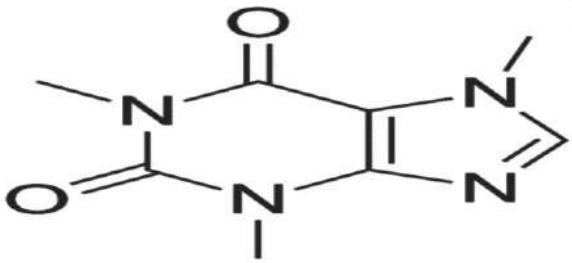
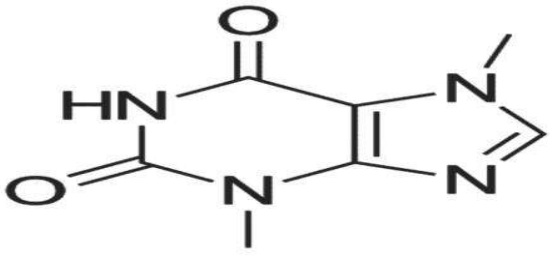
Table 7:Examples form composite Proto alkaloids.

Composite	Chemistry formula
• Ephedrine	
• Adrenaline	

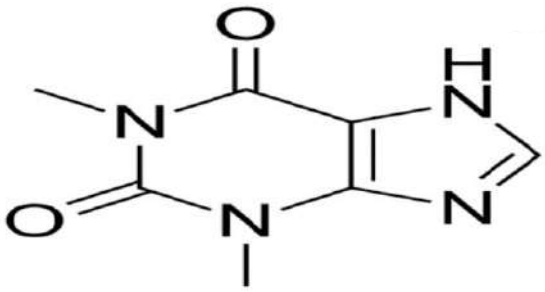
C. Pseudo alkaloids:

Contains heterocyclic ring with Nitrogen but not derived from amino acids. [50]

Table 8:Examples form composite Pseudo alkaloids.

Composite	Chemistry formula
• Caffeine	
• Theobromine	

• **Theophylline**



These metabolites can be divided into different classes according to their precursor,[16]encompassing more than 20 different classes (e.g., pyrrolidine alkaloids, tropane alkaloids, piperidine alkaloids, pyridine alkaloids, quinolizidine alkaloids, and indole alkaloids, among others) .[51]

Chapter (III):

Antioxidant activities

III. Antioxidant activities:

III.1. Oxidative stress:

Oxidative stress is defined as a "state in which oxidation exceeds the antioxidant systems in the body secondary to a loss of the balance between them." It not only causes hazardous events such as lipid peroxidation.[52] Oxidative stress refers to the imbalance between free radicals and their stabilizing agent's antioxidant enzymes in the body. Reactive oxygen species or free radicals can be produced by normal cellular metabolism and react with bimolecular like protein, lipid, and DNA to cause cellular damage and responsible for degenerative changes. At low concentration free radicals play a vital role in the physiological regulation and cellular signaling processes but the high level can cause deleterious changes in the cell.[53]

III.2. Definition of free radical:

Free radicals can be defined as atoms, molecules, or molecular fragments containing one or more unpaired electrons in their atomic or molecular orbitals. Generally it is considered than around 10000-20000 free radicals attacking every cell every day out of which some are good for health which enable human body to fight inflammation, kill bacteria, controls smooth muscles which regulate the proper functioning of internal organs and blood vessels. On the other hand, free radicals play vital role in pathogenesis of various diseases such as heart disease, diabetes mellitus, Alzheimer disease, Parkinson disease, cancer, arthritis etc if produced in large or uncontrolled manner. [54]

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are free radicals generated physiologically in human body which having different types mention in following.

❖ **Reactive oxygen species (ROS)**

- Superoxide $O_2^{\bullet -}$
- Hydrogen peroxide (H_2O_2)
- Hydroxyl radical $HO^{\bullet}$
- Peroxyl radical ($RO_2^{\bullet}$)
- Alkoxyl radical ($RO^{\bullet}$)
- Hydroperoxyl radical ($HO_2^{\bullet}$)
- Singlet oxygen ($^1O_2$)
- Ozone (O_3)

❖ **Reactive nitrogen species (RNS)**

- Nitric oxide ($NO^{\bullet}$)
- Nitrogen dioxide (NO_2)
- Nitrous acid (HNO_2)
- Dinitrogen tetroxide (N_2O_4)
- Dinitrogen trioxide (N_2O_3)
- Peroxynitrite ($ONOO^{\bullet}$)
- Peroxynitrous acid (ONOOH)
- Alkyl peroxynitrites (ROONO)
- Nitroniumcation (NO^{2+})
- Nitryl chloride (NO_2Cl)

III.3.Definition of antioxidant:

An antioxidant is a molecule capable of inhibiting the oxidation of another molecule. Antioxidants break the free radical chain of reactions by sacrificing their own electrons to feed free radicals, without becoming free radicals themselves. [55]

Also defined, An antioxidant can be defined as: "any substance that, when present in low concentrations compared to that of an oxidisable substrate, significantly delays or inhibits the oxidation of that substrate" The physiological role of antioxidants, as this definition suggests, is to prevent damage to cellular components arising as a consequence of chemical reactions involving free radicals. [56]

III.4. Antibacterial activity:

Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents. [52] During the last several decades, natural products with antimicrobial effect were investigated in order to eliminate the use of synthetic antibiotics, which cause the resistance of microorganisms and can exhibit side effects to human health. [53]

III.4.1. Purpose:

The purpose of the Kirby-Bauer disk diffusion, susceptibility test is to determine the sensitivity or resistance of pathogenic aerobic and facultative anaerobic bacteria to various antimicrobial compounds in order to assist a physician in selecting treatment options for his or

her patients. The pathogenic organism is grown on Mueller-Hinton agar in the presence of various antimicrobial impregnated filter paper disks. The presence or absence of growth around the disks is an indirect measure of the ability of that compound to inhibit that organism. [54]

III.5. Determination of the minimum inhibitory concentration (MIC):

The MIC is defined as the lowest concentration of antibiotic that inhibits any visible bacterial growth after twenty-four hours. [55_56]

PRACTICAL PART

Chapter (IV):

Materials and Method

IV. Materials and methods:

IV. 1. Collection of plant material:

The Dr. Tarak MEKHELFIis choose the plant in October of 2018, from the road of Biskra-Algeria, then were dried in the shade, this plant have been identified by professor EDDOD Amar (professor at the University of Ouargla).

IV.2. Preparation of the test samples:

Take 10g of powder plant was macerate with volume (ethanol/water; 3/7; v/v), leave it for a period of 24 h after we havefiltrated.Take 01 ml or 02 ml, and then put the sample in tubes test, we tests initial according to table 9.

Table 9: Protocol tryouts raw. [57]

The family	Methods of detection
Phenols	Add drops of $FeCl_3$ with a concentration (5%)
Flavonoïds	Add chip of Mg + drops of HCl
Saponins	Add water and strongly stirring
Terpens	Add 2 ml $CHCl_3$ + drops of H_2SO_4
Steroids	Add 2 ml$CHCl_3$+ H_2SO_4
Tanins	Add 2 ml HCl (concentrated**)** 1%
Carbohydrate	Add drops of reagent fehling (a+b) with heating
Alkaloids	Add 1 ml of dragedroufwagner reagent
Comarins	Add 3 ml NaOH (10%)
Proteins	Add 5 drops NaOH (10%) + 5 drops $CUSO_4$ (1%)

IV.3. Maceration and extraction:

The protocol extraction is described by *LEBRETON*[58],*BOUTARD*[59]and *GONNET*[60]. At first, we choose system extraction Solid/Liquid; we choose system Ethanol/water, because it is an economic and less toxic. Macerate 1836.8014 g of the plant, in volume (distilledwater/Ethanol; 3/7; v/v), Let macerated for a period of 24 h, then falter. Re-process for

three times.Concentration of the sample at 37^0 C under pressure completely dry, we add water by (1000g/400ml), be the volume of distilled water added 735 ml.The macerate is successively fraction ate by four solvents:

- Add petroleum ether, and separate (X1).
- Add $CHCl_3$, and separate(X3),every time re-process extraction.
- Add AcOE and separate (X3), and every time re-process extraction.
- Add n-butanol, and separate (X11), every time re-process extraction.

The samples are concentrated at 37^o C and weighed as showed in figure 13.

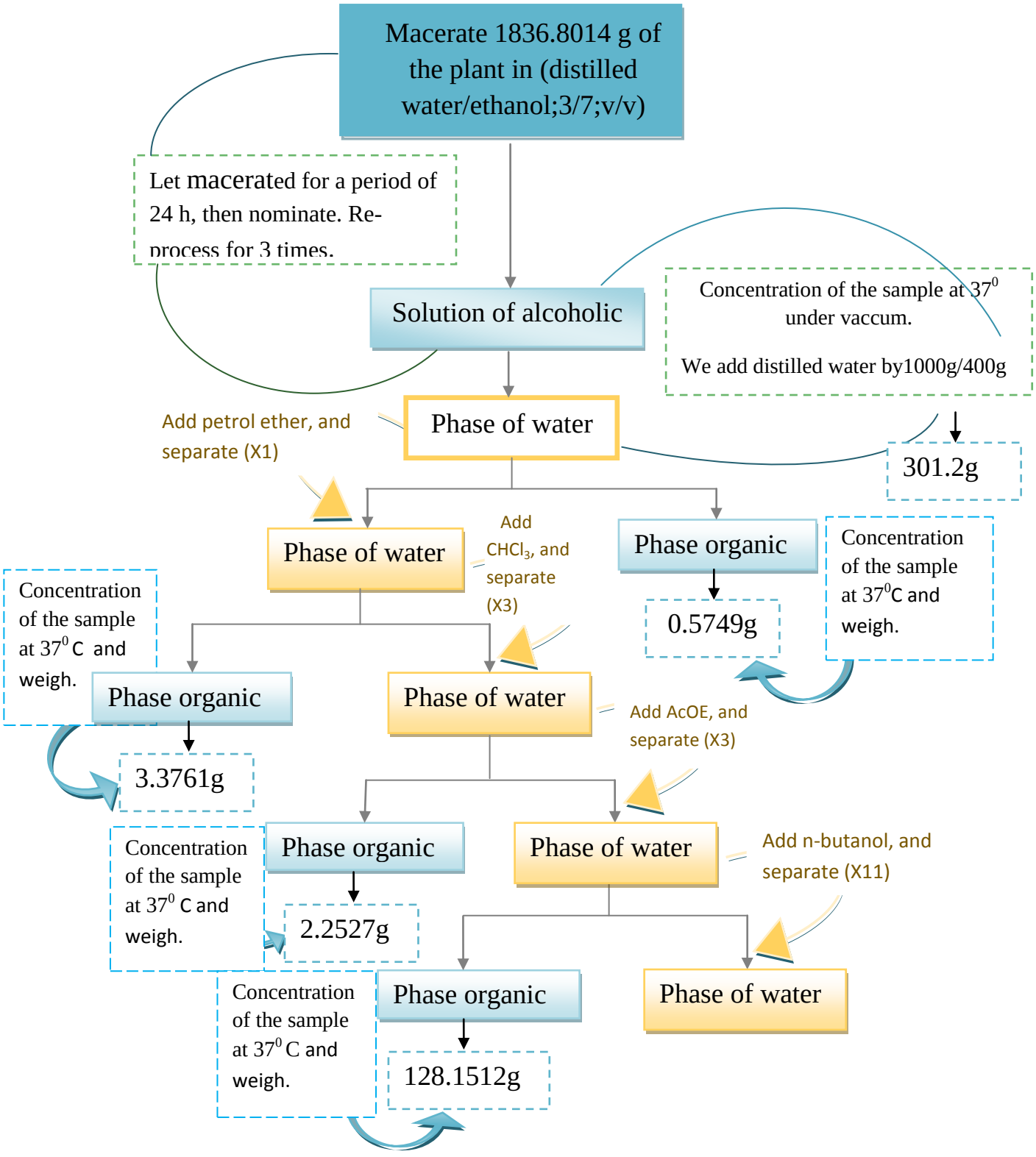


Figure 13: Protocol of extract.

We calculate the yield of each developed by the rule the following:

$$R\% = \frac{m}{M} \times 100$$

R: Return of the phase. / m: Dry weight per phase. / M: The weights of the block dry Solution of alcoholic.

IV. 4. Appreciation quantitative and antioxidant activity:

IV. 4.1. Total phenolic Content assay (TPC):

A. Explain the technical:

The total phenolic content were determined by using the Folin-Ciocalteu assayFolin-Ciocalteau phenol reagent consists of a mixture of the heteropoly acids, phosphomolybdic and phosphotungstic acids in which the molybdenum and the tungsten are in the 6+ oxidation state. On reaction with a reductant, the molybdenum blue. [61]Spectrophotometry is one of the relatively simple techniques for quantification of plant phenolics.

The Folin-Denis and Folin-Ciocalteu methods were the two widely used specrophotometric assays to measure total phenolics in plant materials for many years.[62_63] Both methods are based on a chemical reduction involving reagents containing tungsten and molybdenum. [64] The products of this reduction in the presence of phenolic compounds have a blue color with a broad light absorptionspectrum around 760 nm. The reagents for both methods do not react specifically with only phenols but also with other substances like ascorbic acid, aromatic amines and sugars .[65]

B. Practical terms:

The amount of total phenolics in extracts was determined according to the Folin-Ciocalteu procedure .Samples was introduced into test tubes; 0.5 ml of Folin-Ciocalteu's reagent after 5 min adds 2 ml of sodium carbonate (20%) were added. The tubes were mixed and allowed to stand for 30 min in the dark. Absorption at 760 nm was measured (Systronics UV-vis spectrophotometer). The total phenolic content was expressed as gallic acid (0.03-0.3 g/l) equivalents (GAE) in milligrams per gram dry material.

$$Cmg/g = \frac{\mathrm{nf} \times Abs \times V}{K \times m}$$

$n_{f:}$ The number of extensions /Abs:Absorption / V:Volume/ K: Find the slope of a line of the gallic acid/ m:The total weight of plant.

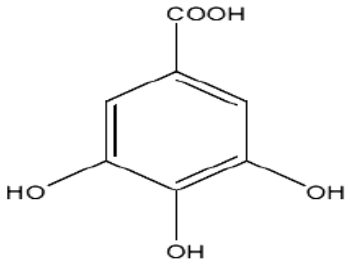


Figure 14: Gallic acid.

IV. 4.2. Total flavonoids content (TFC)*:*

A. Principle:

Formation of acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols in addition with *aluminium chloride. Aluminium chloride* also forms acid labile complexes with the ortho - dihydroxyl groups in the A- or B-ring of flavonoids. For building the calibration curve, quarcetinis used as a standard material. Various concentrations of standard quarcetin solution were used to make a standard calibration curve**.**[66]

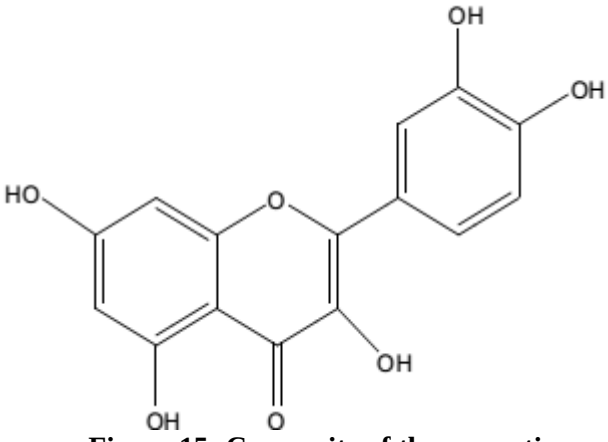


Figure 15: Composite of thequarcetin.

B. Practical terms:

In this method, quercetinwas used to make the calibration curve. 10 mg of quercetinwas dissolved in methanol and then diluted to 6.25, 12.5, 25, 50, 80, and 100 µg/ml. A calibration curve was made by measuring the absorbance of the dilutions at 415 nm (λmax of quercetin) with a Shimadzu UV-1800 spectrophotometer. Aluminium chloride, 1% and potassium acetate, 1M solutions were preparedSamples were introduced into test tubes;.1.5 ml of extracts of Salsolavermiculata.L diluted in methanol add 1.5 ml of Aluminium chloride ($AlCl_3$) (2%) were

added. The tubes were mixed and allowed to stand for 30 min in the dark. Absorption at 430 nm was measured.

IV.4.3.Determination of Condensed Tannin:

This is method was used to make the calibration curve,catechine was used to make the calibration curve.

. Practical terms:

0.4 ml of extracts of Salsolavermiculata.L diluted in methanol and adds 1.5 ml ofHydrochloric acid concentrated (HCl) and adds 3 ml vanillin (4%) and allowed to stand for 30 min in the dark. Absorption at 500 nm.

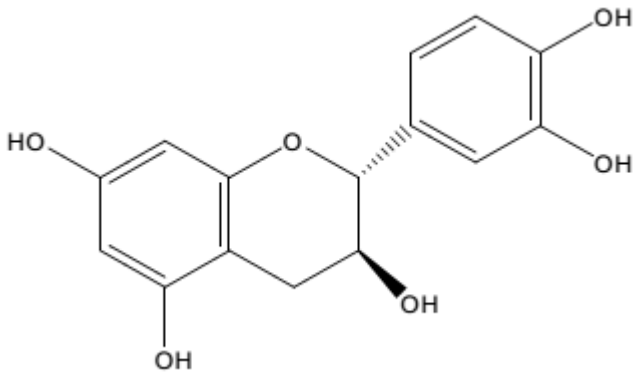


Figure 16: Composite ofthecatechine.

IV.4.4.Antioxidant activities:

IV.4.4.1. DPPH radical scavenging activity:

A. Principle:

DPPH radical is scavenged by antioxidants through the donation of proton forming the reduced DPPH. The colorchanges from purple to yellow after reduction, which can be quantified by its decrease of absorbance at wavelength 517 nm. Radical scavenging activity increased with increasing percentage of the free radical inhibition. The degree of discoloration indicates the free radical scavenging potentials of the sample/antioxidant by their hydrogen donating ability. The electrons become paired off and solution loses colourstochiometrically depending on the number of electrons taken up. [67]

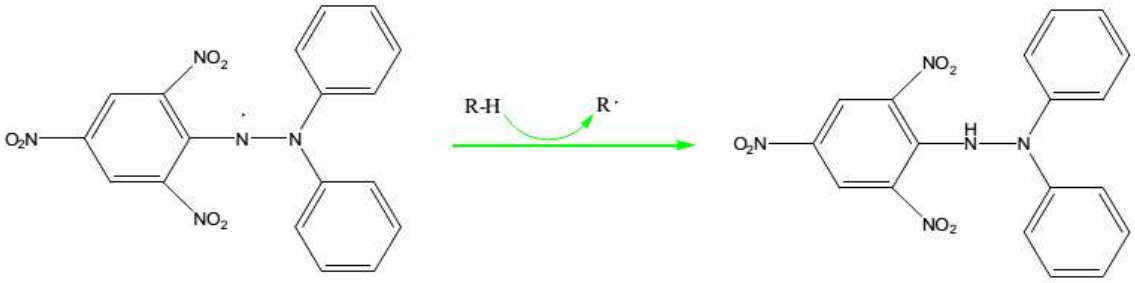


Figure17: The reduced form of the radical DPPH˙.

B. Practical terms:

Samples were introduced into test tubes; 1ml of extracts diluted in H_2O and added 1 ml of **DPPH˙** (0.049 g/l)Solution diluted in methanol or ethanol and allowed to stand for 30 min in the dark. The solution tubes were measured at 517 nm.

The percentage inhibition of DPPH radical was calculated using the following equation:

$$\textbf{Antiradical activity} = \frac{Abs517negativecontrol - Abs517sample}{Abs517negativecontro} * 100$$

The antioxidant activity is expressed by the IC_{50} value, knowing that IC_{50} is the concentration of extract necessary to obtain 50% of the reduced form of the radical DPPH˙.

IV.4.4.2. Determination of total antioxidant capacity:

The total antioxidant capacity of plant was evaluated by phosphomolybdenum method.

To 1 ml of the extract, added 1 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate). The tubes were capped and incubated in a boiling water bath at 95°C for 90 min. After the samples cooled to room temperature, the absorbance of each solution was measured at 695 nm against reagent blank using spectrophotometer. The results were expressed as ascorbic acid equivalent (AEAC) in milligrams per gram dry material.

$$AEAC = \frac{K}{K^*}$$

K: Find the slope of a line of the extract .

K^*:Find the slope of a line of the Vc.

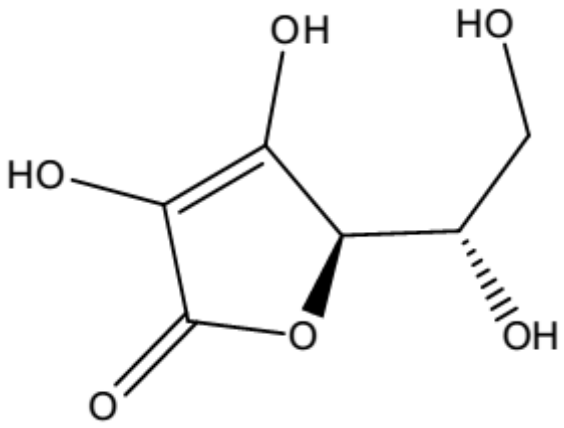


Figure 18: Ascorbic acid.

IV.5.Separation and purification:

Based study structural of flavonoids primarily on the properties of chromatographs, as well as on the methods of analysis of the physiochemical different, by separation:

- TLC
- CC

Methods of spectral analysis:

- UV
- RMN(H^1 _C^{13})
- COSY ,HSQC,HMBC,ROESY and NOESY.

(We will focus in our study at TLC, CC and UV)

A. TLC Chromatography:

A.1. Explanation the technical:

We test initial by chromatography TLC, so to know each phase. At first, equip layer TLC, to cut the widget crisis, and then draw a line launch a 01cm from the edge. Equip Tank, in mixed solvents (mobile phase) v/v, and then we developed in the tank and close up. We develop drops of all the process of the starting line, the dimension of every two drops 0.5 cm , mop up and re-operation to focus the sample. In the latter, we put TLC at the tub close up.

During the migration of phase, moving to the top to TLC pulls his vehicles by the polar. Withdraw TLC of the Tank before the arrival of phase moving to the top edge 0.5cm.

Know the number of vehicles through note spots beneath UV, as well as its kind of vehicles.

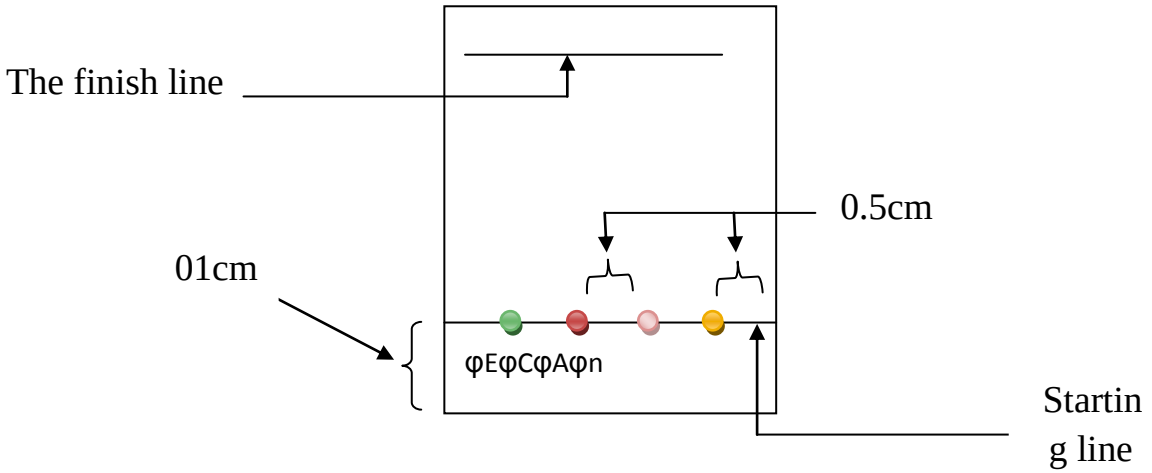
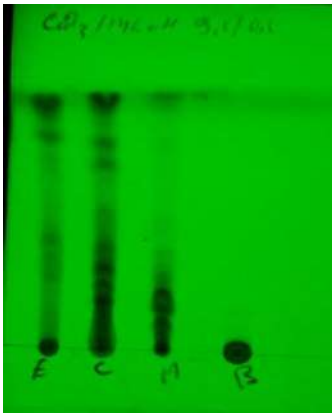
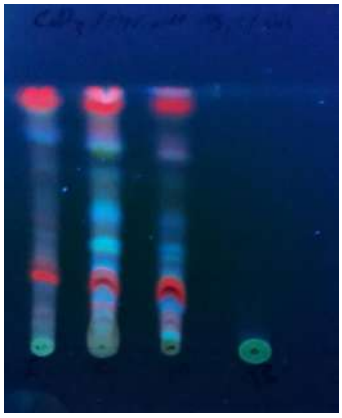


Figure 19: TLC Chromatography.

A.2. Practical terms:

We attended layer TLC **(TLC Silica gel 60 F_{254}, Aluminium sheets 20x20cm)** according to the steps past, and choose phase moving mix solvents ($CHCl_3$/methanol; 9.5/0.5;v/v) and record, we follow TLC by device UV, we get to the following results:

Table 10:Results TLC in lengths wave of different.

Under the length of the waveform:		**In natural light:**
254 nm	365 nm	
		

B. CC Chromatography:

B.1. Explanation the technical:

Conducted as follows:

We choose the column with dimensions of the appropriate, depending on the amount you want to make the process of separation then, for each 01g of the sample to be separation vehicles for the equivalent to 32g of Silica **gel**, then we add solvent least polarity with **Silica gel**, pour mixture in column, until we get to the 2/3. Link column system, nutrition (must not dry and breaking), equip phase moving mixture of solvents, and change ratios by separation of compounds. When equips column separation, prove it then we put the sample to be separations, then we change in phase moving (Separating compounds of least polarity to top polarity).

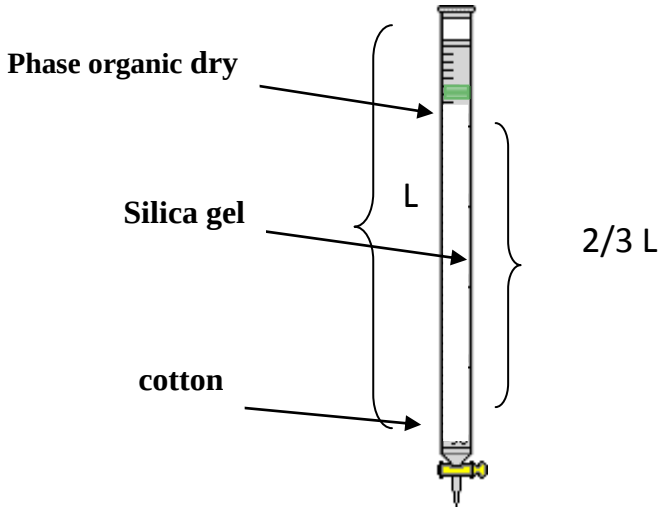


Figure 20:CC Chromatography.

B.2. Practical terms:

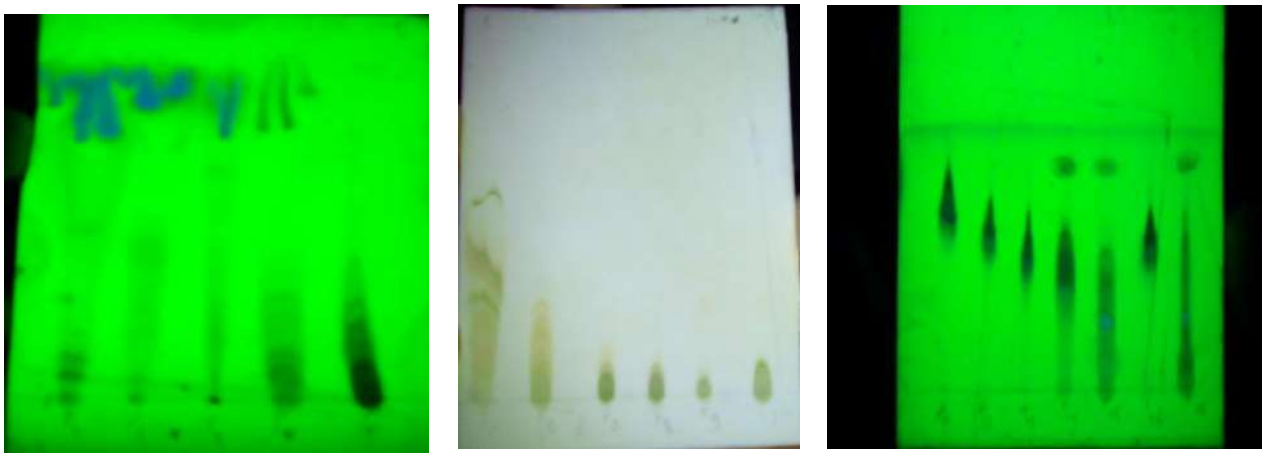
After the appointment of the process of φ$CHCl_3$ to follow up separated, determine the phase moving mix($CHCl_3$/MeOH) according to the table, record results.

Figure 21: CC Chromatography of phase $CHCl_3$.

Table 11:The volume of phase moving and fractions.

Volume(ml)	MeOH%	$CHCl_3$%	Fractions
300	00	100	01-07
400	02	98	08-10
200	03	93	11-12
100	05	95	13
100	07	92	14
100	10	90	15
100	15	85	16
100	20	80	17
100	30	70	18
100	50	50	19
100	00	100	20

We follow results by **TLC**, the results are as follows:



$CHCl_3$ 100%	$CHCl_3$ 100%	$CHCl_3$/MeOH (9.5/0.5)

Figure 22: Results by TLC.

Collect fractions similar, we get to:

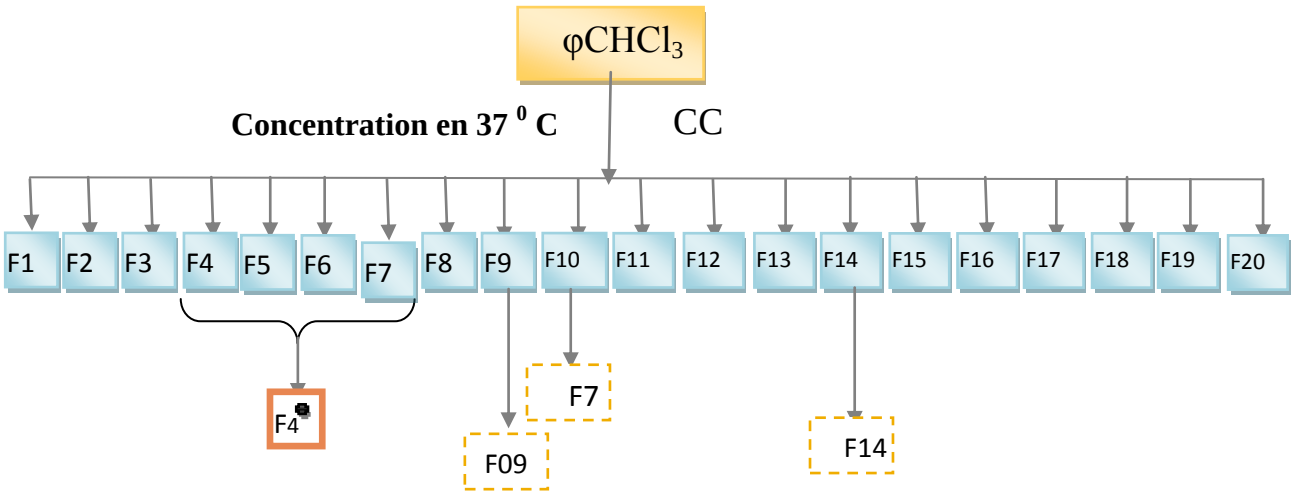
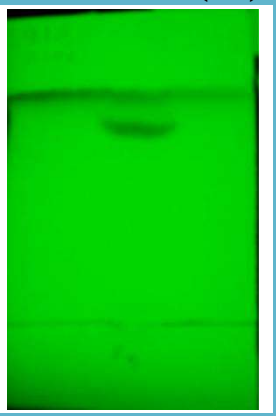


Figure23: fractions of phase $CHCl_3$.

Table 12: Fractions under UV.

Fractions	λ 254 nm /$CHCl_3$%	λ 254 nm $CHCl_3$/MeOH(9/1)
F9		

Determine the compound to be separated, for ways to its color, we choose composite by color flavonoids different depending on the following table 15.

We choose composite shown in F9, then re-separation of flavonoids, we use this time TLC(20cm_05cm) and phase moving ($CHCl_3$100%).In the end, after separation of the boat, purification UV.

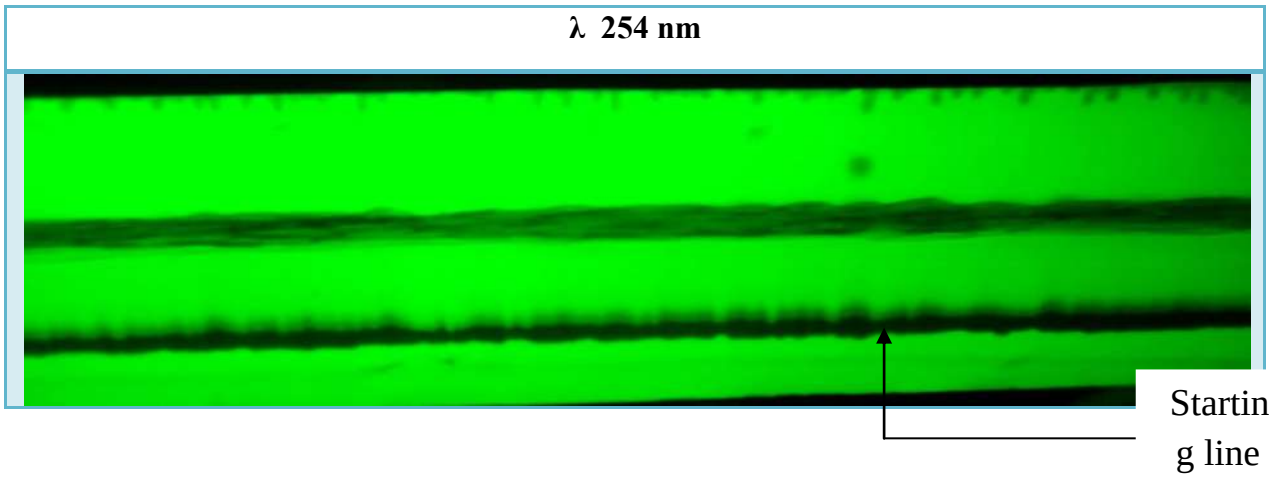


Figure 24: Compound to be purified.

C. **Spectral Characteristics of Flavonoids**:

C.1. **Color of flavonoids under UV:**

The colors of vehicles one of the most important ways to determine flavonoids than others, although it is an initial. That depending on the table 15.

Table13: Color of flavonoids under UV.[68]

Colors	Formats of compose
purple_ black	○ Flavones (5,6,7 or 5,6,8 three-hydroxyl) ○ Flavonol ○ Some Chalcones.
purple_ indigo	○ Flavone (be not5-OH) ○ Flavanone(be not 5-OH) ○ Flavonol (3-R and be /be not 5-OH)
Yellow_ yellowfgfj	○ Flavonol(3-OHfreeand be /be not 5-OH)
Blue Green	○ Flavanone (be not 5-OH)
Orange glossy	○ Isoflavone
Yellow Green	○ Aorone
Green	○ Some Chalcones.

C.2. Absorption of compounds flavonoids:

Studies on flavonoids by spectroscopy have revealed that most flavones and flavonols exhibit two major absorption bands: [21]

- Band I (320–385 nm) represents the B ring absorption.
- Band II (250–285 nm) corresponds to the A ring absorption.

Because the difference in the absorption, to difference in raciness between A, B ring and C ring. Identifies structure according to the table16.

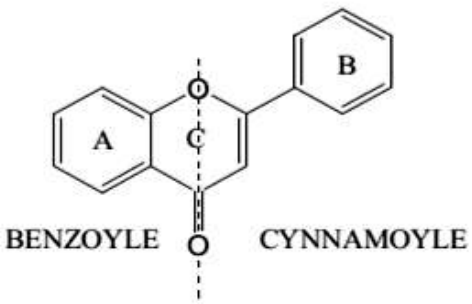


Figure 25: A ring,B and Cring.

Table14: Absorption of Compounds in methanol (solvent).

Compounds	Band I λ(nm)	Band II λ(nm)
Flavone	350-310	280-250
Flavonol(be 3-OR)	360-330	280-250
Flavonol(be 3-OH free)	385-350	280-250
Flavanone / Flavanonol (3 OH)	330-300	295-275
Isoflavone	330-310	275-245
Chalcone	390-340	270-230
Aorone	430-380	270-230

Chapter (V):

Interpretation of analysis results

V.Interpretation of analysis results:

V.1. Preparation of the test samples:

Be the first results as follows:

Table 15: Preliminary results.

Results	
Phenols	++
Flavonoïds	+
Saponins	++
Terpèns	++
Steroids	--
Tanins	+
Carbohydrate	+
Alkaloids	++
Comarins	+
Proteins	--

--: None exist. +: Exist low. ++: Highest exist

V.2.Yield of the phase:

After extraction liquid/liquid, the yield of each extract was calculated as shown in the following table16: **n-butanol > $CHCl_3$ > AcOE > Petroleum ether**

Table 16: Yield of the phase.

M(g)= 301.2, Y%(brut)= 16.40				
Phase	**petroleum ether**	**$CHCl_3$**	**AcOE**	**n-butanol**
m(g)	0.5749	3.3761	2.2527	128.1512
Y%	0.19	1.12	0.75	42.55

V.3. QuantitativeAnalyses :

V.3. 1.Total phenolicContent (TPC):

The result is expressed in mg equivalents of gallic acid per gram of extract (mg EAG / g).

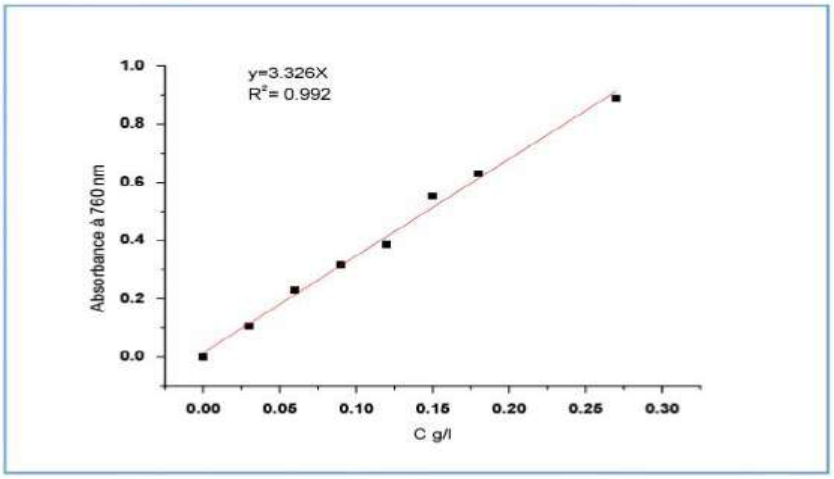


Figure 26: Curve standard of gallic acid of total phenolic content.[69]

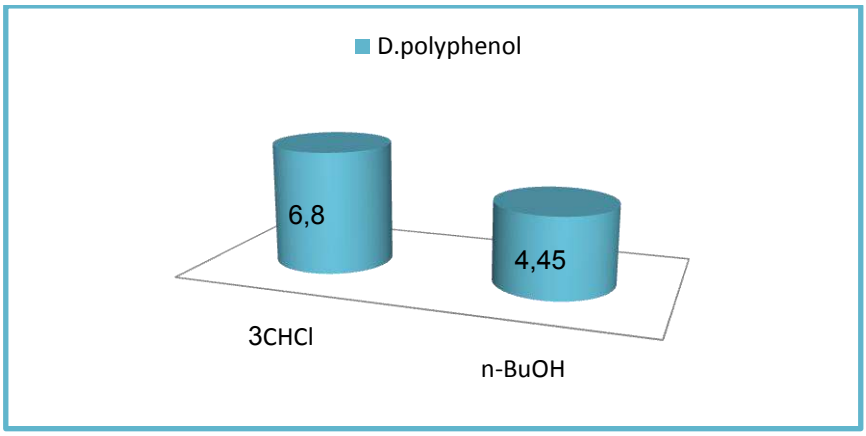


Figure 27: Total phenolic content.

Note through the figure (27) that chloroform phase $CHCl_3$ is more riche 6.8 mg EQE/gthan the phenolic compounds as compared to the n-butanol phase 4.45mg EQE/g.

V.3.2. Total flavonoids content (TFC):

The result is expressed in mg equivalents of*qaurcetin acid* per gram of extract (mg EAG / g).

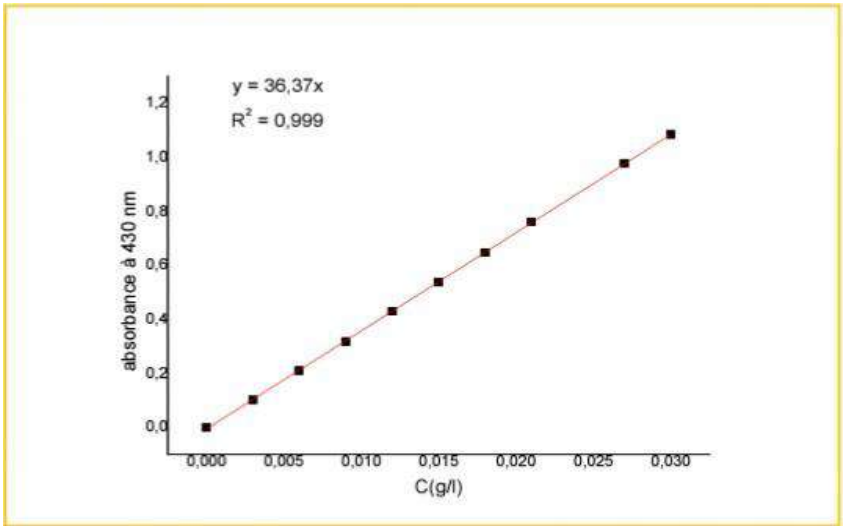


Figure 28: Curve standard of qaurcetin acid of total flavonoids content.[69]

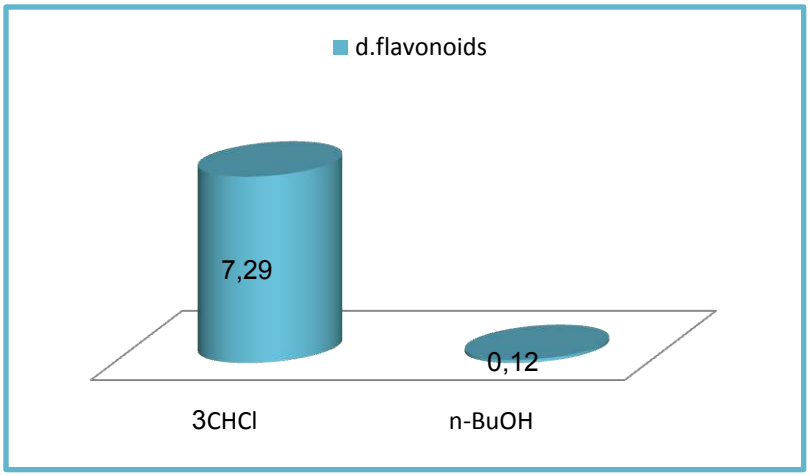


Figure 29: Total flavonoids content.

Note through the figure (29) that chloroform phase $CHCl_3$ is much more riche (7.29mg EAG / g) than the flavonoid compounds as compared to the n-butanol phase is small quantities (0.12mg EAG / g).

V.3.3.Determination of Condensed Tannin:

The result is expressed in mg equivalents of catechine per gram of extract (mg EAG / g).

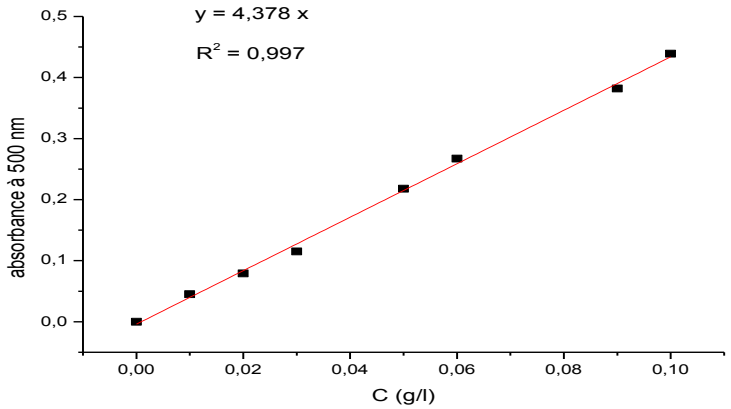


Figure 30: Curve standard of catechineof Determination of Condensed Tannin.[69]

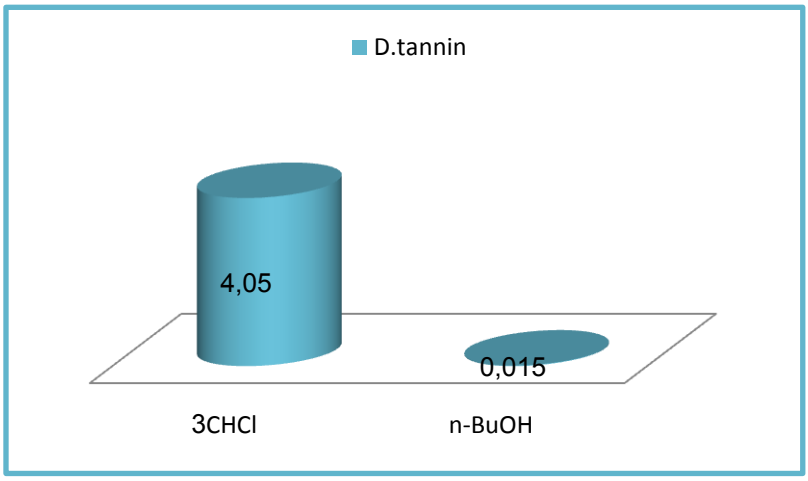


Figure 31: Determination of Condensed Tannin.

Note through the figure (31) that chloroform phase $CHCl_3$ (4.05mg EAG / g) is note riche, also n-butanolphasewhich is virtually non-existent (0.015mg EAG / g).

V.4.Antioxidant activities:

V.4.1.DPPH radical scavenging activity:

The evaluation of the anti-radical activity of our extracts via the DPPH$^{\cdot}$a test leads to the results illustrated by figure 32 .

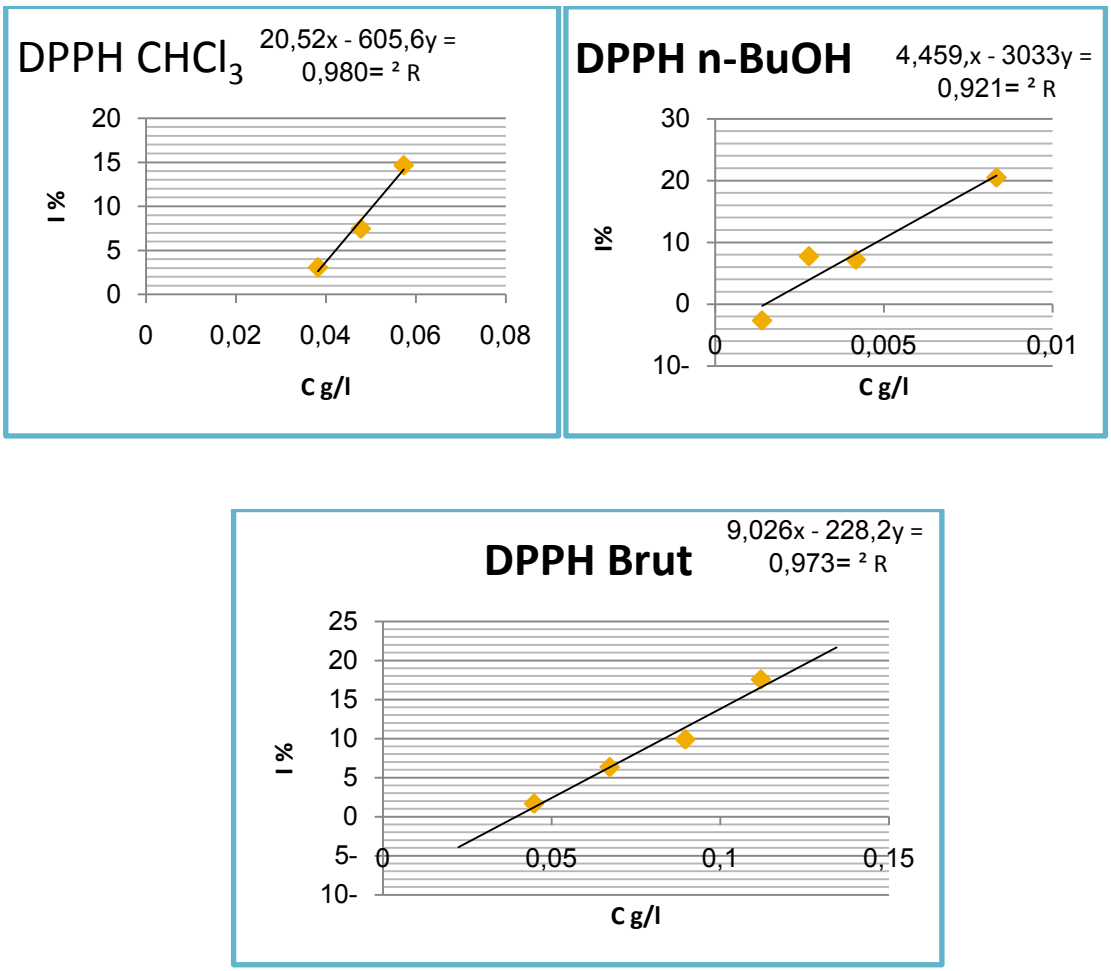


Figure 32: Variation curves of inhibition DPPH of terms concentration extracts.

Table 17: Results of Antioxidant activities (DPPH radical scavenging activity).

Extract	IC_{50} (g/l)
But	0.016
Cl	0.11
Brut	0.24
Vc	0.08±0.001 [57]
BHT	0.356±0.002 [57]
BHA	0.094±0.003 [57]

Note through the results in the table above:

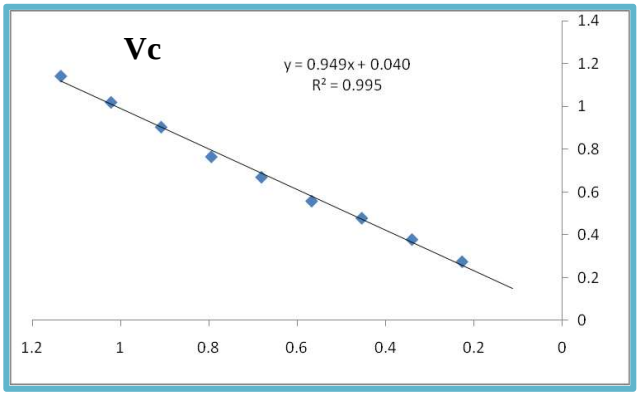
We note that the CI_{50} value of n-butanol phase was the most effective at a value of 0.016 g/l and is highest effective to compared with BHT and BHA .Thanchloroform phase 0.11 g/l , and brut phase is a little effective 0.24 g/l.

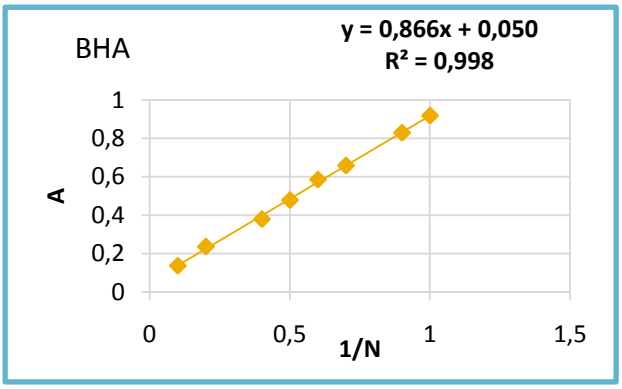
n-BuOH> $CHCl_3$> Brut

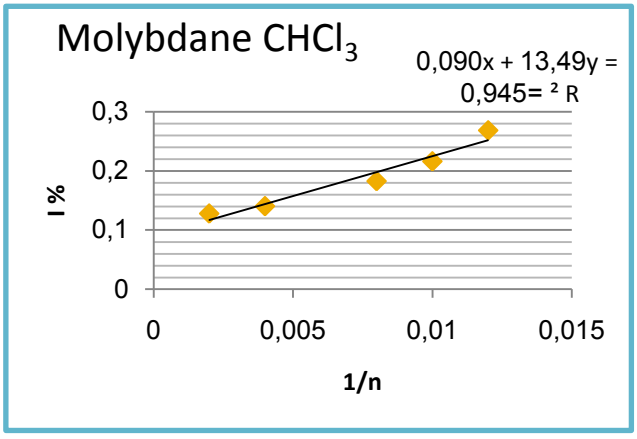
Generally, ***S.Vermiculata*** plant has effective in antioxidant activity of DPPH radical scavenging activity.

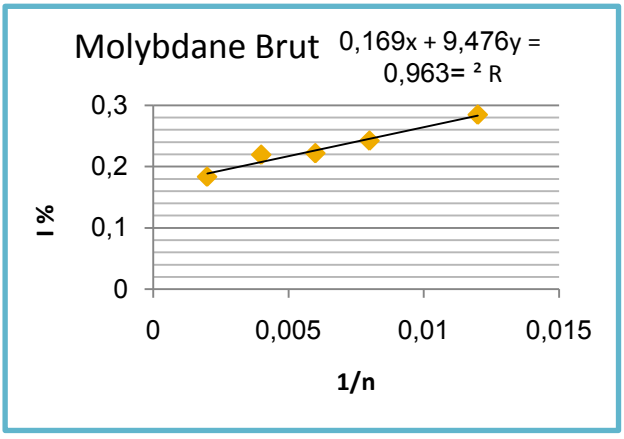
V.4.2. Determination of total antioxidant capacity:

The curves below figure (33) represent the effectiveness of*S.Vermiculata* extracts to reduction of molybdenum Mo (VI).









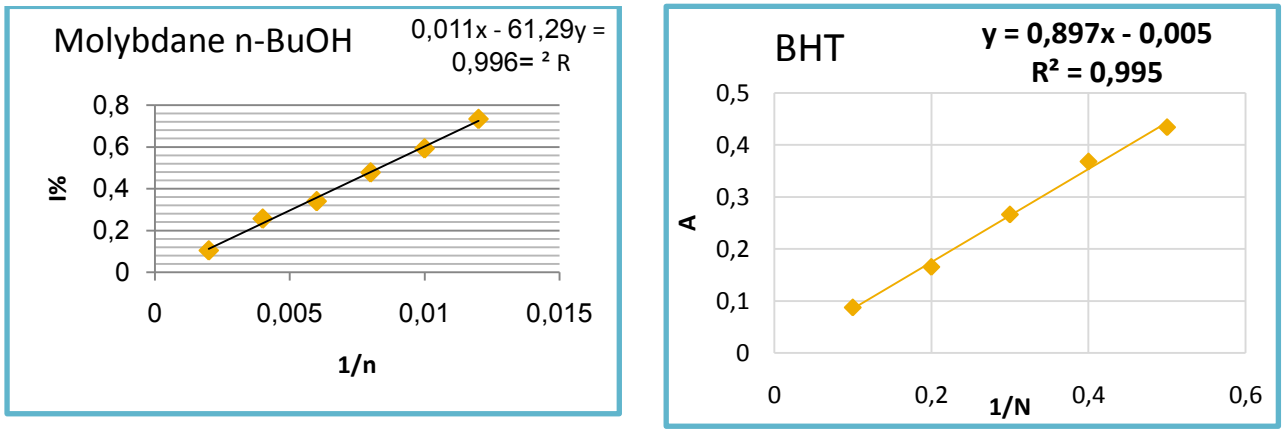


Figure33: Curves representing the reductive power of extracts from s.vermiculata.

Table 18: results of Antioxidant activities(total antioxidant capacity).

Extract	AEAC g/l
$CHCl_3$	142.22±0.017
n-BuOH	645.87±0.277
Brut	99.86±0.042
BHA	0.869±0.065 [57]
BHT	0.881±0.074 [57]

The effectiveness of the reduction of molybdenum Mo (VI) is directly proportional to the value of AEAC, so we notice that highest value to AEAC is 645.87±0.277g/l for n-butanol phase, followed by value chloroform phase 142.22±0.017 g/l, and following value of brut phase 99.86±0.042 g/l.

n-BuOH>$CHCl_3$> Brut

Generally, Generally, ***S.Vermiculata*** plant has effective in antioxidant activity TAC.

At the end of the evaluation of antioxidant activity in SalsolaVermiculata extracts, we can say that these extracts give a good reduction of Molebi (Mo) and a good inhibitory of DPPH

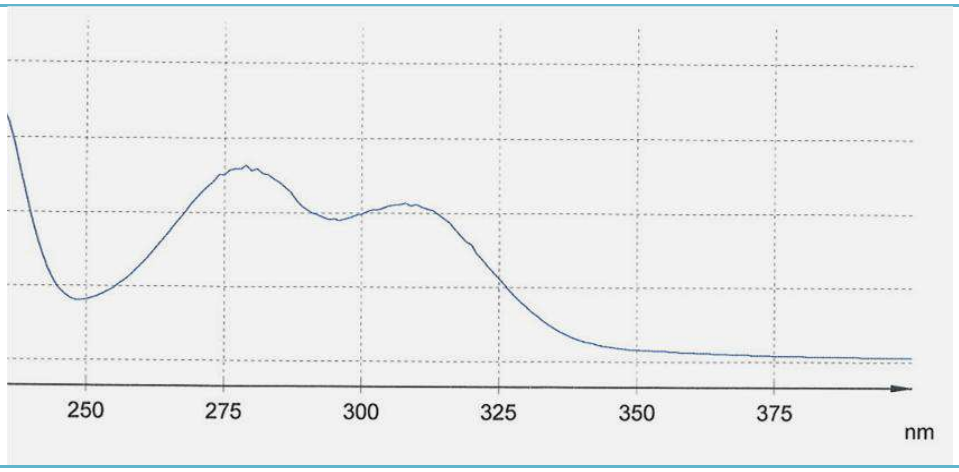
radical compared to the standard antioxidants used. This gives an idea of the reduction mechanism of Mo (VI) or DPPH$^{\bullet}$, at this stage we suggest that biologically active molecules present in the extracts play an electron donor role in the donation of hydrogen and oxygen.

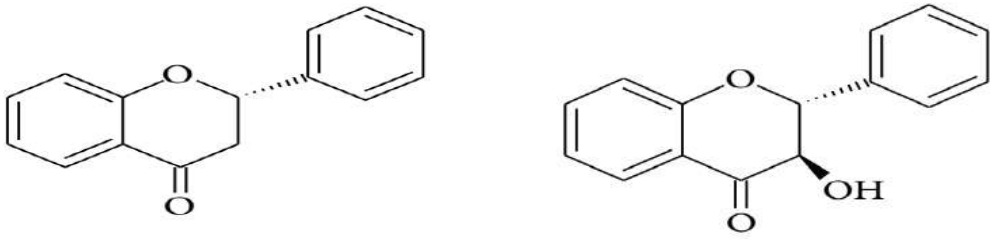
V.5. Identifyof the compounds:

Of spectral analysis by the compound (Results are taken by a UVAnalytikjena AG07745 Jena in the laboratory of the Dr.SIGNI LEGHJEL), we find the following:

Table 19: The results of analysis.

Purple(violet)_ black	
λ 306 nm	**λ 278 nm**





(A) (B)

Figure27: Guess the form of the composite extracted(A)Flavanone family (B)flavanonol family (3 OH).

Conclusion

This is research for *Salsolavemiculata* secondary metabolite products during our study.

We tested the compounds found in this plant and obtained (Flavonoids, comarins, polyphenol, alkaloids…). Thus, a suitable system for the extraction of flavonoids compounds was selected, by an initial separation of column chromatography followed by thin layerchromatography (TLC).

For the structural determination of the severed composites, we will use UV/VIS spectroscopy.

Antioxidant tests were performed for the extracts of $CHCl_3$, n-BuOH . Both of these extracts showed good efficacy in both DPPH and TAC.

We hope to study *Salsolavemiculata* plant to extract alkaloids and study their toxicity.

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Abstract

This work has focused on the phytochemical study of the species ***Salsolavermiculata L.*** belonging to the ***Chenopodiaceae*** family.Our object of this research is identify outputs second metabolism. The antioxidant activity for this plant have highest effective inhibition against free radicals ***DPPH*** and reduction Mo(VI). In this work, we succeeded in the extracting and purifying two compounds. The compounds are illustrated by UV-Vis

Keywords: phytochemical, *Salsolavermiculata*, Flavonoids, antioxidant activity.

الملخص

هذا العمل عبارة عن حصيلة لدراسة فيتو كيميائية لنبتة ***Salsolavermiculata L.*** من عائلة ***Chenopodiaceae.***حيث يهدف هذا العمل للتعرف على نواتج الأيض الثانوي لهذه النبتة خاصة الفلافونيدات.في الفاعلية المضادة للأكسدة لهذه النبتة بينت أنها تملك فعالية عالية في كل من تثبط الجذر الأيوني***DPPH*** و إرجاعالموليبدان***TAC*** .تمكنا في هذا العمل المخبري من استخلاص و تنقية مركبين, حددنا بنية هذين المركبين بطريقة***UV/VIS.***

الكلمات المفتاحية:فيتو كيمياء, فلافونيدات, الفعالية المضادة للأكسدة, ***SalsolaVermiculataL.***

Résume

Ce travail s'est concentré sur l'étude phytochimique de l'espèce *Salsolavermiculata L*. appartenant à la famille des Chenopodiaceae. Notre objet de cette recherche est d'identifier les sorties du deuxième métabolisme. L'activité antioxydante de cette plante présente une inhibition efficace maximale contre les radicaux libres DPPH et la réduction Mo (VI). Dans ce travail, nous avons réussi à extraire et à purifier deux composés. Les composés sont illustrés par UV-Vis

Mots cles:phytochimique**,** flavonoïdes**,** activitéantioxydante**, S*alsolaVermiculataL.***