ALGERIAN DEMOCRATIC AND POPULAR REPUBLIC
MINISTRY OF HIGH EDUCATION AND SCIENTIFIC RESEARCH

UNIVERSITY OF OUARGLA
FACULTY OF SCEINCES AND ENGINEERING
DEPARTEMENT OF ENGINEERING

Graduation project : state engineer diploma

Speciality : Process Engineering
Option : Environmental engineering

Realized by:

Kessal Abdelkader
Bouafia Otmane

## Theme

# Phytoscreening and antibacterial activity of the plants

# *Ephedra alata , Launaea resedifolia and Oudneya africana*

Discussed publicly in front of the jury on October 4th, 2003

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| * Mr. Baamer Lotfi ( University of Ouargla) | President |
| * Mr. Segni Ladjel ( University of Ouargla) | Examiner |
| * Mr. Sakhri Lakhdar( University of Ouargla) | Examiner |
| * Mr. Gherraf Noureddine( University of Ouargla) | Rapporteur |

## Academic year: 2002/2003

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# Acknowledgments

Thanks to God for helping us to achieve our engineering subject in good manner .

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Finally. to everyone helped us during carrying out this work , either by direction , suggestion , or encouraging word even.

# Dedication

**I dedicate this work to :**

- My maitre CHEIKH SIDI ALI ATTABIAI and his pupils.

- My father : KESSAL MOHAMED

- My mother : BOUCHENAFA OMELKHIR

- My brothers, my sisters, and all my family members.

*ABDELKADER KESSAL*

# Dedication

This modest work is dedicated to :

- Every one respects the science and has realized its essentialness
- Martyr's soul who irrigated our nation soil by his blood
- My dear father
- My dear mother
- My family members :KADDOUR, LAMINE ,TAREK, ABD ELGHANI, FATIMA ,KARIMA ,DJAHIDA
- My nephews : NOEH ,MOHAMED, ABD EL SABOUR, AMER ,HAMZA ,JABER, OMAR
- My nieces : KHADIJA ,MASSOUDA ,MARIAM, ROFAIDA,ASMA ,SAMAR ,HANA ,HIBA

*OTMANE BOUAFIA*

# Summary

# Summary

# List of tables

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# Introduction

# Introduction :

For ages, vegetables have always involved many advantages dealing with biological activities in curing and healing the mankind, helping him in endless struggle to survive.

Owing to the richness of these plants with different active principles, The man was able to make use of them in order to overcome different diseases and daily needs problems from the crude use till nowadays prescribed ingredients.

The main evidence that supports the great importance of the vegetables is mainly their active principles i.e : alkaloids, flavonoids, steroids, tannins, sterols, terpenes and cardiotonic glycosides. These principles have been transformed to therapeutic arsenal including several uses such as : antispasmodic, antibiotic , antitumor , analgesic , anesthetic and antibacterial .

In this study, we have focused our attention on the antibacterial effect, since these plants have not been studied so far and moreover this field remains unspoilt in Algeria. Therefore our study is aimed mainly in contributing to the efforts engaged against the spread of new diseases and to provide a useful tool for any subsequent research dealing with the bacterial pollution that may affect our waters in the near future.

Our main objective is to prove the importance of some plants of local growth : *Ephedra alata*, *Launeae resedefolia* and *Oudneya africana*.

This work include five chapters :

ChapterI : phytoscreening

ChapterII : monography

ChapterIII : biological activity

ChapterIV : experimental part

ChapterV : results and discussions

# chapter I

# *phytoscreening*

# part

## I-1-The alkaloids:

### I-1-1-General information:

The term alkaloids has been introduced by W. Meisner in the beginning of 19th century [1].

Alkaloids are all nitrogen heterocycle bases which occur mainly in plants. Their amine character produces an alkaline solution in water and hence the origin of their name Alkaloids [2].

They are thought to be used against animals attack owing to their bitter taste [38].

### I-1-2-Classification and structure:

The usual names often evoke the organism of origin, which terminate by « ine »[3].

The alkaloids have been classified by Hegnanuer to three classes[4]:

- True alkaloids
- Proto alkaloids
- Pseudo alkaloids

## 1-2-1-True alkaloids:

The true alkaloids are toxic with a wide range of physiological activity. They are almost invariably basic. They normally contain nitrogen in a heterocyclic ring. They are derived from amino acids.
They are of limited taxonomic distribution, and normally occur in the plants as salts of an acid. Some exceptions to these "rules" are: colchicines (1), and aristolochic (2) which are not basic and have no heterocyclic ring and the quaternary alkaloids, which are acidic rather than basic[4] .

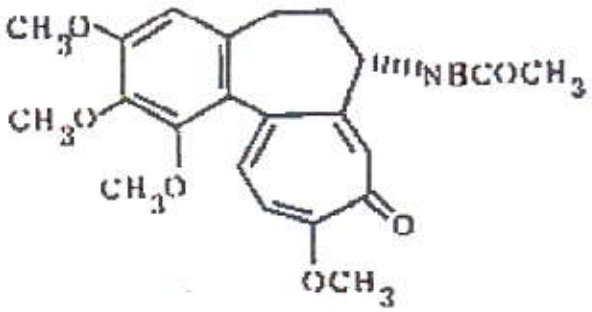



Fig 1.Colchicines

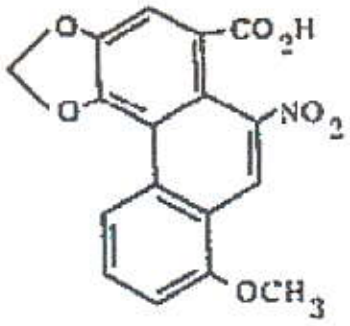



Fig 2. aristolochic

## I-1-2-2-Proto alkaloids:

The proto alkaloids are relatively simple amine in which the amino acid nitrogen is not in a heterocyclic ring. They are biosynthesized from amino acids and are basic. The term "biological amines" is often used for this

group of compounds. Examples are mescaline (3), ephedrine (4), N,N-dimethyltryptamine (5) [4].

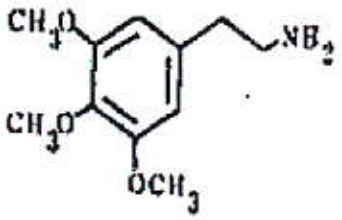


Fig 3. mescaline

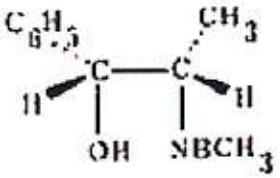


Fig 4. ephedrine

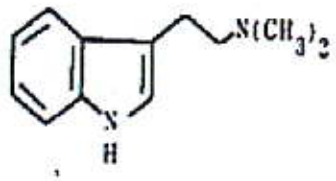


Fig 5. N,N-dimethyl Tryptamine

## I-1-2-3-Pseudo alkaloids:

The pseudo alkaloids are not derived from an amino acid precursor. They are usually basic. There are two important series of alkaloids in this class, the steroidal alkaloids [e.g, conessine (6)] and the purines [e.g., caffeine (7)] [4].

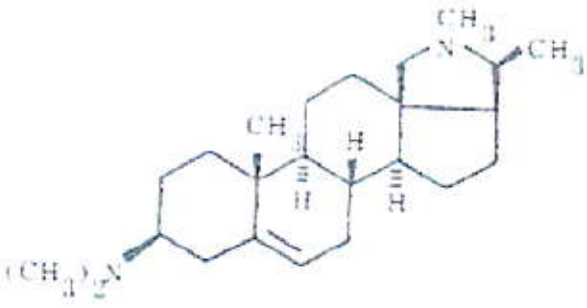


Fig 6.Conessine

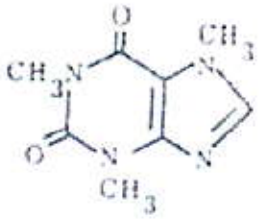
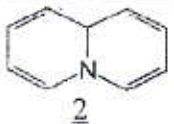
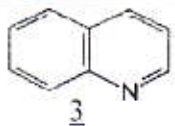
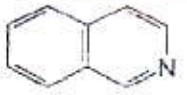
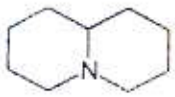
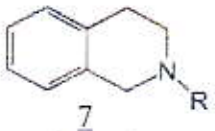
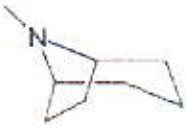
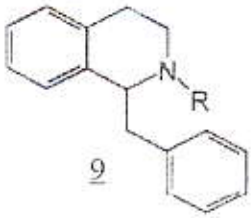
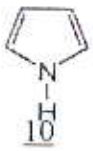
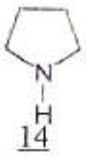
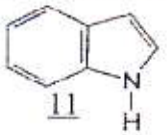
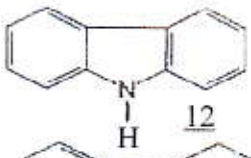
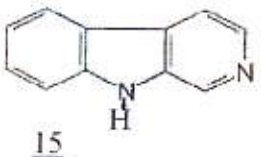
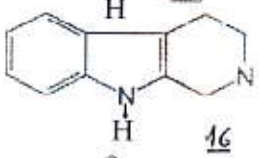
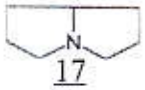
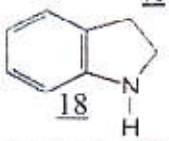
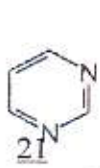
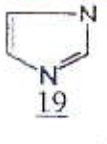
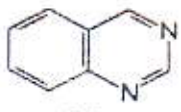


Fig 7. caffeine

Table №1 illustrates the different families of alkaloids.

## Table№1: Principale vegetable alkaloids

| Basic structure | Derivatives |
|---|---|
|  1<br>  5 |  2<br>  3<br>  4<br>  6<br>  7<br>  8<br>  9 |
|  10<br>  13<br>  14 |  11<br>  12<br>  15<br>  16<br>  17<br>  18 |
|  21<br>  19<br>  22 |  20 |

1- pyridine, 2- pyridocaline, 3- quinoleine, 4- isoquinoleine, 5- piperofine, 6- quinolizidine, 7- tetrahydroisoqinoleine, 8- tropane, 9- benzilisoquinolrine, 10- pyrrole, 11- indole, 12- cardozole, 13- pyrroline, 14- pyrroldine, 15- b-carbazoline, 16- tetrahydro b-carbazoline, 17- pyrrolizidine, 18- dihyroindole, 19-imidazoline, 20- quinazoline, 21- pyrimisine, 22 purine.

## I-1-3-Medicinal Action and Uses:

The plants with alkaloids have a considerable importance in herbal medicine. Their physiological actions are varied. Some act on the central nervous system they depress or stimulate, Whereas others act on the autonomous nervous system like sympathomimetic or sympatholytic[3]. There also exist curarizing, antitumor and antipaludic alkaloids [5].

The majority of the cases, they act with low doses, but can have even a strong toxicity with this weak dose [3].

## I-2-Flavonoids:

## I-2-1-General information:

The polyphenols constitute one of the principal classes of secondary metabolism of the plants [6]. Flavonoids are the most important class, this group of phenolic substances is regarded to be the richest class of natural compounds which involves an oxygenated heterocycle [1,8].

Since 1964, several studies have been carried out on the biosynthesis of the flavonoids [11]which showed that the flavonoids especially are abundant and are also diversified at the higher plants [8]. They have a maximum content in the young oranges (leaves, flowers, fruits)[5] and they are responsible for their coloring [10].

## I-2-2-Classification and structure:

The flavonoids have a basic skeleton with fifteen carbon atoms made up of two cycles in $C_6$ connected by a chain in C3 [10]

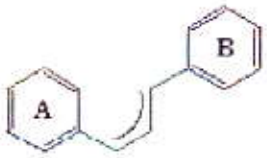



Fig 8.structure of flavonoid

They can be classified as follows [20]:

1 / 2-phenyl benzopyriliums, anthocyans.

2 / Phenyl chromane.

I) Flavones, flavonol and their derivatives.

II) Flavanones and Dihydroflavonole

III) Isoflavanones-isoflavones

3 / 2-phenylechromane

I) Flavane

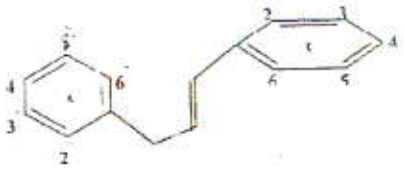
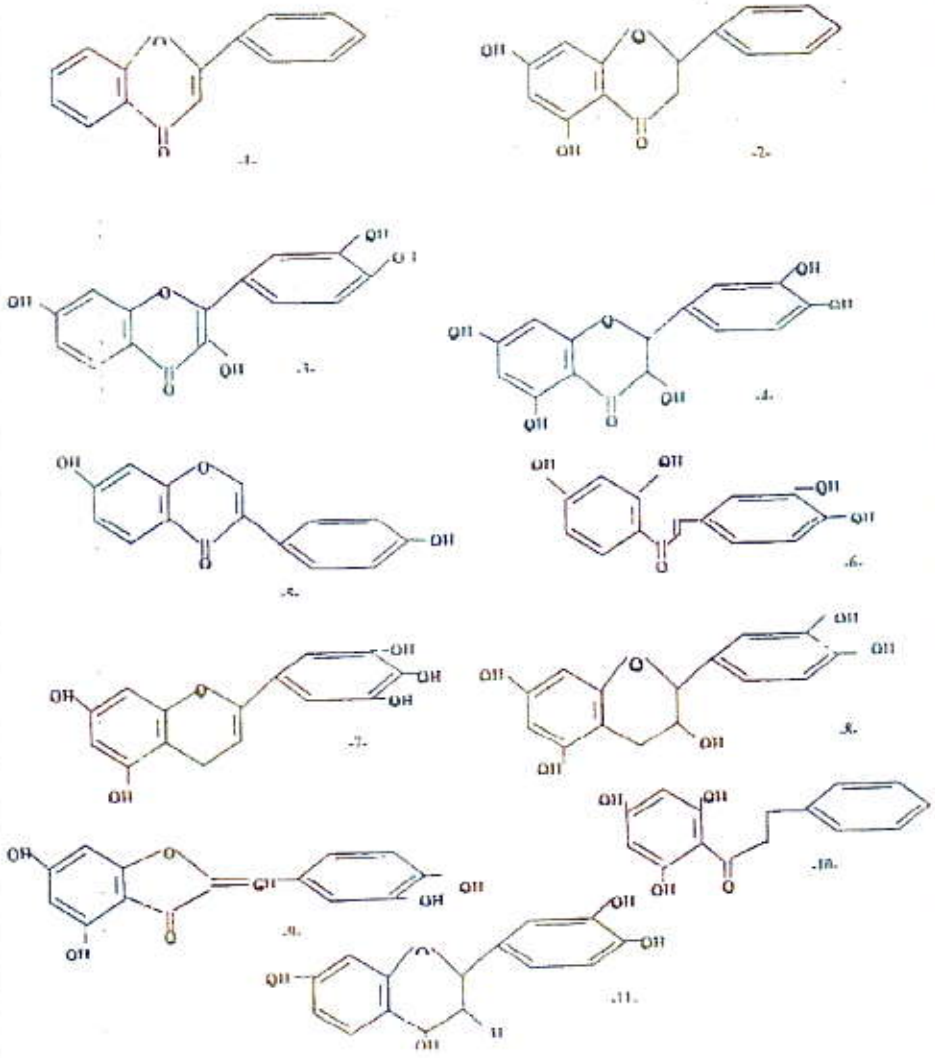
II) Flavan-3-ols, flavan-3, 4-diols

4) Chalcone and dihydrochalcone

5) Benzylidene coumaramones (aurones)

Table№2 illustrates the different families of flavonoids

## Table №2: Different families of flavonoids

| | |
|---|---|
| **Basic skeleton** |  |
| **Derivatives** |  |

-1 flavone, -2 flavonone, -3 flavonal, -4 flavanonol, -5 isoflavone,- 6 chalcone, -7 anthocyanidin, -8 catchine, -9 aurone, -10 dihyrchalcone, -11 flavan-3,4 diol.

## I-2-3-Medicinal Action and Uses:

It is essentially in the capillaro-venous field, where falvonoids are active. They have the usual components of vasculoprotectors, veinotonics and that used in phlebology [5].

Flavonoids are supposed to act as :

- Treatment of the symptoms, which connected with the veinolymphatic insufficiency (heavy legs, pains).
- Treatment of the functional signs related to the haemorrhoids crisis.
- Improvement of fragility capillary troubles on the level of the skin.
- Treatment of the metrorragies during contraception [5].

## I-3-The tannins

## I-3-1-General information:

Actually, it is designated by tannin an heterogeneous group of vegetable phenolic derivatives [9]. This name involves a group of compounds which have certain common properties, but which inevitably do not have analogies of structure [12].

In 1796, Seguin introduced the term tannin [13] in order to designate the pentagallate which constitutes the tannin tea and especially of nut all [14].The tannins are distributed at the higher plants found in the Leaves, the vascular tissues, seeds envelope and stems [9].

The tannins are water soluble. Their molecular weights range between 500 to 3000 [12]. They also have the property to precipitate alkaloids and other proteins [9,12].

## I-3-2-Classification and structure:

It has been usually distinguished between two groups of tannins They are [1]

- Hydrolysable tannins (pyrogallic).
- No hydrolysable tannins (condensed).

**1. Hydrolysable tannins (pyrogallic):**

They are polyesters of glucides and phenolic acids, they are classified according to their nature :

a/ Gallic tannins (gallo-tannins) relating to the gallic acid

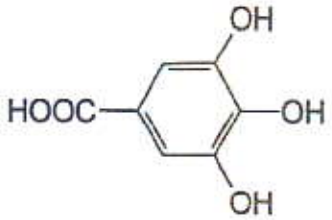



Fig 9. Gallic acid

b/ Ellagic tannins (ellago-tannins) relating to the ellagic acid.

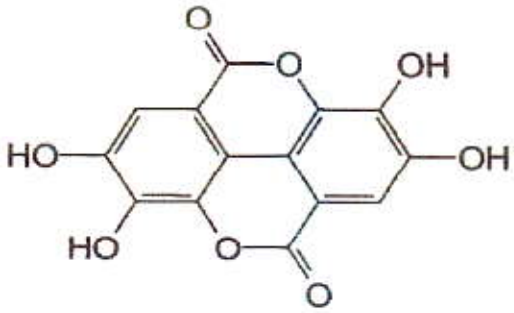


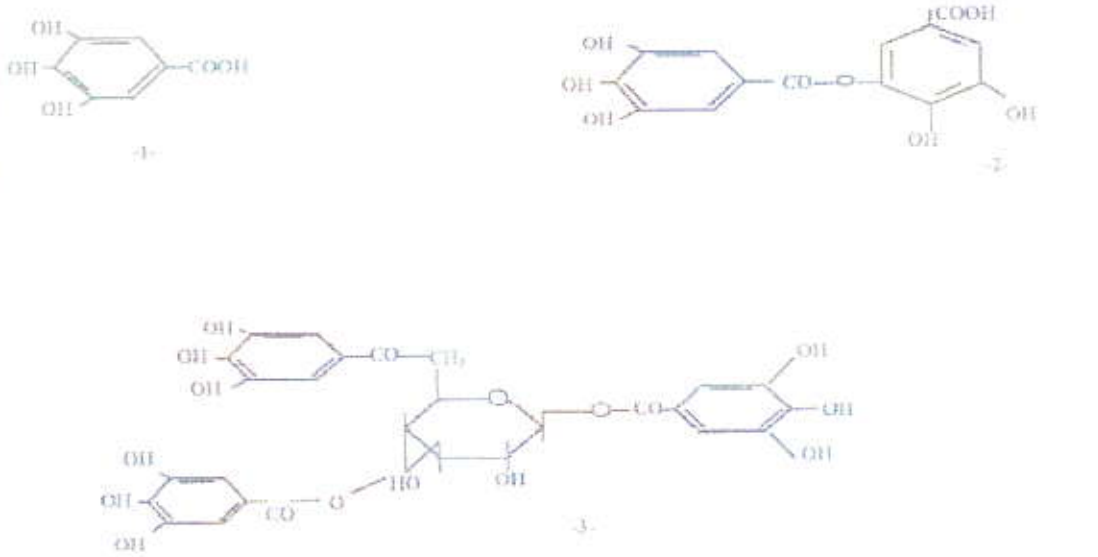
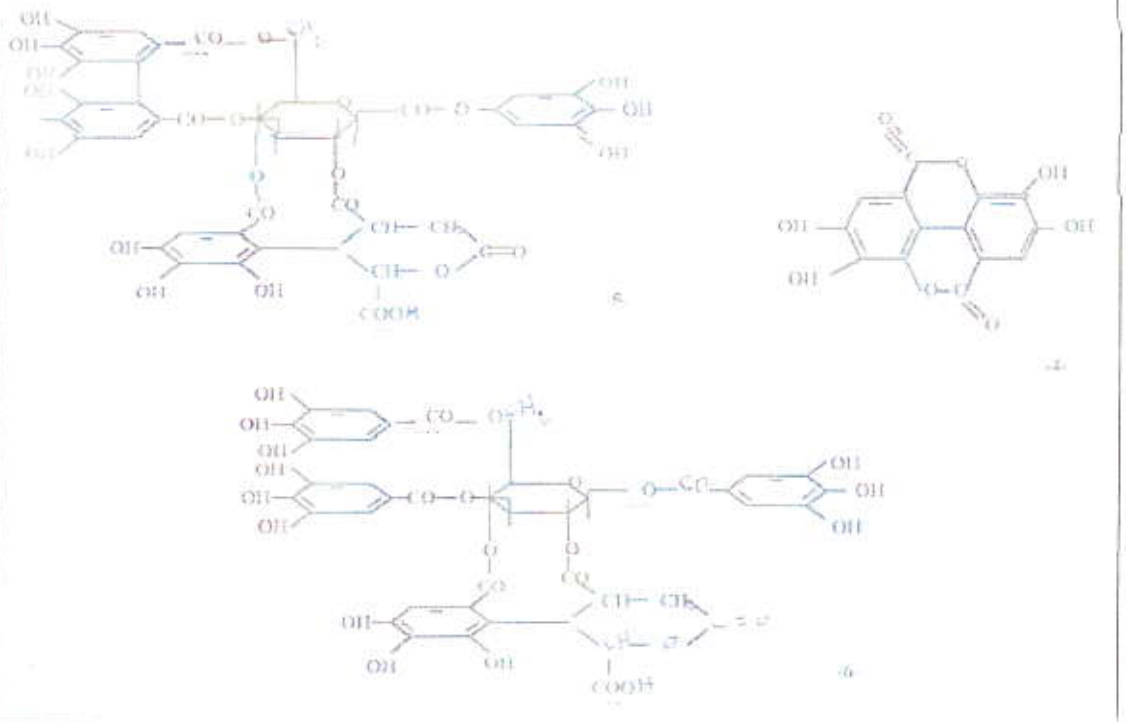

Fig 10. Ellagic acid

**2. No hydrolysable tannins (condensed):**

They are basically different from the first groups. Their structure is close to those of the falvonoid, they do not have sugars in their molecules and could be condensed and hence they have a tendency to be polymerized [1].

Table №3 illustrates important structures of tannins.

**Table №3 : Important structures of tannins**

| Hydrolysable tannins | **Structure** |
|---|---|
| Gallo-tannins |  |
| Ellago-tannins |  |

1- gallic acid, 2- m-digallic acid, 3- trigalloyl-1,3,6 glucose, 4- ellagic acid, 5- chebulagic acid, 6- chebulinic acid.

## I-3-3- Medicinal Action and Uses:

The tannins have a diversity of uses outlined as follows:

- Make the most external layers of the skin and mucous membranes impermeable.
- They have a vasoconstrictor effect on the small vessels surface, In addition, they support the fabrics regeneration in the case of Surface wounds or burns.
- They have an antidiarrheic, antiseptic and antifungic effects[5].

In addition to their roles in the medical field, the tannins have commercial uses and the world annual production is about 500.000 tons. They play an important role in the oil field [5].

## I-4-Saponosids:

## I-4-1-General information:

These substances are ordinarily surface-actives compounds that have the capacity to form colloidal solutions and to generate foam in contact with water like soaps [13] and hence the name saponosids, which means in Latin soap.

## I-4-2-Classification and structure:

From a structural viewpoint, the saponosids are terpenic glycosides [15] whose genuine is named sapogenin [1].

According to this genuine the saponosids are classified in two groups:

- Saponosids with steroidic genuine.
- Saponosids with triterpenic genuine.

**1. Saponosids with steroidic genuine:**

This group has a whole skeleton with 27 carbon atoms, which contains usually six cycles [5].

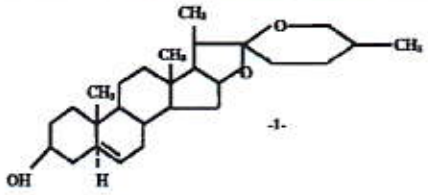
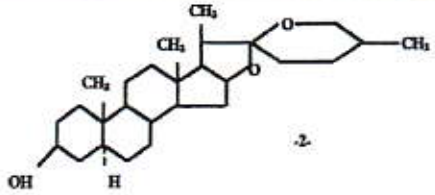
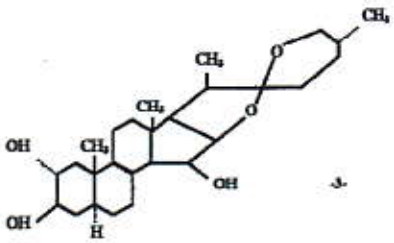
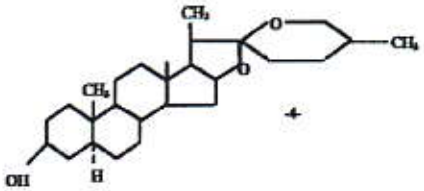
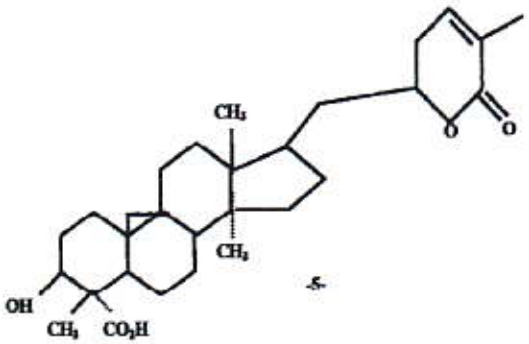
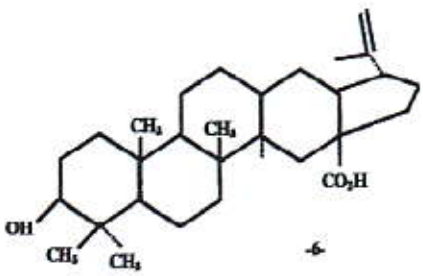
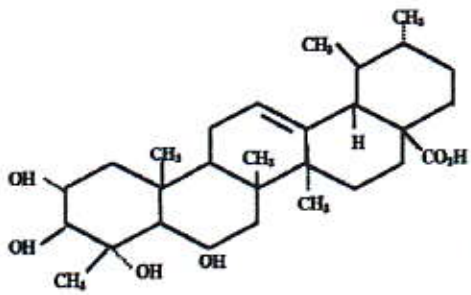
**2.Saponosids with triterpenic genuine**:

This group represents the great majority of vegetable saponins [15]. They are pentacyclic molecules more rarely tetracyclic[15,5].

There is a third category of saponosids, those of heterosides of steroidic amines, which for other authors are considered as pseudo-alkaloids because of their behavior [5].

Table №4 illustrates the principal skeletons of the steroidic and triterpenic genuines of saponosids.

**Table№4: principal skeletons of the steroidic and triterpenic genuines of saponosids.**

| Saponosids | Structure |
|---|---|
| **Steroidic Saponosids** |     |
| **Triterpenic Saponosids** |    |

1-yamoginine –2- tigogenine –4- similagenine –5- abrussogenine –6- betulimign acid –7- madecassic acid

## I-4-3-Medicinal Action and Uses:

Several drugs with saponosids have a great medicinal effect such as anti-inflammatory. In the case of the liquorice it has a gastric anti-ulcerous activity.

Drugs with saponosids are also used in phlebology and proctology for their vasculoprotectress properties and veinotonic effect as well as for their interest for the improvement of the functional symptomatolology of the venous insufficiency and the haemorrhoids crisis [5].

## I-5-Terpenes:

## I-5-1-General information:

The terpenes, otherwise known, as terpenoids are cyclic or acyclic hydrocarbons, derived from the isoprene [7]. They are secondary metabolites and their precursor is the mevalonic acid [8,7].

It was Kekule who gave the name of terpene to hydrocarbons contained in the essential gasoline or oils [7].

Around 20.000 terpenoids were isolated from the plants at present, and characterized by their volatility and their intense prickly odor [9].

## I-5-2-Classification and structure:

All terpenes may be broken up formally into units of isoprene that leads to polymerizations:

**1 / Monoterpenes:**

They comprise two units $C_5H_8$; they do not have a known biochemical Function but they have a biological function in nature by their strong Odor [16].

**2 /Sesquiterpenes:**

They are a combination of three units of isoprene, they group:

- The linear sesquiterpenes, which are largely, frequent in the plants [12].
- The cyclic sesquiterpenes whose monocyclic and bicyclic compounds are more frequent [12].

**3 /Diterpenes:**

They are a combination of four units $C_5H_8$. Phytole is one of acyclic terpenes which constitute the base of chlorophyll [2,12].

**4 /Triterpenes:**

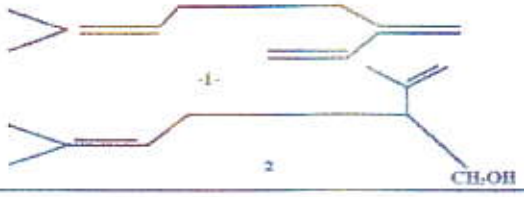
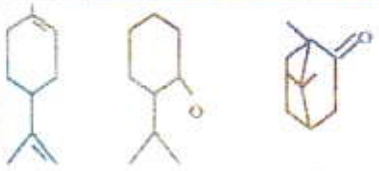
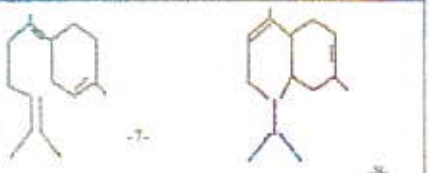
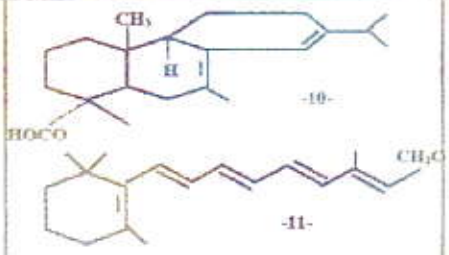
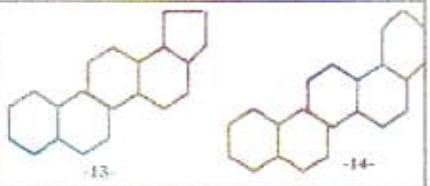
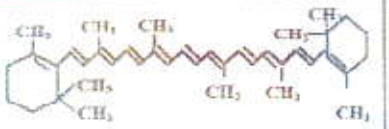
They are a combination of six units $C_5H_8$, they include several groups of substances and many important compounds in the biological field

**5 /Tetraterpenes:**

The most important biological compounds of this group are the carotenoids and the carotenoic acids; Their cyclic and acyclic derivatives contain eight units $C_5H_8$.

Table№5 illustrates types of terpenes in the plants.

## Table№5: Different types of terpenes in the vegetable reign

| | | |
|---|---|---|
| Isoprene |  | |
| Terpenes | | |
| Monoterpenes |  |  |
| Sesqui-terpenes |  |  |
| Diterpene |  |  |
| Triterpenes |  |  |
| Tetraterpenes |  |  |

1- myrcene, 2- lavandula, 3- limonene, 4- menthol, 5- comphre, 6-farnesol, 7-zingiberene, 8- cardinene, 9- phytol, 10- obetic acid, 11- vitamin A, 12- squaline, 13-lupeol, 14- anyrine, 15-lycopene, 16- B carotene.

## I-5-3-Medicinal Action and Uses:

Several drugs with terpenes are employed in several fields:

- Drugs of monoterpenes are used as antispasmodic and hypoallergenic. They enter in the formulation of recommended regulations in the treatment of atopic eczema and that of sapoings.

- Drugs of diterpenes exert a positive inotrope action on the myocardium and by decreasing vascular and peripheral resistance, have anti-hypertensive action [5].

## I-6-Sterols:

## I-6-1-General information:

It pointed out under sterol designation every group of solid substance comprising in their molecule one or more hydroxylated functions [17].

In 1908, Tanret discovered the ergosterol. The oldest vegetable sterol was isolated from the rye pin [18,19].

Several sterols were defined after the ergosterol such as the stigma sterols Isolated from broad beans of Calabar. The sitosterol, which seemed to be in the higher plants and other sterols, which are badly known like brassicastérol [18].

## I-6-2-Classification and structure:

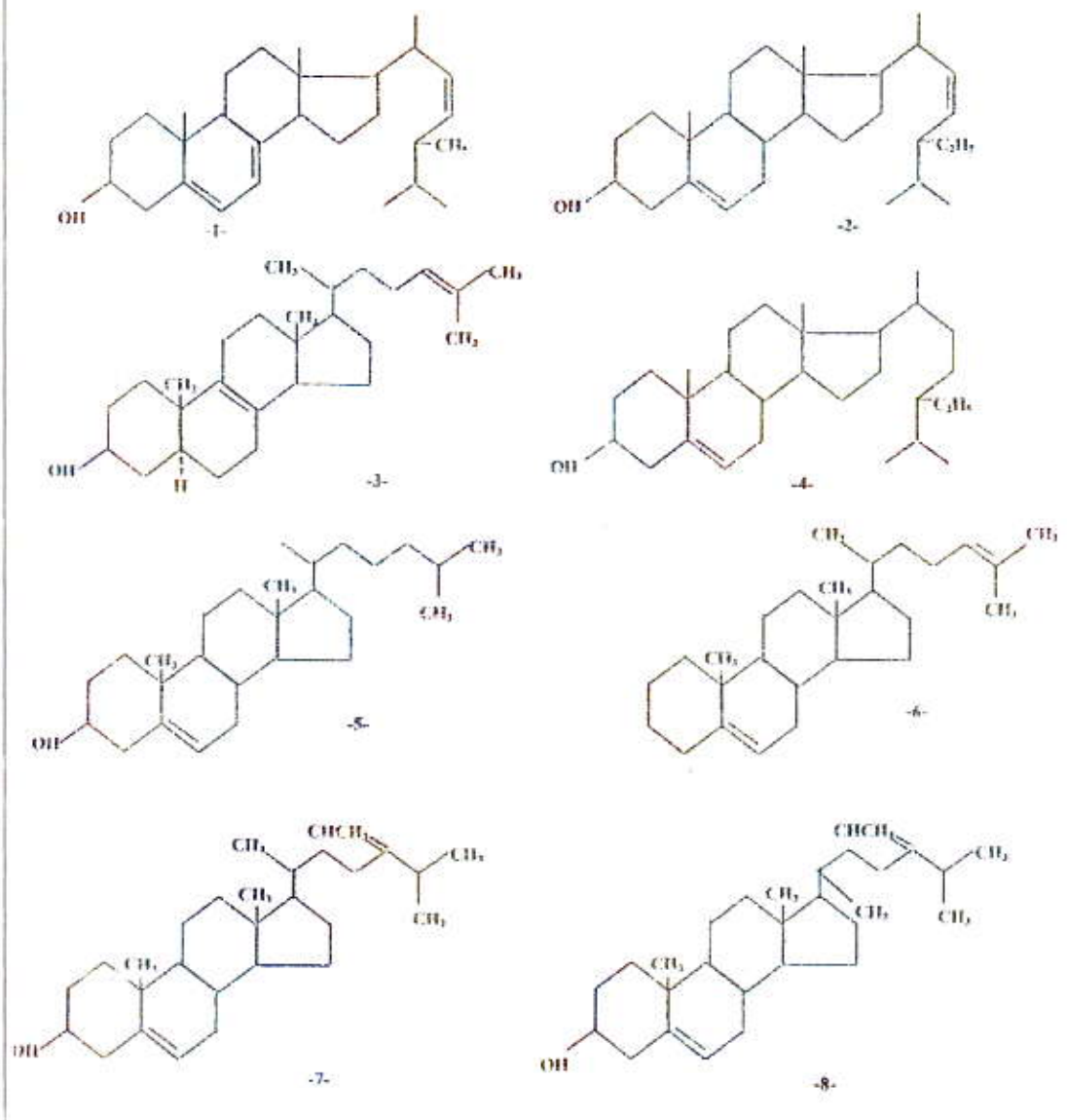
Sterols of vegetable origin are not pretty known, among those:

- The ergosterols which has as a crude formula $C_{28}H_{44}$, it has three double bonds, it gives all the colored reactions of sterols [18].

- The stigmasterols, whose structure was elucidated by Femholz and Guiteras.
- The sitosterol: in Greek language sitos means grain is the equivalent of cholesterols in animals [18].
- The cholesterol, which was isolated from (red algae) by Isuda and al. in 1958 and from tomato by Johnson and al. in 1963 [18].

Table №6 illustrates principal vegetable sterols.

## Table №6: The principal vegetable sterols

| Vegetable sterols |
| --- |
|  |

-1- ergosterol, 2- stigmasterol, 3- aymosterol, 4- sitosterol,

5-cholesterol, 6-desmosterol, 7-fucosterol, 8- sargosterol.

## I-6-3-Medicinal Action and Uses:

Sterols involve a wide range of therapeutic effects including the antitumor properties. They increase the capacity of T-cells to divide, thus increase the number of T-cells in our defenses. Sterols also help to regulate the imbalance of B-cells, starting a disorder antoimmum dysfunctional to function normally.

They stimulate the proliferation of T-cells, reduce the cholesterol LDL And remove coristol secretion by adrenalin gland [32].

## I-7- The cardiotonic glycosides:

## I-7-1-General information:

The cardiotonic glycosides are heterosides, Whose structure is very similar to that of saponosids [12]. These compounds have an action on the work and rhythm of cardiac muscle [21].

The cardiotonics glycosides have enough restricted distribution in the plants, whereas they are accompanied by saponins in many plants families, particularly in Digitalis and the strophanthus [11].

## I-7-2-Classification and structure:

From a structural viewpoint, these compounds are glyco-Stéroides because of the structure that is slightly different from the aglycone [1].
Two types of this group are distinguished below:

- Cardenolides
- Bufadienolides

**1 /Cardenolides:**

They have 23 carbon atoms and a lactone cycle with 5 chains in Position C17 carrying only one unsaturated bond [1].

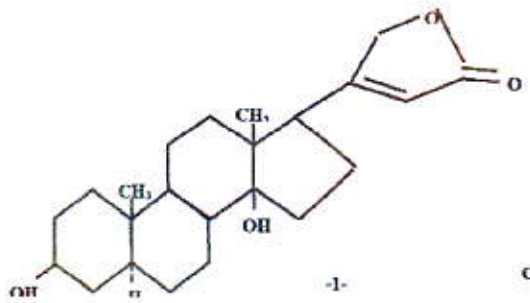
**2 /bufadienolides:**

They have 24 carbon atoms and a lactone cycle with 6 chains Which carries two double unsaturated bonds on C17 [12,1].

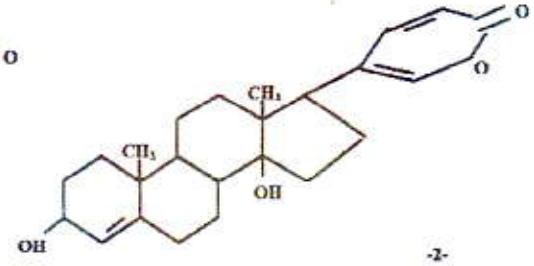
Table №7 illustrates the principal skeletons of the glycosides cardiotonic.

## Table №7: principal skeletons of the glycosides cardiotonic

Genuine

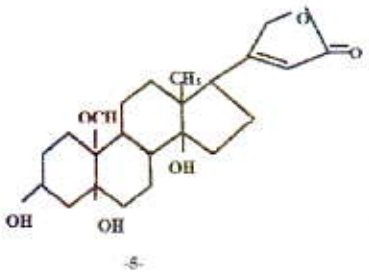


-1-

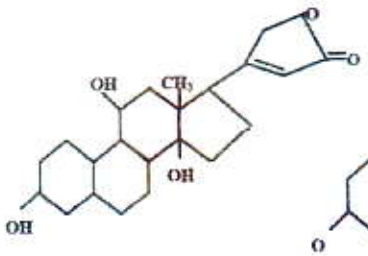


-2-

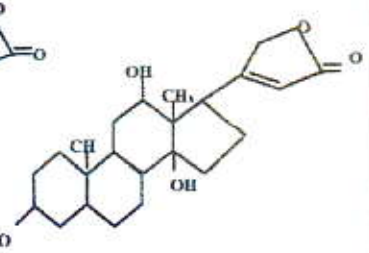
Derivatives



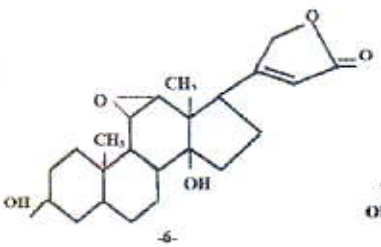
-5-



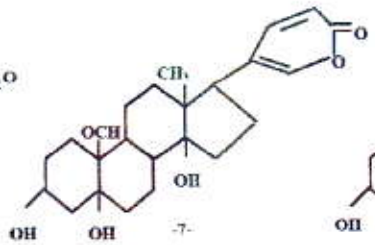
-3-



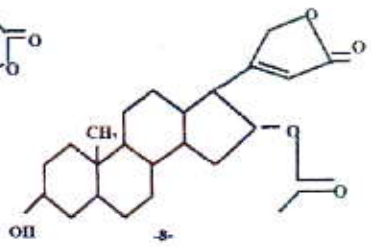
-4-



-6-



-7-



-8-

1- cadenolide, 2- bufadienolides, 3- sarmentogenine, 4-digoxigenine, 5-cymarigenine 6-cerbertigenine, 7- hellebrigenine, 8- oleandrigenine.

## I-7-3-Medicinal Action and Uses:

At present, the cardiotonic glycosides are used for the following cases:

- The cardiac insufficiency with flow beats, generally in association with the diuretic one, in particular when there is an auricular fibrilation [5].
- The troubles of supra ventricular rate, deceleration or reduction of auricular fibrillation [5].

However, counter indications were noted because of the use of these Substances such as the ventricular hyperexcitability and the Auriculo-ventricular blocks.

# chapter II

# Monography

**Fig.11.** ***Ephedra alata***

# *Ephedra Alata(Dec. )*

## Nomenclature:

**Vernaculaire name** : Alanda [23,40].
**French name** : ephédres[24]

## 1-Taxonomy:

| | |
|---|---|
| reign | : eucaryote vegetable [25,26] |
| Sub reign | : cormophyte[25,26] |
| Embranchement | : spermaphyte [25,26] |
| Sub Embranchement | : chlamydosperme [25,26] |
| Class | : saccovuleae[26] |
| Order | : gnetal[26] |
| Family | : epheddraceae [27,26,40], gnetaceae[23] |
| Genus | : ephedra [28,27,26,40,23]. |
| Species | : Alata [28,27,26,40,23] |

## 2-Botanical Description:

Shrubs with joined rowers, carrying on the level of nodes a small opposed leaves, alternating from one node to another; flowers in small cones, males and females generally on different feet, the cones females with bracts growing during maturation. [28].

## 3-Biogeography:

Common in all Western and Northern Sahara and the Sandian Sahara [28].

## 4-Therapeutic uses:

Ephedra Alata is used in the cases of cold, flux and respiratory diseases. Its instructions for use require a maceration or mixture [29].

**Fig.12. *Launaea resedifolia***

# *Launaea resedifolia (L)*

## Nomenclature:

**Vernacular name:** adaid.[28,30]

## 1-Taxonomy:

| | |
|---|---|
| Sub Junction | : angiosperm [25,26,40] |
| Reign | : eucaryote vegetable [25,26] |
| Sub reign | : cormophytes [25,26] |
| Junction | : spermaphyte [25,26,40] |
| Class | : dicotyledon [25,26,40] |
| Sub class | :gamopetale [26] |
| Series | : inférovarieae tetracyclic [26] |
| Order | : asterale [26], synantherale [25,38]. |
| Family | : composeteae[25,28,26,38,40,30]. |
| Sub family | : liguliflorous [40] |
| Genus | : launaea [28,40,30] |
| Species | : resedifolia [28,40,30]. |

## 2-Botanical Description:

Robust plant, clambering with stems very glabrous rowers, culinary leaves not embracing, lower cut out in linear lobes. Ovoid or subcylindric flowerheads, lengthened. Akenes of 4-7 mm, smooth or velvety, with dimension prolonged at the base in 4 acute spurs [29].

## 3-Biogeography:

Common in the Sahara northern and central Medit [29].

## 4-Therapeutic uses:

The only indication of this plant is in the case of scorpion puncture , the patient chews some leaves of launaea resedifolia and swallows the juice[29].

**Fig.13.** ***Oudneya africana***

# *Oudneya africana* (R.Br)

## Nomenclature:

**Vernacular name:** hannet elbel [24]

## 1-Taxonomy:

| | |
|---|---|
| Reign | : eucaryote vegetable [25,26] |
| Sub reign | : cormophytes [25,26] |
| Junction | : spermaphyte [25,26] |
| Sub Junction | : angiosperme [25,26] |
| Class | : dicotyledones [25,26] |
| Sub class | : dialypetale [25,26] |
| Series | : thalamieflore [26], liliflore [25] |
| Order | : parietal [26] |
| Sub order | : rhoedale [26] |
| Family | : crucifere [23,26,27,31,41]. |
| Genus | : oudneya[23,27, 28, 31]. |
| Species | : africana [23,27,28,31]. |

## 2-Botanical description:

Shrub very branching whole leaves, fleshy spatulates. Flowers in short bunches. purple rose, large enough 10 - 25 mm [27]. cylindrical and narrow fruits containing 12-20 winged seeds inserted on two rows.

## 3-Biogeography:

Common in the Northern Sahara, OUED M'zab, El Golea, Ouargla, Biskra and Tunisian South [28], and also in gypseous and rocky grounds [31].

## 4-Therapeutic uses:

**Skin disease:** *Oudneya africana* is mixed with *Lawsonia Inermis*, the whole is reduced to very fine powder in moistening with water in order to make a paste, applying to the feet and hands [29].

# chapter III

# *Biological activity*

## I- Antimicrobial Activity:

The protocol used for antibacterial analysis is as follows:

The bacterial culture medium is MUELLER-Hinton's agar, the antimicrobial activity tests have been realized in the CHU Constantine's bacteriology laboratory. The bacterial stubs come from the same laboratory. For the current tests, Nine stubs are chosen:

*Escherichia coli, staphylococcus aureus , Proteus mirabilis, Serratia sp Enterobacter sp, Pseudomonas aerogenosa, Proteus vilgaris, Staphylococcus aureus, Sanetta sp and shigella flexneri.* By lack of availability of sufficient plants extracts quantities to carry out well inhibition zones test as the MIC, two limit concentrations were selected 1 mg/ml and 10 mg/ml so as to valorize the possible antimicrobial activity of the three used plants extracts, *Oudneya africana*, *Launaea resedifolia* and *Ephedra alata.* [36].

## II-Measurement Methods of Antimicrobial Activity:

The antimicrobial activity of the plants is detected by the development degree observation of the varied microorganisms which the plant extracts put in contact with.

Three methods of antimicrobic activity detection are available, namely: diffusion, dilution and bioautography method. These methods are used just to have an idea about the presence or the absence of substances having an antimicrobic activity in the extract, the potential of the active compounds is given only on pure compounds using a standards methodology[36].

### II-1-Diffusion Method:

It is called also the disc-diffusion method, or discs method, or paper disc method. This method is most suitable for the sifting of fungicides, as well as for other antimicrobic agents. Indeed, the Agar diffusion method is used in the antifungal sifting; it has been also used to measure the antimicrobial activity .

In diffusion technique, discs with paper (5 to 7 mm of diameter) are impregnated by plants extracts and deposited on the agar-agar medium surface that is contained in Petri dishes. After incubation from 2 to 4 days at a suitable temperature (25 to 40 °C), the diameter of the clear zone around the disc (inhibition diameter) is measured[36] .

This method has several advantages:

- It is fast, and gives excellent results for the current practice [11].
- Several antifungal compounds were discovered via this Method.
- The tiny quantity extract use and the possibility of testing up to six compounds by petri dishes against only one micro-organism[36] .

## II-2-Dilution Method:

A series of dilution for raw plant extract is carried out; afterwards, the same protocol as previous is adopted. The advantages of these technique are the followings:

- Allows to evaluate the MIC (minimum inhibitory concentration).
- Allows to carry out curves of tested micro-organism growth.
- Several and different microorganisms are tested simultaneously in the same dilution.
- It is very appreciated to test pure samples, in particular when a high degree of sensitivity is recommended[36] .

## II-3-Bioautography

The bioautography, as a method of bacterial activity localization in a chromatogram, is largely applied in the searching field for new antibiotics starting from the microorganisms. This method has been implemented to mitigate the problems due to the differential diffusion starting from chromatogram towards dish of Agar by direct autobiographical detection on the chromatographic layer. However this method requires more

complex microbiological equipment in spite of the facility with what this method makes to test highly active antibiotics. It is not promising for plants extracts tests which generally contain antimicrobial agents much less active in comparison with available antibiotics [36].

# chapter IV
# Experimental part

# I-Materials:

## I-1-Equipment:

1. Standard drying oven Memmert.
2. Grinder of the karlkolb type.
3. Magnetic Agitator standard compound Gallenthamp.
4. Balance of the Sartorius type of precision 0.001 g.

## I-2-Reagents:

### Acids:

| | |
|---|---|
| HCl | chlorohydric Acid. |
| $H_2SO_4$ | sulphuric acid |
| $CH_3COOH$ | acetic Acid |

### Alcohols:

| | |
|---|---|
| $CH_3OH$ | methanol |
| $C_2H_5OH$ | ethanol |

Amylic alcohol

### Bases:

| | |
|---|---|
| $NH_3$ | ammonia |
| $NH_4OH$ | ammonia Salts: |

| | |
|---|---|
| $FeCl_3$ | iron chloride |
| $HgCl_2$ | mercury chloride |
| KI | potassium iodure |

### Organic Solvants:

| | |
|---|---|
| $CHCl_3$ | chloroform |

Ether of oil

### Reagent of Mayer:

Solution A: 13.55 g of $HgCl_2$ dissolved in 20 ml of $H_2O$.
Solution B: 49.80 g of dissolved KI in 20 ml of $H_2O$ .
The two solutions are mixed and diluted with distilled water up to 1 liter.

## II-Sampling:

### 1-Picking of Plants:

When the plants are used for their medicinal properties, it is necessary to understand that their active principles are often volatile and their freshness plays an important role [33].

The best period of picking the plants is carried out at the beginning of afternoon in dry and sunny weather when their active principles reach their maximum [34].

### 2-Drying of Plants:

The medicinal plants have been generally dried before their use. The drying of our plants is carried out under shade in open air during a week.

### 3-Powdering of Plants:

The Powdering of our plants have been carried out in a gauges grinder (1 mm).

### 4-Conservation of Plants:

So as to be well protected, our medicinal plants were preserved in containers of well-sealed glass in order to keep their color, odor, taste and mainly their power.

## 5-Picking Area:

Hassi Ben Abdellah is one of the largest municipalities in Northern Ouargla by a distance of 20 Km, it has a surface of 3060 $Km^2$. Its confines are as follows :

Northerly by : N'gousa municipality.

Southerly by : Ain Beida municipality.

Easterly by : Hassi Messaoud and Hidjira municipality.

Westerly by : Sidi Khouiled municipality[35].

According to its special situation it is considered as a small and typical example of ecoregion in Sahara as far as the vegetal cover, animals and weather are concerned. Thus it has been chosen as a sample of picking our plants.

## III-Active Principle Detection:

### 1-Volatil oils:

1. Subject the plant to steam distillation.
2. Remove the condensed water.

**Positive observation:**

-Appearance of a thin oily layer with a pleasant odor.

### 2-Alkaloids:

1. Dissolve 10 g of dry powder in 50 ml of diluted HCl (1 %).
2. Make the extract basic with $NH_3$.
3. Extract the mixture 3 times with 20 ml of $CHCl_3$ .
4. Evaporate the organic phase then dissolve the precipitate in 2 ml of diluted HCl (1 %).
5. Add to the solution 3 drops of the Mayer reagent.

**Positive observation:**

-Appearance of white precipitate.

### 3-Flavonoids:

-Macerate 20 g of dry powder in 150 ml diluted HCl (1 %) during 24 hours.

## Positive observation:

1. Appearance of a clear yellow color in the higher part of test tube.
2. After filtration, return 10 ml of basic filtrate with $NH_4OH$.

## 4-Free Flavonoids:

1. Take 5 ml of previous filtrate.
2. Add 2.5 ml of pentanol.

## Positive observation:

-The alcoholic phase, which is in top of test tube, is coloured in clear yellow.

## 5-Tannins:

1. Extract 10 g from dry powder with an aqueous solution of $C_2H_5OH$ 1%.
2. Filter and test the filtrate with drops of $FeCl_3$ solution.

## Positive observation:

-Appearance of green color.

## 6-Saponosids:

1. Put 2 g of powder at boiling with 80 ml of distilled water.
2. Filter and cool the solution.
3. Stir the filtrate.

## Positive observation:

-Appearance of foam, which lasts a few moments.

## 7-Cardenolides:

1. Macerate 1 g of dry powder in 20 ml of distilled water and filter it.
2. Take 10 ml of filtrate and extract it with a mixture of 10 ml of ($HCl_3$, $C_2H_5OH$).
3. Evaporate the organic phase and dissolve the precipitate in 3 ml of icy $CH_3COOH$.
4. Add some drops of $FeCl_3$ followed by 1 ml of $H_2SO_4$ concentrated on the walls of test tube with much of attention.

## Positive observation:

-Appearance of a green-blue color in the acid phase.

## 8-Sterols and triterpenes:

1. Dissolve 5 g of dry powder in 20 ml of oil ether, and filter.
2. Evaporate till dryness, the residue is dissolved in 0.5 ml of acetic anhydric acid and then in 0.5 ml of $HCl_3$.
3. The solutions are transferred in a test tube then add 1 ml from concentrated $H_2SO_4$.

## Positive observation:

-The formation of a purple or brown circle, then becomes gray in the zone of contact between the two liquids.

## 9-Coumarins:

1. 3 ml of the ether extract are evaporated to dryness, the residue is dissolved in hot water.
2. After cooling, the solution is divided in two tubes : one tube will contain the reference and the aqueous solution of the second tube is made alkaline with 0.5 ml of ammonia solution (10 percent).

## Positive observation:

-The occurrence of an intense fluorescence under UV light indicates the presence of Coumarins and its derivatives.

## IV-The alcohol extract:

The ethanol or methanol extracts from the defatted vegetal products May contain important groups of natural constituents, as for example:

- Polyphenols (tannins, etc....)
- Reducing compounds
- Alkaloid salts
- Polyphenolic glycosides
- Sterol glycosides (cardiotonic saponosids)
- Triterpene glycosides

Very good result may be also obtained by extraction with alcohol (70-30 per cent) [39].

## Plants Extracts:

The extracts of our plants was carried out by the following method :

-According the table mentioned below take the quantities of different organs from each plant.

-For each plant the mixture powder soaked in solution of ethanol and water (70-30 percent).

-After 24 hours, the mixture is taken to be filtered.

-The filtrate is evaporated under vacuum, to obtain our dry extract.

**Table №8: Extract table**

| | *Oudneya africana* | | *Launeae resedifolia* | | *Ephedra alata* | |
|---|---|---|---|---|---|---|
| | B.E | A.E | B.E | A.E | B.E | A.E |
| Roots (g) | 15 | 2.80 | 15 | 2.50 | 15 | 2.20 |
| Stems (g) | 42.5 | 6.30 | 70 | 7.50 | 70 | 6.8 |
| Leaves (g) | 42.5 | 5.5 | / | | / | / |
| Flowers (g) | / | / | 15 | 2.40 | 15 | 2.1 |

B.E : before extraction, A.E: After extraction.

## V-The Antibacterial Method (diffusion method):

The disc of Wattman's sterile paper N 3 (5 mm of diameter) is impregnated 0.1 ml of the methanolic solution then dried at low temperature. The technique has been repeated till all the quantity (0.1 ml) is concentrated in the disc and to obtain a concentration equals 0.1 m g / disc as well. Each extract is tested with the same micro-organism within three repetition to have the most probable average of its activity, then the disc is set down under aseptic conditions on the Inoculated medium surface, containing each specific micro-organism (bacterium or mushroom). After 24 hours of incubation at 37 °C for the bacteria, the positive results are assessed by the measurement of the clear inhibitor zone around paper disc; this zone corresponds to the intrinsic extract activity[36].

# chapter V
# Results
# and
# Discussion

# V-Results:

## V-1-Active principles detection results :

The results of phytochemical tests relating with each active principle foreach plant, are illustrated in the table below.

**Table №9 : Active principles in each plant**

| Active principle | *Oudneya africana* | *Launeae resedefolia* | *Ephedra alata* |
|---|---|---|---|
| **Alkaloids** | + | + | + |
| **Flavonoids** | + | + | + |
| **Free Flavonoids** | - | - | + |
| **Tannins** | + | + | + |
| **saponosids** | + | + | + |
| **cardenolids** | + | + | + |
| **Terpenes** | + | + | + |
| **Sterols** | + | + | + |

The sign ( + ) presence of active principle.
The sign ( - ) absence of the active principle.

## V-1-Bacteria results:

## V-1-1-*Escherichia coli*

**Table №10 : *Escherichia coli* inhibition zone diameter**

| Plant | O.A | | L.R | | EA | |
|---|---|---|---|---|---|---|
| Concentration(mg/ml) | (1) | (10) | (1) | (10) | (1) | (10) |
| Diameter of inhibition zone (mm) | 0 | 5.67 | 0 | 6.66 | 1.82 | 9.63 |


Chart.1. *Escherichia coli* DIZ

## Discussion

According to the chart above it is obvious that *E.alata* is more efficient than *L.resedefolia* and *O.africana* on the activiy of *Escherichia coli* through the diameter of inhibition zone which equals to 9.63mm and 1.82mm at both cases of limit concentration 1mg/ml and10mg/ml.

DIZ : Diameter of inhibition zone
O.A : *Oudneya africana*
L.R : *Launeae resedefolia*
EA : *Ephedra alata*

## *V-1-2-Proteus mirabilis*

**Table №11 : *Proteus mirabilis* inhibition zone diameter**

| Plant | O.A | | L.R | | EA | |
|---|---|---|---|---|---|---|
| Concentration(mg/ml) | (1) | (10) | (1) | (10) | (1) | (10) |
| Diameter of inhibition zone(mm) | 1.21 | 6.48 | 0 | 7.31 | 2.31 | 10.59 |

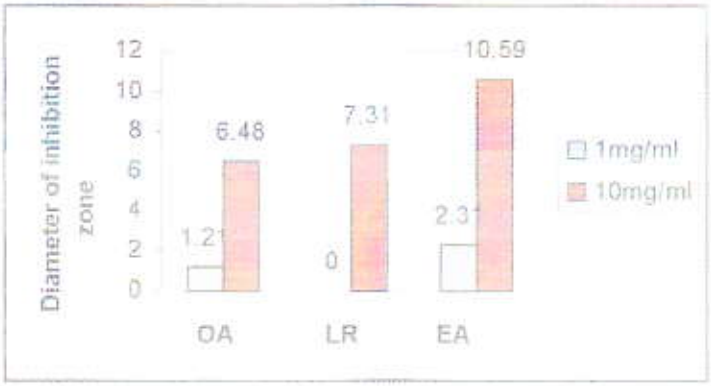



Chart.2. *Proteus mirabilis* DIZ

## Discussion :

Through the chart again, it has been regarded that *E.alata* has the highest inhibition zone diameter. Thus means has a consider effectiveness against *Proteus mirabilis* by 10.59mm, in contrary, to *O.africana* and *L.resedifolia*, which they are less antibacterial effectiveness whose 6.48 mm and 7.31 mm in this case of 10 mg/ml . even in the case of 1 mg/ml.

## V-1-*3-Pseudomonas aerogenosa:*

**Table №12 : *Pseudomonas aerogenosa* inhibition zone diameter**

| Plant | O.A | | L.R | | EA | |
|---|---|---|---|---|---|---|
| Concentration(mg/ml) | (1) | (10) | (1) | (10) | (1) | (10) |
| Diameter of inhibition zone(mm) | 0 | 8.83 | 0 | 3.82 | 0 | 7.31 |


Chart.3. *Pseudomonas aerogenosa* DIZ

## Discussion :

Contrary to the cases above, with *Pseudomonas aerogenosa* the chart shows that *O.africana* has the highest antibacterial effect through its inhibition zone diameter, which equals 8.83 mm meanwhile there is no indication of this activity with 1 mg/ml, for all plants extract .

## *V-1-4-Serratia sp*

**Table №13 : *Serratia sp* inhibition zone diameter**

| Plant | O.A | | L.R | | EA | |
|---|---|---|---|---|---|---|
| Concentration(mg/ml) | (1) | (10) | (1) | (10) | (1) | (10) |
| Diameter of inhibition zone(mm) | 0 | 4.36 | 0 | 4.05 | 0 | 8.11 |

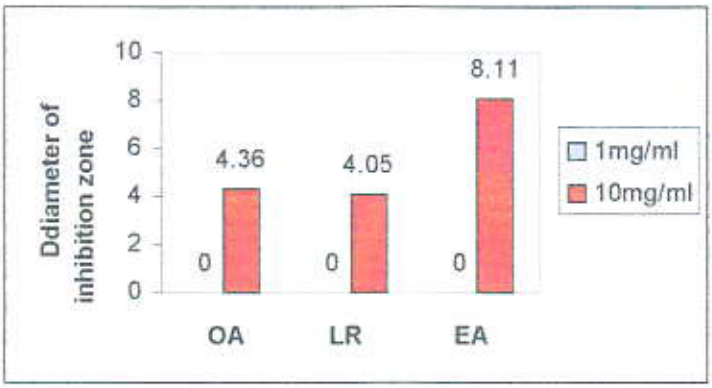



Chart.4. *Serratia sp* DIZ

## Discussion :

Once again the *E.alata* extract test proves that has high effectiveness, here against *Serratia sp.* This effectiveness may get at the sum of both other extracts of *O.africana* and *L.resedifolia.* Through the inhibition zone diameter that equals 8.11 mm. Whereas in the case of 1 mg/ml and likewise the former test, all the extracts have shown no antibacterial activity.

## *V-1-5-Staphylococcus aureus*

**Table №14 : *Staphylococcus aureus* inhibition zone diameter**

| Plant | O.A | | L.R | | EA | |
|---|---|---|---|---|---|---|
| Concentration(mg/ml) | (1) | (10) | (1) | (10) | (1) | (10) |
| Diameter of inhibition zone(mm) | 0 | 8.08 | 0 | 6.36 | 3.02 | 15.32 |


Chart.5. Staphylococcus aureus DIZ

## Discussion :

The obvious deference appears again with *Staphylococcus aureus* through the deference between the inhibition zone diameter values of different plant. Whereas *E.alata* has a diameter equal the sum of both other plants with 15.32 mm , in opposite of O.africana 8.08 mm

And *L.resedefolia* 6.36 mm. while for 1mg/ml the value 3.02 mm is just as additional proof.

## *V-1-6-Sanetta sp:*

**Table №15 : *Sanetta sp* inhibition zone diameter**

| Plant | O.A | | L.R | | EA | |
|---|---|---|---|---|---|---|
| Concentration(mg/ml) | (1) | (10) | (1) | (10) | (1) | (10) |
| Diameter of inhibition zone(mm) | 0 | 2.94 | 0 | 3.26 | 0 | 6.08 |

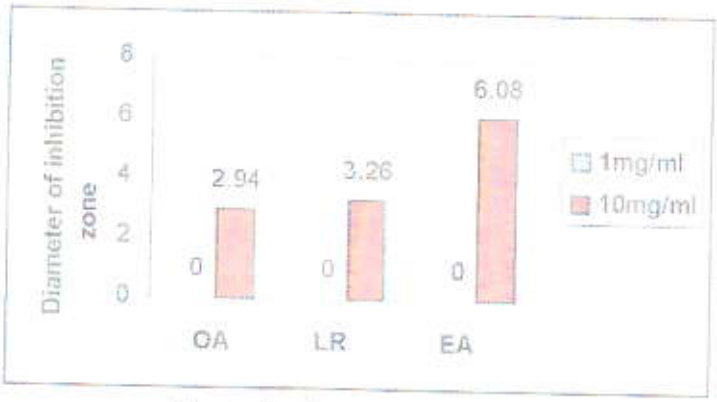



Chart.6. *Sanetta sp* DIZ

## Discussion :

In the current case and with *sanetta.sp* the test has confirmed the effectiveness of *E.alata* with inhibition zone diameter equal 6.08mm as tallest one in opposite to3.26mm for *L.resedefolia* and *O.africana* , meanwhile the plants extracts have not exerted any significant effect.

## *V-1-7-Proteus vulgaris* :

**Table №16 : *Proteus vulgaris*_inhibition zone diameter**

| Plant | O.A | | L.R | | EA | |
|---|---|---|---|---|---|---|
| Concentration(mg/ml) | (1) | (10) | (1) | (10) | (1) | (10) |
| Diameter of inhibition zone(mm) | 7.01 | 11.73 | 0 | 5.09 | 0 | 7.01 |


Chart.7. *Proteus vulgaris* DIZ

## Discussion :

The antibacterial activity in this case due to *O.africana* effect has marked the largest inhibition zone diameter against *Proteus vulgaris* that is Estimated to be 11.73 mm for 10mg/ml while in the second case of 1mg/ml the value 7.01 mm supports the previous results .

## *V-1-8-Shigella flexeri* :

**Table №17 : *Shigella flexeri* inhibition zone diameter**

| Plant | O.A | | L.R | | EA | |
|---|---|---|---|---|---|---|
| Concentration(mg/ml) | (1) | (10) | (1) | (10) | (1) | (10) |
| Diameter of inhibition zone(mm) | 0 | 0 | 0 | 2.25 | 0 | 9.33 |


Chart.8. *Shigella flexeri* DIZ

## Discussion :

Through the last chart , it has been noted that *E.alata* extract has the highest column that equals 9.33 mm in the case of 10 mg/ml , and 2.25mm for *L.resedefolia*, while no indication in the case of 1mg/ml, thus *E.alata* extract is more efficient towards *shigella flexnire* than the rest of plants extracts.

## *V-1-9-Enterobacter sp:*

**Table №18 : *Enterobacter sp* inhibition zone diameter**

| Plant | O.A | | L.R | | EA | |
|---|---|---|---|---|---|---|
| Concentration(mg/ml) | (1) | (10) | (1) | (10) | (1) | (10) |
| Diameter of inhibition zone(mm) | 2.11 | 9.51 | 0 | 0 | 0 | 9.44 |

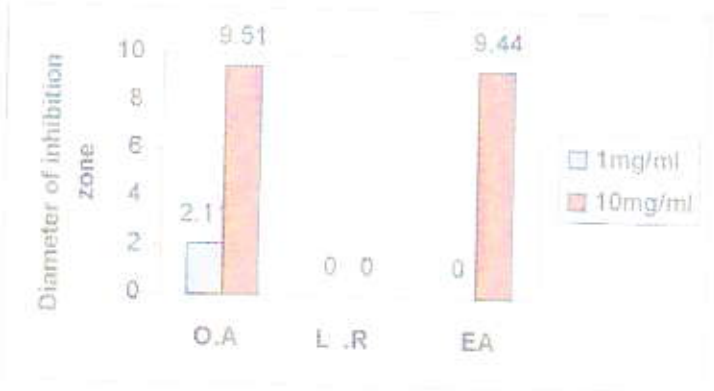



Chart.9. *Enterobacter sp* DIZ

## Discussion :

For the second time and through the chart, *O.africana* has an effectiveness more than *E.alata* and *L.resedefolia* against *Enterobacter sp* in this test, but by slight deference between them nearly estimated as 0.06 mm..

# Conclusion

# Conclusion

In the sight of aforementioned results through charts and tables , and as comparison between different plants : *Ephedra alata, Launeae resedefolia* and *Oudneya africana* , it seems that *Ephedra alata* is rich in most active principles especially with alkaloid which may has a great importance in the self-defense against insects and other bacterial and fungal diseases as already mentioned in theoretical part. *Launeae resedefolia* that has several active principles, whereas *Oudneya africana* shows the least presence of those principles.

In other side and according to the charts where the evolution of antibacterial activity is illustrated , it could be said that *Ephedra alata,* with the highest active principles rates especially alkaloids and flavonoids has a considerable antibacterial effectiveness against the majority of bacteria such as *Escherichia coli*, *Proteus mirabilis*, *Serratia sp*, *Staphylococcus aureus*, *Sanetta sp*, *Shigella flexnire*. And *Oudneya africana* has an effectiveness against the remaining three. Whereas *Launeae resedefolia* has a less effect in comparison with the others.

We do recommend deep studies supported by analytical methods in order to identify the active principles qualitatively and to sort out the active compounds responsible for such activities in different organs of each plant .

Generalization of these tests should be conducted on other kinds of Bacteria so as to confirm the previous results.

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**Abstract:**

Medicinal plants has several uses in different therapeutic fields as antibiotics, therein, three plants has been studied *Ephedra alata*, *Oudneya africana* and *Launaea resedifolia* so as to detect the active principales. The mothod has been followed is the ethanol extracion, followed by antibacterial activity test against nine bacteria : *Escherichia coli, Proteus mirabilis,Pseudomonas aerogenosa, Serratia sp, Staphylococcus aureus, Sanetta sp, Proteus vulgaris, Shigella flexnire, Enterobacter sp.*

As a conclusion and throughout experimental results, it is clear that *Ephedra alata* shows a significant antibacterial activity against these bacteria, in comparison with *Oudneya africana* and *Launaea resedifolia,* that's may due to Ephedra alata richeness in alkaloids.

**Key worde:** ***Medicinal plants, Antibacterial activity, Active principales,*** *Ephedra alata, Oudneya africana and Launaea resedifolia*

**Résumé**

Les plantes médicinales sont utilisées aux plusieurs domaines therapeutiques, comme l'Antibiotique. Pour cela, trois plantes ont été etudées : *Ephedra alata, Oudneya africana* et *Launaea resedifolia. Des* testes des principes actifs et d'activité antibactérienne contre neuf : *Escherichia coli, Proteus mirabilis, Pseudomonas aerogenosa, Serratia sp, Staphylococcus aureus, Sanetta sp, Proteus vulgaris, Shigella flexnire, Enterobacter sp.*

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**Mots clés** : Les plantes médicinal, Activité antibactérienne, Principes actifs, *Ephedra alata, Oudneya africana* et *Launaea resedifolia.*

**ملخص:**

للنباتات الطبية استعمالات في شتى الميادين مثل ميدان المضادات الحيوية، لهذا الغرض تم دراسة ثلاث نباتات طبية: *Ephedra alat*، *Oudneya africana* ، *Launaea resedifolia* بهدف معرفة محتوياتها من المواد الفعالة وكذا تأثيرها على تسعة بكتيريات هي:

*Escherichia coli, Proteus mirabilis, Pseudomonas aerogenosa, Serratia sp, Staphylococcus aureus, Sanetta sp, Proteus vulgaris, Shigella flexnire, Enterobacter sp* .

وكنتيجة لهذه الدراسة وجد أن نبتة Ephedra alata لها تأثير كبير على البكتيريات مقارنة مع النباتات الأخري *Oudneya africana* و *Launaea resedifolia* السبب الذي قد يرجع إلى وجود القلويدات بكمية معتبرة في تلك النبتة.

**الكلمات المفتاحية**: النباتات الطبية، النشاط المضاد للبكتيريا، المواد الفعالة ، *Launaea resedifolia* ، *Oudneya africana* ، *Ephedra alata* .

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